Advancing the utility of palynology: Scope, breadth and novelty of approaches

Julia Webb

A thesis submitted to the University of Gloucestershire in accordance with the requirements of

June 2022

Word count (excluding tables, figures, reference list and papers submitted in this portfolio): 14,661

Abstract

Palynology – the study of pollen and spores – is a multi-disciplinary tool. It has been utilised for many years in: (1) traditional palaeoecology for reconstructing Quaternary and deep-time vegetation communities; (2) within archaeology to trace cultural and agricultural developments; (3) in contemporary ecology to inform management and conservation; (4) in studies of pollination biology and pollen dispersal; and (5) as a tool to determine the location or provenance of items and animals, including in forensic settings. In this thesis, ten published* papers are presented for a retrospective PhD by publication, all of which advance the utility of palynology in different ways. After a general introductory chapter, these ten papers are grouped into four chapters thematically. Firstly, two papers are presented that focus on local scale vegetation reconstructions over the last three millennia. These papers provide information on vegetation history that informs both ongoing archaeological research and contemporary ecological management. Secondly, and moving to a wider spatial scale, two papers are presented where multi-site palaeoecological data are mined to reconstruct the impacts of past global climate perturbations (c.600 and 8,200 years ago). These papers demonstrate the importance of the use of palaeoecology for predicting and managing vegetational responses to human-accelerated climate change events of a similar magnitude. Thirdly, to advance the use of palynology to inform contemporary ecology, an overarching review paper is presented on the range of ways that palaeoecology can benefit applied neoecology (including non-native management, in-situ conservation, reintroduction and rewilding). This is then followed by two papers that use palaeoecological data to test general ecological theory. The final group of papers demonstrates the application of palynology in tracing the location and provenance of subjects within forensic settings and ecological contexts. This thesis thus showcases the considerable scope and breadth of palynology as a research tool and highlights novel ways that it has been applied by the candidate to make important contributions to the fields of palaeoecology, archaeology, contemporary ecology and forensics.

* One paper is in review with *Community Ecology* (submitted April 2022)

Author declaration

I declare that the work in this thesis was carried out in accordance with the regulations of the University of Gloucestershire and is original except where indicated by specific reference in the text. No part of the thesis has been submitted as part of any other academic award. The thesis has not been presented to any other education institution in the United Kingdom or overseas.

Any views expressed in the thesis are those of the author and in no way represent those of the University.

Signed … Date ……01.06.2022……………………

doi: 10.46289/CC1483TE

Contents

List of figures

List of tables

Acknowledgements

Firstly, I'd like to thank Dr Mike Fowler (University of Portsmouth) for planting the seed many moons ago now, that a PhD by retrospective publication was possible. Secondly, my family need a medal in recognition of their support for my PhD. Particularly given the somewhat odd practice of me moving out of the family home and living in a caravan in a field (my "writing retreat"!) for several weeks while the overarching chapters were completed. My husband, Charlie, has risen to the extra challenges that two small boys bring when solo parenting, particularly when illness hits. He is my hero, and I will always be grateful for his unwavering support. Thirdly, thanks to my work colleagues who have enabled this PhD completion by juggling timetables, picking up extra tasks and holding the fort while I took myself away to write up. Completing research in teaching-focussed Universities will always need flexibility and resourcefulness, and colleagues deserve thanks for collaborating to reach this goal for me. Fourthly, my supervisors Prof. Anne Goodenough and Dr Lucy Clarke. They have been inspirational throughout this process, and I cannot express my gratitude enough. Finally, I would like to acknowledge and thank my external examiners, Prof. Chris Hunt and Dr Eline Van Asperen not only for agreeing to examine, but also for accepting the tight timescales associated with this thesis submission and viva. I am very grateful for them fitting it in between their own restrictions on time.

1. Brief foreword: Biography

I felt that this PhD by publication would benefit from a short foreword to allow the reader to understand how this portfolio of ten papers combine with my academic journey to form a cognate set of research publications that meet the professional standards of a doctorate award.

Following the completion of my Masters degree, I joined the University of Gloucestershire as a Research Technician in 2003. I was keen to become involved in laboratory projects and fieldwork linked to many research and consultancy projects that were taking place within the then School of Environment. Many of these early research projects involved me developing methods or undertaking the practical work needed to acquire data. The diversity of skills that I had already gained from undergraduate and postgraduate courses were key to the successful delivery of many of these projects. I was also able to add to my repertoire by learning new techniques that further developed my existing skills. Although direct involvement with writing papers was rare at this early stage in my career, I took every opportunity to read and comment upon draft papers before submission. In this way, I was able to develop my scientific writing skills alongside my empirical work. At this time, I was mainly engaged in work that involved preparing palaeoecological samples for humification analysis, identification and counting of pollen and plant macrofossils, and dating techniques (radiocarbon, Luminescence, lead 210 and Spheroidal Carbonaceous Particle analysis). Other projects involved quantification of dust from landfill sites, analysing particle size of archaeological sediments, and developing novel methods for geochemically fingerprinting sediments. Some of this work is reflected in my earliest publications listed in Table 1.1: Sillasoo et al. (2007) showcases work on the ACCROTELM (Abrupt Climate Changes Across The European Land Mass) project, an EU funded multi-disciplinary and multi-institution research venture; Fowler et al. (2010) investigates the geochemical signature of dust from a hazardous waste site in the UK; Pirrie et al. (2009) reflects on a novel technique for testing the similarity of sediment samples in forensic settings. Note my maiden name (Newberry) in these early publications.

In 2006, I was recruited as a part-time lecturer to teach practicals on undergraduate programmes in biosciences, geography and environmental science, and I became passionate about teaching students industry-standard techniques. In the laboratory, this included geochemical analyses of flora and sediment, colourimetry of water and soil washes, and simple pH techniques; while on fieldwork this included coring bogs and lake sediments for palaeoecological investigations, using liquid nitrogen to freeze and retrieve river bed samples, and vegetation and non-invasive faunal surveys. This position came with no research activity time but I continued working on projects in my role of Research Technician, which included the development of consultancy in addition to primarily research work in the laboratory.

I was given the opportunity in late 2006 to assist in forensic cases as a consultant to UK and Irish police forces. I worked in conjunction with the well-known forensic botanist Professor Patricia Wiltshire in obtaining palynological evidence from a range of exhibits. I worked at crime scenes, in forensic and pathology laboratories, at mortuaries and police-owned vehicle garages to obtain pollen and spores from soil, sediment splashes on vehicles, clothing, footwear and from within human orifices. As an expert witness I completed witness statements, took responsibility for tracing continuity of exhibits in my possession, and testified in court. This consultancy work spanned 48 operations from 21 police forces, plus two forensic consultancies and the RSPCA. While much of this work could not be published, the experience of being an expert witness became the springboard for four publications (Hart and Webb (2013) and Webb et al. (2018), included within this PhD, as well as Pirrie et al. (2009) and Pirrie et al. (2013) detailed in Table 1.1). The economic downturn of the late 2000s, coupled with the increasing internalisation of police resources, reduced the number of cases that were available and my time spent in the forensic laboratory diminished. This coincided with my teaching contract with the University of Gloucestershire being increased, which came with allocation in my workload for research. I relinquished my research technician role when I returned from maternity leave in 2013 to become employed solely as a research-active academic (albeit part-time until 2019).

Awards of small internal grants (detailed in Table 1.2) enabled me to return to the relative sanctuary (certainly when compared to Crown and magistrate courts!) of academic palynology. During this time, I embarked on fieldwork to the small Pembrokeshire islands of Skomer and Skokholm to unravel the vegetation history of the islands during the late Holocene (the last c. 4,000 years). These islands had been of interest to me since attending an undergraduate geological field trip to Newgale, where I saw sub-fossil tree stumps in the sound between the Welsh Coast mainland and the islands. Literature was never clear as to the timing and cause of the loss of Mesolithic woodland in this area and when colleagues were heading onto Skomer for summer sea bird censuses, I jumped at the chance to take the corer and undertake sampling to investigate further. The subsequent paper from this trip (Webb et al. 2017), which is included in this PhD, was my first experience of lead authorship and engagement with the entire publication process from conception through to writing, reviewing, editing and submission.

Having time to develop my own research for the first time enabled me to branch out and collaborate with colleagues, both internally and externally, and I was able to offer my palaeoecological skills to enhance a number of active research projects. This included work ultimately published as Charman et al. (2012) and van der Plicht et al. (2013) (Table 1.1), and a novel study investigating the routes taking by migrant birds prior to their arrival in the UK (Wood et al. 2014), which is included within this PhD. Researching while employed in a post-92 teaching-focused University has its challenges, with limited time allocation designated for research and resources being the most significant of these challenges. It requires my colleagues and me to be resourceful in ways to increase both time and funding for research, and one mutually beneficial approach is to work collaboratively with students/graduates to expand undergraduate dissertation projects to become academic publications. This process not only enables more research to take place, but also emphasises my ability to select appropriate projects and guide students through the research activity. Publications Webb et al. (2018) (included in this PhD) and Goodenough et al. (2019) both reflect this student-staff collaboration approach, which also gives graduates valuable research experience as they embark on careers or further study.

The series of publications led by McCarroll (McCarroll et al. 2016; McCarroll et al. 2017; McCarroll et al. 2016) and Jarman (Jarman et al. 2017; Jarman et al. 2018; Jarman et al. 2019a; Jarman et al. 2019b; Jarman et al. 2019c;) in Table 1.1 reflect a step change in my career and an academic opportunity to supervise PhD students. I took every opportunity to encourage my students to publish early in their academic journey (and as first authors) to ensure they gain this valuable experience. Jarman eventually submitted his PhD by the publication route rather than the traditional route given the number of publications he produced. I have now co-supervised three PhD students to completion, and I have examined one internal PhD candidate.

Following a second period of maternity leave and a move to a full-time academic post, the most recent phase of my publications reinforces my career as an effective researcher. While in some early papers (not included in this portfolio) my contribution was solely in data acquisition, the latter papers onwards reflect my development as I take a leading role in these publications. Since 2013 I have taken a lead role in conceptualisation, extracting primary and manipulating secondary data, and writing, as well as being corresponding author for several publications. This progression is summarised in Figure 1.1. Recent publications have been well received in academia and in the public domain, which enabled me to develop an expanding research network of internal and external collaborators (for example Webb and Goodenough (2018) received public interest and Webb et al (2022a) led onto external collaboration, both papers are included in this PhD). Not only do I lead on publications, but I also develop research ideas and discuss these with collaborators nationally and internationally. Additionally, during this latter phase of my academic development I began to act as a reviewer for articles, including for *Journal of Forensic Science.*

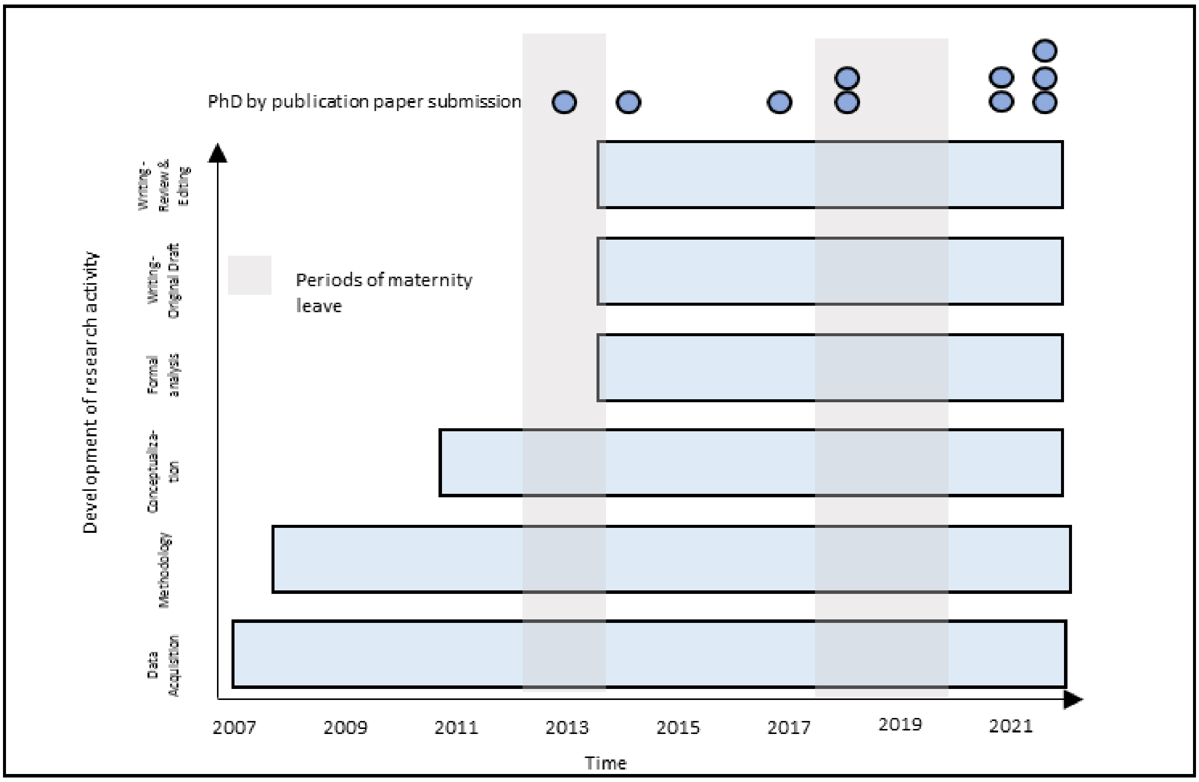


Figure 1.1 Julia Webb's academic progression from early-career researcher acquiring data to underpin publications to more advanced leadership of publications.

Table 1.1. Table of all published papers by Webb (née Newberry) presented in chronological order, with number of citations (according to Google Scholar as at February 2022). Those highlighted in yellow are included within this PhD portfolio.

Publication reference	Cited by	Year
Webb, J.C. McCarroll, J. and Morrow, D. (2022a) Palynological contributions towards the Archaeology of Skokholm Island, south west Wales. *Archaeology in Wales* 60, 45-48.	0	2022
Webb, J.C. McCarroll, J. Chambers, F.M. and Thom, T. (2022b) Evidence for the Little Ice Age in upland north-western Europe: multiproxy climate data from three blanket mires in northern England. *The Holocene* 32(5), 451 – 467.	0	2022
Goodenough, A.E. and **Webb**, J.C. (2022) Learning from the past: opportunities for advancing ecological research and practice using palaeoecological data. *Oecologia*. (online first)	0	2022
Goodenough, A.E. and **Webb**, J.C. Understanding spatiotemporal variation in taxonomic richness and rate of within-site turnover in vegetation communities across Eurasia over the last 4,000 years. Submitted to *Community Ecology*.	0	2022
Webb, J.C. and Goodenough, A.E. (2021) Vegetation community changes in European woodlands amid a changing climate: a palaeoecological modelling perspective. *Community Ecology* 22(3), 319-330.	0	2021
Jarman, R. Hazell, Z. Campbell, G. **Webb**, J.C. and Chambers, F.M. (2019a) Sweet Chestnut (*Castanea Sativa* Mill.) in Britain: Re-assessment of its Status as a Roman Archaeophyte. *Britannia* 50, 49-74.	7	2019
Jarman, R. Mattioni, C. Russell, K. Chambers, F.M. Bartlett, D. Martin, M.A. Villani, F. **Webb**, J.C. and Cherubini, M. (2019b) DNA analysis of *Castanea sativa* (sweet chestnut) in Britain and Ireland: Elucidating European origins and genepool diversity. *PLoS ONE* 14(9), 1-26.	6	2019
Goodenough, A.E. **Webb**, J.C. and Yardley, J. (2019) Environmentally-realistic concentrations of anthelmintic drugs affect survival and motility in the cosmopolitan earthworm *Lumbricus terrestris* (Linnaeus, 1758). *Applied Soil Ecology* 137, 87-95.	6	2019
Jarman, R. Chambers, F.M. and **Webb**, J.C. (2019c) Landscapes of sweet chestnut (*Castanea sativa*) in Britain - their ancient origins. *Landscape History* 40(2), 5-40.	0	2019
Webb, J.C. Brown, H.A. Toms, H. and Goodenough, A.E. (2018) Differential retention of pollen grains on clothing and the effectiveness of laboratory retrieval methods in forensic settings. *Forensic Science International* 288, 36-45.	20	2018
Webb, J.C. and Goodenough, A.E. (2018) Questioning the reliability of "ancient" woodland indicators: Resilience to interruptions and persistence following deforestation. *Ecological Indicators* 84, 354-363.	9	2018
Jarman, R. Moir, A.K. **Webb**, J.C. Chambers, F.M. and Russell, K. (2018) Dendrochronological assessment of British veteran sweet chestnut (*Castanea sativa*) trees: successful cross-matching, and cross-dating with British and French oak (*Quercus*) chronologies. *Dendrochronologia* 51, 10-21.	6	2018
McCarroll, J. Chambers, F.M. **Webb**, J.C. and Thom, T. (2017) Application of palaeoecology for peatland conservation at Mossdale Moor, UK. *Quaternary International 432*, 39-47.	24	2017
Jarman, R., Moir, A.K. **Webb**, J.C. and Chambers, F.M. (2017) Sweet chestnut (*Castanea sativa* Mill.) in Britain: its dendrochronological potential. *Arboricultural Journal* 39(2), 100-124.	7	2017

Chambers, F.M. Crowle, A. Daniell, J.R. Mauquoy, D. McCarroll, J. Sanderson, N. Thom, T. Toms, P. and **Webb**, J.C. (2017) Ascertaining the nature and timing of mire degradation: using palaeoecology to assist future conservation management in Northern England. *AIMS Environmental Science 4*(1), 54-82.	3	2017
Webb, J.C. McCarroll, J. Carpenter, W.S. Chambers, F.M. Toms, P. and Wood, M.J. (2017) Apparent lack of woodland and abundance of woodland indicator species: the role of humans, birds and rabbits on the changing vegetation of Skomer Island, Wales. *Archaeology in Wales* 56, 90-99.	Not fully indexed	2017
McCarroll, J. Chambers, F.M. **Webb**, J.C. and Thom, T. (2016a) Using palaeoecology to advise peatland conservation: An example from West Arkengarthdale, Yorkshire, UK. *Journal for Nature Conservation* 30, 90-102.	20	2016
McCarroll, J. Chambers, F.M. **Webb**, J.C. and Thom, T. (2016b) Informing innovative peatland conservation in light of palaeoecological evidence for the demise of Sphagnum imbricatum: the case of Oxenhope Moor, Yorkshire, UK. *Mires and Peat* 18, 1-24.	8	2016
Wood, M.J. Morgan, P.J. **Webb**, J.C. Goodenough, A.E. Chambers, F.M. and Hart, A.G. (2014) Exploring the prevalence and diversity of pollen carried by four species of migratory Old-World warbler (Sylvioidea) on arrival in the UK. *Bird Study* 61(3), 361-370.	4	2014
Monckton-Smith, J. Adams, T. Hart, A.G. and **Webb**, J.C. (2013*) Introducing Forensic and Criminal Investigation*. Sage. ISBN 978-0857027528	48	2013
van der Plicht, J. Yeloff, D. van der Linden, M. van Geel, B. Brain, S. Chambers, F.M. **Webb**, J.C. and Toms, P.S. (2013) Dating recent peat accumulation in European ombrotrophic bogs. *Radiocarbon* 55(3-4), 1763-1778.	23	2013
Pirrie, D. Rollinson, G.K. Power, M.R. and **Webb**, J. (2013) Automated forensic soil mineral analysis; testing the potential of lithotyping. *Geological Society, London, Special Publications* 384(1), 47-64.	19	2013
Webb, J.C. and Stafford, R. (2013) Location-based mobile phone applications for increasing student engagement with field-based extra-curricular activities. *Planet* 27(1), 29-34.	5	2013
Charman, D.J. Hohl, V. Blundell, A. Mitchell, F. **Newberry**, J. and Oksanen, P. (2012) A 1000-year reconstruction of summer precipitation from Ireland: Calibration of a peat-based palaeoclimate record. *Quaternary International* 268, 7-97.	19	2012
Kirkhope, C.L. Williams, R.L. Catlin-Groves, C.L. Rees, S.G. Montesanti, C. Jowers, J. Stubbs, H. **Newberry**, J. Hart, A.G. Goodenough, A.E. and Stafford, R. (2010) Social networking for biodiversity: the BeeID project. In: *2010 International Conference on Information Society*, 625-626	16	2010
Fowler, M, Datson, H and **Newberry**, J. (2010) Quantitative assessment of dust propogation from hazardous waste landfill. *Journal of Environmental Monitoring* 12(4), 879-889.	8	2010
Pirrie, D. Power, M.R. Rollinson, G.K. Wiltshire, P.E. **Newberry**, J. and Campbell, H.E. (2009) Automated SEM-EDS (QEMSCAN®) mineral analysis in forensic soil investigations: Testing instrumental reproducibility. In: Ritz, K., Dawson, L., Miller, D. (eds) Criminal and Environmental Soil Forensics. Springer, Dordrecht, 411-430.	61	2009
Sillasoo, Ü. Mauquoy, D. Blundell, A. Charman, D. Blaauw, M. Daniell, J. R. G. Toms, P. **Newberry**, J. Chambers, F M. and Karofeld, E. (2007) Peat multi-proxy data from Männikjärve bog as indicators of late Holocene climate changes in Estonia. *Boreas* 36, 20-37.	126	2007

1.1 Author contribution statement

The author contribution statement for this PhD is provided in the form of a grid (Table 1.2), where contributions are denoted by ✓ (for minor contributions) and ✓✓ (for major contributions). The grid has been developed based on the CRediT (Contributor Roles Taxonomy) author statement system (Brand et al. 2015) and definitions for each of the terms used are provided in Table 1.3.

All co-authors of papers submitted for this PhD were asked to agree with the candidate's contribution to the individual publications in which they were involved. A shared online spreadsheet was used to collect the information, and co-authors were requested to either insert their electronic signature, or if unable to do that, to insert 'agreed' in the relevant cell. No co-author responded to disagree with the contribution, the gaps in the table represent co-authors that did not respond. Importantly, where I, as the PhD candidate, am not first author of the paper, the first author is always in agreement regarding the author contributions for that paper. The spreadsheet which collated agreement is provided as Table 1.4.

Table 1.2 Author contribution statement for the publications included within this PhD by Publication

Paper	JW contribution	Conceptualization	Methodology	Statistical analysis	Formal analysis	Validation	Investigation	Resources	Data Curation	Writing - Original Draft	Writing - Review & Editing	Visualization	Supervision	Project admin	Funding acquisition
Goodenough, A.E. and Webb, J.C. (2022) Learning from the past: opportunities for advancing ecological research and practice using palaeoecological data. *Oecologia*	50%	✓✓	✓✓	N/A	N/A	✓✓	N/A	N/A	N/A	✓✓	✓	✓✓	N/A	✓	N/A
Goodenough, A.E. and Webb, J.C. Understanding spatiotemporal variation in taxonomic richness and rate of within-site turnover in vegetation communities across Eurasia over the last 4,000 years *submitted to Community Ecology*	35%		✓	✓		✓	✓	✓	✓✓	✓✓	✓	✓	N/A		N/A
Hart A.G and Webb, J.C. Forensic Science Specialisms: palynology, entomology and DNA. In: Monckton-Smith, J , Adams, T, Hart, A.G and Webb, J.C (2013) Introducing Forensic and Criminal Investigation. Sage. ISBN 978-0857027528	50%	✓✓	N/A	N/A	N/A	✓	N/A	N/A	N/A	✓✓	✓✓	✓✓	N/A	✓✓	N/A
Webb, J.C., McCarroll, J. and Morrow, D. (2022) Palynological contributions towards the Archaeology of Skokholm Island, south west Wales. *Archaeology in Wales,* 60, 45-48	80%	✓✓	✓✓	N/A	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓
Webb, J.C., McCarroll, J., Chambers, F.M., and Thom, T. (2022) Evidence for the Little Ice Age in upland north-western Europe: multiproxy climate data from three blanket mires in northern England. *The Holocene* 32:5 451-467	20%	✓									✓	✓	N/A	✓	N/A
Webb, J.C and Goodenough, A.E. (2018) Questioning the reliability of "ancient" woodland indicators: Resilience to interruptions and persistence following deforestation. *Ecological Indicators* (84) 354-363.	50%	✓✓	✓✓		✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	N/A	✓✓	N/A
Webb, J.C and Goodenough, A.E. (2021) Vegetation community changes in European woodlands amid a changing climate: a palaeoecological modelling perspective. *Community Ecology* 22 (3) 319-330	65%	✓✓	✓✓		✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	N/A	✓✓	N/A
Webb, J.C. Brown, H.A. Toms, H. and Goodenough, A.E. (2018) Differential retention of pollen grains on clothing and the effectiveness of laboratory retrieval methods in forensic settings. Forensic Science International, 288. pp. 36-45.	65%	✓✓	✓✓		✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓
Webb, J.C. McCarroll, J. Carpenter, W.S. Chambers, F.M. Toms, P. and Wood, M.J. (2017) Apparent lack of woodland and abundance of woodland indicator species: the role of humans, birds and rabbits on the changing vegetation of Skomer Island, Wales. *Archaeology in Wales* , 56. 90-99.	80%	✓✓	✓✓	N/A	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓
Wood, M.J. Morgan, P.J. Webb, J.C. Goodenough, A.E. Chambers, F.M. and Hart, A.G. (2014) Exploring the prevalence and diversity of pollen carried by four species of migratory Old World warbler (Sylvioidea) on arrival in the UK. *Bird Study*, 61 (3) 361-370.	15%	✓							✓		✓		N/A		N/A

Table 1.3 Terms and definitions used in the author contribution statement for this PhD by publication (Brand et al. 2015).

Term	Definition
Conceptualization	Ideas; formulation or evolution of overarching research goals and aims
Methodology	Development or design of methodology
Statistical analysis	Application of statistical techniques
Formal analysis	Synthesis of study data
Validation	Verification of the overall replication/ reproducibility of results/experiments
Investigation	Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection
Resources	Provision of study materials, reagents, materials, laboratory samples, instrumentation, computing resources, or other analysis tools
Data Curation	Management activities to annotate (produce metadata), scrub data and maintain research data for initial use and later reuse
Writing - Original Draft	Preparation, creation and/or presentation of the published work, specifically writing the initial draft
Writing - Review and Editing	Preparation, creation and/or presentation of the published work, specifically critical review, commentary or revision – including pre-or post-publication stages
Visualization	Preparation, creation and/or presentation of the published work, specifically visualization/ data presentation
Supervision	Oversight and leadership responsibility for the research activity planning and execution
Project administration	Management and coordination responsibility for the research activity planning and execution
Funding acquisition	Acquisition of the financial support for the project leading to this publication

Table 1.4 Co-author contribution agreement

Paper	JW contribution	Author agreement											
		Anne Goodenough	Hannah Toms	Harriet Brown	Adam Hart	Julia McCarroll	Frank Chambers	Phil Toms	Darrien Morrow	Matt Wood	Will Carpenter	Peter Morgan	Tim Thom
Goodenough, A.E. and Webb, J.C. (2022) Learning from the past: opportunities for advancing ecological research and practice using palaeoecological data. *Oecologia*	50%	Anne Goodenough											
Goodenough, A.E. and Webb, J.C. Understanding spatiotemporal variation in taxonomic richness and rate of within-site turnover in vegetation communities across Eurasia over the last 4,000 years *submitted to Community Ecology*	35%	Anne Goodenough											
Hart A.G and Webb, J.C. Forensic Science Specialisms: palynology, entomology and DNA. In: Monckton-Smith, J , Adams, T, Hart, A.G and Webb, J.C (2013) Introducing Forensic and Criminal Investigation. Sage. ISBN 978-0857027528	50%				Agreed								
Webb, J.C., McCarroll, J. and Morrow, D. (2022) Palynological contributions towards the Archaeology of Skokholm Island, south west Wales. *Archaeology in Wales,* 60, 45-48	80%					Agreed							
Webb, J.C., McCarroll, J., Chambers, F.M., and Thom, T. (2022) Evidence for the Little Ice Age in upland north-western Europe: multiproxy climate data from three blanket mires in northern England. *The Holocene* 32:5 451-467	20%					Agreed	Agreed						T.J. Thom
Webb, J.C and Goodenough, A.E. (2018) Questioning the reliability of “ancient” woodland indicators: Resilience to interruptions and persistence following deforestation. *Ecological Indicators* (84) 354-363.	50%	Anne Goodenough											
Webb, J.C and Goodenough, A.E. (2021) Vegetation community changes in European woodlands amid a changing climate: a palaeoecological modelling perspective. *Community Ecology* 22 (3) 319-330	65%	Anne Goodenough											
Webb, J.C. Brown, H.A. Toms, H. and Goodenough, A.E. (2018) Differential retention of pollen grains on clothing and the effectiveness of laboratory retrieval methods in forensic settings. Forensic Science International, 288. pp. 36-45.	65%	Anne Goodenough	Agreed	Agreed									
Webb, J.C. McCarroll, J. Carpenter, W.S. Chambers, F.M. Toms, P. and Wood, M.J. (2017) Apparent lack of woodland and abundance of woodland indicator species: the role of humans, birds and rabbits on the changing vegetation of Skomer Island, Wales. *Archaeology in Wales*, 56. 90-99.	80%					Agreed	Agreed	Agreed		Agreed	Agreed		
Wood, M.J. Morgan, P.J. Webb, J.C. Goodenough, A.E. Chambers, F.M. and Hart, A.G. (2014) Exploring the prevalence and diversity of pollen carried by four species of migratory Old World warbler (*Sylvioidea)* on arrival in the UK. *Bird Study*, 61 (3) 361-370.	15%	Anne Goodenough			Agreed		Agreed			Agreed			

2. Fundamentals of palynology

Palynology is the study of pollen grains that are produced from seed generating plants (angiosperms and gymnosperms), as well as spores that are produced by bryophytes, pteridophytes, fungi and algae (the term 'pollen' is used hereafter to refer both to pollen and spores, unless spores are mentioned specifically). The cell or cluster of cells forming an individual pollen grain is very small, averaging around 30 microns (Moore et al. 1991). These reproductive cells are protected from degradation and microbial degradation by a tough sporopollenin outer layer (the exine). The sporopollenin is so effective that the exine of pollen grains that do not reach their intended recipient can be preserved for thousands (and in some cases, millions) of years (Batten and Dutta 1997). The structure of pollen grains and the sculpturing on the exine evolved to support the reproductive function, but it also enables palynologists to identify the grains to family or generic level; in some rare cases identification to species level is possible (for example, *Hyacinthoides non-scripta* (bluebell) and *Mercurialis perennis* (dog's mercury)).

Pollen grains that do not reach their intended recipient plant for reproduction (whether transported by air currents, insect or animal vector, or water) can become incorporated into sediments or biological products (such as peat or within honey), or adhere to surfaces. Retrieving the pollen from these materials can be used to answer a diverse array of questions that are specific to the discipline in which palynological investigation is being applied.

The earliest evidence of studies of pollen under the microscope was in the 1640s by botanists seeking understanding of sexual reproduction in flowers (Bradbury 1967). Since then there has been a steady rise in interest in palynology, principally to quantify past vegetation changes (Birks and Berglund 2018), but the concept has been used successfully in other contexts, including the study of plant pathogens, archaeology, allergy, and forensics (Peglar and Birks 1993; Burge 2002; Bunting and Farrell 2018; Ochando et al. 2018). Figure 2.1 summarizes the range of disciplines that employ palynology; this is an indicative range rather than exhaustive as more novel uses of palynology continue to evolve.

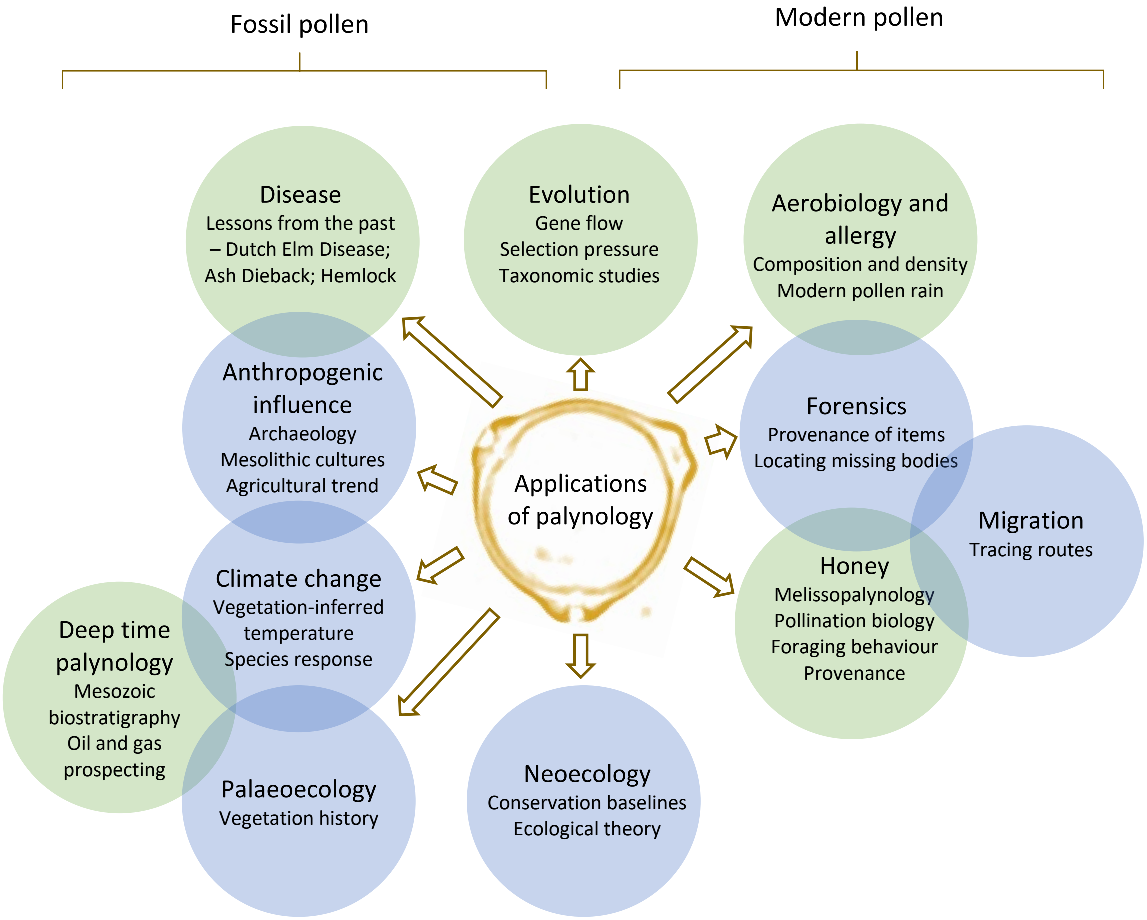


Figure 2.1. The breadth of palynological studies. Note the interrelated areas indicated by overlapping circles (the degree of overlap does not represent the strength of these links). Blue circles are subject areas included within this thesis. Author's own image.

Regardless of the discipline or the aims of a specific study, retrieval of pollen from the sediment or material of interest requires careful processing to avoid damage to the pollen grains. While pollen is robust in natural environments that are anaerobic or acidic (preserving well in waterlogged environments such as peat, lake sediments and acidic soils), the oxidising chemicals that are used to separate pollen from the sediments or artefacts to which they are entrained need be used carefully. Standard techniques discussed by Moore et al. (1991) cover the methods used to remove internal structures of pollen and as much of the surrounding matrix as possible for ease of identification of the pollen through microscopy. Standard extraction techniques have been adapted by the many sub-disciplines of palynology to suit different research questions; for example, Webb et al. (2018) removed pollen from fabrics using a mild surfactant, and Wood et al. (2014) describes the adaption to the method to retrieve pollen from bird bills. The standard extraction process, and variations within that, is summarised in Table 2.1.

Table 2.1 Preparation protocols for palynological samples. After: Faegri and Iverson (1989); Moore et al. (1991); Munsterman and Kerstholt (1996); Chambers et al. (2010); Rodrigues et al. (2020).

* indicates author's own adaptations from standard protocols. **+ [plus sign]** indicates where the procedure can affect the pollen grain by causing swelling and **— [minus sign]** indicates where shrinkage can occur.

<table>
<tr><th colspan="2">1. Retrieval</th></tr>
<tr><td>Fossil pollen
(Soft sediments, rock, pottery)</td><td>Modern pollen
(Comparator samples, honey/nectar, clothing)</td></tr>
<tr><td colspan="2">All samples should be processed in conical centrifuge tubes with leak-proof screw-on lids.
It is advised that labels are produced with a label sticker machine as permanent ink will be easily removed by chemicals in step 7*
Target 1 ml volume of sediment</td></tr>
<tr><td>Lake/bog/soil: sample 1 cm^3 sediment
Pottery: wash the sediment from the inside
Rock: mechanically disaggregate and start at step 5</td><td>Clothing: wash in mild surfactant (e.g. Teepol™), then centrifuge to form a pellet*
Disaggregate honey/nectar/stamen in warm HCl, then centrifuge to form a pellet*</td></tr>
<tr><td>Insert exotic marker grains (if used) at a ratio of 1:5 exotic: fossil</td><td></td></tr>
<tr><td colspan="2">2. Removal of humic acids and further disaggregation (use 50 ml tube)</td></tr>
<tr><td>Samples containing humic acids (particularly peat) will benefit from 10 minutes of reflux* in 10 ml of 8 % NaOH or KOH (author preference on chemical choice) +
Whirlymix™ before, during and after treatment
* boiling can damage thin walled grains</td><td>Gently warm for 5 minutes in 8% NaOH or 10 % KOH +
Whirlymix™ before, during and after treatment</td></tr>
<tr><td colspan="2">3. Sieve through 180µm mesh - use new mesh each time for forensic cases, in other instances, wash carefully.</td></tr>
<tr><td colspan="2">Centrifuge (3000 rpm) and safely discard supernatant* *Can be used to neutralise some acids later in the procedure</td></tr>
<tr><td colspan="2">4.Wash with distilled water, centrifuge and discard supernatant
Repeat until supernatant is clear</td></tr>
</table>

5. Add c.5 ml of HCl to remove carbonates (warm if necessary) and Whirlymix™ (use 15 ml tubes from here)

Centrifuge and discard supernatant carefully according to the establishment's discharge consent.

6. Remove minerogenic element

THIS STEP TRADITIONALLY USES HYDROFLUORIC ACID SO ALTERNATIVES ARE SUGGESTED

Option 1:	Option 2:	Option 3:
It is possible to separate the lighter polleniferous aliquot from the heavier minerogenic constituent by consciously allowing the post-sieving fluid to partially settle before gently swirling and tipping off the flot (and discarding the heavier mineral grains settled at the bottom of the vessel)	Using heavy liquids to assist in density separations. 1) Sodium polytungstate ($3Na_2WO_4 9WO_3 H_2O$) is easy to use and effective, but is very expensive 2) Zinc Chloride (ZnCl) has been used with good results (Bromoform is highly toxic and is no longer considered safe to use (Munsterman and Kerstholt 1996))	HF treatment. Hydrofluoric acid causes severe burns and subsequent complications which can be fatal. Suitable PPE must be worn and antidote gel available, as well as a safety shower. HF resistant fume hood and equipment must also be used.
During step 3. Physically separate lighter material from heavier material using the technique described above	After step 5, use Sodium Polytungstate or ZnCl at a specific gravity of 2.1 to facilitate removal of lighter fraction	Resuspend pellet in a few drops (<1 ml) of 10 % HCl by Whirlymixing* *This step is introduced to negate the need to mechanically mix the pellet in HF
	Centrifuge and wash samples. Recover and reuse heavy liquid (after careful screening to avoid pollen contamination between samples)	Add c.5 ml of 40 % HF, replace leak-proof lid, and lie the tube flat in a sealed container (lined with powdered chalk in case of leakage). Leave overnight or for several days depending on the sample * — *Lying the sample flat enables a larger surface area for the HF to attack
		Centrifuge, discard supernatant carefully, and wash in warm HCl to remove silicofluorides

7. Remove soluble cellulose via acetolysis

Dehydrate sample in c.5 ml concentrated glacial acetic acid. Centrifuge and discard supernatant carefully. This stage is vital as the acetylation mixture reacts violently with water

Prepare fresh acetylation mixture (Erdtman's solution) in a 9:1 ratio of concentrated acetic anhydride and concentrated sulphuric acid.

Reflux* in c.5 ml of solution for no more than 5 minutes. Care must be taken not to over-acetolyse samples as surface structures can be lost. *Sphagnum* and *Pteridium* are easily damaged over 3 minutes of reflux (Chambers et al. 2011) and wind pollinated tree and grass pollen are also easily overacetolysed. Under acetolysis may leave cellulose residues that affect successful identification (Moore et al. 1991). +

Hunt (1985), Hesse and Waha (1989) and Lenlfer and Boyd (2000) suggest use of acetolysis in palaeopalynology should be reconsidered given the potential for damage during the process. They also argue that acetolysis is only needed in preparation of fresh samples. Mudie and Lulievre (2013) suggest using Ammonium Hydroxide (NH_4OH) as a suitable (and less hazardous) alternative. van Asperen (2016) agree this procedure needs refining to uniformly preserve fungal spores.

End the acetolysis reaction by filling the tube with glacial acetic acid
Centrifuge and discard supernatant carefully

To avoid crystallisation, resuspend in glacial acetic acid Whirlymix™, centrifuge and discard supernatant carefully

8. Staining

Staining is very much personal preference, but when used, add a few millilitres of safranin or fuchsin in 50 ml of alkaline (8 % NaOH) solution (to help the stain take more effectively). Add 5 ml of the solution and Whirlymix™ centrifuge and decant. The supernatant should not be pink (an indicator of overstaining), but the pellet should have pink/red colouration to it (indicating organic material has taken the stain). Adjust concentrations of NaOH and stain to suit if uptake is not satisfactory. Be careful not to overstain.

9. Mounting on slides

Glycerol jelly (+) or silicone oil are the two most frequently used mounting media. Both have suitable refractive indices. While there appears to be more advantages of using silicone oil, the stability of glycerol jelly in temperate climates outweighs its disadvantages. Slides are normally 1.2 mm thick (to avoid issues with using high magnification objectives).

Glycerol jelly #		**Silicone oil**	
Advantages	Disadvantages	Advantages	Disadvantages
- Excellent optical properties - Easy to handle - Produces stable slides (glycerol is solid at room temperature)	- Over time glycerol jelly absorbs water and pollen grains swell	- Viscosity can be altered depending on preference - Pollen remains mobile and can be turned (by poking with a seeker) to aid identification - No pollen size inflation	- Even when slides are sealed seepage can occur - Flat storage is essential

Glycerol Jelly Recipe (makes approximately 150 ml): Gelatine (10 g); Distilled water (60 ml); Glycerine (70 ml); TCP™ (1 ml). To make: Warm the distilled water and slowly add the gelatine. Stir until dissolved. Add the glycerine and TCP (which acts as a preservative). Store in 250 ml conical screw-top flasks with label and date. Shelf life unknown (>1 year). *

The morphological characteristics of pollen prepared and mounted on simple microscope slides and visible under a simple light microscope with 100 – 400 x magnification allow identification of the taxa via the use of keys – See Figure 2.2 for examples of pollen viewed under a light microscope. Faegri and Iverson (1989) and Moore et al. (1991) provide comprehensive identification keys for pollen and some common spore types in book form, while Hooghiemstra and van Geel (1998) offer a list of all available physical databases.

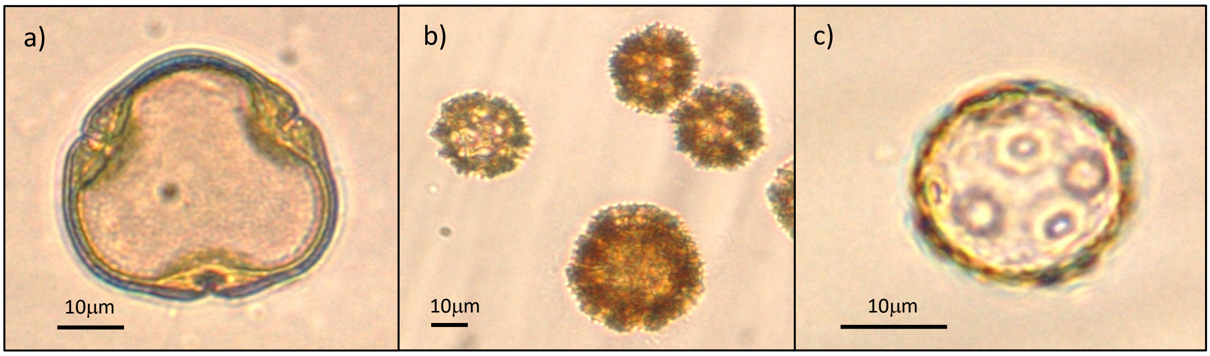


Figure 2.2 Light microscopy photographs of a) *Tilia cordata*; b) *Taraxacum officinale* and c) *Plantago lanceolata.* Magnifications vary between images, note 10μm bar for scale. Author's own images.

More recent online resources, such as the Global Pollen Database (GPD), have reinvented physical type slide collections held at museums and universities, as well as physical photographic databases. By virtue of being online, the GPD (https://globalpollenproject.org/) is easily accessible and constantly evolving with new images being added continually. It also has impressive search functions as well as crowd-sourced identification capabilities (Martin and Harvey 2017).

Attempts have been made to automate pollen identification (see review by Holt and Bennett (2014) and sources therein). Artificial intelligence is yet to replicate the reliability of human palynologists, although as computing powers and machine learning algorithms continue to improve it may only be a matter of time before automated processes become commonplace. Yamada et al. (2021) have had some success with sorting pollen grains via a cell counter for use in radiocarbon dating, but the technique is not routinely used due to variable results. One example of an often-overlooked challenge with automating pollen counts is the uneven spread of pollen grains on a slide. For example, care needs taken when counting around the edge of slides, as smaller pollen grains tend to migrate and clump and layer at the edge of slides when the coverslip is added (Brooks and Thomas 1967). This is comparatively straightforward for a palynologist to assess and account for, but adds an important confounding variable into automated processes that affects reliability.

Until the precision and accuracy of human palynologists can be replicated by computers, the identification and quantification of microscopic pollen and spores via light microscopy remains a labour-intensive, manual approach. The reliability of the data produced often depends on the skill of the palynologist and also the number of individual pollen grains that need to be counted in order to make meaningful inferences. For example, in investigations of fossil pollen, sums of between 300 and 500 grains per sample are commonplace (Lowe and Walker 2015), despite Weng et al. (2006) stating that there may be statistically significant errors with counts lower than 1000. More recently, Djamali and Cilleros (2020) demonstrate that counts of just 150 pollen grains can be sufficient for 90 % and 300 for 95 % repeatability to be attained, such that the benefit of counting higher numbers is questionable. Moreover, in deciding the appropriate count total, the question that palynology is being employed to answer is also important. For example, in forensic palynology the target is not reaching a particular number for the total grain count, but rather to count the entire slide, or until no new pollen types are identified in two short slide transects if multiple slides are counted (Wiltshire et al. 2015).

In all palynological disciplines striving for a reconstruction of a source environment (whether a past environment using fossil pollen or a contemporary environment using modern pollen), consideration needs to be given to the pollen taphonomy – the behaviour of pollen arriving at, and being preserved within, the samples under question. The number of pollen grains produced by the parent plant, and the dispersal technique that species had evolved, will both affect the frequency with which pollen grains are found in samples. Studies examining pollen productivity and dispersal are numerous (for example Calcote (1995); Bunting et al. (2005); Theuerkauf et al. (2013); Theuerkauf and Couwenberg (2021)). Generally, pollen production in plants relying on insects for dispersal (entomophilous taxa) is relatively lower than for wind pollinated plants (anemophilous taxa). There are, of course variations, within each taxon too. For example, *Quercus* relies on wind pollination, but productivity is much lower than for *Fraxinus* (Bunting and Farrell 2018), while insect-pollinated *Calluna*, is often over represented within samples due to its comparatively prolific pollen production (Chambers et al. 2010). Other taphonomic considerations such as sinking speed of pollen (which itself depends on size and morphology), air currents, obstructions (such as buildings/terrain) and weather conditions have an influence on the dispersal of pollen and so also need to be considered when interpreting results

(Theuerkauf et al. 2013). For example, large grains (e.g. *Cerealia*) will settle rapidly close to the parent plant, but air bladders (as with *Pinus*) will aid buoyancy meaning that grains can be carried for vast distances. Given warm air currents providing uplift, no obstructions and clear skies, *Pinus* has been known to travel considerable distances; up to hundreds of kilometres in some cases (Hjelmroos and Franzén 1994; Tipping 2008; Bajpai and Kar 2018).

In fossil and forensic pollen studies, the rate of degradation of the pollen also needs to be considered when analysing samples. When pollen is recovered from sub-optimal materials (dry, high-energy or alkaline environments), grains can have a thinned exine, or be crumpled or broken, which often removes their identifying features (Tipping 2000). Not only can environmental factors degrade pollen, grains from different taxa will also exhibit different levels of resistance to damage. Twiddle and Bunting (2010) investigated the preservation of four pollen types in laboratory conditions (*Alnus, Secale, Dactylis* and *Ambrosia)*. They subjected the pollen to chemical oxidation (with hydrogen peroxide) or physical agitation (in water with sand/pebbles) and demonstrate that the four taxa studied had differing resistance. However, pollen can also be surprisingly robust. In an unpublished laboratory experiment, *Castanea sativa* was subjected to oxidation in differing concentrations of household bleach (a commonly used product for cleaning laboratory benches between examining exhibits in forensic settings) to: (1) test a hypothesis that *C. sativa* is under-represented in profiles from the Holocene (last c.11,700 years) owing to it being fragile; and (2) investigate the hypothesised reliability of household bleach as an appropriate decontamination solution in forensic palynology (Julia Webb, Unpublished). The results of this investigation refuted both of these hypotheses.

To avoid taphonomic biases within applications of palynology, a detailed understanding of pollen-vegetation relationships is needed. This is achieved in most instances with comparator samples, either to modern pollen rain to help reconstruct provenance or vegetation communities, or to pertinent places (crime scenes, for example). Therefore, to overcome this aspect of variability within palynological data, extensive quantitative modelling can be employed in studies of fossil pollen (Bunting and Middleton 2005; Sugita 2007b, 2007a). See further analysis of taphonomic considerations of fossil pollen and the proposed published resolutions later in Table 2.2.

2.1 Fossil pollen studies

One of the most explored areas in palynological research is focused on vegetation reconstruction using fossilised pollen from lakes and bogs, within the Quaternary period (Birks and Berglund, 2018). The Quaternary is the most recent sub-division of geological history. It extends from around 2.6 million years ago (the start of the Quaternary has been contested in recent years (Lowe and Walker 2015)) to encompass the modern day. It is characterised by fluctuating climate between cold periods (glacial stages) and warm phases (interglacial stages). The intensity, frequency and environments formed by these stages have been of great interest to researchers in the last 200 years (Lowe and Walker 2015), and has resulted in numerous studies investigating biological evidence fossilised (or sub-fossilised) within Quaternary sediments (see Lowe and Walker (2015) for a range of examples).

Fossil pollen studies grew from early investigations in the 1910s where Scandinavian researchers sought to use pollen as a stratigraphical and relative dating tool by matching bog, mire and lake sequences with known past events (Birks and Berglund, 2018). The utility of palynology quickly became apparent following a seminal lecture by von Post in 1916 outlining the principles of pollen analysis for seeking past vegetation trends. By the mid-1920s palynology was being used in many parts of the world; for example, von Post and Granlund (1926) published vegetation reconstructions from southern Sweden, Auer (1927) in Canada, and Erdtman (1928) in the British Isles. These papers were the first to use palynology as a true palaeoecological technique, and identified broad trends of changing climate inferred from temporal shifts in terrestrial vegetation. The publication of the first *Textbook of Pollen Analysis* in 1950 (Fægri and Iversen 1950) and the development of radiocarbon dating in the 1940s and 1950s that enabled robust dating of peat sequences, meant that palynology in palaeoecological studies could be released from its early use as a relative dating tool and be used solely as a vegetation reconstruction tool. The subsequent rise in publications of spatial and temporal studies, in particular high-resolution studies of vegetation dynamics, have provided insights into human impact on vegetation, impact of disease and climate change (examples of these early studies include Goodwin 1940; Aario 1944; Andersen 1954; Iversen 1954).

Advances in the application of palynology have not come solely from the publication of Fægri and Iversen’s (1950) textbook, and the development in radiocarbon dating. Continual improvements in light and electron microscopy have assisted in the ease of identification, as well as access to suitable microscopes in schools and colleges promoting further education and training in microscopy. Advances in coring technology for wetlands, bogs and lakes have made palynology one of the most universally used and valuable tools in palaeoecology over the 20th Century. Finally, the increased use in desk (and lap)-based computers through the 1980s to the present day have given palynologists access to data handling, graphical and statistical packages to further analyse sequences of vegetational change. As such, the number of and resolution within Quaternary pollen studies has risen steeply from 1990 to 2012, Figure 2.3 plots the rise in number of publications.

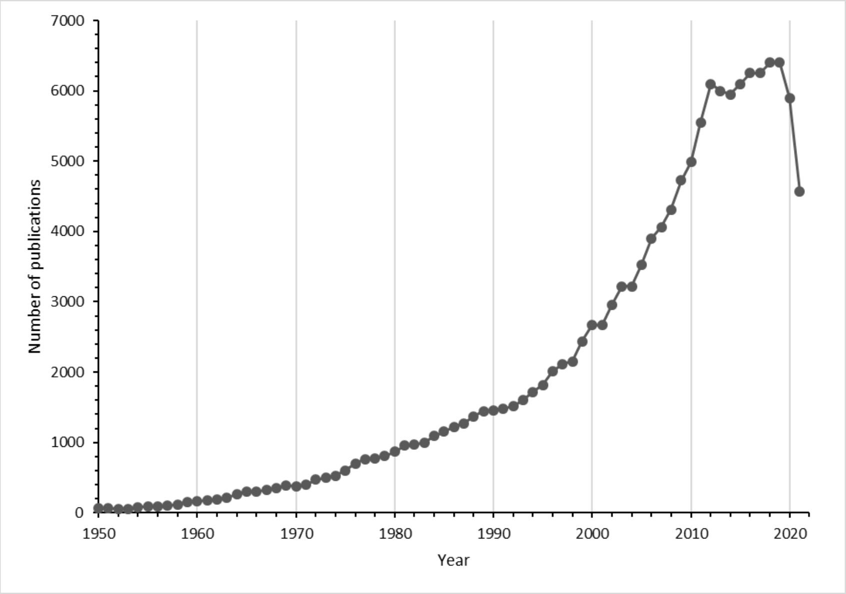


Figure 2.3. Authors own Google Scholar search output as total number of records for “Quaternary pollen” in yearly increments from 1950 – 2021. Note the steep growth in number of papers published on the topic between 1990 and 2012 and also the impact of the global pandemic on published outputs in the field. Care needs to be taken when analysing this Google record, as there is likely to be uncertainty in these figures, but it is useful to note the trend.

2.1.1 Applications of fossil pollen

Fossil pollen records have been used to reconstruct broad changes in past climate, including glacial-interglacial cycles (Tzedakis 1994; Miyoshi et al. 1999; Langgut et al. 2011; Camuera et al. 2019; Marden et al. 2022), as well as more nuanced trends in climate. Examples of these subtler climate research papers include evolution of monsoonal patterns (Wan et al. 2022) and the Little Ice Age perturbation in Britain (Webb et al. 2022b, in this thesis). While palynology is often used solely to describe climate patterns, interpretations have been made with quantitative data, for example estimating July temperatures (Huntley and Prentice 1988), annual average temperatures (Davis et al. 2003) and seasonal rainfall quantities (Vincens et al. 2007). In the early phase of the development of palynology, a large proportion of published climate reconstructions were based on single site analyses providing discussion on a local level, and such approaches are still used today to answer site-specific questions (modern examples of single site reconstructions include Litt et al. (2012) and Wan et al. (2022)). However, with advances over the 20th Century, research has increasingly spanned multiple sites to quantify regional and continental-scale patterns in climate change (for example Elenga et al. (2000) reconstruct biomes at continental scale and Davis et al. (2003) map temperatures across Europe).

Not only are climate reconstructions inferred from pollen, but the application of palynology can be used to study other events that happened in the past, such as faunal population dynamics, disease outbreaks and human occupation. Peglar and Birks (1993) used vegetation reconstructions in Norfolk to unravel the timing of the decline in *Ulmus* around 4,000 years ago. They concluded that the loss of *Ulmus* was not solely owing to forest clearance by humans (for agriculture), but that disease spread by the elm bark beetle (*Scolytus multistriatus*) was fundamental in the rate of the decline. The influence of early farmers on the landscape was noted by Gauthier et al. (2010) and Magyari et al. (2012) who both noted woodland clearance in their pollen records. Webb et al (2018) (in this thesis) also note the change in farming practices on Skomer Island with the use of coprophilous fungi from herbivores (assumed as cattle on Skomer) alongside pollen records. Coprophilous fungi have also been used in combination with pollen to reconstruct pre-historic herbivore density (Davies, et al. 2022), and pollen and plant macrofossils have been used successfully to reconstruct the diet of an extinct forest rhinoceros (*Stephanorhinus kirchbergensis*) (Stefaniak et al. 2021).

Many palynological studies are now made possible by the global sharing of pollen (and other proxy) data via large online data repositories. For example, the Canadian Pollen Database; the Pollen Database for the Russian and Far East; and the European Pollen Database (EPD) (Fyfe et al. 2009) to name a few. These are all now available, together with many others, in one large central repository of data (Neotoma) (Williams et al. 2018). As a result, this collaborative venture gives endless opportunities for desk based palaeoecological studies.

2.1.2 Taphonomic considerations when using fossil pollen

Palynology is not a “silver bullet”. One criticism of fossil pollen studies is that environmental reconstructions are often qualitative, and use broad climatic zones such as “temperate”, “cool”, “arctic” and so on (Birks et al. 2011). This, coupled with taphonomic variables means that the palynologist should question the representativeness of a fossil pollen sample (Chevalier et al. 2020). Palynologists must have awareness of both the taphonomy, and the statistical techniques employed to attempt to resolve these critiques, to produce reliable data. Some taphonomic challenges and proposed solutions are summarised in Table 2.2, although given the number of taphonomic considerations and the fact that many are unique to individual species, studies, or locations, this list should not be considered to be exhaustive.

Table 2.2 Taphonomic considerations in fossil pollen research

Taphonomic consideration		
Pollen productivity and dispersal Each plant species has evolved a specific reproductive strategy. In simple terms, for example, entomophilous taxa put energy into flowers, scent, and nectar to attract insect pollinators to directly fulfil their duty. In contrast, anemophilous taxa expend energy in producing pollen en-masse to ensure that some pollen is likely to reach the intended destination (the recipient plant). As such, there is a notable (but variable) amount of 'surplus' pollen that becomes incorporated in deposits that can be later analysed by palaeoecologists. Thus, the relationship between plant abundance and pollen abundance is not uniform and thus finding the same number of pollen grains in a sample for two different taxa does not necessarily mean the taxa themselves were equally abundant.	Alongside an understanding of pollination ecology, interpretations of pollen results can be assisted by applying relative pollen productivity corrections which are generated from modern pollen rain samples.	The Prentice-Sugita-Sutton (PSS) model (Prentice, 1985) is the most widely used model to simulate pollen loading to a site from species in the surrounding area. A number of wider landscape models use the PSS equation (POLLSCAPE (Sugita 1993; Gaillard et al. 2008); HUMPOL (Bunting and Middleton 2005); LOVE (Sugita 2007a); REVEALS (Sugita 2007b) to reconstruct former landscapes more accurately.
Dispersal mechanisms While wind and insects are the two primary mechanisms for pollen dispersal, water and animals (particularly in Australia) also play a role. Dispersal can thus be complicated by weather generating positive conditions (up drafts) that can carry grains further than the research in sinking speeds of pollen suggest (Siska et al. 2001). Animals can also play a role in postdepositional mixing.		

Preservation When preservation conditions are not optimal, damage in the form of exine thinning and loss of surface features, crumpling and complete loss can skew pollen profiles. There is also differential preservation between taxa.	This is a relatively understudied area. Twiddle and Bunting (2010) produce a very small study of the preservation of 4 pollen taxa. More research in this area that could strengthen landscape models would be beneficial. Techniques in fluorescence microscopy (such as Yeloff and Hunt (2005) and Hunt et al (2007)) could be useful in exploring degradation in sporopollenin.	
Environmental setting The original environmental setting of a pollen archive can influence the dispersal of pollen rain. Small lakes, mires and forest hollows will contain geographically restricted pollen rain and therefore (largely) represent a local vegetation signal, whereas larger lakes, blanket bogs and marine sediments reflect a wider (regional) pollen signal. Separating the local taxa from regional taxa is a goal of many palynologists in interpreting fossil pollen results.	There is somewhat a circular argument here in attempting to assign a past environmental setting to a location to be able to interpret the pollen profile, given that, to some extent, pollen interpretation is needed to be able to characterise the setting. For example, a present-day forest hollow may have previously been part of a different environment. Given the vast environmental changes in the Holocene, a forested area may well have been a more open landscape in the past, or even a small lake. Nevertheless, several landscape models incorporating modern pollen dispersal calculations attempt to resolve this issue to refine vegetation interpretations. Consideration of comparator sampling of modern pollen rain is needed in specialist environments, for example, caves (Hunt and Fiacconi 2018) as models are not able to reconstruct environments from these settings.	POLLSCAPE (Sugita 1993; Gaillard et al. 2008); HUMPOL (Bunting and Middleton 2005); LOVE (Sugita 2007a); REVEALS (Sugita 2007b); Mosaic (Middleton and Bunting 2004).

Taxonomic resolution Pollen grains are rarely identifiable to species level and so pollen records often contain a mix of taxonomic levels. This can generate problems with collaborative studies, and also decreases the accuracy of interpretations (for example, *Betula nana* and *Betula pubescens* have slightly different environmental distributions, but are often combined into one pollen count (although identification is sometimes possible by using size and pore depth as distinguishing features (Karlsdóttir et al. 2008)). Therefore, the accuracy of the climate interpretation that could be made in this instance would be reduced).	Improvements in identification and differentiation of indicator species from a higher taxonomic resolution. Bush and Weng (2007) indicate improvements are developing from sharing of large online pollen atlases. Identification capabilities may improve future studies, but taxonomic smoothing will be needed to match the resolution of pre-existing records in collaborative projects.	
Variable pollen counts Inevitably, there is huge variability in total pollen count sums. This can lead to weaker interpretations when only small total counts are achieved, and disconnects in some collaborative projects where vast differences in total pollen counts are noted.	Consideration of the question in the study and the minimum pollen count sum is important to be able to assess the utility of the data (Djamali and Cilleros 2020).	Rarefraction can be conducted to remove the perceived problems with inconsistent count numbers (Birks and Line 1992). (Mottl et al. 2021) introduces a new method (RRatepol) for dealing with inconsistent counts in palaeoecological sequences and enabling rates of change over time to be established.

In recent years, fossil pollen studies have been informed by indicator species modelling, assemblage approaches, and multivariate calibration methods. What has evolved is a diverse array of techniques to transform pollen data, to overcome (or at least reduce the influence of) many taphonomic variables, and to better reconstruct past landscapes, environments and human impacts (Bunting and Middleton 2005; Sugita 2007a, 2007b; Hellman et al. 2008a; Hellman et al. 2008b). However, it is still important to acknowledge that these models are all based on assumptions of uniformitarian principles and that the mathematical models involved need to be flexible enough to represent the complexity of the ecological record. There must therefore be continual refinement and challenges to these models (Edwards et al. 2015).

2.2 Modern pollen studies

Besides the academic challenge and satisfaction of reconstructing past vegetation (and, ergo, environments) from fossil pollen, there are significant justifications for using palynology for understanding long-term ecological processes. Understanding the response of vegetation in the past to changing climate and anthropogenic pressures can be used to predict and mitigate (or at least buffer) detrimental future changes.

Palaeoecology is an ecological discipline, but networking between scientists of both neoecology and palaeoecology has historically been poor (Birks 1993). This divide is potentially driven by the two disciplines traditionally sitting within different departments within Universities. Neoecology (or simply ecology) frequently sits in Biology departments, whereas palaeoecology is studied in many Geography or Earth Science departments. The discipline of ecology often refers to near-present day studies, those that are monitored within weeks, months and years, but rarely decades. These modern-day studies are completed with direct human observations and measurements (Rull 2012) and are rarely able to consider temporal processes that span centuries (or longer). Fossil pollen studies can provide a valuable insight into ecological baselines, past vegetation distributions, vulnerability to disease, and ecological thresholds, and there is untapped potential for use of these techniques within applied ecological contexts of monitoring, management and conservation. Willis and Birks (2006) suggests this is mainly because palaeoecological studies are considered too descriptive and imprecise by ecologists. However, more recent high-resolution palaeoecological records with spatial and temporal records quantified with modelling and supported by robust dating have enabled a recent paradigm shift to encourage greater synergies between palaeoecology and neoecology (Willis and Birks 2006).

2.2.2 Applications of modern pollen

In a novel study in tropical Africa, Gillson (2015) used pollen to identify natural shifts in woodland to grassland in the Tsavo National Park, Kenya. She demonstrated that managing elephant populations to suppress perceived risks of desertification was not needed as feedback loops ensured long-term stability. Numerous reviews on the utility of palynology to answer conservation questions have been published (Birks 1993, 1996, 2019; Willis and Birks 2006; Willis et al. 2007, 2010; Froyd and Willis 2008; Vegas-Vilarrúbia et al. 2011; Rull 2012; Dietl et al. 2015; Jackson and Blois 2015; Nogué et al. 2017 and the recent dedicated PAGES (Past Global Changes) periodical (Gillson et al., 2022) but comparatively few applied ecologists have taken up the available opportunities to use palaeoecological data (see Goodenough and Webb (2022) in this thesis). Birks (2012) suggests this is because many published palaeoecological papers are not easily found by ecologists. The lack of key terms in titles, abstracts, keywords or within the text mean that electronic searches struggle to locate these papers, although this might be changing with more online full text articles.

The application of modern pollen is not restricted to resolving challenges and improving temporal trends in conservation ecology. Modern pollen can be used to understand evolutionary theory through plant-pollinator studies, taxonomic studies and plant genetic diversity (see the review of key questions in pollination ecology by Mayer et al. (2011)).

In contemporary applications of palynology, a key function that pollen can play is in understanding the provenance or source location of items. In aerobiology this may be understanding the composition and density of airborne biological particulate matter, their routes in air currents and receptors (including human nasal cavities). As climate change alters pollen distribution, predictive modelling to forecast long- and short-term changes in pollen concentrations is essential (Burge 2002). In studies of honey (melissopalynology), the analysis of pollen loads on bees and the pollen content of honey is of value to apiarists and the food industry alike (Jones and Bryant 1992; Sniderman et al. 2018). Understanding the plants and habitats that honeybees visit is also critical in the challenge to protect and conserve key foraging areas (Jones and Bryant 2014). Moreover, in commercial honey production, the use of palynology in the provenance of honey has become important in the verification of source location. Sawyer (1985) describes an incident whereby a honey vendor was found guilty of misrepresenting European honey as 'Yorkshire Clover Honey' on the basis of the discovery of pollen from species rarely found in Yorkshire contained within the honey; Bryant and Mildenhall (1998) also describe a similar case from the USA where honey was sold for higher value than its true worth.

It is not just the spatial foraging patterns of bees that modern pollen studies can be used for. Geolocating animals and tracking their movement can be important in answering invasion ecology questions or studying species dispersal and migration. This can be especially valuable for species that are too small or light for GPS trackers to be fitted. For example, Hendrix and Showers (1992) track the long-range movement and food plants used by two species of moths, while Wood et al. (2014, in this thesis) uses pollen on the bills of small warblers to indicate the importance of stopover points on migratory routes. In other novel approaches, Jones (2012) uses pollen to identify the origin of an infestation of weevils in the USA, and Bjune (2000) show temporal and spatial variation in the diet and grazing behaviour of reindeer (*Rangifer tarandus platyrhynchus).* These few (and very specific) examples from the literature suggests that this is an under-utilized area in palynology.

Using pollen to provenance items is also used in forensic palynology. Forensic palynology has been used as a law enforcement tool since the 1950s; use was limited at first but increased through the latter part of the 20th Century and into the 21st Century (Bryant et al. 1990; Mildenhall 1990, 2006; Szibor et al. 1998; Horrocks and Walsh 2001; Mildenhall et al. 2006; Riding et al. 2007)**.** The approach is used in many different areas of the world, with cases reported from as far afield as Nigeria (Walter et al. 2019), Spain (Ochando et al. 2018)**,** New Zealand (Mildenhall 1990), India (More and Bera 2015) as well as the UK (for example, Wiltshire 2006a). However, the prevalence of use is not spatially uniform, for example Bryant and Jones (2006) note the comparatively fewer instances of use in the USA compared to colleagues in the UK.

Published case histories, and the PhD candidate's own experiences, demonstrate that forensic palynology can be used to assist in a range of situations extending from wildlife crimes (such as illegal fishing or badger baiting) to serious crimes such as assault and robbery, murder and terrorism (Mildenhall et al. 2006). In all cases, the aim is to link the pollen assemblage retrieved from key items in a forensic investigation to suspects or interested places. An unpublished case in the UK that gives a good example of the use of palynology in forensic settings was where a suspect was apprehended on suspicion of murder, although no body was found. Police seized the suspect’s shoes and clothing and they were subjected to palynological investigation. Soles and uppers of shoes and lower cuffs of trousers (and other fabric areas if they have been in contact with the ground – e.g. knees) can be used to create a palynological profile from areas visited. In this particular investigation a full range of pollen and spores were isolated, indicative of several areas visited – as you would expect on a pair of shoes worn frequently for daily use. Amongst the array of pollen, were *Sphagnum* and *Polypodium* spores, and pollen from *Tsuga* and *Betula*, and this assemblage was indicative of the edge of a forestry plantation. The police used this information to narrow the search for the missing body, which was indeed found in one of the few areas of Forestry Commission plantations in the area.

2.2.3 Taphonomic considerations in modern pollen studies

The utility of pollen in forensic cases can assist in: (1) relating suspects to a pertinent place; (2) relating suspects to objects and drugs; (3) prove or disprove alibis; (4) help locate missing items or bodies, or determine the routes items or people have taken (Mildenhall et al. 2006; Monckton-Smith et al. 2013; Wiltshire 2016)**.** The discipline relies strongly on comparator samples, rather than vegetation surveys from surrounding areas, as there are known disconnects between vegetation and pollen rain (Quamar et al. 2021). In addition to the challenge of reading mixed pollen profiles from multiple areas, forensic palynology is also confronted by similar taphonomic issues raised in traditional palynology, although taphonomic considerations seem amplified in this challenging field. Wiltshire (2006a) describes some taphonomic considerations and emphasises them with case study examples. Figure 2.4 highlights the main considerations unique to forensic palynology.

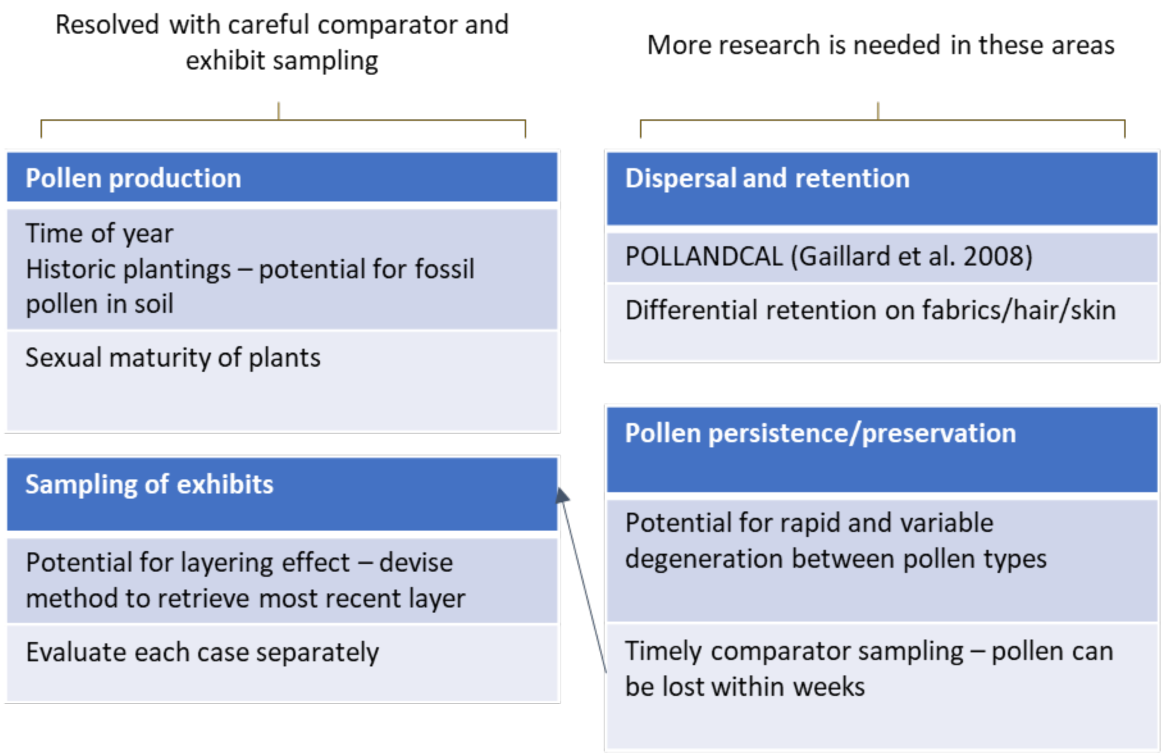


Figure 2.4 A summary of important taphonomic considerations unique within forensic palynology (Author's own).

Given the importance of correctly attesting pollen results in courts of law, it is incredibly important that these taphonomic variables are given priority in research. Webb et al. (2018) (in this thesis) was the first to document retention of pollen on different fabric weaves (before and after washing), and how this can alter interpretations of results. Hunt and Morawska (2020) continued this theme of understanding pollen retention and reports pollen remaining on hands after standard washing techniques. In forensic cases where a suspect attempts to hide their illicit journey, it is vital to understand how washing impacts the pollen assemblage. It also lends questions to cleanliness and contamination on hands in forensic laboratories. Without further research into areas identified in Figure 2.4, palynological expert witnesses in courts could be easily challenged.

The utility of palynology in contemporary science is vast, and only recently has the significance of the potential been realised. Recent advances in concepts and methods across ecology and forensic science has elevated a traditionally palaeoecological discipline to a higher importance. This is owing to palynology often being the only source of baseline data of past ecosystem composition which is essential to inform restoration management. Likewise, palynology can also be the only line of evidence in forensics or provenance studies, further highlighting the importance of the discipline.

2.3 Objective of this thesis

In this thesis, I will demonstrate my ability as an effective researcher by presenting a synthesis of ten papers and book chapters in modern and fossil palynological studies. Nine of these papers/chapters are published in peer-reviewed journals and books, and one is in review (see Table 1.1). These papers fill gaps in the previous literature and attempt to answer questions within the field which advance knowledge and support practitioners (in ecology and forensic science).

The thesis is structured so that Chapter 1 has given an introduction to my academic journey and how the papers within the thesis have arisen, while Chapter 2 (this chapter) has introduced the overall topic of palynology covering uses, history, methods, challenges and opportunities. Moving forward, Chapter 3 presents two papers that document local-scale single site vegetation reconstructions from fossil pollen assemblages, while Chapter 4 moves from single site reconstructions into multi-site regional and continental scale data analysis of known climate perturbations. The thesis then shifts from presenting papers covering traditional palaeoecology to considering modern pollen in contemporary contexts. In Chapter 5, three papers are presented that use palaeoecological approaches to inform contemporary ecological practice and test ecological theory. Finally, Chapter 6 is focused on the utility of pollen to understand provenance or movement, with two papers considering forensic contexts and one paper using similar skills to trace movement of birds on migration.

3. Single-site vegetation reconstructions: informing archaeological research and local vegetation management

This chapter introduces two short papers linked by topic, location and publishing journal and discusses their importance, impact, and implications:

Webb, J.C. McCarroll, J. Carpenter, W.S. Chambers, F.M. Toms, P. and Wood, M. J. (2017) Apparent lack of woodland and abundance of woodland indicator species: the role of humans, birds and rabbits on the changing vegetation of Skomer Island, Wales. *Archaeology in Wales*, 56, 90-99.

Webb, J.C. McCarroll, J. and Morrow, D. (2022a) Palynological contributions towards the Archaeology of Skokholm Island, south west Wales. *Archaeology in Wales*, 60, 45 – 48.

The islands of Skomer and Skokholm sit off the west coast of Pembrokeshire, Wales (Figure 3.1). Their maritime climate and location mean that they were not affected by the ice advances of the Last Glacial Maximum (Clark et al. 2018). Sediments have potentially accumulated on the islands for many thousands of years and contain preserved pollen documenting a vegetation history that is key to solving unanswered questions on the baseline vegetation and archaeology of the Islands.

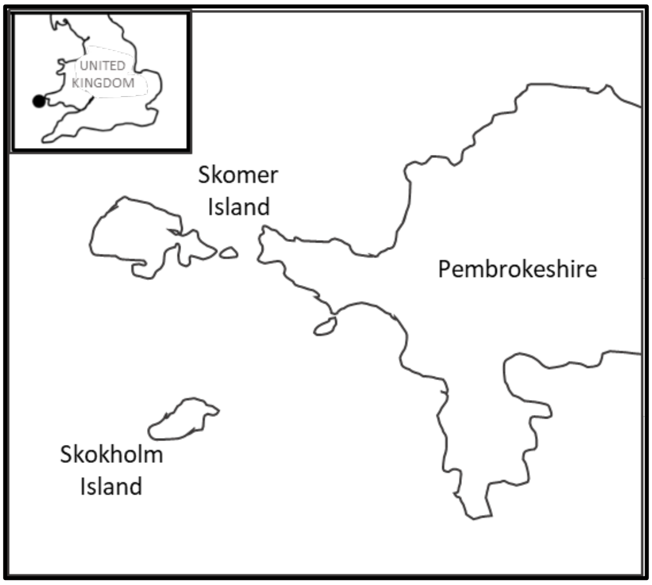


Figure 3.1 Location of Skomer and Skokholm islands in relation to the mainland and the UK setting.

Both islands have a documented history of inhabitation by humans from the 14th Century onwards, but Driver (2007) and The Wildlife Trust of South and West Wales (2021) have documented archaeological finds on the islands that push further back in time. For example, a Late Mesolithic tool was found on Skomer and dated to between 6000 – 9000 years ago, and pottery from 3700 years ago was discovered recently on Skokholm Island (The Wildlife Trust of South and West Wales (2021)). This pottery was the first archaeological find on Skokholm, and the island has not been subject to any archaeological research to date. By contrast Skomer has been the subject of several archaeological digs along lynchets, cairns, huts and field margins (Evans 1990; Barker et al. 2015) and the artefacts from these are mainly stored in the Island's library. Both Skokholm and Skomer have prehistoric field systems evident from airborne laser scanning (Driver 2007).

Although early archaeological investigations on Skomer (Evans 1990) were successful, it was considered that the soils were not acidic enough to preserve pollen. However, there was no attempt to test that assumption and personal communications with Judy Webb (co-author of Moore et al (1991)) in the mid-2000s suggested that, based on her own unpublished preliminary investigation on the vegetation history of Skomer, fossil pollen was not only present but actually reasonably well preserved.

As the role of an archaeologist is to reconstruct and explain the prehistoric context of their archaeological site, it is natural that reconstructing the former vegetation via pollen analysis is important. While many of the principles of pollen analysis remain the same as for any fossil pollen investigation, there are a number of differences to be aware of: (1) the preservation of pollen in archaeological settings, such as excavations into soil, can be very poor (in contrast ditches and moats tend to be anaerobic from waterlogged conditions, so preservation is reasonable); (2) extraction of pollen from archaeological artefacts (such as pottery) uses similar techniques to those used in forensic palynology; and (3) the questions archaeologists are keen to answer are likely to relate to human occupation altering vegetation through agricultural practices rather than natural vegetation communities.

In the two studies presented in this chapter, simple adaptations were made to standard palynological processing techniques listed in Chambers et al. (2010) and Moore et al. (1991)

– see Table 2.1, to guard against deterioration of pollen grains during the chemical extraction phase. Time spent washing and heating in sodium hydroxide (to remove humic acids) was also reduced (this was also sensible given that the soils were not heavily humified) and agitation was kept to a minimum. Pollen preservation was found to be good, with little degradation to the exine, but it is, of course, impossible to assess if any vulnerable grains were lost without evidence. Both papers provide insights into the vegetation that benefited the ongoing archaeological investigations on Skomer (Webb et al., 2017) and Skokholm (Webb et al., 2022). *Cerealia* – type and other indicators of human cultivation (especially arable weeds) were found on both islands in two periods of cultivation, indicating arable farming (and by association, ploughing) was taking place in the late Bronze Age (3000 years ago) and again in the 14th and 15th Centuries.

Palynological profiles for Skomer Island (Webb et al. 2017) were valued highly by archaeological teams working on the island, and led to the University of Gloucestershire being invited to become stakeholders in the cross-disciplinary EU-funded CHERISH (Climate, Heritage and Environments of Reefs, Islands, and Headlands) project. In addition to the CHERISH project, the work completed on Skomer has been included in public lectures by the Royal Commission on the Ancient and Historic Monuments of Wales (RCAHMW) (Christmas Lecture 2016) and subsequent work leading on from Webb et al. (2017) has been published alongside the RCAHMW team (Barker et al. 2015).

Not only do these two papers form contributions towards understanding the archaeology of the islands, they also provide a prehistoric baseline of vegetation for future management decisions. Mike Alexander and Thomas Faulkner (both long-term volunteers on Skomer who are charged with reporting on the vegetation) work tirelessly to undertake annual vegetation surveys. These surveys are considered regularly and inform Island vegetation management plans. While the taxonomic resolution is not the same with the reconstructed vegetation from fossil pollen, it certainly offers insight into broad vegetation communities. In particular for Skomer, the results confirm that no woodland has been present on the island for over 2,800 years. As such, proposals to manage (cull) the rabbit populations and return the vegetation to previous woodlands, are not supported by the palaeoecological data.

The mystery of the missing Mesolithic woodland does, however, continue. Since Leach noted the submerged forest in 1918 off the Pembrokeshire coast (Leach 1918), it has not been studied (there are no scientific publications on the topic, although the submerged forest receives press attention after heavy storms reveal more fossil stumps). Curiosity into the ancient submerged forest led the initial inspiration for work on Skomer (Webb et al. 2017), as the submerged forest can be seen at low tide at Newgale (Newgale is itself close enough to be visible from Skomer on clear days). It is also notable that the origin of the names ‘Skomer’ and ‘Skokholm’ are Norse and mean ‘pole’ or ‘wooded’ isle. Disappointingly, erosion on both Skomer and Skokholm have both created a hiatus in sediment (and pollen) accumulation in the Mesolithic and the evidence of a widespread forest that could have covered the islands and the land bridge between the islands and the mainland is unfortunately missing. Future work to investigate and extend knowledge of the submerged forest and the cause of the demise of the woodland would be beneficial to the palaeoecological community. Understanding the vegetation response to the rising sea level would be of interest, as would human and vegetation interactions around that critical time. Other islands (Grassholm, Gateholm or Ramsay) on the Pembrokeshire peninsula may yield suitable locations for coring and pollen preservation, as might the slacks behind Marloes Sands on the mainland.

Projects involving single site and/or single proxy reconstructions, such as these presented here, are often now rejected by funders and journals as the feeling can be that they do not bring anything new to the discipline. However, while such studies are not able to answer some of the broader questions within palaeoecology, they are useful in answering specific questions raised about particular sites, and showcase how palynological research is useful within archaeology. Seddon et al.’s (2014) paper identifying 50 priority research questions in palaeoecology highlights the need for further clarification on the human-environment interactions in the late Quaternary (question 2). While this question can be answered more broadly in multi-proxy studies, there remains value in these smaller single-site studies, especially when the environments are unique, complex, or there is an archaeological context.

APPARENT LACK OF WOODLAND AND RELATIVE ABUNDANCE OF WOODLAND INDICATOR SPECIES: THE ROLE OF HUMANS, BIRDS AND RABBITS IN THE CHANGING VEGETATION OF SKOMER ISLAND, WALES

Julia C Webb, Julia McCarroll, William Carpenter, Frank M Chambers, Phillip S Toms, and Matt J Wood[1]

To date, the interactions between small mammals, birds and humans have not been explored in a small island environment which is under strong environmental pressure in its exposed maritime position. Skomer is a small island off the coast of south-west Wales; its present vegetation includes a range of grasses, sedges and herbaceous species, and a notable lack of trees. A shallow (460 mm) soil core spanning c.14 ka was retrieved from the South Valley on the Island. Interpretation of the lithology suggests that sediment accumulation rates are slow, and vegetation takes a long time to establish, probably due to thin soils and high rates of erosion. The vegetation inferred from the pollen record is dominated by grasses and herbs, similar to the present day, with periods of greater influence of Ericaceae. There is no evidence for woodland throughout the vegetation record preserved in the soil of the island, although 74 woodland indicator species are currently present. Woodland is likely to have been present during the early Holocene, but has been absent for at least 2800 years. Skomer Island provides an example of the persistence of woodland indicator species in the absence of woodland. The authors suggest that Pteridium provides the surrogate protection from grazing and competition from grasses to allow bluebells and other woodland indicator species to thrive. Birds, humans and small mammals have influenced the vegetation through forest clearance, and then by restricting re-growth through grazing, burrowing disturbance and an altered nutrient load from associated animal faeces.

Introduction

Globally, there is a clear record of a range of anthropogenic influences on the environment on both small and large scales (Auffret, 2014; Azuara *et al*, 2015; Rose *et al*, 2016): for example, Holocene changes in vegetation brought about by actions of human clearance of woodland to farm, to build dwellings and for industrial use (Tallis & McGuire, 1972; Karlıoğlu, 2016; Robinson, 2014) are well documented. Current farming and grazing pressures in many areas of Europe prevent cleared areas from becoming re-wooded (Edwards *et al*, 1989; Hope *et al*, 1996; Kuiters & Slim, 2002; 2003); in most instances these areas are grazed by large ruminants (predominantly sheep and cattle), but there is some evidence that smaller mammals can also prevent mature woodland becoming established at the expected rate of natural succession (Sumption & Flowerdew, 1985; Gill, 1992). Separating anthropogenic effects from climatic impacts is a challenging issue, more so when the environment is in a fragile location such as a dry steep slope; an area of extreme temperature; or exposed maritime position, which are all highly responsive to minor changes in climate. The interactions between small mammals, birds and humans have not been explored to date in a small island environment that by its nature is under more environmental pressure in its exposed maritime position.

Skomer Island is a small (2.92 km²) island off the Pembrokeshire coast in South West Wales, UK (SM 7242 0990). It is designated as a Site of Special Scientific Interest (SSSI), National Nature Reserve and a Special Protection Area owing to the bird life, and a Scheduled Ancient Monument owing to the archaeological remains and relict agricultural landscape in the centre of the island. The archaeology of the island is of interest (Grimes, 1950; Evans, 1986 and 1990; Barker *et al*, 2012; Scanning Skomer, 2012) with current theories suggesting the island has been visited by humans for 35,000 years (evidenced by flint tools) and occupied by settlers since around 5000 years ago (3000 years BC) (Evans, 1986; Roden *et al,* 2011). Remains of huts, field boundaries and cairns (possibly burial cairns) can be identified as evidence of human occupation (Driver, 2007).

Evans (1986) and Driver (2007) suggest the natural vegetation of the island consisted of low shrub oak (*Quercus*), similar to the present-day coastal wooded areas on the nearby mainland, with inclusions of elm (*Ulmus*), birch (*Betula*) and hazel (*Corylus*), with willow (*Salix*) and alder (*Alnus*) in the wetter areas and valleys of the island. It is hypothesised that the natural vegetation would have been altered by the initial settlers as the native woodland was cleared to create valuable grazing land (Roden *et al*, 2011), and the thin soils ploughed for arable use (Barker *et al*, 2012).

---

[1] Centre for Environmental Change and Quaternary Research, School of Natural and Social Sciences, University of Gloucestershire, GL50 4AZ, UK.
Corresponding author: Email: jwebb@glos.ac.uk

The early settlers may have benefited from a mixed economy of livestock and cereals to supplement coastal and marine resources (Driver, 2007). Fields of barley and primitive forms of wheat (spelt and emmer) could have been cultivated on the visible ancient terraces (Evans, 1986).

Current vegetation does not include that associated with arable farming, and the vegetation is not returning to native woodland since arable farming ceased after the Second World War. Ground conditions in many areas are unstable owing to the activity by ground nesting birds and rabbits. Maritime cliff communities form the predominant current vegetation, including National Vegetation Classification MC1 (Rodwell, 2000), which comprises rock samphire (*Crithmum maritimum*), greater sea-spurrey (*Spergularia rupicola*), red fescue (*Festuca rubra*), thrift (Armeria maritima) with the Aster tripolium subcommunity, and MC12, which comprises red fescue (*Festuca rubra*), bluebell (*Hyacinthoides non-scripta*), common sorrel (Rumex acetosa) and Yorkshire fog (*Holcus lanatus*) with subcommunities of lesser celandine (*Armeria maritima and Ranunculus ficaria*) (Rodwell, 2000). Large areas of red campion (*Silene dioica*), wood sage (*Teucrium scorodonia*) and sea campion (*Silene uniflora*) are also present. The bluebell, often regarded as a woodland field-layer plant, is prolific on Skomer, carpeting much of the island with its distinctive flowers during May before blankets of bracken (*Pteridium*) dominate the vegetation. The presence of the bluebell, wood sage and bracken have been thought to indicate Skomer Island's native woodland past by Harris, (2011) and 72 other vascular plants with a strong affinity to woodland settings are also present. Bluebells when found in conjunction with other forest field layer vascular plants is regarded as an ancient woodland indicator species in the British Isles (Rose, 1999), but in parts of western Britain bluebells are considered a plant of open moorland (Rodwell, 2000).

The timing and length of occupation of humans on Skomer Island remains the subject of debate and research. Evans (1986) identifies the colonization period by humans during the Iron Age (2800 – 1900 years BP) as very short, perhaps only a few generations or a century at maximum. Barker *et al* (2012), using LiDAR (Light Detection And Ranging) optical remote sensing technology has identified overlapping field systems and interprets these as evidence for longer occupation. If occupation was relatively short-lived, or seasonal, evidence for changing vegetation would be very rapid, with perhaps only a brief period(s) when arable crops were grown. Questions therefore arise as to why the native woodland has not returned to the island. It is suggested by Harris (2011) that this might be due to rabbits destroying saplings and to harsh maritime conditions.

This study investigates the vegetation history of Skomer

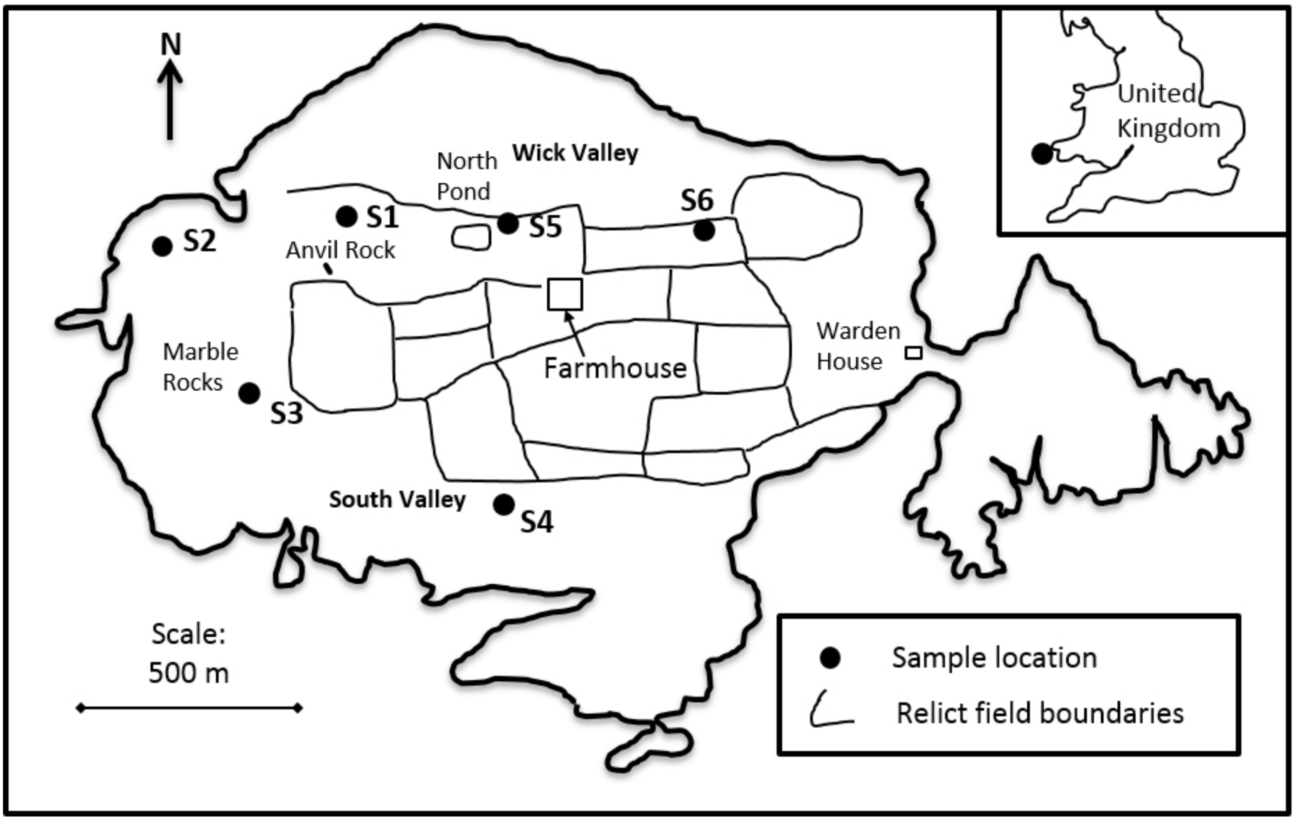


Figure 1. Location of soil samples collected for pollen analysis on Skomer Island.

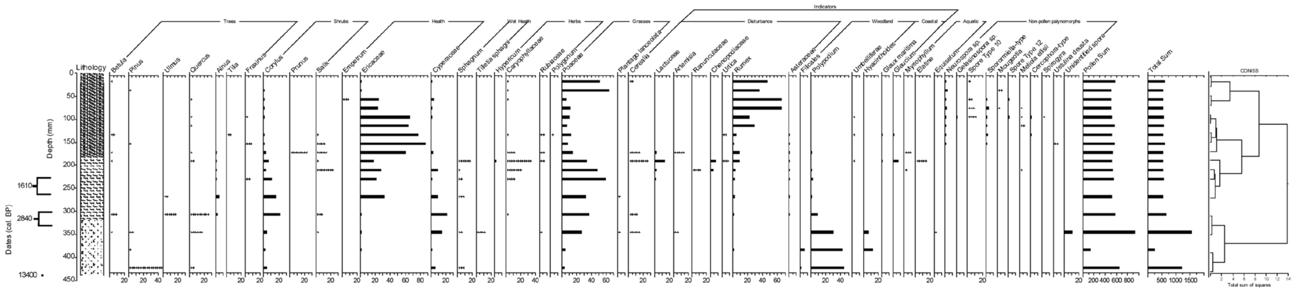


Figure 2. Lithography, palynology and associated dates for Skomer Island

Island with the aim to document the role of humans, birds and small mammals on the changing vegetation. It is intended that this study will enable future conservation plans to acknowledge the natural vegetation history of the island, by representing plant life in the history of Skomer. It is also valuable to recover palaeoenvironmental data from Skomer Island to enable archaeological evidence to be placed in its environmental context.

Methods

Site selection

The soil types of Skomer Island fall into three main categories: stagnogley, stagnopodzol and peaty ranker (Jenkins and Owen, 1995), all of which provide suitable conditions for the preservation of pollen. Until now, pollen analysis has not been successful on the island owing to poor site selection and degradation of fossil pollen (Jenkins and Owen, 1995). A basal topographic survey of the Island, following detailed soil maps (Jenkins and Owen, 1995), was completed to locate a suitable site for sampling (Figure 1) where pollen preservation was better in anaerobic conditions. Evans (1986) noted that the south of the Island may yield suitable locations, although peat there is generally very thin. Coring the soil using a Russian corer (50 mm chamber) revealed that up to 400 mm of soil matter had accumulated at the surface, and in places seemed to be undisturbed either by burrowing or surface trampling by birds and rabbits. Soil erosion was evident in several coastal areas (predominantly in the south and west of the island), so these areas were avoided. In total six sites (S1 to S6) were sampled and soil retrieved from five sites for palynological (pollen) analysis (S5 was disregarded owing to disturbance by plant rootlets). The valley in the north of the island provided more suitable coring locations than the rockier south, although the greatest depth of soil was found in the south of the Island at location S4.

Field and Laboratory Practices

After extraction of the soil cores, the profile was described using Troel-Smith's system (1955) for unconsolidated sediments (Figure 2). This was completed in detail to ascertain whether the soil sequences retrieved represented an undisturbed sediment accumulation, or whether hiatuses were present. The colour variation throughout the cores was also noted by comparing soil samples against a Munsell Color Chart.

In the laboratory, 10 mm cubed aliquots of soil were taken as sub-samples throughout the core at 20 mm intervals from surface to 240 mm then at 40 mm intervals from 280 mm to the base. The aliquots were then exposed to standard palynological chemical extraction techniques (Moore et al, 1991; Chambers et al, 2011), before mounting onto separate slides. At least 500 pollen grains were identified and recorded for each sub-sample, and all non-pollen palynomorphs (NPPs) seen during the pollen count were also noted and identified where possible. The pollen diagram is produced in accordance with the format suggested in Bennett and Willis (2001). Two bulk AMS (Accelerator Mass Spectrometry) radiocarbon dates were obtained from depths based on the results of the pollen analysis (no visible plant macrofossils were available) and an optically-stimulated luminescence date was retrieved from the basal inorganic sediments. AMS dates from above 240 mm depth were not possible owing to a lack of suitable material.

Results

Pollen assemblages from 17 subsamples at stated depths are provided in Figure 2 and are zoned using CONISS (an agglomerative cluster analysis technique which compares the total pollen assemblage of each sample with that of its stratigraphic neighbours). The pollen sum is displayed alongside lithographic representation and calibrated dates presented as calendar years before present.

Radiocarbon dates from the soil/peat deposits shown in Figure 2 and the Age Depth model of Figure 3 [BETA 333440 - 333441] have been calibrated against the IntCal13 Northern Hemisphere radiocarbon calibration curve (Reimer *et al*, 2013), using the INTCAL13 database (Heaton *et al*, 2013).

Basal sediments mainly consisted of clay (Argilla steatodes), coarse sand (Grana suburralia) and small to medium sized gravel particles (Grana glareosa). The top of this basal section of sediment has been luminescence dated to 13.4 ± 0.9 ka placing it within the Lateglacial (ca. 15,000–11,500 cal BP), an interval of rapid climatic shifts of interstadial/stadial amplitude (Walker *et al.* 2003). The date and inorganic nature of the sediments suggest they were deposited during a cold stage and the shallow depth of these clays and gravel indicate either very low accumulation rates, or that erosional processes may have occurred following deposition. The sediments over-lying the clay, sand and gravel consist of moss peat (Turfa bryophytic) with some grass, sedge

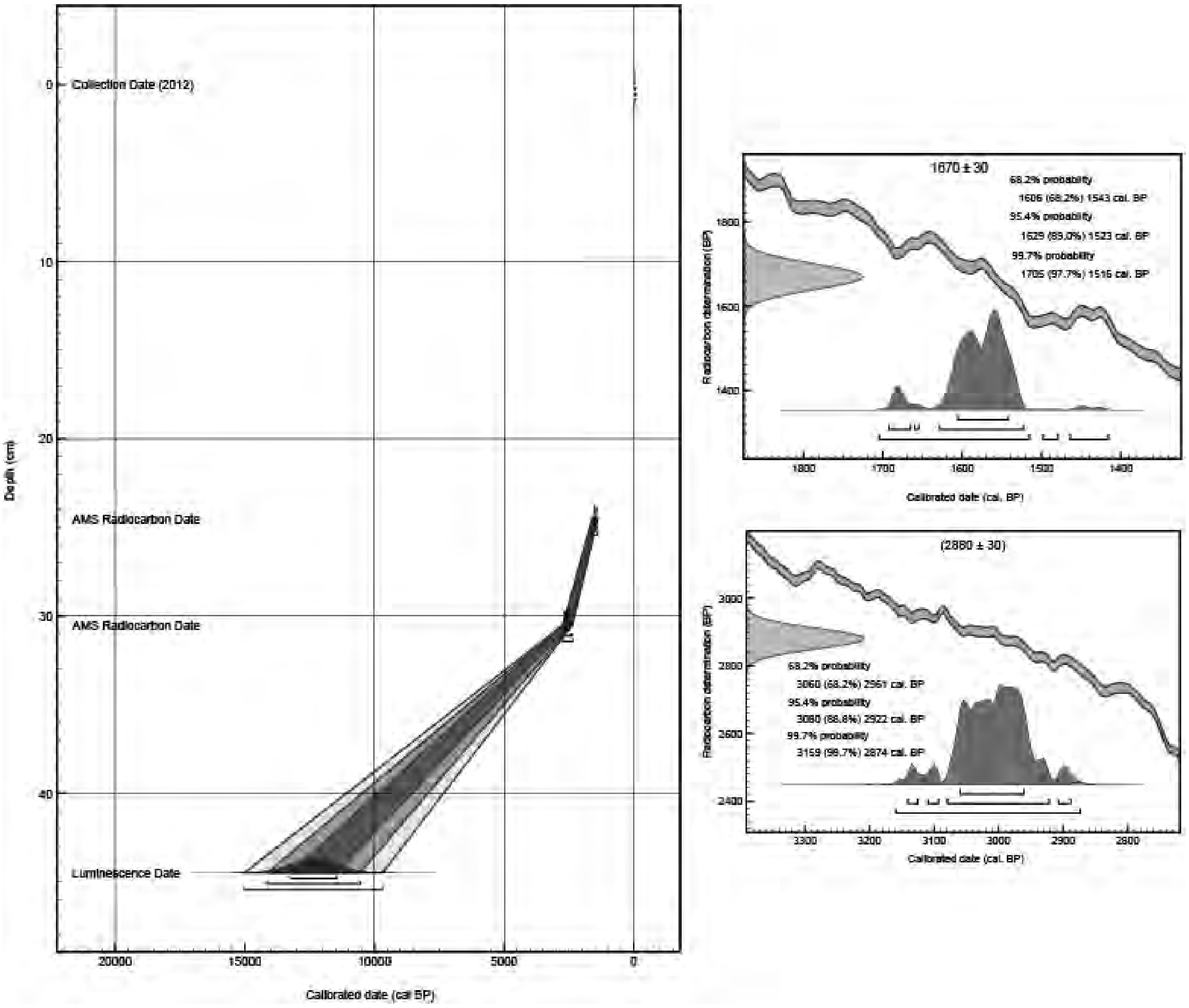


Figure 3. Age–depth model for Skomer Island.

and fern peat (Turfa herbacea) with herbaceous remains (Detritus herbosus). The radiocarbon dates imply either that the soil formation took place at some point after 13.4 ka and indicate a slow accumulation rate, or that a hiatus is present and the early Holocene (Mesolithic) is missing as a result of erosion.

Preservation of pollen was good, with some degradation evident in the lower sediments; however, it did not affect the identification process. Poaceae species were found to be the main component and concentrations fluctuated with heathland shrub and herbs in association throughout the sequence. *Polypodium* is present at the base of the sequence and declines as Ericaceae and Poaceae increase around 300 mm. The highest percentage of tree pollen was noted between the depths of 240 (c.1610 years BP) and 320 mm. *Alnus, Corylus, Pinus* and *Salix* combined accounted for 12.2 % of the assemblage at 240 mm, 21% at 280 mm and 24.7 % at 320 mm, with *Corylus* being the predominant species. There was some evidence of pollen from cereal crops at 180 – 200 mm and at 320 – 360 mm (c.2840 years BP).

NPPs presented in Figure 2 are phaeospores (dark spores) of fungi. van Asperen (2016) demonstrated dark fungal spores are more resistant to acetylation (owing to thick cell walls) than clear spores and so are more likely to be represented in pollen samples that have been subject to standard palynological preparation techniques than clear spores. Table 1 details the ecological preferences for these fungi.

Numbers of NPPs varied from 1 – 16 spores (per 500 pollen grains counted) between 240 mm (1610 years BP) and the modern surface. The absence of recorded NPPs below 240 mm may be a function of poor preservation with increasing age of the sediment. Presence of *Sporomiella* is likely to indicate a high concentration of herbivore dung in the area at that time (Davis, 1987; David and Shafer, 2006; Baker *et al*, 2013). Coprophilous fungi occur at the transition in lithology at 180 mm and their presence continues until 60mm depth from the surface. Wet and stagnant water indicator spores are present throughout the near-surface sediments.

Morphotype	**Taxamonic group**	**Indicator value**	**Reference**
Cercophora (Order: Sordariales)	Ascomycota	Coprophilous fungi	van Geel *et al* (2011)
Kretzchmaria deusta (Ustulina deustra)	Ascomycota	Plant pathogen decaying trunk and roots of broadleaved living trees (particularly Fagus, Quercus, Tilia and Acer) "Brittle Cinder" Cannon et al (1985) reports particularly *common on Fagus in British Isles.*	Cannon et al (1985); Rogers & Ju (1998)
Meliola ellisii	Meliolaceae	Associated with Calluna and Vaccinium	van Geel *et al* (2006)
Mougeotia	Zygnematacae	Freshwater algae: typically found in stagnant water, although some occupy moving water habitats.	John *et al* (2002)
Neurospora and gelasinospora	Ascomycota	Cosmopolitan (Neurospora crassa could be an indicator of local fires)	García *et al* (2004); van Geel and Aptroot (2006)
Spirogyra	Zygnematacae	Filamentous green alga (inc. blanket weed)	John *et al* (2002)
Spore type 10	Unknown	Ecological preferences not known	
Spore type 12	Unknown	Ecological preferences not known	
Sporormiella	Ascomycota	Coprophilous fungi. Associated with herbivore dung	Davis (1987); David and Shafer (2006)

Table 1. Ecological preferences of non-pollen palynomorphs counted in acetylated pollen slides on Skomer Island.

Discussion

This project has enabled a vegetation record to be established dating back longer than any historical record for the Pembrokeshire islands.

Skomer Island since the Ice Ages

Skomer and areas of the current south-west Wales coastline are likely to have escaped glaciation during the Last Glacial Maximum around 20,000 years ago (Lowe and Walker, 1997); periglacial conditions would have been widespread in this cold, windy environment (Bromwich, 1989). Owing to the mass of water held as ice on land, sea levels would have been much lower than today, and Skomer and surrounding areas would have been connected to the mainland UK via land bridges. Models of ocean bathymetry, isostatic rebound and postglacial sea level rise (Evans, 1986, Bell, 2007, UKHO, 2011) predict that the Pembrokeshire islands remained connected until the mid-Holocene when the depth of the basin in Jack Sound would have caused Skomer to be cut off from the mainland (Evans 1986). This geographic separation divided the landscape, and lead to the evolution of the endemic sub-species of Skomer Vole (*Clethrionomys glareolus skomerensis*) (Adler and Levins, 1994). The work here documents sedimentation from 13,400 years ago, long after ice retreated from the Welsh mainland, but before the islands were cut off from the mainland.

Presence of woodland

Evidence indicative of woodland communities during the Holocene was not found in the soil profile, as no individual tree taxon accounted for more than 4.6 % of the total pollen assemblage. *Corylus* was found in significant (25%) number, but is likely to be growing as part of the shrub community rather than arboreal. The significance of the presence of a species varies based on the production and dispersal of pollen for that species. For example, 5 % *Salix* pollen in a sample would suggest a more substantial presence than 5 % *Pinus* pollen (Faegri and Iverson, 1989; Peeters and Totland, 1999) due to the buoyancy and associated travel distances allied with *Pinus*. At such low concentrations it is likely that arboreal pollen taxa derive from woodland on the mainland. Prevailing wind conditions on Skomer are from the south-west, and usually strong, which would usually blow mainland pollen away from the island. However, during spring, winds can be from the north east, which corresponds to the peak period of pollination of the majority of woodland trees (NPARU, 2012). These winds could disperse small amounts of mainland tree pollen towards Skomer Island, accounting for the findings of this study without requiring local tree cover.

Bluebell as an indicator species

Pollen grains from the bluebell were identified in the soil cores, but few near the core surface. Bluebells are entomophilous, producing pollen to be dispersed by insects. Species that rely on insects as distributors for reproduction do not produce as much pollen as those that are anemophilous (wind pollinators). The fossilised pollen in soil cores reflects this and species with entomophilous pollen are under-represented in the record. Analyses of results need to treat these abundance data with care as absence of pollen does not necessarily indicate an absence of the associated species. Faegri and Iversen (1989) and Birks and Line (1992) suggest the use of calculations to compensate for the variation in pollen productivity. Applying this calculation has not been performed in the case presented here; however, because of the present species abundance on the island coupled with low contemporary pollen record, the bluebell pollen noted in the cores can be given a greater weighting within interpretation.

Pollen of bluebell (*Hyacinthoides non-scripta; Endymion non-scripta; syn. Endymion non-scriptus; Scilla non-scripta*) has rarely been recorded in sediments in northwest Europe, although it was recorded from horizons dating from the Bronze Age at the stone-circle site of Cefn Gwernffrwd, Mid-Wales (Chambers , 1983), at a time when woodland was apparently cleared, possibly for agriculture. Despite the perception that the presence of bluebell indicates the former presence of woodland in the history of the island (Evans 1986; Rose, 1999; Harris, 2011), another scenario is that bluebells became established on Skomer Island as a maritime cliff community (Rodwell, 2000). The current canopy of heather, *Pterdium* and similar species act as surrogate tree cover enabling the bluebells to thrive as they do now on the cliff-top vegetation of Pembrokeshire in the absence of woodland. UK distribution maps document bluebells becoming more widespread in the west of the UK, occupying non-woodland locations such as grassland, heath, scrub, hedge banks and sea cliffs (Preston, *et al*, 2002). Blackman and Rutter (1950) describe moist soil in spring with good drainage and low mineral status for the successful growth of bluebell, as present on Skomer. Malloch (1971) describes the cliff-top communities of the Lizard and Land's End Peninsulas (Cornwall, UK) and documents bluebells present in areas of high organic contents, again similar to the conditions on Skomer. At 360 and 400 mm depth in this sequence bluebell pollen appears to be more abundant, indicating perhaps a greater presence of the species blanketing the island than today. Little research has been completed on the nature of competition in bluebell stands since Blackman and Rutter's work in the late 1940s (Blackman and Rutter, 1950). Their work documents the effect of grassland management and grass species on the bluebell vigour. They conclude that the addition of nitrogen decreased bluebell growth when the grass was not cut, but conversely when the grass was cut and nitrogen added the bluebells appeared to respond positively. These results could explain the pattern of increased bluebell occurrence before 2800 years ago on Skomer Island, where the introduced livestock kept the grasses short, but provided the nitrogen in faeces to produce the boom in bluebell population.

Potential for coppice management

A plausible scenario for the presence of bluebells in the absence of woodland is the practice of hazel coppice by early inhabitants. Waller *et al* (2012) state that bluebell is a species characteristic of growing in coppiced woodland areas. The practice of coppicing has existed from the Mesolithic period onwards, and was commonly used to manage *Corylus* (hazel) and *Alnus* (alder). This implies that inhabitants may have been present alongside regularly cut woodland and is consistent with the data, which show that *Corylus* and *Alnus* comprise 82% of the tree pollen found. Low overall levels of pollen may be explained by regular coppicing not allowing the coppice stems to reach maturity before cutting (a coppice interval of 5 years would produce poles, but it is unlikely that the regrowth would flower (Embling, 2014; pers comm). Waller also states that bluebell is heavily under-represented in the modern pollen record, which would also explain the paucity of modern bluebell pollen on the island.

Other indicators of woodland

Neighbouring Skokholm island (a few kilometres to the south) carries a Norse name, thought to translate as 'wooded' or 'pole' island (Morgan, 1887; Goodman and Gillham, 1954 p.300), perhaps indicating the management of hazel coppice in prehistory. Archaeological evidence also supports the coppicing scenario on Skomer Island, where field boundaries in Wick Valley indicate an unenclosed seaward area, which may have been reserved for coppiced woodland (Barker *et al*, 2012) to grow the building materials essential for the construction of roundhouse roofs.

Current vegetation surveys of the island demonstrate that species which have a strong affinity with woodland settings thrive on Skomer Island. 74 woodland indicator species are present on the island (species from ancient woodland indicator lists provided by Peterken (1974), The Woodland Trust (2007), Durham County Council (2008) and Glaves (2009)), a significant number given the lack of current arboreal vegetation. Historic evidence of many woodland ground flora genera cannot be identified owing to the difficulties in separating species during fossilised pollen identification, but considering woodland is likely to have existed during the early Holocene, and indicator species are currently present, it seems reasonable to assume that these indicator species have been persistent since the demise of the woodland.

Pteridium is thought to be a woodland genus, as it was originally described as a component of open forest (Huntley and Birks, 1985; Marrs and Watt, 2006). However, it occurs in many different plant communities, including open grassland, heath and maritime habitats (Marrs and Watt, 2006). The presence of *Pteridium* on Skomer cannot therefore be used as an indicator for the presence of prehistoric woodland on the island. The pollen diagram is devoid of *Pteridium* spores throughout the sequence suggesting a recent expansion of *Pteridium* to its current local dominance. Marrs and Watt (2006) show that across the British Isles *Pterdium* abundance increases with recent land management changes, which suggests that the area has experienced significant anthropogenic interference. Pterdium does provide a symbiotic partnership to woodland indicator species, particularly bluebell, enabling survival by providing late spring shade and protection from overgrazing (Blackman and Rutter, 1950). This does, however, leave a question as to what was providing the surrogate canopy before the recent *Pteridium* expansion.

Direct and indirect influence of humans on vegetation

A conspicuous detail in the sediment sequence on Skomer Island is the slow accumulation or hiatus of sediment spanning the Mesolithic period. Subsequently, Neolithic evidence of humans is apparent with evidence for construction of dwellings (Driver, 2007; Barker *et al*, 2012) and introduction of farming. These interventions may have caused an imbalance in Skomer's fragile maritime landscape and potentially led to soil erosion creating a hiatus in the sediment sequence.

The alternating fluctuations of heath to grasses can be attributed to a number of factors. Environmental changes such as increased grazing pressures and burning are thought to benefit certain grass species at the expense of heathland species (Yeloff *et al* 2006; Ramchunder et al 2009). *Ericaceae* seems to be replaced by *Poaceae* at 40 mm depth which may be attributed to increased grazing pressures from rabbits and previously from sheep and goats on Skomer Island. The synchronous decrease in *Ericaceae* and rise in *Poaceae* at 180 mm depth could be attributed to wildfire or anthropogenic burning. Current conservation management plans for Skomer's vegetation consider that the shrinkage of heathland and expansion of grassland in recent decades may be due to increased soil nitrogen input from the increasing seabird populations (from their droppings and predated remains) (Alexander, 2014), the primary feature for Skomer's many designations as a nature reserve. The people of the St Kilda archipelago (Outer Hebrides, Scotland) harvested seabirds for food on the inhabited island of Hirta and neighbouring islands, and it is difficult to imagine that Skomer's residents or visitors did not do the same on Skomer, currently home to an estimated 300,000 pairs of Manx shearwaters *Puffinus puffinus* (Perrins *et al*, 2012) and 20,000 Atlantic Puffins *Fratercula arctica* that nest in accessible burrows. As seabird populations were reduced by human exploitation, nitrogen input would have decreased, perhaps promoting the spread of heathland as soils became more nutrient poor. If the human population abandoned the island once seabirds were over-exploited, or soils became too poor for crops, the situation would have been reversed as seabird populations recovered and grassland returned, rendering the island suitable for the next human colonisation.

The decline of *Polypodium* after 320 mm depth (c. 2840 cal. BP) could be related to a change in moisture conditions on the island, as the true ferns prefer sites of thin rocky soils in moist to mesic conditions and are much less common in drier areas (Clapham, 1962). An ageing population of *Polypodium* with a high population density and low light intensity causing a decrease in germination could also explain the decline (Smith and Robinson, 1975); however, with *Polypodium* only making up 25% of the total assemblage, the more likely explanation would be that conditions became too dry.

The species represented in a pollen assemblage are those whose plants have reached the flowering stage, and can be an incomplete view of the plant spectrum that existed in the past. This is particularly so where humans have been present and may have cut plants before they reached the flowering stage, resulting in an absence of pollen grains for species that may have lived alongside the island's earlier inhabitants (Faegri and Iversen, 1989). This is not the case for arable cereals. Although cereal pollen can be sparse (Bower, 1992), the pollen should be present in greater number within the soil profile if the settlers of Skomer were using the fields for cultivation of crops (Evans, 1986). The absence of this evidence suggests that cereals did not form a significant portion of Skomer islander's crops, and that the stems of the cereals were not used as thatching for dwellings. However, bearing in mind the possibility of short-lived human occupation, the resolution of the pollen study (20 mm intervals) could have overlooked the presence of cereals.

Evidence of grazing

Areas of modern-day high grazing intensity (Davis, 1987; David and Shafer, 2006; van Asperen *et al*, 2016) report coprophilous fungi in similar concentrations to those seen in the section 60 – 180 mm on Skomer Island. It is unlikely that these fungi are an indicator of small mammal grazing presence, because van Asperen notes in her modern-day experiments (van Asperen, 2016) that dry dung does not give rise to fungal growth to produce spores. It is hypothesised therefore that the small size of rabbit faeces would dry rapidly and not yield fungal spores. van Asperen (2016) also notes a seasonal variation in coprophilous spore production whereby fungal growth is limited in very wet (winter) and very dry (summer) conditions. On this basis, coprophilous spore presence in Skomer sediments can be interpreted as evidence for introduced large herbivore grazing.

Conclusion

Separating anthropogenic effects from climatic responses and small mammal impacts has been challenged on Skomer Island in a project that has enabled an ecological record spanning longer than any historical record for the Pembrokeshire islands to be established. Reconstruction of the vegetation history did not reveal that extensive woodland existed on the island in the last 2800 years, although regional evidence suggests woodland was present in the early Holocene. Instead broad fluctuations in shrubs and heathland species dominate the vegetation history sequence, and woodland field layer vascular species have been persistent on the island without the assumed woodland canopy. Bluebell continue to be a feature of the island in late spring, supported by suitable soil conditions and favourable conditions bought about by grazing from large herbivores; however, their present-day abundance may in part be a consequence of grazing pressure by naturalised rabbits on other plant species. Human occupation of the island has provided both direct and indirect influences on the environment, by altering the vegetation through farming, grazing and potentially causing extensive soil erosion; and exploiting the seabird populations, possibly changing the nutrient status of the thin soils.

Acknowledgements

Thanks to the then Warden of Skomer Island, Chris Taylor and the Wildlife Trust for South and West Wales for logistical support; and Toby Driver (RCAHMW) and to Mike Alexander and Eline van Asperen for fruitful discussions.

References

Adler, GH and Levins, R (1994) The Island Syndrome in Rodent Populations. *The Quarterly Review of Biology* Vol. 69, No. 4 pp. 473-490.

Alexander, M (2014) Skomer Island vegetation. Unpublished report. Available from Mike Alexander: mike_alexander@btinternet.com.

Auffret, A, Berg, J & Cousins, S (2014) The geography of human-mediated dispersal *Diversity & Distributions*, 20, 12: 1450-1456.

Azuara, J, Combourieu-Nebout, N, Lebreton, V, Mazier, F, Müller, S, & Dezileau, L (2015) Late Holocene vegetation changes in relation with climate fluctuations and human activity in Languedoc (southern France), *Climate Of The Past*, 11, 12: 1769-1784.

Baker, AG, Bhagwat, SA & Willis KJ (2013) Do dung fungal spores make a good proxy for past distribution of large herbivores? *Quaternary Science Review*, 62: 21–31

Barker, L, Davis, O and Driver, T (2012) Puffins amidst prehistory : reinterpreting the complex landscape of Skomer Island. *In*: Reflections of the Past. Essays in honour of Frances Lynch. Welshpool: Cambrian Archaeological Association.

Bell, M (2007) Prehistoric coastal communities: the Mesolithic in western Britain. CBA Research Reports

Bennett, KD and Willis, KJ, (2001). Pollen. Tracking Environmental Change Using Lake Sediments. Volume 3: Terrestrial, Algal, and Siliceous Indicators: 5 – 32

Bevins, R (1980). Skomer Island. *Geological Conservation Review* (17) 6: 2731

Birks, HJB and Line, JM (1992) The use of rarefaction analysis for estimating palynological richness from Quaternary pollen-analytical data. *The Holocene*, 2: 1–10

Blackman, GE and Rutter, AJ (1950) Physiological and ecological studies in the analysis of plant environment. V. An assessment of the factors controlling the distribution of the bluebell (Scilla nonscripta) in different communities. *Annals of Botany* 14: 487-520

Bower, M (1992) Cereal Pollen Dispersal: A Pilot Study. *Cambridge Archaeological Journal*, 2: 236-241

Bromwich, DH (1989) Satellite Analyses of Antarctic Katabatic Wind Behavior. *Bulletin of the American Meteorological Society,* 70: 738–749

Cannon, PF, Hawkesworth, DL and Sherwood-Pike, MA (1985) The British Ascomycetes. An annotated checklist. *Commonwealth Mycological Institute*. UK. 30

Chambers, FM (1983) The palaeoecological setting of Cefn Gwernffrwd – a prehistoric complex in Mid-Wales, *Proceedings of the Prehistoric Society* 49: 303–316.

Chambers, FM and Daniell, JRG (2011). Conservation and habitat restoration of moorland and bog in the UK uplands: a regional, paleoecological perspective. *PAGES Newsletter*, 19, pp. 45-47.

Chambers, FM, van Geel, B and van der Linden M (2011). Considerations for the preparation of peat samples for palynology, and for the counting of pollen and non-pollen palynomorphs. *Mires and Peat* 7 (Article 11): 1–14.

Clapham, AR, Tutin, TG and Warburg, EF (1962). *Flora of the British Isles*. Cambridge University Press, Cambridge.

Driver, T, (2007). Pembrokeshire: Historic Landscapes from the Air. RCAHMW, 77-83

David, OK and Shafer, DS (2006) Sporormiella fungal spores, a palynological means of detecting herbivore density. *Palaeogeography, Palaeoclimatology and Palaeoecology* 237: 35 – 50

Davis, OK (1987). Spores of the dung fungus Sporormiella: increased abundance in historic sediments and before Pleistocene megafaunal extinction. *Quaternary Research* 28: 290-294.

DURHAM COUNTY COUNCIL, (2008) The County Durham Landscape Guidelines: Woodland and Forestry, Durham County Council.

Edwards, PJ, Putman, RJ, Hill SDD, Hill, R How C, and Mann, JCE (1989). Vegetational and faunal changes in an area of heavily grazed woodland following relief of grazing. *Biological Conservation* 47: 1: 13.

Etienne, D, and Jouffroy-Bapicot, I (2014) Optimal counting limit for fungal spore abundance estimation using *S porormiella* as a case study. *Vegetation History and Archaeobotany* 23: 743-749.

Evans, JG (1986). Prehistoric farmers of Skomer Island: An archaeological guide. In: *Nature conservation*. Dyfed: West Wales Trust. 13-14.

Embling, B (2014) Personal Communication: Email to Barry Embling, 2 April.

Faegri, K and Iversen, J (1989). The interpretation of pollen diagrams. In: *Textbook of pollen analysis*. 3rd ed. Oxford: Blackwell Scientific Publications. 144-153.

García, D, Stchigel, AM, Cano, J, Guarro, J and Hawksworth, DL (2004). A synopsis and re-circumscription of Neurospora (syn. Gelasinospora) based on ultrastructural and 28S rDNA sequence data. *Mycological Research*, 108: 1119-1142.

Gill, RMA (1992) A Review of Damage by Mammals in North Temperate Forests. 2. Small Mammals. *Forestry* 65 (3): 281-308.

Glaves, P, Handley, C, Birbeck, J, Rotherham, I and Wright, B, 2009. A survey of the coverage, use and application of ancient woodland indicator lists in the UK. Hallam Environmental Consultants Ltd., Biodiversity and Landscape History Research Institute/Geography, Tourism and Environment Change Research Unit, Sheffield Hallam University, Sheffield.

Godwin, H (1975) *History of the British Flora* (2nd edition) Cambridge University Press, Cambridge.

Goodman, GT and Gillham, ME (1954) Ecology if the Pembrokeshire Islands: II. Skokholm, Environment and Vegetation. *Journal of Ecology* 42 (2): 269 - 327.

Grimes, WF (1950). The Archaeology of Skomer Island. *Archaeologia Cambrensis*, 101, 1-20.

Grimm, EC (2004) TILIA 1.7.16 Illinois State Museum, Springfield

Harris, J (2011) Skomer Frequently Asked Questions: Responses from the Assistant Warden. The Wildlife Trust of South and West Wales.

Heaton, TJ, Blackwell, PG and Buck, CE (2009). A Bayesian approach to the e stimation of radiocarbon calibration curves: the IntCal09 methodology. *Radiocarbon* 51(4):1151–64.

Hope, D, Picozzi, N, David C & Moss, R (1996), Effects of Reducing Sheep Grazing in the Scottish Highlands, *Journal of Range Management*, 4: 301.

Huntley, B & Birks, HJB (1983). *An Atlas of Past and Present Pollen Maps for Europe: 0–13000 Years Ago*. Cambridge University Press, Cambridge, UK.

Jenkins, DA and Owen A (1995). *The soils of Skomer*. Contract Science Report for Countryside Commission for Wales (CCW) 157.

John, DM, Whitton, BA & Brook, AJ (eds) (2002) The Freshwater Algal Flora of the British Isles. *An identification guide to freshwater and terrestrial algae*. pp. 479-593. Cambridge: Cambridge University Press.

Karlıoğlu, N, Caner, H, Rauh, N, Akkemik, Ü, Köse, N & Connor, E (2016), Palynological evidence for human occupation in western Rough Cilicia (southwest Turkey), *Quaternary International,* 401.

Kuiters, A, & Slim, P (2002) Regeneration of mixed deciduous forest in a Dutch forest-heathland, following a reduction of ungulate densities, *Biological Conservation*, 105: 65-74.

Kuiters, A, & Slim, P (2003). Tree colonisation of abandoned arable land after 27 years of horse-grazing: the role of bramble as a facilitator of oak wood regeneration. *Forest Ecology And Management, 181.*

Lowe, JJ and Walker, MJC (1997) *Reconstructing Quaternary Environments*. Longman: London.

MacCallum, R (2011) *Pembrokeshire coast through time*. Amberley Publications: Stroud.

Malloch, A (1971) Vegetation of maritime cliff tops of the Lizard and Land's End Peninsulas, West Cornwall. *New Phytologist*, 70, 6: 1155–1197.

Marrs, R, & Watt, A (2006) Biological Flora of the British Isles: Pteridium aquilinum (L.) Kuhn, *Journal of Ecology,* 94, 6: 1272-1321.

Met Office (no date) Wales: climate. Available from: http://www.metoffice.gov.uk/climate/uk/[Accessed 12.12.12].

Morgan, T (1887) *Handbook of the origin of place-names in Wales and Monmouthshire*. Available online http://llennatur.com/files/u1/on__Place-names_ThosMorgan_Pr_HWSouthey_1887.pdf accessed 1.8.14

Moore, PD Webb, JA Collinson, ME (1991) *Pollen analysis*. 2nd ed. Oxford: Blackwell Scientific.

NPARU (2012) Pollen Calendar. National Pollen and Aerobiology Research Unit. Available from http://www.worcester.ac.uk/discover/pollen-calendar.html [Accessed 12.12.12]

Peeters, L and Totland, O (1999). Wind to insect pollination ratios and floral traits in five alpine Salix species. *Canadian Journal of Botany* 77(4): 556-563

Perrins CM, Wood MJ, Garroway CJ, Boyle D, Oakes N, Revera R, Collins P & Taylor C (2012) A whole-island census of the Manx Shearwaters *Puffinus puffinus* breeding on Skomer Island in 2011. *Seabird* 25:1–13.

Peterken, GF (1974) A method of assessing woodland flora for conservation using Indicator Species. *Biological Conservation*, 6, 239-245.

Preston, CD, Pearman, DA & Dines, TD (2002) *New atlas of the British and Irish flora*. Oxford: University Press.

Ramchunder, SJ, Brown, LE and Holden, J (2009) Environmental effects of drainage, drain-blocking and prescribed vegetation burning in UK upland peatlands. *Progress in Physical Geography*, 33: 49-79.

Reimer, PJ, Baillie, MGL, Bard, E, Bayliss, A, Beck, JW, Blackwell, PG, Bronk.

Ramsey, C, Buck, CE, Burr, GS, Edwards, RL, Friedrich, M, Grootes, PM, Guilderson, T.P., Hajdas, I, Heaton, TJ, Hogg, AG, Hughen, KA, Kaiser, KF, Kromer, B, McCormac, FG, Manning, SW, Reimer, RW, Richards, DA, Southon, JR, Talamo, S, Turney, CSM, van der Plicht, J, and Weyhenmeyer, CE (2009) IntCal09 and Marine09 radiocarbon age calibration curves, 0-50,000 years cal BP. *Radiocarbon* 51: 1111-1150.

Robinson, M (2014) The ecodynamics of clearance in the British Neolithic, *Environmental Archaeology*, 19, 3: 291-297.

Roden, J and others (2011). Skomer Island History Factsheet. Welsh Wildlife Factsheet. Available from: http://www.welshwildlife.org/wp-content/uploads/2011/05/History-of-Skomer-including-timeline.pdf

Rodwell, JS (2000). Maritime communities and vegetation of open habitats. In: *British plant communities*, volume 5. UK: Cambridge University Press. 264-267, 310-314.

Rogers, JD, and Ju, Y-M (1998) The genus Kretzschmaria. *Mycotaxon* 68: 845-893

Rose, F (1999) Indicators of ancient woodland. *British Wildlife*, 10: 241-251.

Rose, R, Monteith, D, Henrys, P, Smart, S, Wood, C, Morecroft, M, Andrews, C, Beaumont, D, Benham, S, Bowmaker, V, Corbett, S, Dick, J, Dodd, B, Dodd, N, Flexen, M, McKenna, C, McMillan, S, Pallett, D, Rennie, S, Schäfer, S, Scott, T, Sherrin, L, Turner, A, & Watson, H (2016) Evidence for increases in vegetation species richness across UK Environmental Change Network sites linked to changes in air pollution and weather patterns, *Ecological Indicators*, 68, Assessing ecosystem resilience through Long Term Ecosystem Research: observations from the first twenty years of the UK Environmental Change Network, pp. 52-62.

Scanning Skomer (2012) *Current Archaeology*, (268), 7.

Smith, DL and Robinson, PM (1975) The Effects of Spore Age on Germination and Gametophyte Development in Polypodium vulgare L. *New Phytologist*, 74: 101-108.

Sumption, KJ, & Flowerdew, JR (1985) The ecological effects of the decline in Rabbits (Oryctolagus cuniculus L.) due to myxomatosis. *Mammal Review*, 15(4): 151.

Tallis, J, & McGuire, J (1972), Central Rossendale: The Evolution of an Upland Vegetation: I. The Clearance of Woodland, *Journal of Ecology*, 3: 721

The Woodland Trust (2007) Back on the Map: An inventory of ancient and long-established woodland for Northern Ireland. Available from www.backonthemap.co.uk Accessed 26.10.2016

Troels-Smith, J (1955) Characterisation of unconsolidated sediments. *Danmarks Geologiske Undersøgelse 4 Række*, 3: 1-73.

UKHO (2011) INSPIRE Portal and Bathymetry DAC. Available from: http://aws2.caris.com/ukho/mapViewer/map.action Accessed: 31 August 2016

van Asperen, E (2016) Reconstructing past herbivore abundance using dung fungal spores: a taphonomic approach, paper presented to Quaternary Research Association Discussion Meeting, National Museum Wales, 6 – 7 September 2016.

van Asperen, E, Kirby, J, and Hunt, C (2016) The effect of preparation methods on dung fungal spores: Implications for recognition of megafaunal populations. *Review of Palaeobotany and Palynology* 229: 1-8.

van Geel, B and Aptroot, A (2006) Fossil ascomycetes in Quaternary deposits. *Nova Hedwigia* 82: 313-329.

van Geel, B, Aptroot, A, Mauquoy, D (2006) Sub-fossil evidence for fungal *hyperparasitism* (*Isthmospora spinose* on *Meliola ellissii* on *Calluna vulgaris*) in a Holocene intermediate ombotrophic bog in Northern England. *Review of Palaeobotany and Palynology* 141: 121-126.

van Geel, B, Gelorini, V, Lyaruu, A (2011) Diversity and ecology of tropical African fungal spores from a 25,000-year palaeoenvironmental record in southeastern Kenya.

Review of Palaeobotany and Palynology 164 (3-4):174-190

Walker, M, Coope, G, Sheldrick, C, Turney, C, Lowe, J, Blockley, S & Harkness, D (2003) Devensian Lateglacial Environmental Changes In Britain: A Multi-Proxy Environmental Record From Llanilid, South Wales, UK. *Quaternary Science Reviews*, 22: 475-520.

Waller, M, Grant, MJ, Bunting, MJ (2012) Modern pollen studies from coppiced woodlands and their implications for the detection of woodland management in Holocene pollen records. *Review of Palaeobotany and Palynology.* 1-63.

Wolf, FA (1969) Disintegration of Fungus Spores. *Journal of Elisha Mitchell Scientific Society* 85:92-94.

Yeloff, DE, Labadz, JC and Hunt, CO (2006) Causes of degradation and erosion of a blanket mire in the southern Pennines, UK. *Mires and Peat*, 1: 1-18.

PALYNOLOGICAL CONTRIBUTIONS TOWARDS THE ARCHAEOLOGY OF SKOKHOLM ISLAND, SOUTH WEST WALES

Julia Webb[1], Julia Mccarroll and Darrien Morrow

Site details

Skokholm (SM 736 048, 51° 41' N, 05° 17' W, 40m asl) is a small island (1.05km^2) off the Pembrokeshire coast in south west Wales, U.K. “Skokholm” is a Norse name, thought to translate as ‘wooded’ or ‘pole’ island (Morgan 1887), and perhaps indicating the early management of hazel coppice on the island in prehistory. Along with neighbouring islands, Skokholm is designated as a Site of Special Scientific Interest (SSSI) and a Special Protection Area owing to its importance as a seabird breeding ground, and a Marine Reserve (Thompson 2007).

The region is characterised with a temperate maritime climate of cool summers and mild winters. The average annual rainfall is 929 mm. The rather flat topography is underlain by Devonian Old Red Sandstone on top of which up to 1300 mm of brown earth soil has developed. There are three wet areas on the island, East Bog (51° 41' 52.4" N 05° 16' 22.9" W), South Pond (51° 41' 49.9" N, 05° 17' 09.1" W) and North Pond (51° 42' 00.5" N, 05° 16' 41.9" W).

The vegetation communities of the island are thought to be fairly unique (Thompson 2007) and comprise: sub-maritime grasslands and wet heath, with notable inclusions of eagle fern (*Pteridium aquilinum*), bluebell (*Hyacinthoides non-scripta*), common sorrel (*Rumex acetosa*), and Yorkshire Fog (*Holcus lanatus*) with the sub-communities of sea thrift (*Armeria maritima*), lesser celandine (*Ranunculus ficaria*) and sea campion (*Silene maritima*). The dominant suppressant for maintaining the vegetation is the intense grazing pressure from rabbits (*Oryctolagus cuniculus*) and the manuring and trampling by seabirds.

Neighbouring islands are well-known for their rich archaeological history (Webb *et al* 2017). Skokholm, in contrast, until recently (The Wildlife Trust of South and West Wales 2021) is thought to be relatively uninhabited until more modern times, where documented evidence notes the island was a rabbit farm in the 14th and 15th centuries (Thompson 2007). Given the interrupted history of neighbouring Skomer Island (Webb *et al* 2017), Skokholm’s vegetation history has been explored in this paper with the aim to extend the sequence back further into the Holocene and to answer questions relating to recent early archaeological finds (The Wildlife Trust of South and West Wales 2021) and the Mesolithic decline of woodland in the area.

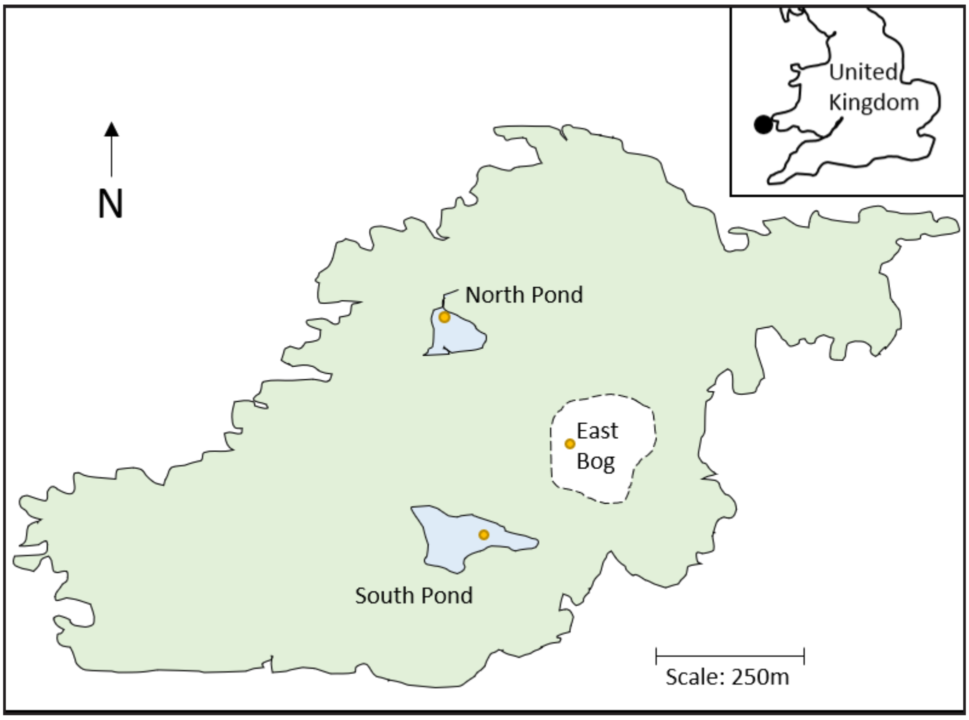


Figure 1: Skokholm Island with three areas located as possible sampling options. North Pond was selected as the most suitable location.

---

[1] School of Natural and Social Sciences, University of Gloucestershire, Francis Close Hall, Swindon Road, Cheltenham GL50 4AZ, UK. jwebb@glos.ac.uk

Methods

Site selection

The soil type of Skokholm Island is a uniform brown earth, meaning the soil type across the island does not change, except for topographically led variation where peaty soil develops. Until now, pollen analysis has not been attempted on the island. A basal topographic survey of the island located suitable sites for sampling (Figure 1) where pollen preservation was likely in anaerobic conditions. Coring the sediment using a Russian corer (50 mm chamber) revealed that up to 680 mm of accessible sediment had accumulated at the surface, and in places seemed to be undisturbed either by burrowing or surface trampling by birds and rabbits. North Pond was selected for detailed palynological analysis owing to its undisturbed nature

Field and Laboratory Practices

After extraction of the soil core, the profile was described using Troel-Smith's system (1955) for unconsolidated sediments. This was completed in detail to ascertain whether the soil sequences retrieved represented an undisturbed sediment accumulation, or whether hiatuses were present. The colour variation throughout the cores was also noted by comparing soil samples against a Munsell Colour Chart.

In the laboratory, 10 mm cubed aliquots of soil were taken as sub-samples throughout the core at 20 mm intervals from surface to the base. The aliquots were then exposed to standard palynological chemical extraction techniques (Moore *et al* 1991; Chambers *et al* 2011), without exotics, before mounting onto separate slides. A minimum of 500 pollen grains were identified in each sample between 0 – 480 mm depth. There was no evidence of fossilised pollen between 500 – 680 mm. The pollen sum includes all pollen grains, but not spores or non-pollen palynomorphs (NPPs). Taxon frequencies were calculated as percentages of the total terrestrial pollen sum. One AMS ^{14}C measurement was performed on bulk organic sediment at 240-260 mm depth by the Beta Analytic Radiocarbon Dating Laboratory, Florida. The ^{14}C age was converted into calendar years BP using the Intecal 13 database (Reimer *et al* 2013) [Beta–444232: North Pond 240–260 mm 320 ±30 BP (cal AD 1470 to 1650; cal BP 480–300)].

Sediment description

A short (680 mm) sediment core was retrieved from the deepest section of North Pond using a narrow-gauge Russian corer. The lithostratigraphic units are the following (Colours coded to Munsell Soil Colour Chart):

0–250 mm Very dark brown organic material, poorly decomposed 2.5Y 2.5/1 (black)

250–500 mm Lighter brown, gradually more clay 2.5Y 3/2 (brown)

500–530 mm Lighter brown inorganic (clay) matter 2.5Y 4/2 (dark greyish brown)

530–610 mm Lighter coloured clay 2.5YR 6/4 (light yellowish brown)

610–650 mm Organic lake mud 2.5YR 3/3 (dark olive brown)

650–680 mm Organic lake mud 2.5YR 4/3 (olive brown)

Interpretation

The pollen diagram (Figure 2) was divided into four Local Pollen Assemblage Zones (LPAZ) 1–4, using total sum of squares method via the CONISS software (Grimm 1987)

LPAZ 1 (390–480 mm) Human Cultivation Phase 1

In this zone, the pollen assemblage is dominated by grasses and sedges (90%) with minor contributions of herbs (notably *Malvaceae* and *Chenopodioideae*). *Plantago lanceolata* and pollen of cultivated cereals (Cerealia-type) are present at low levels, suggesting an element of human disturbance on the island at this time. The frequency of arboreal pollen is very low, and overall tree and shrub pollen decreases throughout the zone.

LPAZ 2 (340–390 mm) Open Grassland

Notably in this zone the island vegetation is unusually characterised almost solely by grasses. Minor elements of sedges and ferns suggest an impoverished landscape of low species diversity.

LPAZ 3 (120–340 mm) Human Cultivation Phase 2

A much richer diversity of species on the island is present in this zone, still dominated, however, by grasses. There is evidence of anthropogenic disturbance with a rise in *Plantago lanceolata, Taraxacum* and *Cerealia*-type. It is likely that this horizon corresponds to the existing rabbit farm in the 14th and 15th Century following the radiocarbon date (AD 1470 to 1650). Coprophilous NPPs peak in this zone, supporting evidence of human imported herbivore mammals on the island (David and Shafer 2006). Pollen of Cyperaceae declines in number throughout this phase, as Chenopodioideae pollen increases. There is a rise in pollen of trees and shrubs at 180 mm depth, although still only representing 8 % of the total pollen sum.

LPAZ 4 (0–120 mm) Present day

The island vegetation has remained stable since the 14th Century regardless of human occupation on the island. There is a range of flowering herbs, a dominance of grasses and minor inclusions of Ericaceae, Cyperaceae and ferns, similar to the current island vegetation community.

Conclusion

Much like neighbouring Skomer Island, no evidence of Mesolithic woodland is present in the preserved vegetation history on Skokholm. Instead, the vegetation reflects changes in the more recent occupation of the island with phase shifts between periods of greater diversity associated with disturbance events. Vegetative evidence of early occupation on the island by humans corroborates recent archaeological evidence, prehistoric pottery and stone tools, reported by The Wildlife Trust of South and West Wales.

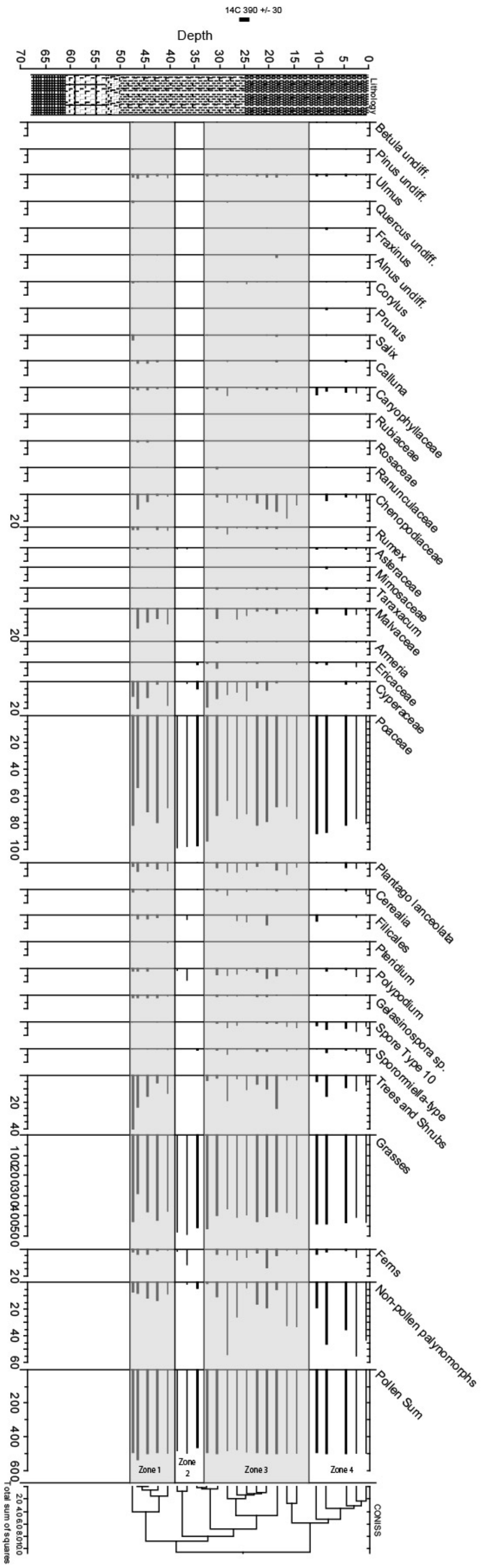


Figure 2: Pollen diagram from North Pond, Skokholm Island.

Acknowledgements

The authors would like to thank Sarah Davies and Henry Lamb, Aberystwyth University, Aberystwyth, UK for assistance with fieldwork and to the Skokholm Island Wardens Giselle Eagle and Richard Brown for being so accommodating.

References

Chambers, F M, van Geel, B, and van der Linden, M, 2011, Considerations for the preparation of peat samples for palynology, and for the counting of pollen and non-pollen palynomorphs. *Mires and Peat* 7 (Article 11), 1–14

David, O K, Shafer, D S, 2006, Sporormiella fungal spores, a palynological means of detecting herbivore density. *Palaeogeography, Palaeoclimatology and Palaeoecology* 237, 35–50

Grimm, E, 1987, CONISS: a FORTRAN 77 program for stratigraphically constrained cluster analysis by the method of incremental sum of squares, *Computers and Geosciences,* 13, 13-35

Moore, P D, Webb, J A, Collinson, M E, 1991, *Pollen analysis*. 2nd ed. Oxford: Blackwell Scientific.

Morgan, T, 1887, *Handbook of the origin of place-names in Wales and Monmouthshire*. Available online http://llennatur.com/files/u1/on__Place-names_ThosMorgan_Pr_HWSouthey_1887.pdf accessed 1.8.14

Reimer, P J, Bard, E, Bayliss, A, Beck, J W, Blackwell, P G, Bronk Ramsey, C, Grootes, P M, Guilderson, T P, Haflidason, H, Hajdas, I, Hatt'e, C, Heaton, T J, Hoffmann, D L, Hogg, A G, Hughen, K A, Kaiser, K F, Kromer, B, Manning, S W, Niu, M, Reimer, R W, Richards, D A, Scott, E M, Southon, J R, Staff, R A, Turney, C S M, and van der Plicht, J, 2013, IntCal13 and Marine13 Radiocarbon Age Calibration Curves 0-50,000 Years cal BP. *Radiocarbon*, 55(4), 1869–1887.

The Wildlife Trust of South and West Wales, 2021, Lucky finds from rabbit hole re-write prehistory of Skokholm Island! Available from https://www.welshwildlife.org/pressreleases/lucky-finds-from-rabbit-hole-re-write-prehistory-of-skokholm-island/ Accessed: 26th July 2021.

Thompson, G V F, 2007, *The Natural History of Skokholm Island.* Trafford Publishing: Bloomington, IN. USA.

Troels-Smith, J, 1955, *Characterisation of unconsolidated sediments.* Danmarks Geologiske Undersøgelse 4 Række, 3, 1-73.

Webb J C, McCarroll, J, Carpenter, W, Chambers, F M, Toms, P S, Wood, M J, 2017, Apparent lack of woodland and relative abundance of woodland indicator species: the role of humans, birds and rabbits in the changing vegetation of Skomer Island, Wales. *Archaeology in Wales*, 56, 90–99

4. Multi-site reconstructions: national and continental advances in understanding climate change

While Chapter 3 focussed on single-site reconstructions, this chapter features wider-scale reconstructions. Here, two papers are presented that provide evidence of two well-recognised palaeoclimatic events in the Holocene and, in the case of the second paper, uses this as a palaeoecological analogue of current human-accelerated climate change.

Webb, J.C. McCarroll, J. Chambers, F.M. and Thom, T. (2022b) Evidence for the Little Ice Age in upland north-western Europe: multiproxy climate data from three blanket mires in northern England. *The Holocene* 60, 45-48

Webb, J.C. and Goodenough, A.E. (2021) Vegetation community changes in European woodlands amid a changing climate: a palaeoecological modelling perspective. *Community Ecology* 22 (3), 319-330

The Holocene is the current geological epoch, extending from around 11,700 years before present (11.7 ka) and encompasses the modern day (Wanner et al. 2011). Following the intensely fluctuating climate of the preceding Pleistocene epoch, the Holocene is characterised by relatively stable climate, albeit with some fluctuations. Within this relatively stable epoch climatically, human population grew and (unlike in any other epoch) humans have had a dramatic impact on the vegetation and environment.

The dynamic environments within the Holocene are extensively researched (see reviews by Lowe and Walker 2015; Roberts 2015; Wanner et al. 2008, 2011) and the climate perturbations within are well-explored (Bond et al. 2001; Mayewski et al. 2004; Debret et al. 2007; Davis et al. 2009; Wanner et al. 2011). The two papers presented in this chapter build on this knowledge and use two putative global climate events from within the Holocene to explore the environmental impact of these episodes on a broad spatial scale. These events are the Little Ice Age - a period of c. 400 years commencing around 600 years ago – studied in Webb et al. (2022b); and the 8.2 ka event, comprising c. 300 years of cooling and subsequent warming c. 8,200 years ago, studied in Webb and Goodenough (2021).

Not only are these papers linked by topic, they both also demonstrate the step change from the papers presented in Chapter 3 by moving away from single-site reconstructions to using data from multiple locations within a single study. For Webb et al. (2022b) the paper provides evidence for a regional signal of vegetation change in relation to the Little Ice Age climate perturbation across Yorkshire, while Webb and Goodenough (2021) explores change in vegetation during the 8.2 ka event at a continental scale.

It is only in more recent years that has there been a focus on, and ability to study, palaeoecological data from multiple sites to address regional, continental and global change (Jones et al. 2009; Birks and Berglund, 2018; Githumbi et al. 2022). A seminal work on vegetation change across Europe (and one of the first studies to truly consider spatiotemporal patterns in palaeoecology) is provided by Huntley and Birks (1983). The maps they produced are evidence of a “mammoth task” (Barber 1984), particularly so at the time of publication when computer digitisation was in its infancy. The lack of early synthesis between multiple palaeoecological sites may well be due to the enormous manpower needed to collate the information.

The growth in number of outputs using spatial data (Figure 4.1) over recent years is likely suggestive of greater sharing of data, higher computing powers, and the development of online data repositories; for example the EPD (Fyfe et al. 2009) and Neotoma (Williams et al. 2018). As such, Giesecke et al. (2017) were able to use hundreds of sites with data held on the EPD to map patterns of vegetation change over the last 15,000 years and Kujawa et al. (2016) mined the Neotoma database and retrieved 127 samples to investigate changing land cover in the American Midwest.

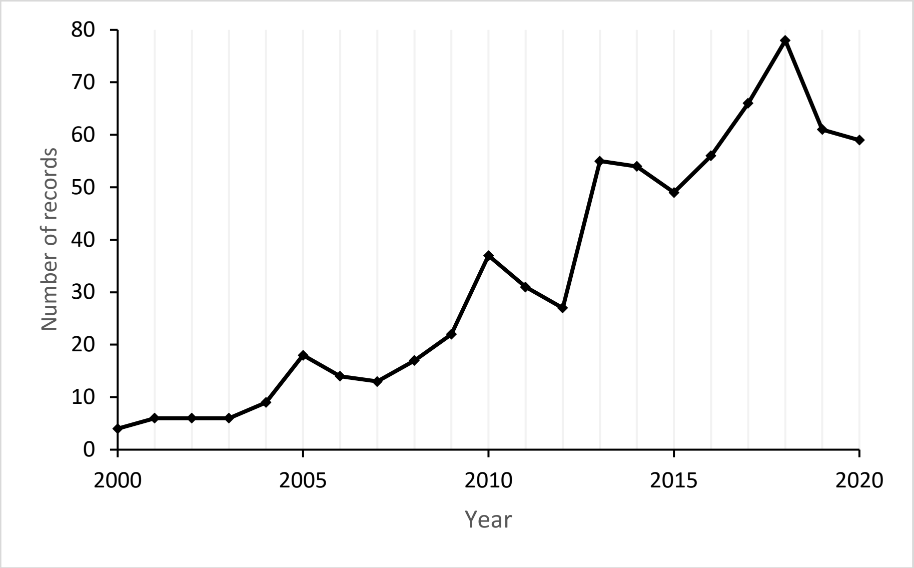


Figure 4.1

Author’s own Google Scholar search result for publications using spatial data from multiple palaeoecological sites published over the last 20 years in annual increments, using “multi-site” and “palaeoecology” as Boolean search terms. Care needs to be taken when analysing this Google record, as there is likely to be uncertainty in these figures, but it is useful to note the trend.

In order to investigate regional, continental or global phenomena, and investigate the forcing of those phenomena, the spatial resolution of studies must be compatible with the scale of inferred impacts (Jones et al. 2009; Davies et al. 2018), i.e. multiple study sites must span regional, or continental scales. Williams et al. (2004) successfully achieved this by collating data from 700 sites in the USA to reconstruct the limits of former biomes across the continent, a task that could not be completed without analysing numerous sites across a wide spatial scale. However, this does not always happen. For example, the ACCROTELM project (see Chapter 1) aimed to test the synchronicity of rapid climate changes across Europe (Chambers 2006) but although the project was a collaboration of several European universities, and the overall project utilized data from seven sites spanning Europe, only two of the published outputs used data from multiple sites in their analyses (Charman and Blundell 2007; Mauquoy et al. 2008). This is changing, with more recent work increasingly using multi-site analyses. For example, Miller et al. (2008) and Seppä et al. (2009) had more success in exploring temporal and spatial shifts in vegetation across Scandinavia using data from multiple sites, and other examples showcasing the use of multiple data collections can be seen in Hultberg et al. (2015), Trondman et al. (2015) and Githumbi et al. (2022).

The potential of multi-site data to explore the role of climate, disturbance events and disease across wide areas is considerable. Many climate models also rely on spatial data to "test" scenarios where the outcomes are already known through previous rigorous assessment of palaeoclimate data in a form of hindcasting (Jones et al. 2009). It is therefore important that palaeoecologists provide data in the form of multi-proxy spatial reconstructions to inform future climate models.

4.1 The Little Ice Age

The Little Ice Age (LIA) was a short-lived (c. 400 year) period of abrupt climate change. Although there is evidence that this occurred globally, the timing of the event is variable, although Mann et al. (2009) suggest on average it spanned AD 1400 – 1700. While named the Little Ice Age, the climatic changes were not globally uniform, with the temperature shifts and changes in the moisture regime observed being spatially variable. Reiter (2000) lists farming practices altering in China and Europe in response to cooler and wetter conditions, arctic pack ice extending so far south that Innuits arrived in Scotland, flooding in a usually dry Timbuktu, and violent storms and loss of low-lying land in northern continental Europe and Malaria spreading widely amidst wetter conditions.

Webb et al. (2022b) used multi-proxy data (including fossil pollen) across three sites to conclude the regional signal of the LIA as a wet phase in blanket peatlands in upland Britain. While the palynological data provide context to the land use at the time, the more revealing proxies were plant macrofossils and humification, which provided evidence for increasing wetness during the LIA, but no firm evidence for cooler conditions. These results provide a contrast to Harrison et al.'s (2014) data to support their claims for glacial advance in the Cairngorms (Scotland, UK) demonstrating much colder temperatures in northern Britain. Compare this to Rhodes et al. (2012) who provided evidence of up to 1.6 ^{o}C cooling in Antarctica, Chambers et al. (2014) found drier conditions in Argentina, and Lee-Thorp et al. (2001) documented drier and colder conditions in the African interior during the LIA. The strong evidence base supported by multiple-site palaeoecological data demonstrates the variability of the climate event, and the importance of research to understand the drivers of the LIA. This is critical to be able to apply knowledge to future events of a similar magnitude.

4.2 The 8.2 ka event

Similar to the LIA, the 8.2 ka event is a short-lived rapid cooling and warming event, albeit longer ago. Chambers (2016) notes the similarity between the events, but suggests that the onset of the 8.2 ka event could be even more rapid than that of the LIA. In contrast to many papers reporting on the impact of the 8.2 ka event (Alley et al. 1997; Olsson et al. 2010; Collins et al. 2012; Ghilardi and O'Connell 2013), Webb and Goodenough (2021) report on community level vegetation changes before, during and after the climate event, with a focus on woodlands. As such, they sought to understand the resilience of woodland vegetation communities amid a changing climate and discovered a surprising resilience in richness through the interval, with comparatively little change in the number of taxa throughout the focal period. However, measures of vegetation community turnover were high, indicating only 24 % of species remained at some sites after the short-lived cooling phase. This paper bridges the gap between palaeoecology and ecology, as whilst the data presented are palaeoecological, the implications of the work are important for applied practitioners in ecology and forest management. The results of this paper urge ecological researchers and practitioners to focus on broad spatial (gamma) diversity and focus on landscape-scale approaches to manage woodlands over centuries, rather than focusing on single sites.

4.3 The importance of multi-site studies

Both the LIA and 8.2 ka event studies demonstrate the importance of using multiple sites in understanding long-term environmental change. Microclimate and other specialised local variables can prevent the magnitude and extent of rapid climate events being determined, and so value should be placed on multi-site studies to understand broadscale patterns to ensure research has general applicability in a way that is useful for informing current management. While the utility of single-site palaeoecological reconstructions are of benefit in understanding site-specific patterns and processes (as per Chapter 4), understanding species and community response on a broad spatial scale can reveal regional trends that are hidden by dominant local habitat signals in single site analysis.

Research Paper

The Holocene
1–17

DOI: 10.1177/09596836221074036
journals.sagepub.com/home/hol
SAGE

Evidence for the Little Ice Age in upland northwestern Europe: Multiproxy climate data from three blanket mires in northern England

Julia C Webb,[1] Julia McCarroll,[1] Frank M Chambers[1] and Tim Thom[2]

Abstract

The Little Ice Age (LIA) is a well-recognised palaeoclimatic phenomenon, although its causes, duration and severity have been matters of debate and dispute. Data from a wide range of archives have been used to infer climate variability before, during and after the LIA. Some published proxy-climate data from peatlands imply that two particularly severe episodes within the LIA may be contemporaneous between hemispheres; these echo a previous climatic downturn ca. 2800 cal BP of similar severity but lesser duration. Here, we present palaeoclimate data from the mid- to late-Holocene, reconstructed from three blanket peats in Yorkshire: Mossdale Moor, Oxenhope Moor and West Arkengarthdale. Multiproxy techniques used for palaeoclimatic reconstruction were plant macrofossil, pollen and humification analyses. Dating was provided by a radiocarbon-based chronology, aided by spheroidal carbonaceous particles (SCPs) for all sites, and ^{210}Pb dates for one. The LIA presents as a distinct climatic event within each palaeoenvironmental record at the three sites. These indications are compared with terrestrial datasets from northwest Europe and elsewhere. A broad degree of synchronicity is evident, signifying that the LIA is one of the most pronounced downturns in global climate in the last ca. 6000 years, and arguably the most routinely recorded within the Holocene.

Keywords

blanket peat, Holocene, Little Ice Age, palaeoclimate, palaeoecology, peatlands

Received 27 December 2021; revised manuscript accepted 27 December 2021

Introduction

The Little Ice Age (LIA) is one of the most renowned climate instabilities of the Holocene, with causes being attributed variously to reduced solar activity (Blackford and Chambers, 1995; Grove, 2001; Mauquoy et al., 2002, 2004), volcanic eruptions (Miller et al., 2012) and even to human impact (Ruddiman, 2003). However, Turner et al. (2016) note that solar-type signals in peatlands can be the product of random variations alone, and caution that a more critical approach is required for the vigorous interpretation of such signals. The coldest temperatures of the LIA have been inferred for the interval AD 1400–AD 1700 (Mann et al., 2009), although there is disagreement on the start and end dates for this climatic phenomenon (Fagan, 2001). Matthews and Briffa (2005) suggest that LIA 'climate' (as opposed to glacier advance) is defined as a time interval of about 330 years (ca. AD 1570–1900) when Northern Hemisphere summer temperatures (land areas north of 20°N) fell significantly below the AD 1961–1990 mean, whereas in the North Atlantic, the LIA onset is said to be as early as before the early 14th century (Grove, 2001). However, some regional heterogeneity is apparent for northwestern European peatlands, with Mauquoy et al. (2002) identifying the LIA from Lille Vildmose, Denmark and Walton Moss, Cumbria between AD 1449–1464 and AD 1601–1604; De Vleeschouwer et al. (2009) from Slowinskie Blota, Poland AD 1200–1800; the beginning of the LIA AD 1250–1350 as identified by Barber et al. (2004) from Dosenmoor, Germany and Svanemose, Denmark; AD 1410–1540 and AD 1660–1720 from Letterfrack, Ireland (Blackford and Chambers, 1995); and the 'last pulse' of the LIA as AD 1650–1850 from Fallahogy, Northern Ireland and Moine Mhor, Scotland (Barber et al., 2000). The geographical variation in these timings is evidence for the multiphase characteristic of the LIA, with possible attribution to the Wolf, Spörer, Maunder and Dalton Minima (Blackford and Chambers, 1995): periods of reduced sunspot activity that occurred at AD 1320 (Wolf), AD 1400–1540 (Spörer), AD 1645–1715 (Maunder) and 1790–1830 (Dalton) (McCracken and Beer, 2014). However, cold temperatures have been recorded outside of these timings, yet still within the LIA, with the early Maunder Minimum (ca. AD 1600), being described as one of the coldest phases of the LIA, with

---

[1]Centre for Environmental Change and Quaternary Research, School of Natural and Social Sciences, University of Gloucestershire, UK
[2]Yorkshire Peat Partnership, Yorkshire Wildlife Trust, UK

Corresponding author:
Frank M Chambers, Centre for Environmental Change and Quaternary Research, School of Natural and Social Sciences, University of Gloucestershire, Francis Close Hall Campus, Swindon Road, Cheltenham GL50 4AZ, UK.
Email: fchambers@glos.ac.uk

temperatures considered as extreme (Mauquoy et al., 2004). Overall, temperature estimates for the LIA suggest that this event must be considered a time of modest cooling of the Northern Hemisphere, with temperatures dropping by approximately 0.6°C during the 15th–19th centuries (Mann, 2002), which provides motivation behind much academic interest and the focus of the present paper.

The above records all derive from the Northern Hemisphere, but whether the LIA can be regarded a global event has been disputed, largely owing to a relative lack of terrestrial archives in the Southern Hemisphere (Chambers, 2016). A recent paper provides new evidence, supporting the claim that the LIA may be a global phenomenon: using identical methods on a peatland in Tierra del Fuego as have been used for peatlands in northwest and central Europe, the most pronounced episodes of the LIA (at ca. cal AD 1460 and from ca. cal AD 1675 to cal AD 1770 in the Tierra del Fuego mire) were shown to be contemporaneous between hemispheres (Chambers et al., 2014). The bog response in the Southern Hemisphere site (to surface dryness) was opposite to site responses in the Northern Hemisphere (increased surface wetness), which the authors interpreted as indicating globally contemporaneous equatorward shifts of moisture-bearing airmasses during the severest decades.

Proxy-climate data can and have long been inferred from late-Holocene peat stratigraphy (Amesbury et al., 2012a, 2012b; Anderson et al., 1998; Barber, 1981; Barber et al., 1994, 2000, 2003, 2013; Bingham et al., 2010; Blackford, 2000; Blackford and Chambers, 1991, 1995; Blundell and Barber, 2005; Chambers et al., 1997; Charman, 2010; Chiverrell, 2001; Daley and Barber, 2012; Hughes et al., 2000; Sillasoo et al., 2007), with many studies suggesting an association between the visible stratigraphy of peatlands and past climatic change (Barber et al., 2003; Blackford and Chambers, 1991; Stoneman, 1993; Xie et al., 2004). Peatlands contain within them a detailed archive of local and regional vegetation history, making them ideal for researching Holocene environmental and climatic changes (Blackford, 2000).

In the case of blanket mires, such as those in the present study, peat can appear homogenous with little or no stratigraphy; this deterred proxy-climate research in previous decades, as did the perception that some blanket mires contain few identifiable macrofossils (Chambers et al., 1997). This apparent unsuitability was falsified by Chambers (1984) and Blackford and Chambers (1991) using humification data and Tallis (1995) and Mauquoy and Barber (1999) using plant macrofossil data, with blanket peat being used to reconstruct Holocene palaeoclimate in a number of recent studies (Blundell and Holden, 2015; Castro et al., 2015; Swindles et al., 2015).

A strategic way of indicating a regional forcing factor, such as climate change, is to be able to demonstrate a synchronous response in separate mires (Hughes et al., 2000). However, the dating of the LIA can be problematic in peatlands, given that the most widely used dating method is conventional radiocarbon dating, which may have limitations in providing sufficient precision and accuracy for the last few hundred years. Conversely, using techniques such as wiggle-matching and bomb-spike dating at a high enough resolution, it is possible to use radiocarbon alone to produce chronologies to date events such as the LIA (Chambers et al., 2014). Other dating methods are available for peatlands covering the later part of the LIA, for example, tephra, ^{210}Pb, ^{137}Cs and relative dating using Spheroidal Carbonaceous Particles (SCPs) (van der Plicht et al., 2013), but these methods are not without their flaws. van der Plicht et al. (2013) highlight that ^{210}Pb age estimates can be too old, and as such, age estimates of peat samples based only on ^{210}Pb should be used with caution. SCPs can only provide relative age estimates, which are subject to geographical variation (Swindles, 2010).

The LIA has previously been identified in palaeoclimatic reconstructions from peatlands in continental Europe, including Denmark (Aaby, 1976; Barber et al., 2004) and Germany (Barber et al., 2004), and in Britain, including Temple Hill Moss, Scotland (Langdon et al., 2003), Talkin Tarn, Cumbria (Barber and Langdon, 2007), the North York Moors, northern England (Chiverrell, 2001), Greater Manchester and Cheshire, northwest England (Davis and Wilkinson, 2004), Northumberland, northern England (Charman et al., 1999), Cumbria and the Scottish Borders, northern Britain (Mauquoy and Barber, 1999), northern England and Scotland (Stoneman, 1993), the northern Pennines (Swindles et al., 2015) and Yorkshire (Turner et al., 2014). This paper contributes to the record of the LIA in the uplands of northern England with the addition of palaeoclimate data from three Yorkshire upland blanket bogs.

The present study aims to examine the LIA in northern England, based on palaeoclimatic reconstructions using pollen, plant macrofossil and humification data from three Yorkshire blanket bogs: Mossdale Moor (MDM), Oxenhope Moor (OXM) and West Arkengarthdale (ARK). The long-term ecological (palaeoecological) records of these sites were published elsewhere (McCarroll et al., 2016a, 2016b, 2017); in the present paper palaeoclimate data for the LIA are compared with the overall palaeorecords from the three sites, and with proxy-climate data from sites in northwest Europe and elsewhere, to evaluate the LIA compared with previous climatic downturns in the Holocene.

Site descriptions

Site descriptions of MDM, OXM and ARK have previously been provided by McCarroll et al. (2016a, 2016b, 2017) in studies using palaeoecology to advise conservation. For the purpose of this study, only a summary of each site is provided here.

Mossdale Moor

Mossdale Moor is a degraded blanket mire at 550 m altitude located in Upper Wensleydale, North Yorkshire, UK (Figure 1). The modern-day peat supports species characteristic of National Vegetation Classification (NVC) M19 (*Calluna vulgaris-Eriophorum vaginatum* blanket mire) (Rodwell, 1998). Various management practices have altered the vegetation characteristics of the moor from typical blanket mire towards heathland communities.

Oxenhope Moor

Oxenhope Moor is a degraded blanket mire at 430 m altitude located north of Hebden Bridge in West Yorkshire (Figure 1). The modern-day peat supports species characteristic of NVC types M20 (*Eriophorum vaginatum* blanket and raised mire) and M25 (*Molinia caerulea-Potentilla erecta* mire as surveyed by Natural England in 2008) (Rodwell, 1998).

West Arkengarthdale

West Arkengarthdale is a blanket mire at 380 m altitude located north-west of Reeth in North Yorkshire (Figure 1). A vegetation survey conducted during fieldwork identified that the modern-day peat supports species characteristic of NVC type M20 (*Eriophorum vaginatum* blanket and raised mire) (Rodwell, 1998).

Methods

Field sampling strategy

The field sampling strategy was a modified version of the method used in the ACCROTELM Research Project (Chambers, 2006;

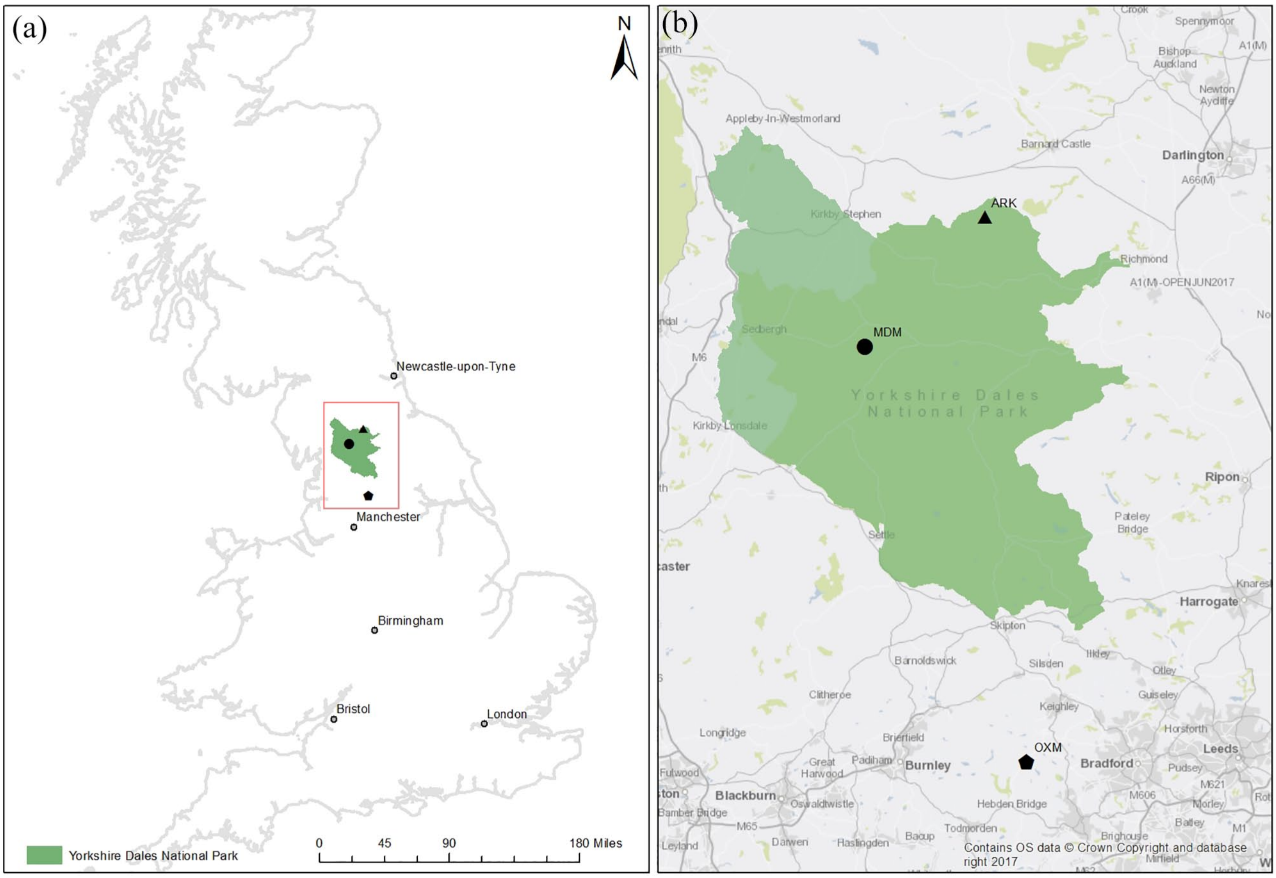


Figure 1. (a) Map of United Kingdom showing the site locations of Mossdale Moor, Oxenhope Moor and West Arkengarthdale. (b) Map of Yorkshire Dales National Park showing site locations of Mossdale Moor (MDM: circle), exact coring location at Latitude: 54.300292°N, Longitude: 2.315507°W; Oxenhope Moor (OXM: pentagon), exact coring location at Latitude: 53.793759°N, Longitude: 1.977952°W; and West Arkengarthdale (ARK: triangle), exact coring location at 54.458815°N, Longitude: 2.067067°W

De Vleeschouwer et al., 2010). First, site morphology was established by measuring peat depths across each site to allow the deepest ombrotrophic zone closest to the highest point of the bog to be identified. By describing multiple cores using the Troels-Smith (1955) method, the gross site stratigraphy was determined, in turn allowing the identification and selection of the master core, taken in a zone between a hummock and a hollow, as recommended by De Vleeschouwer et al. (2010). Using a 5 cm diameter Russian corer, overlapping adjacent cores were extracted, described, photographed for the full length of the profile before being placed in labelled plastic guttering, wrapped in airtight carbon-stable bags and transported to the laboratories at the University of Gloucestershire where they were stored at 4°C.

Laboratory methods

Laboratory methods of analysis were the same at all three sites, with the exception of Mossdale Moor, where ^{210}Pb dating was also undertaken. Radiocarbon dates were obtained from plant macrofossils where possible (11 of 27 samples – see Table 1). Where this was not possible, bulk peat samples were sent to Beta Analytic Miami for analysis, where following pre-treatment, the plant fraction (as opposed to bulk organic carbon) of the sample was selected for dating. The depths selected for radiocarbon dating were chosen following pollen analysis and their positioning at particular points of interest or on boundaries selected by CONISS (an agglomerative cluster analysis technique that compares the total pollen assemblage of each sample with that of its stratigraphic neighbours) and by eye. The pollen preparation method used in this study does not degrade SCPs (Chambers et al., 2011b) and therefore SCPs were counted alongside pollen. ^{210}Pb samples from Mossdale Moor were analysed every 1 cm for the first 32 cm depth at the University of Exeter using alpha spectrometry (rather than gamma spectrometry; see Zaborska et al. (2007) for comparison), which measures the decay of ^{210}Po, a daughter product of ^{210}Pb. As a chemical yield tracer, a Polonium spike (^{209}Po) was added before Po was extracted from the sample using acid digestion and electroplated onto a silver disc. Ages were calculated using the Constant Rate of Supply (CRS) model (Appleby and Oldfield, 1978).

Peat humification analysis has been subject to criticism in recent decades (Hughes et al., 2012; Yeloff and Mauquoy, 2006). Studies show that changes in humification may not be influenced solely by climate and that plant species composition can also be a contributor to observed changes (Hughes et al., 2012; Yeloff and Mauquoy, 2006). Yeloff and Mauquoy (2006) suggest that the assumption that the major influence on peat humification is the surface wetness of the bog (and therefore climate) should be tested. Hughes et al. (2012) found that, overall, humification data are validated, but that the species signal is sometimes sufficient to change the timing and number of events and trends recorded in the data. Furthermore, the role of secondary decomposition during dry periods may also influence the humification signal (Borgmark and Schoning, 2006). Provided the shortfalls are considered, humification analysis can aid palaeoclimatic reconstructions when incorporated as part of a multiproxy palaeoclimate study; this method remains a straightforward and fast way to produce high-resolution, contiguous datasets.

Table 1. Dates for MDM, OXM and ARK including ^{210}Pb (MDM only), SCPs and radiocarbon[1].

Depth (cm)	Radiocarbon Date (yr BP); SCP/^{210}Pb date (AD)	Lab Number	Calibrated Age (95.4%) (cal yr BP) from-to	Material/method
MDM				
1	2008 ± 2			^{210}Pb
1.5	1980 ± 10			SCPs
2	2001 ± 2			^{210}Pb
3	1991 ± 3			^{210}Pb
3.5	1950 ± 10			SCPs
4	1982 ± 4			^{210}Pb
5	1975 ± 4			^{210}Pb
6	1968 ± 5			^{210}Pb
7	1959 ± 6			^{210}Pb
8	1949 ± 8			^{210}Pb
9	1941 ± 8			^{210}Pb
10	AD 1933 ± 10			^{210}Pb
11	AD 1925 ± 12			^{210}Pb
12	AD 1919 ± 14			^{210}Pb
13	AD 1906 ± 17			^{210}Pb
13.5	AD 1850 ± 10			SCPs
14	AD1891 ± 22			^{210}Pb
15	AD1878 ± 26			^{210}Pb
34.5	530 ± 30	BETA-327196	645–502	*Polytrichum commune*
51.5	770 ± 30	BETA-426568	734–668	Bulk plant material
58.5	480 ± 30*	BETA-327197	625–469	Monocot leaves
65.5	1070 ± 30	BETA-385275	1055–929	Bulk plant material
69.5	930 ± 30*	BETA-426569	925–785	Bulk plant material
74.5	350 ± 30*	BETA-327198	503–306	Monocot leaves
94.5	1340 ± 30	BETA-327199	1334–1180	Monocot leaves
118.5	1520 ± 30	BETA-327200	1528–1314	Monocot leaves
134.5	2610 ± 30	BETA-385276	2775–2720	Bulk plant material
150	5310 ± 30	BETA-364579	6267–5944	Charred material
OXM				
0.5	1990 ± 10			SCPs
8.5	1945 ± 10			SCPs
24.5	1850 ± 10			SCPs
28.5	350 ± 30	BETA-382650	503–306	Bulk plant material
108.5	1300 ± 30	BETA-382651	1304–1088	Bulk plant material
212.5	2270 ± 30	BETA-382652	2354–2152	Bulk plant material
310.5	3170 ± 30	BETA-382653	3480–3253	Bulk plant material
380.5	3910 ± 30	BETA-382654	4512–4160	Plant macrofossils
436.5	4730 ± 30	BETA-382655	5588–5322	Bulk plant material
479.5	6090 ± 30	BETA-382656	7159–6799	Plant macrofossils
ARK				
16.5	1850 ± 10			SCPs
12.5	1950 ± 10			SCPs
8.5	1970 ± 10			SCPs
4.5	1990 ± 10			SCPs
39.5	300 ± 30	BETA-381604	485–156	Plant macrofossils
100.5	1780 ± 30	BETA-385277	1822–1570	Plant macrofossils
141.5	2710 ± 30	BETA-426566	2859–2758	Bulk plant material
147.5	3410 ± 30	BETA-379805	3828–3563	Wood
155.5	3430 ± 30	BETA-444008	3830–3578	Bulk plant material
159.5	3640 ± 30	BETA-385278	4089–3841	Plant macrofossils
182.5	3320 ± 30*	BETA-379806	3685–3450	Bulk plant material
199.5	3970 ± 30	BETA-426567	4524–4299	Bulk plant material
260.5	4820 ± 30	BETA-379807	5645–5468	Bulk plant material
289.5	5610 ± 30	BETA-379808	6487–6300	Bulk plant material

[1] Initial radiocarbon chronologies of MDM and ARK are discussed in McCarroll et al. (2016b, 2017). However, further radiocarbon dates have been obtained for the purpose of the present paper for these sites, so interpretation is briefly revised.

*Date rejected as an outlier.

The preparation and quantification of humification samples follows a modified methodology based on that described by Chambers et al. (2011a). The protocol used differs where 0.1 g of sediment is used as opposed to 0.2 g. Humification was analysed contiguously at every centimetre for each site, which equates to a 10-year sampling resolution. 1 cm^3 of peat was sampled and oven-dried for 48 h at 60°C before being ground and returned to the weighing boat. 0.1 g of sample was accurately weighed, recorded

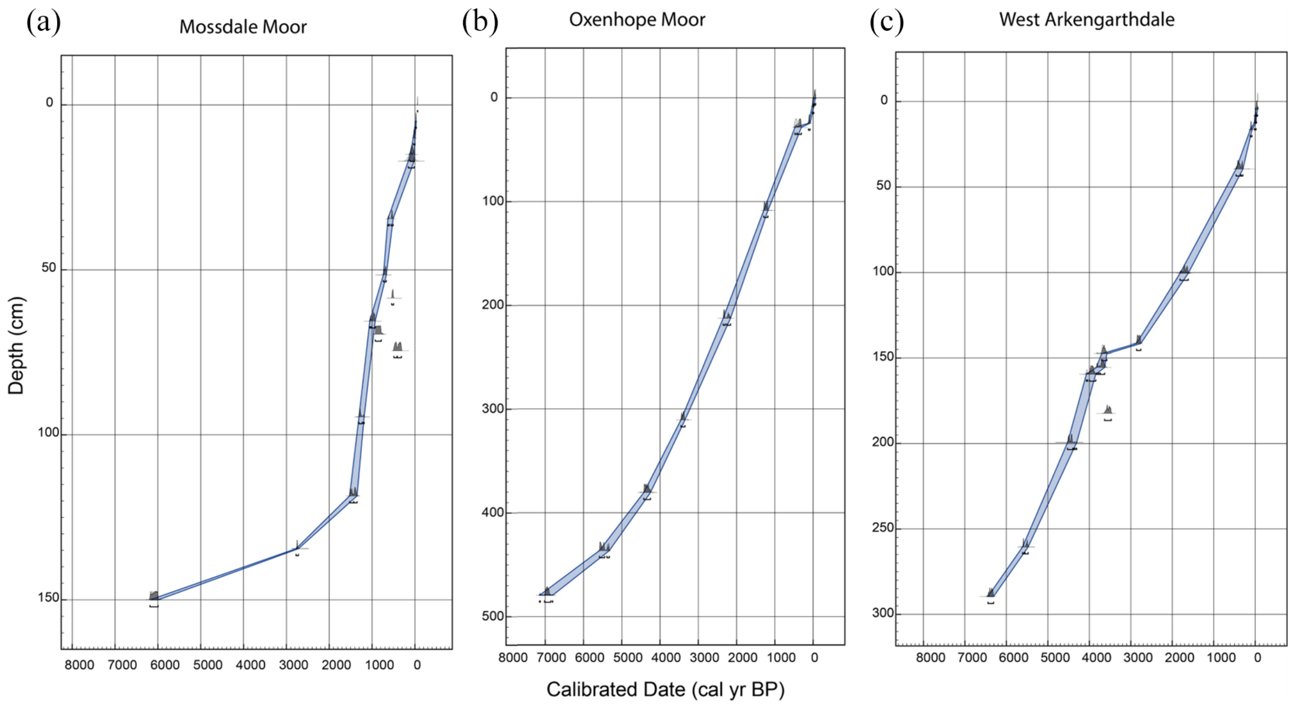


Figure 2. Full sequence age-depth models for (a) MDM, (b) OXM and (c) ARK using radiocarbon, SCP and ^{210}Pb dates (MDM only).

and then transferred into a 150 mL beaker where 100 mL of 8% NaOH solution was added to each beaker before the samples were simmered on a hotplate for 1 h. The contents of each beaker were then poured into a 200 mL labelled volumetric flask before being filtered into separate 50 mL labelled volumetric flasks. Only 50 mL of filtrate was transferred. The samples were then measured in a spectrophotometer (set at a wavelength of 540 nm). Measurements were repeated three times and an average recorded.

The preparation and quantification of pollen samples follows a modified methodology based on that of van Geel (1978) in Chambers et al. (2011b). The sampling resolution was a minimum of 8 cm at Oxenhope Moor, and 4 cm at Mossdale Moor and Arkengarthdale, with a maximum resolution of 2 cm at points of interest, equating to a minimum of a 40-year sampling resolution over the LIA. *Lycopodium clavatum* tablets were added to the samples in order to calculate microfossil concentrations. Pollen grains were identified using Moore et al. (1991) and a reference collection of type slides at the University of Gloucestershire. The pollen sum (500 grains) is Total Land Pollen, which includes the total number of pollen from trees, shrubs and terrestrial herbaceous plants.

For the analysis of plant macrofossils, a minimum sampling resolution of 8 cm at OXM and 4 cm at MDM and ARK was employed. Sub-samples measuring 4 cm^3 were taken using a scalpel and sieved through a 125 µm mesh with a standard 5 L volume of tap water. The samples were transferred to three glass petri dishes and spread out to form a monolayer, before quantification using the quadrat and leaf count (QLC) method of macrofossil analysis described by Barber et al. (1994). Plant macrofossils were identified using type collections and with reference to modern plant material sampled from the study sites. In addition, Daniels and Eddy (1985) was used to identify *Sphagna* and Smith (2004) was used for non-*Sphagnum* bryophytes.

An adapted version of the weighted-average Dupont Hydroclimatic Index (DHI) (Dupont, 1986) was applied to the macrofossil data. Weights were assigned to species based on those used in Daley and Barber (2012) and Mauquoy et al. (2008) and the DHI scores were calculated in Excel using plant macrofossil percentages.

Results and interpretation

The data presented in this paper have previously been published elsewhere (McCarroll et al., 2016a, 2016b, 2017) as individual sites with a focus on using palaeoecology to inform conservation. These records have now been brought together, with the addition of seven radiocarbon dates (four for MDM; three for ARK) to revise chronologies. The data are now used to tackle a different palaeoenvironmental research question, providing insight into palaeoclimatic changes in upland Yorkshire blanket bogs over the span of the LIA.

Basis of chronology

Four of the 27 radiocarbon dates obtained did not show increasing age with depth (three from MDM, one from ARK), suggesting that the accumulation of peat may have ceased or that some material may have been eroded (Table 1). At both sites, these anomalies occur approximately at the mid depths of the profiles, at a time predating the LIA. Ages were modelled in OxCal version 4.2 (Ramsey, 2009), to include dates from Spheroidal Carbonaceous Particles (SCPs) and ^{210}Pb (site MDM) (Figures 2 and 3); those that show increasing age with depth were accepted. Calibration of the radiocarbon ages to calendar years BP was conducted using INTCAL13 (Reimer et al., 2013) in OxCal version 4.2.

^{210}Pb dating

Depth profiles for ^{210}Pb typically show exponential decline with depth throughout the core (Figure 3). The Constant Rate of Supply (CRS) model was used to develop an age-depth model from ^{210}Pb data using the method described by Appleby (2001). The CRS model is thought to be the most appropriate in ombrotrophic peats as ^{210}Pb inputs are dominated by atmospheric inputs (Appleby, 2008). Uncertainty was calculated by colleagues at Northeast Normal University, China using analytical uncertainty and the error propagated using the CRS model. The level of activity decreases below 17 cm depth (see Supplemental Material) and at this point, the error margin becomes unreliably larger, and the dates calculated exceed beyond the 130-year

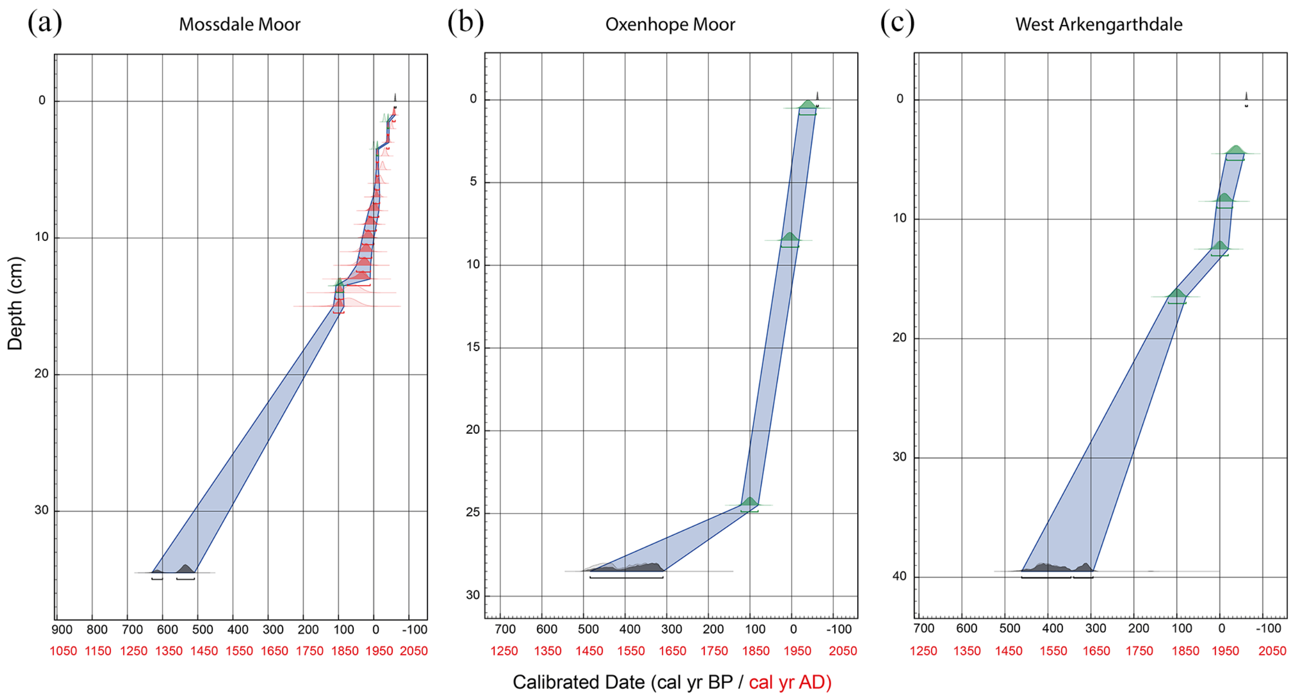


Figure 3. LIA age-depth models for (a) MDM, (b) OXM and (c) ARK using radiocarbon (grey), SCP (green) and ^{210}Pb dates (red – MDM only).

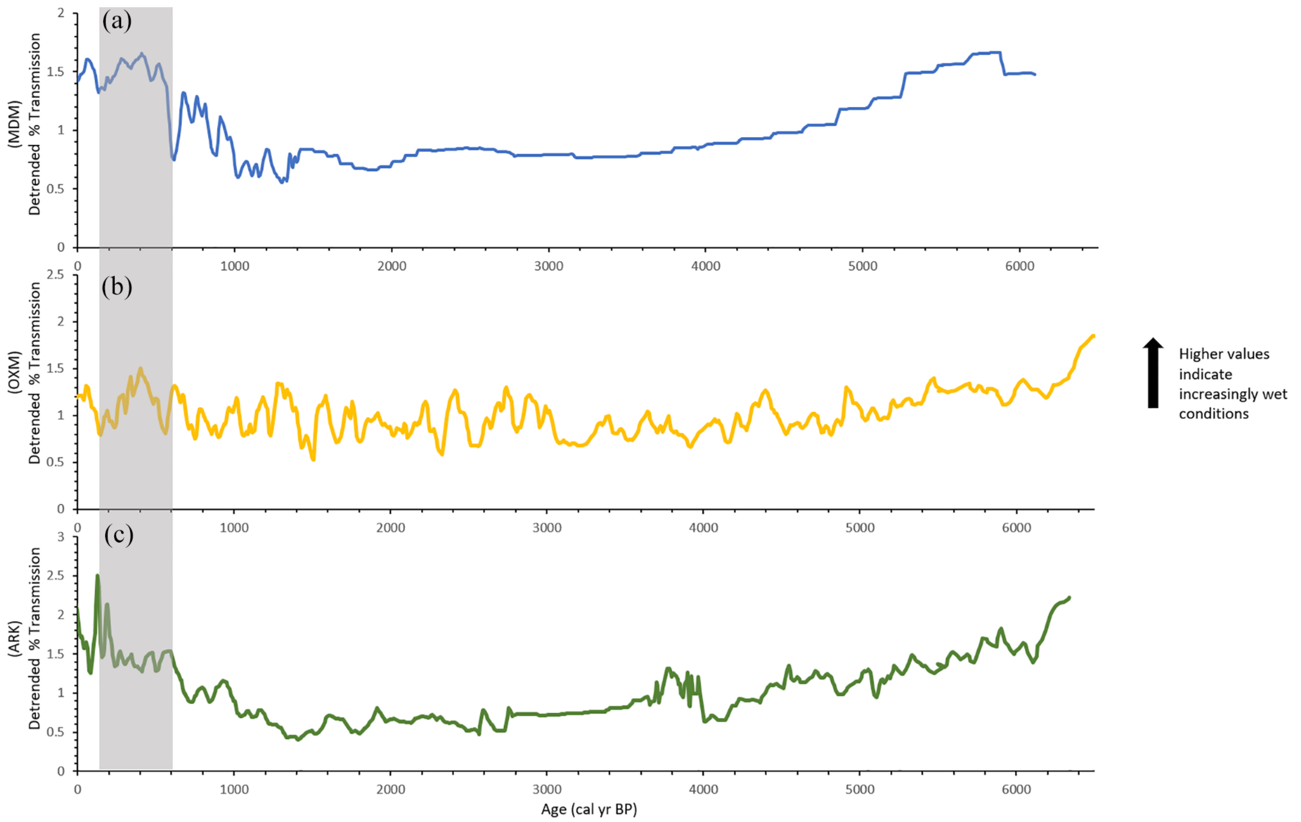


Figure 4. Humification data plotted against age for (a) MDM, (b) OXM and (c) ARK. %T values have been detrended and are therefore displayed as residual values from a linear trendline. The shaded bar approximates the LIA, for visual guidance only.

range of ^{210}Pb dating (Appleby, 2008) and so were excluded from the age-depth model.

Humification

Mossdale Moor. At the base of the profile, high T values (detrended % transmitted light) suggest low humification (ca. 6000–5000 cal yr BP) (Figure 4a). Following this, humification is higher between 5000 and 1600 cal yr BP, as indicated by low T values. T values then fluctuate, suggesting alternations between more and less humification occurred, but the peat appears more humified between ca. 1400 cal yr BP and 700 cal yr BP (cal yr AD 1250), which overlaps with and could be interpreted as the climatic amelioration known as the Mediaeval Warm Period (MWP).

Between ca. 600 and 140 cal yr BP, generally high T values suggest low humification. However, the earlier date may reflect the low decomposition rates of the acrotelm (5–10 cm depth) and that this plant material has not had as long to decompose as the lower layers of peat in the catotelm. The T values are particularly high between ca. 500 and 300 cal yr BP (beneath the acrotelm), which is interpreted likely as the cooling associated with the Little Ice Age (LIA).

Oxenhope Moor. The T curve is highly fluctuating and could be evidence of cyclicity. There is less humification taking place between 7000 and 5000 cal yr BP when compared with the rest of the profile, with notably high T values, the highest in the bottom half of the profile, between 7000 and 6000 cal yr BP. More humification occurred between 5000 and 3000 cal yr BP (Figure 4b), followed by lower humification identified by higher T values between 3000–2600 cal yr BP and 1300–1100 cal yr BP. Furthermore, there are particularly high T values towards the surface of the profile particularly between 500 and 300 cal yr BP consistent with the Little Ice Age. The values here are higher than the rest of the profile (with the exception of high T values between 7000 and 6000 cal yr BP). T then briefly drops before rising again towards the surface, interpreted as fresh peat in the acrotelm that has comparatively less time to decompose than the peat below.

West Arkengarthdale. Towards the base of the profile, between 6200 and 4200 cal yr BP, T values increase with age suggesting low humification, reaching the highest T values at ca. 6200 cal yr BP (Figure 4c). T values are low suggesting more humification at 4000 cal yr BP but less between 3900 and 3600 cal yr BP. Lower T values indicate more humification taking place between 3500 and 1100 cal yr BP, gradually becoming less towards the surface of the profile. T values are highest between 600 and 150 cal yr BP (being particularly high between 300 and 150 cal yr BP) and is interpreted as the Little Ice Age (LIA). T values are then briefly lower subsequently up until approximately cal yr AD 1960 with a return to less humified conditions. Despite this, peat at this depth has had much less time to decompose than the layers of peat below and therefore the high values here are likely a reflection of this.

Pollen and charcoal

Each of the sites was likely surrounded by mixed woodland as indicated by high percentages of tree and shrub pollen: at MDM, between ca. 6100 and 5000 cal yr BP; at OXM, between ca. 7000 and 5500 cal yr BP; and at ARK, between ca. 5600 and 4400 cal yr BP (Figure 5 and Supplemental Material). Mixed mire communities then succeed at each of the sites, evidenced by *Sphagnum* spores and Ericales species at MDM, between ca. 5000 and 1500 cal yr BP; OXM, between ca. 5500 and 3400 cal yr BP; and ARK, between ca. 4400 and 3700 cal yr BP.

At OXM, between ca. 3400 and 2300 cal yr BP, anthropogenic influence is suggested by an introduction of arable and ruderal species but percentages of tree and shrub pollen are still high at this stage. At ARK, between ca. 3700 and 600 cal yr BP, a mixed mire community (indicated by Ericales pollen and *Sphagnum* spores) prevails on site, likely surrounded by mixed woodland as tree and shrub pollen are still high. Charcoal is high, indicative of burning, possibly deliberately by human activity.

At MDM, between ca. 1500 and 1300 cal yr BP, pollen of trees and shrubs is lower than preceding sections, as are *Sphagnum* spores. A higher percentage of Ericales suggests drier conditions, perhaps attributable to the Mediaeval Warm Period. At OXM, between ca. 2300 and 1200 cal yr BP, evidence suggests either a climatically dry environment or clearance by fire (high percentage of grasses and sedge pollen, high charcoal and fluctuating tree and shrub pollen, low *Sphagnum* spores). A brief move to wetter conditions is suggested by a peak in bog spores at ca. 2250 cal yr BP.

At MDM, between 1300 and 600 cal yr BP, wet conditions are suggested by low charcoal, and high percentages of *Sphagnum* and bog spores. At OXM, between ca. 1200 and 350 cal yr BP, wet conditions are also identified by a high number of *Sphagnum* and bog spores. Anthropogenic activity is also evidenced by a high percentage of ruderal and arable pollen.

Between ca. 600 and −60 cal yr BP (cal yr AD 1350–2010) at MDM and ARK, percentages of trees and shrub pollen are low in comparison to preceding sections, consistent with high charcoal numbers, indicative of clearance by man. However, at OXM, between ca. 350 and −60 cal yr BP (cal yr AD 1600–2100), charcoal fragments are low and *Sphagnum* spores are high, indicative of wet conditions.

Plant macrofossils

At OXM between ca. 7000 and 5200 cal yr BP, the presence of *Sphagnum* along with monocots and Cyperaceae (including *Eriophorum vaginatum*) suggests a mixed *Sphagnum*, sedge and graminoid mire (Figure 6 and Supplemental Material). At ARK, between ca. 6400 and 5600 cal yr BP, standing water is suggested by the presence of aquatic species (including *Nymphaea*), before the formation of peat. Once peat is established, Ericaceae, monocot roots and Cyperaceae species suggest the presence of a mixed ericaceous, sedge and graminoid community.

At MDM, between ca. 6100 and 2400 cal yr BP, UOM reaches 90%, owing to the very degraded nature of the blanket peat at this depth. At OXM between 5200 and 3200 cal yr BP, the percentage of UOM is high, Ericaceae (*Calluna vulgaris*) are present and the percentage of identified *Sphagnum* is lower than above, suggestive of drier conditions. At ARK, between ca. 5600 and 4400 cal yr BP, shallow water is indicated by the presence of aquatic species (including *Equisetum fluviatile*). However, a predominantly sedge community exists (consisting of *Trichophorum cespitosum, Eriophorum vaginatum* and *E. angustifolium*). Following on from this, between ca. 4400 and 3700 cal yr BP, wet conditions are indicated by the presence of *Sphagnum cuspidatum* and low UOM.

At MDM between ca. 2400 and 1300 cal yr BP, UOM is still high. Aquatic species (including *Equisetum fluviatile* and *Scheuchzeria palustris*) suggest shallow water and the presence of charred remains signifies local fire, before giving way to a sedge-graminoid community between ca. 1300 and 1100 cal yr BP, indi-cated by Cyperaceae and monocot species. At OXM, between ca. 3200 and 1000 cal yr BP, wet conditions are indicated by low UOM and a high percentage of *Sphagnum* Section A*cutifolia*, mainly *S. austinii*. A *Sphagnum*-sedge-Ericales-graminoid com-munity is present, identified by the presence of Ericales rootlets, monocot roots, and Cyperaceae species. At ARK, between ca. 3700 and 500 cal yr BP, shallow water is indicated by the presence of aquatic species (*Scheuchzeria palustris* and *Equisetum fluviatile*). The community consisted of Cyperaceae (*Eriophorum vaginatum*), monocots and Ericaceae (*Erica tetralix*), with the presence of birch shrubs growing on site, indicated by *Betula* bark.

At MDM, between 1100 and 50 cal yr BP, wet conditions are indicated by the presence of *Sphagnum* Section *Acutifolia* and *Polytrichum commune*. A *Sphagnum*-sedge-graminoid community prevails, evidenced by identified Cyperaceae (*Eriophorum vaginatum*) and Monocot leaves. Following on from this, between ca. 50 and −60 cal yr BP (cal yr AD 1910–2100), a low percentage of UOM, high percentage of Ericaceae (*Vaccinium oxycoccus* stems) and the presence of *Polytrichum commune* are suggestive of wet conditions. However, the percentage of Monocot roots is high, suggesting dry or unfavourable conditions. At OXM, between ca. 1000 and −60 cal yr BP, a low percentage of UOM and the presence of *Sphagnum* (including *S. papillosum* and *S. cuspidatum*) are suggestive of wet conditions. Monocots are also present, as is Cyperaceae (*Trichophorum cespitosum*), reaching its highest in the profile, indicating a mixed *Sphagnum* and sedge

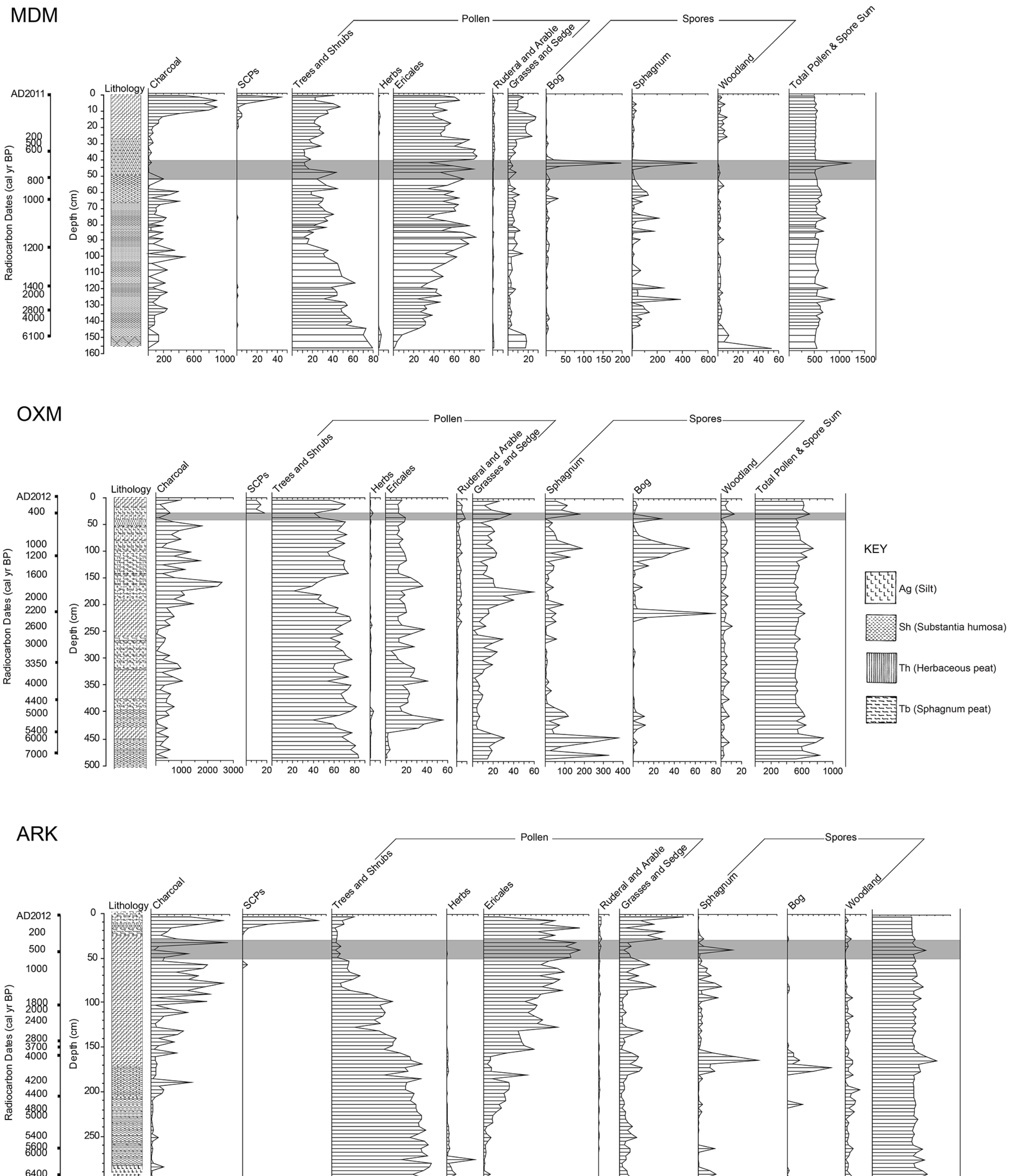


Figure 5. Summary pollen diagram for MDM, OXM and ARK. Shaded bar represents LIA. Full pollen diagrams available from McCarroll et al. (2016a, 2016b, 2017).

mire. At ARK, between ca. 500 and −60 cal yr BP, a mixed mire community is present including *Polytrichum commune*, some Cyperaceae (*Eriophorum vaginatum* and *E. angustifolium*), and Ericaceae (*Calluna vulgaris*). There is a high percentage of identified *Sphagnum*, including *S. papillosum* towards the surface, indicative of the presence of hummocks.

DHI

DHI (Dupont, 1986) has been applied to the plant macrofossil data to provide a qualitative indication of changes in water table (Figure 7). DCA was applied to the plant macrofossil data but was unsuccessful, perhaps owing to the very infrequent presence of certain species, or rare species, such as *Nymphaea*, occurring only once in a profile. Despite a recent favoured use of Detrended Correspondence Analysis (DCA) in studies on late-Holocene *Sphagnum*-dominated peats, DHI has some benefits over DCA and other ordination options (Daley and Barber, 2012). Unlike DCA, DHI does not require any portion of the data to be removed for it to function properly. One of the criticisms of DCA is that samples with rare species, such as is the case with the present datasets, can have a large effect on the resulting ordination (Daley and Barber,

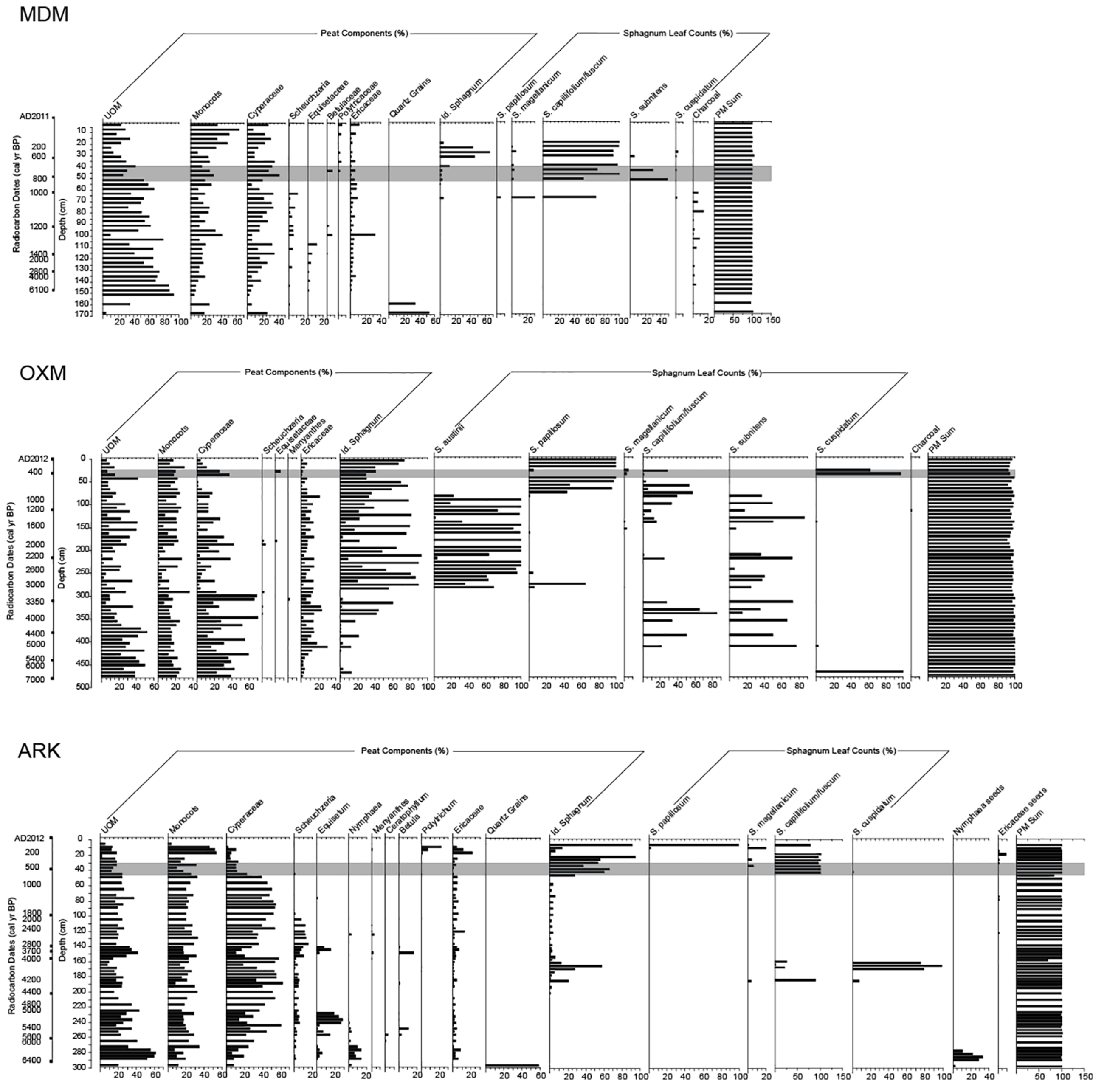


Figure 6. Summary plant macrofossil diagram for MDM, OXM and ARK. Shaded bar represents LIA. Full plant macrofossil diagrams indicating species within the summary groups Cyperaceae (*Eriophorum vaginatum*, *E. angustifolium* and *Trichophorum cespitosum*) and Ericaceae (*Calluna vulgaris*, *Erica tetralix* and *Vaccinium oxycoccus*) available from McCarroll et al. (2016a, 2016b, 2017).

2012). When applying DHI, the full range of data can be used. Furthermore, in DCA, the resulting axis may not be influenced by a single environmental variable. In DHI, weightings are assigned to species based on ecological knowledge of response to specific environmental variables and so despite the apparent subjectivity of the method, it is probable that DHI provides important results (Daley and Barber, 2012).

The indices used were Unidentified Organic Matter (UOM) 8, *Calluna vulgaris* 8, Ericaceae undifferentiated 8, *Betula* 7, *Polytrichum commune* 7, *Eriophorum vaginatum* 6, monocots undifferentiated 6, *Trichophorum cespitosum* 6, *Vaccinium oxycoccus* 5, *Sphagnum papillosum* 4, *Sphagnum* Section *Acutifolia* 4, *Sphagnum magellanicum* 3, *Menyanthes* 3, *Eriophorum angustifolium* 2, *Scheuchzeria palustris* 2, *Ceratophyllum* 1, *Nymphaea* 1, *Sphagnum cuspidatum* 1, based upon the weights used in Daley and Barber (2012) and Mauquoy et al. (2008) and knowledge of ecological tolerances and habitat preferences of each species.

Mossdale Moor. Low values indicate wet conditions, implying that the mire was wet at 1300, 950 and 600–350 cal yr BP (LIA) (Figure 7), although single-sample examples must be treated with caution. The DHI curve largely agrees with the humification curve for the LIA. However, the reliability of the DHI curve is problematic, given that there seems to be a disappearance of *Sphagnum* in the plant macrofossils below 65 cm depth, perhaps owing to decomposition. *Sphagnum* may have been present at the site despite the absence in the plant macrofossils, given the presence of *Sphagnum* spores counted in pollen analysis.

Oxenhope Moor. The DHI results are plotted against age rather than depth (cp. McCarroll et al., 2016a). At 6400 cal yr BP, UOM is high, owing to higher decomposition levels at this depth in the catotelm. The high score attributed from a high percentage of UOM dilutes the wetness signal indicated by the presence of *Sphagnum cuspidatum* at this depth. Low values indicate wet conditions, suggesting that the mire was wet at 2800 cal yr BP, 1700

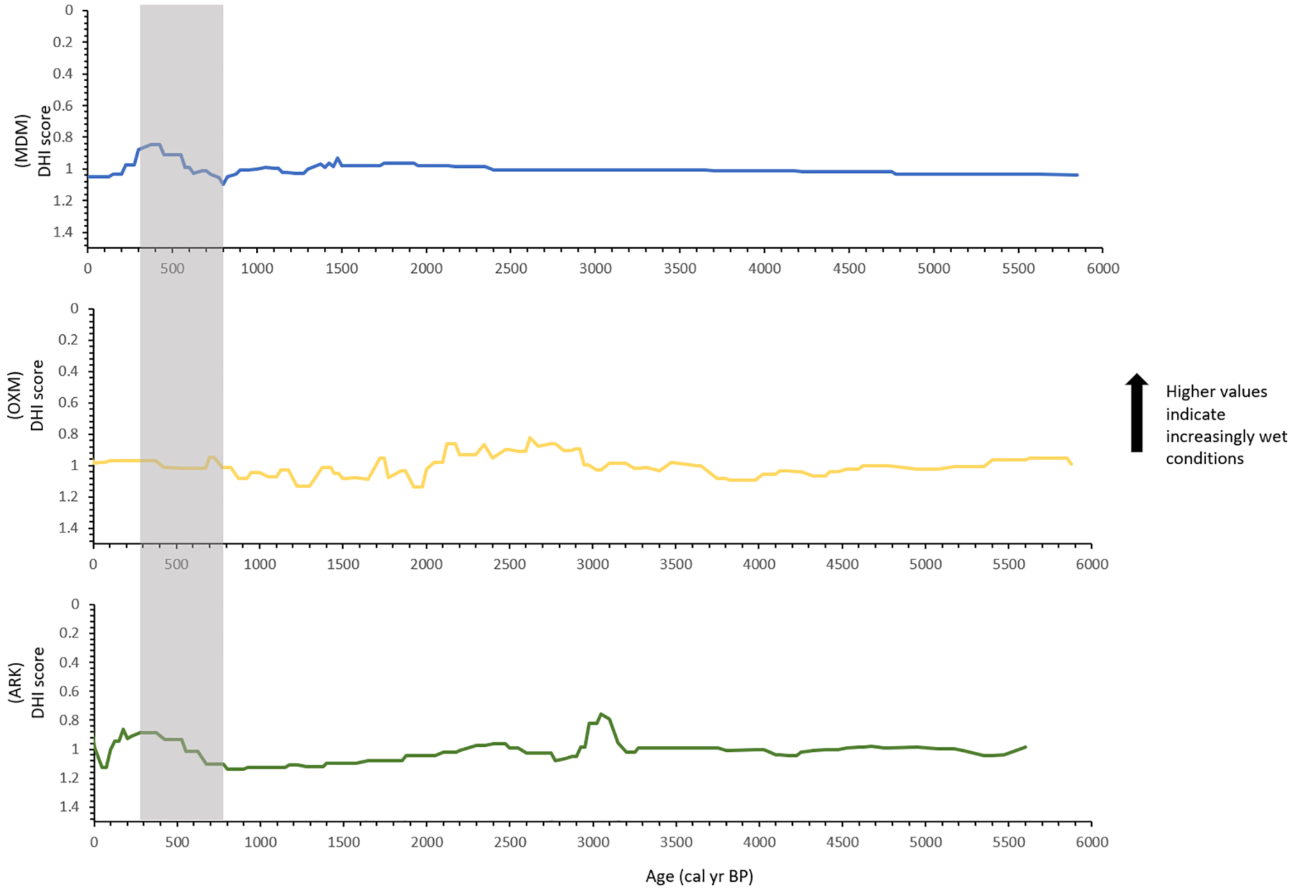


Figure 7. DHI scores plotted against age for MDM, OXM and ARK. The shaded bar represents the LIA, for visual guidance only.

cal yr BP, 1350 cal yr BP, 1000 cal yr BP and between 750–300 cal yr BP (LIA).

West Arkengarthdale. A summary plotting the DHI data against age as opposed to depth is provided here. Low values indicate wet conditions, and these suggest that the mire was wet at 3900 cal yr BP, 2600–2100 cal yr BP, 1800 cal yr BP and 450–200 cal yr BP (LIA).

Palaeoenvironmental evidence for the LIA from Yorkshire blanket peat

At Mossdale Moor, regional wet conditions are identified after 660 cal yr BP, indicated by rising T values and large numbers of *Sphagnum* and associated *Tilletia sphagni* spores, and by low levels of charcoal and *Calluna* pollen, which would be indicative of dry conditions. Upon re-examination of the data published by McCarroll et al. (2017), including DHI scores using a revised chronology, we identify a reduction in charcoal fragments and a reduction in tree and shrub pollen from pollen and spore data between 700 and 600 cal yr BP (Figure 5). A sharp increase in bog and *Sphagnum* spores is present between 650 and 600 cal yr BP, suggestive of wet conditions. The plant macrofossil data show remains of *Sphagnum*, mainly *S. capillifolium* and *S. subnitens* and low levels of UOM, suggesting wet conditions locally.

The lowest degree of peat humification in the profile is shown where % T reaches its highest peak at ca. 340 cal yr BP (ca. cal yr AD 1610), suggesting the most marked of climatic deteriorations (to wet and/or cold) observed throughout the profile at MDM, concluding by 135 cal yr BP (ca. cal yr AD 1815). From ca. 700 to 250 cal yr BP (cal yr AD 1250–1700), DHI scores are lower, with the lowest DHI score throughout the profile observed at ca. 430 cal yr BP/cal yr AD 1520).

The combined data suggest a climatic deterioration between 600 and 300 cal yr BP (cal yr AD 1350–1650), consistent with the LIA, defined as occurring between cal yr AD 1200 and 1850 from the peatland palaeoenvironmental evidence from northwestern Europe.

At Oxenhope Moor, evidence for the LIA is apparent at 400 cal yr BP/cal yr AD 1550 from humification data (high T values) and at ca. 500 cal yr BP from the identification of *Sphagnum cuspidatum* from plant macrofossils. Between 45 and 40 cm depth, 100% of 35% identified *Sphagnum* is attributable to *S. cuspidatum* and pollen and spore data show low charcoal fragments, comparatively lower tree and shrub pollen than the rest of the profile, increasing bog species and *Sphagnum* spores. Low DHI values are observed and the highest T values throughout the profile are evident between 600 and 200 cal yr BP (cal yr AD 1350–1750), consistent with the LIA as defined within this paper.

Similarly, at West Arkengarthdale, ca. 450 years ago, the LIA is evidenced by low humification, low UOM and low DHI values (from 600 to 200 cal yr BP). The pollen and spore data show low tree and shrub and grass and sedge pollen and a high number of *Sphagnum* spores. From the plant macrofossils, a return of *Sphagnum* is evident at 45 cm depth (450 cal yr BP) consisting of *S. capillifolium* and *S. fuscum*.

T values are at their highest between 300 and 130 cal yr BP. DHI values reduce from ca. 500 cal yr BP and are at their lowest in the profile at ca. 150 cal yr BP. All proxies agree on a climatic deterioration between 700 and 250 cal yr BP (cal yr AD 1250–1700), consistent with the LIA as defined within this paper.

Discussion

Differences in the onset and duration of the LIA in Yorkshire

The multiproxy evidence (from pollen, plant macrofossil and humification analysis) suggests that the LIA has been identified at each of the sites but with slightly differing ages (Mossdale Moor: 600–300 cal yr BP (cal yr AD 1350–1650); Oxenhope Moor: 600–200 cal yr BP (cal yr AD 1350–1750); West Arkengarthdale: 700–250 cal yr BP (cal yr AD 1250–1700). This may be owing to differing response times of the vegetation communities at each of the sites: for instance, there is a high percentage of *Sphagnum papillosum* at Oxenhope Moor compared to *Sphagnum* Section *Acutifolia* at Mossdale Moor and West Arkengarthdale, although both have the same DHI scores and can form lawns, so it is not immediately apparent how this should delay response. Monocots and *Eriophorum vaginatum* are present at all three sites in varying amounts. *E. vaginatum* is ecologically tolerant in a range of environments (Atherton et al., 2010) and therefore perhaps has varying response times at different locations. An additional contributing factor may be variations in geographical location. For instance, West Arkengarthdale sees the earliest onset of the LIA and is located furthest north; however, it is situated at the lowest altitude of the three sites. Dating uncertainties may also play a role in the differences seen between sites, given that ^{210}Pb dates were obtained for MDM but not for OXM and ARK. A possible way to resolve this would be to obtain ^{210}Pb dates from OXM and ARK.

At Malham Tarn Moss, Yorkshire, a marked period of increased effective precipitation and a prolonged phase of increased Bog Surface Wetness (BSW) is apparent in palaeohydrological proxies (testate amoebae, plant macrofossil and humification analysis) between ca. AD 1460 and 1850 (490–100 cal yr BP) (Figure 8 and Table 2) and is synchronous with the LIA climate deterioration (Turner et al., 2014). The LIA starts earlier at the sites investigated in the present study, where the onset is identified as AD 1350 at MDM and OXM and AD 1250 at ARK. Furthermore, the duration of the LIA at Malham Tarn Moss is longer, ending AD 1850, compared to AD 1650 at MDM, AD 1750 at OXM and AD 1700 at ARK.

A testate amoebae profile and water-table reconstruction from Moor House blanket peat in Northern England shows a transition from near surface to deeper water tables at the boundary of the LIA (Swindles et al., 2015). However, Swindles et al. (2015) suggest that the magnitude of the reconstruction is too dry and therefore, these data have been excluded from Figure 8 and Table 2.

In the North York Moors in northern England, Chiverrell (2001) identifies a shift to wetter or cooler climatic conditions at May Moss after cal yr AD 1400–1620, which is contemporaneous with the onset of the LIA (Figure 8, Table 2). At May Moss, this deterioration lasts from ca. AD 1350 to 1450 to ca. AD 1900. The LIA is more long-lived at May Moss, ending AD 1900, compared to AD 1650 at MDM, AD 1750 at OXM and AD 1700 at ARK. This is interesting given that May Moss and the sites in the present study are all located in Yorkshire. The differences may be owing to varying sensitivity of the sites, perhaps as May Moss is located closer to the coast, therefore making the site susceptible to cold easterly winds from the North Sea.

The LIA in northwestern Europe

At Talkin Tarn, Cumbria, wetter conditions have been identified at 600 BP by low chironomid-inferred July temperatures and wet-shifts in plant macrofossils and testate amoebae (Barber and Langdon, 2007) (Figure 8, Table 2). Charman et al. (2006) also recognise 600 BP as being a period of higher water tables in northern Britain. The same deterioration is identified by Baker et al. (1999) at 600–500 BP and is claimed to correlate with Maunder and Spörer sunspot minima. The LIA is also identified by Langdon et al. (2003) from Temple Hill Moss, Pentland Hills, southeast Scotland and in southern Scotland from Talla Moss, where Chambers et al. (1997) recognise a cool and wet episode commencing at ca. 540 BP from pollen and humification analysis. Peat sequences in Denmark (Aaby, 1976; Barber et al., 2004) show wet shifts of an equivalent age and Barber et al. (2004) recognise the LIA from northern Germany and Denmark from peat macrofossil investigations. These data overlap with the age ranges proposed for the LIA in Yorkshire in the present study, with the onset identified as 600 cal yr BP for MDM and OXM although slightly earlier at ARK at 700 cal yr BP.

Davis and Wilkinson (2004) identify *Amphitrema* spp. from testate amoebae analysis at ca. 300 BP (cal yr AD 1488–1796) at Astley Moss, Greater Manchester and Danes Moss, Cheshire, northwest England, which the authors suggest likely corresponds to the LIA. Again, each of these suggested date ranges overlaps with the LIA as identified at MDM, OXM and ARK.

Charman et al. (1999) recognise prominent wet peaks at Coom Rigg Moss, Northumberland, northern England from the reconstructed water-table record using testate amoebae at cal yr AD 1400–1500 and cal yr AD 1650–1900. These wet periods are also well replicated in the plant macrofossil record at the same site. In the present study, one wet period is identified at each site for the LIA, as opposed to two as identified by Charman et al. (1999). This is perhaps owing to differences in the location of the sites, or perhaps the difference in methods used to reconstruct palaeoclimatic changes. However, Mauquoy and Barber (1999) also studied Coom Rigg Moss and only report one wet shift from this site, associated with the decline and local extinction of *Sphagnum austinii* between cal yr AD 1395 and 1485. This does overlap with the dates for the LIA from the sites in the present study. Mauquoy and Barber (1999) provide further evidence supporting the occurrence of climatic deteriorations synchronous with the LIA in northern Britain. There are wet shifts associated with the decline and local extinction of *S. austinii*, dated to cal yr AD 1160–1400 from Raeburn Flow and Bell's Flow (Scotland) and cal yr AD 1030–1400 from Bolton Fell Moss and Walton Moss (England), which incorporates the onset of the LIA. As well as the aforementioned Coom Rigg Moss, they also studied Felecia Moss, where wet shifts are associated with the decline and local extinction of *S. austinii* between cal yr AD 1395–1485, again synchronous with the earlier dates for the LIA.

Stoneman (1993) found evidence from northern England and southern Scotland from 10 raised peat bogs (Walton, Bolton Fell, Glasson, Carsegowan, Ellergower, Cranley, Dogden, Drone, Letham and Blairbech Mosses) for a climatic deterioration at ca. cal AD 1335–1615 from plant macrofossil and humification data. These dates closely overlap with the dates proposed in the present study, with the LIA beginning at ca. AD 1350 for MDM and OXM and AD 1250 for ARK, ending AD 1650 at MDM, AD 1750 at OXM and AD 1700 at ARK. It would be useful to revisit each of the sites studied by Stoneman (1993) and the sites in the present study to understand whether testate amoebae water table depth reconstructions would confirm or deny these propositions, particularly given the age ranges proposed by Charman et al. (1999) for Coom Rigg Moss, where testate amoebae were used and how these differed from the ages proposed by Mauquoy and Barber (1999) for the same site, without testate amoebae analysis. Furthermore, many of these studies were conducted in the 1990s and early 2000s; revisiting these datasets with advances in dating may refine results. It may also be the case that sites at higher altitude respond earlier, owing to decreasing temperature with height.

The global record of the LIA

Increasing evidence allows the LIA to be regarded as a global event, with differing responses dependant on location. For example, Chambers et al. (2014) argue that the most severe episodes of the LIA are contemporaneous between hemispheres but with opposite site responses at a mire in Tierra del Fuego in the Southern Hemisphere when compared to those in a mire in north-central Europe in the Northern Hemisphere.

The LIA is also identified in Australasia; for example, Winkler (2000) identifies a LIA glacier maximum at Mueller Glacier and

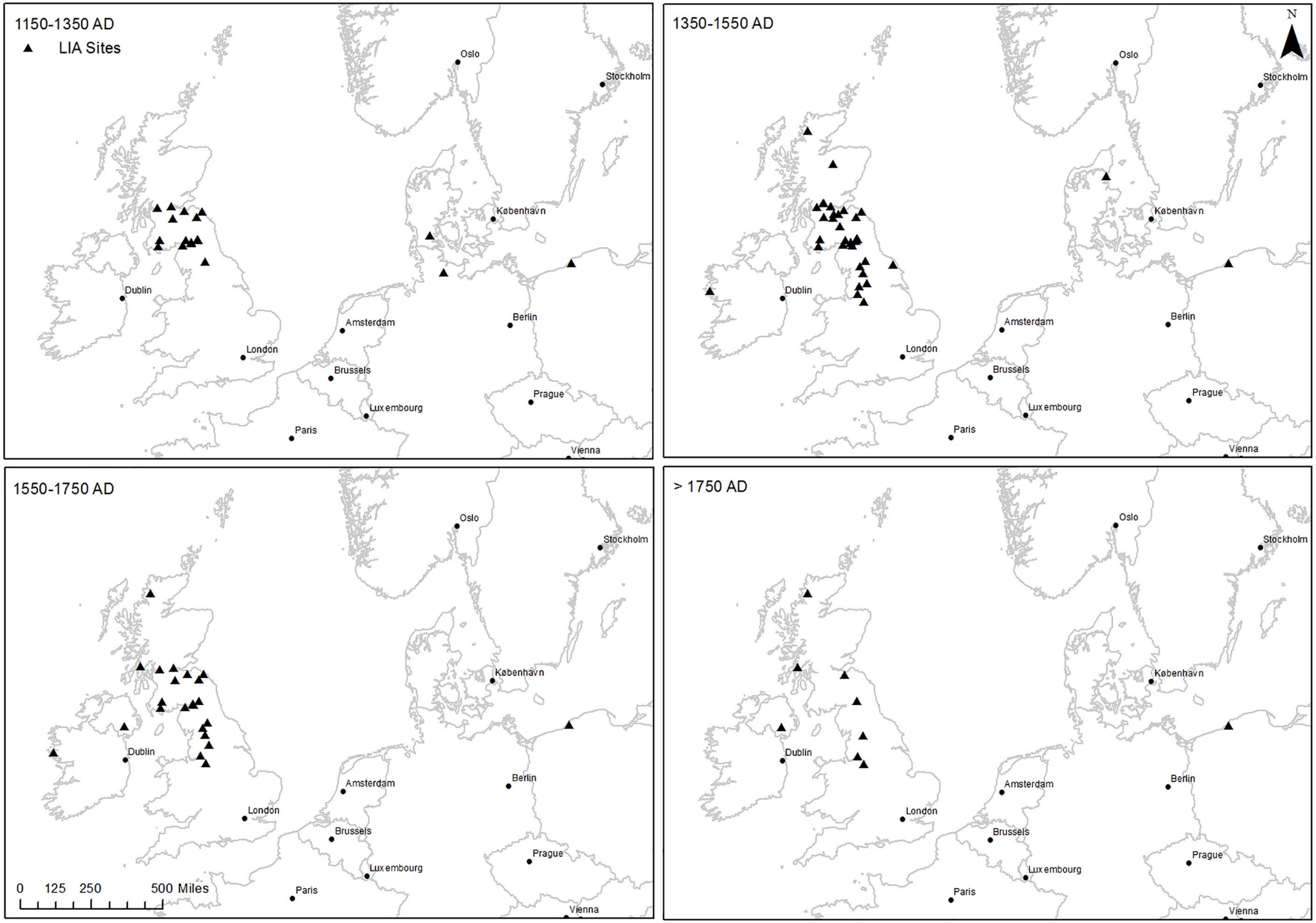


Figure 8. Geographical and temporal patterns of the LIA across northwestern Europe. See Table 2 for sites included and age ranges.

Table 2. Geographical and temporal patterns of the LIA across northwestern Europe including site names, start and end dates for the LIA, country and coordinates and authors.

Site name	LIA start date (AD)	LIA end date (AD)	Country	Latitude (°N)	Longitude (°E; −ve: °W)	Authors
Bolton Fell Moss	1160	1400	England	55.012925	−2.7990421	Mauquoy and Barber (1999)
Walton Moss	1160	1400	England	54.992401	−2.7767524	Mauquoy and Barber (1999)
Coom Rigg Moss	1160	1400	England	55.108665	−2.48507	Mauquoy and Barber (1999)
Raeburn Flow	1160	1400	Scotland	55.933171	−3.1300138	Mauquoy and Barber (1999)
Felecia Moss	1160	1400	England	55.092938	−2.4386886	Mauquoy and Barber (1999)
Bells Flow	1160	1400	Scotland	55.073781	−3.0665036	Mauquoy and Barber (1999)
Slowinske Blota	1200	1800	Poland	54.399925	16.483501	De Vleeschouwer et al. (2009)
Dosenmoor	1250	1350	Germany	54.136281	10.019157	Barber et al. (2004)
Svanemorse	1250	1350	Denmark	55.22	9.3	Barber et al. (2004)
West Arkengarthdale	1250	1700	England	54.458815	−2.067067	Present paper
Walton Moss	1335	1615	England	55.012925	−2.7990421	Stoneman (1993)
Bolton Fell Moss	1335	1615	England	55.012925	−2.7990421	Stoneman (1993)
Glasson Moss	1335	1615	England	54.932438	−3.189822	Stoneman (1993)
Carsegowan Moss	1335	1615	Scotland	54.900419	−4.455654	Stoneman (1993)
Ellergower Moss	1335	1615	Scotland	55.087103	−4.384487	Stoneman (1993)
Cranley Moss	1335	1615	Scotland	55.710083	−3.699848	Stoneman (1993)
Drone Moss	1335	1615	Scotland	55.895281	−2.249653	Stoneman (1993)
Dogden Moss	1335	1615	Scotland	55.73942	−2.506115	Stoneman (1993)
Letham Moss	1335	1615	Scotland	56.0545	−3.7993760	Stoneman (1993)
Blairbech Moss	1335	1615	Scotland	56.018719	−4.5155371	Stoneman (1993)
Talkin Tarn	1350	1350	England	54.922164	−2.711438	Barber and Langdon (2007)
Tore Hill Moss	1350	1350	Scotland	53.70463	−2.378667	Charman et al. (2006)
Mallachie Moss	1350	1350	Scotland	57.234743	−3.717148	Charman et al. (2006)
Shirgarton Moss	1350	1350	Scotland	56.1392	−4.179245	Charman et al. (2006)
Killorn Moss	1350	1350	Scotland	56.137636	−4.220133	Charman et al. (2006)
Temple Hill Moss	1350	1350	Scotland	55.837639	−3.4178588	Charman et al. (2006)
Langlands Moss	1350	1350	Scotland	55.734948	−4.176072	Charman et al. (2006)
Longridge Moss	1350	1350	Scotland	55.839884	−3.6686636	Charman et al. (2006)
Butterburn Flow	1350	1350	England	55.056049	−2.509428	Charman et al. (2006)
Coom Rigg Moss	1350	1350	England	55.108665	−2.48507	Charman et al. (2006)
May Moss	1350	1350	England	54.3532	−0.6531684	Charman et al. (2006)
Traligill Basin	1350	1450	Scotland	58.148555	−4.977197	Baker et al. (1999)
May Moss	1350	1450	England	54.3532	−0.65316849	Chiverrell (2001)
Mossdale Moor	1350	1650	England	54.300292	−2.315507	Present paper
Oxenhope Moor	1350	1750	England	53.793759	−1.977952	Present paper
Coom Rigg Moss	1400	1500	England	55.108665	−2.48507	Charman et al. (1999)
Letterfrack	1410	1540	Ireland	53.550965	−9.958596	Blackford and Chambers (1995)
Talla Moss	1410	1410	Scotland	55.463916	−3.34104	Chambers et al. (1997)
Lille Vildmose	1449	1464	Denmark	56.889176	10.211426	Mauquoy et al. (2002)
Malham Tarn	1460	1850	England	54.097024	−2.168611	Turner et al. (2014)
Astley Moss	1488	1796	England	53.476002	−2.433262	Davis and Wilkinson (2004)
Danes Moss	1488	1796	England	53.237879	−2.143975	Davis and Wilkinson (2004)
Traligill Basin	1550	1800	Scotland	58.148555	−4.977197	Baker et al. (1999)
Walton Moss	1601	1604	England	54.987636	–2.778144	Mauquoy et al. (2002)
Fallahogy	1650	1850	Northern Ireland	54.345	−6.336	Barber et al. (2000)
Moine Mhor	1650	1850	Scotland	56.103303	−5.501073	Barber et al. (2000)
Coom Rigg Moss	1650	1900	England	55.108665	−2.48507	Charman et al. (1999)
Letterfrack	1660	1720	Ireland	53.550965	−9.958596	Blackford and Chambers (1995)
Temple Hill Moss	1700	1800	Scotland	55.883372	−3.1025082	Langdon et al. (2003)

Yan et al. (2015) document a retreat of the East Asian Summer Monsoon and the Australian Summer Monsoon into the tropics. In south central Alaska, Barlow et al. (2012) identify glacial advance; yet, Fritz et al. (1994) provide evidence for aridity and drought during the LIA from Devils Lake, North Dakota, USA. Dry intervals have also been suggested by Platt Bradbury (1988) from Elk Lake, Minnesota, again suggesting that for this part of the globe, an 'ice age' does not appropriately describe the climatic event.

Drought is identified in Québec, Canada by Archambault and Bergeron (1992) but despite this, Bégin and Payette (1988) show that snowfall increased towards the end of the LIA in the same province; Petersen (1994) notes that the LIA is cold and dry in the southern Rocky Mountains, U.S.A., differing from the wet and cold conditions observed in northern England and Europe. Regardless of the climatic variation of the LIA across the globe, the identification of this event at this scale is evidence for its severity.

Evidence for other climatic deteriorations in this study

Other climatic deteriorations identified in the present study and from the wider literature are indicated in Table 3. These events were identified in two of the sites from the present study as well

Table 3. Evidence for other climatic deteriorations in terms of wetness/coldness.

Climatic deterioration (time period)	Sites	Authors	Evidence
3200–2100 cal yr BP	Mossdale Moor, Oxenhope Moor, Bolton Fell Moss, Butterburn Flow, Walton Moss, Talkin Tarn, Bigland Tarn (northern Britain), Mongan Bog, Abbeyknockmoy Bog (Ireland), Temple Hill Moss (southeast Scotland), North Atlantic Ocean core, ^{14}C calibration curve.	Present paper, Barber et al. (2003, 2013), Langdon et al. (2003), Bond et al. (1997), van Geel et al. (1996), van Geel and Renssen (1998).	MDM: *Sphagnum* spores present, low humification. OXM: *Tilletia sphagni* spores present, low humification, low DHI values, low *Calluna* pollen, low charcoal.
4400 cal yr BP	Oxenhope Moor, West Arkengarthdale, Walton Moss (northern Britain), Temple Hill Moss (southeast Scotland).	Present paper, Hughes et al. (2000), Langdon et al. (2003).	OXM: *Sphagnum* spores present, low *Calluna* pollen, low humification. ARK: low humification, low charcoal, low *Calluna* pollen, presence of *Scheuchzeria palustris* in plant macrofossils.
5000 cal yr BP	Oxenhope Moor, West Arkengarthdale, Walton Moss, Talkin Tarn (northern Britain), Eilean Subhainn, Glen Tarridon, Glen Carron (northwest Scotland)	Present paper, Barber and Langdon (2007), Anderson et al. (1998).	OXM: *Sphagnum* spores present, low humification, low charcoal. ARK: Low humification, presence of *Equisetum fluviatile* in plant macrofossils.

as by other authors. None of these events is present at all sites in the present study, none is found routinely in other studies, and therefore, perhaps none of these events can be regarded as pronounced as the LIA.

The widely discussed and globally identified 4.2 ka event (Bond et al., 2001; Booth et al., 2005; Drysdale et al., 2006; Liu and Feng, 2012; Thompson et al., 2002) has not been identified in the present study, with the possible exception of at ARK, where *S. cuspidatum* is seen to increase around this time; however, this is not enough evidence alone to suggest the presence of this climatic event. The identification of this event is somewhat contested in northwest European peatlands, particularly in the UK (Roland et al., 2014). Roland et al. (2014) attribute this to the possibility that either the dominant forcing mechanisms of this period lie outside of the North Atlantic, or any changes in the region may not have been severe enough to be recorded in peatlands. This is a possible explanation for the absence of this event from the climatic changes identified in the present study.

The significance of the LIA

While there is evidence for other climatic deteriorations recorded in the data from the sites in the present paper, the LIA is the only such event recorded by the proxy-climate data from all three sites. It is the most widely recorded climatic instability in many other peatlands and other records of palaeoclimate across northern England and continental Europe, suggesting that the LIA is the most noticeable palaeoclimate downturn here in the latter half of the postglacial. The humification data from MDM, OXM and ARK show the most pronounced shift towards wetter conditions in the entirety of the ca. 6000-year records at ca. 500 cal yr BP. Similarly, the DHI records for MDM and ARK show that the harshest climatic conditions in terms of wetness/coldness were present during this time. In particular, there was a vegetation shift at OXM from *Sphagnum papillosum* dominated peat before the event to wet-loving *S. cuspidatum* dominance during the LIA. The dominance then reverts to *S. papillosum* following the end of the LIA. A comparable shift in *Sphagnum* dominance is not present in the rest of the profile. A climatic cause for this vegetation shift is also supported by a decrease in humification values and a signal for a wider than local climatic deterioration in the pollen signal. The palaeoclimatic changes observed throughout the rest of the profiles at each site appear more modest in comparison to the apparent wetness of the LIA. This is perhaps the most pronounced downturn in climate observed in the northern England terrestrial record, as potentially in Europe.

Conclusions

The Little Ice Age has been identified in the form of a wet phase from three blanket peats in Yorkshire using pollen, plant macrofossil and humification data, providing new palaeoclimate data for this area of Britain. Although other climatic events are apparent at each site, this is the only event clearly evident in all three in the last ca. 6000 years. The data are insufficiently detailed to identify multiple phases within the LIA clearly. Slightly differing ages are apparent for the LIA between sites, although this is in keeping with differences between other sites in the literature and may be attributed either to the limitations in radiocarbon dating spanning across this time or to the individual sensitivity of each site to changes in climate. The LIA has previously been identified in published literature for northern England and northwest Europe from terrestrial records and from various other sources across the globe, highlighting the severity of this event. The present paper adds further evidence in support of this. It is perhaps the most widespread and routinely recorded climatic event evident in peatlands in northern England over the mid- to late-Holocene.

Acknowledgements

We thank the Yorkshire Peat Partnership for their financial support; the Quaternary Research Association for additional financial support; John Daniell, Hongyan Zhao, Oliver Pritchard, James Dracott, Briony Eastabrook, Briony Overs, Rachel Leven and Will Carpenter for assistance with fieldwork; Hongkai Li and Angus Mackenzie for assistance with the modelling of ^{210}Pb dates; Briony Eastabrook and Will Carpenter for laboratory assistance and Oliver Pritchard for help with maps.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Author JM received funding support from the Yorkshire Peat Partnership, Quaternary Research Association and held a university-funded research studentship at the University of Gloucestershire during field and laboratory research.

ORCID iD

Frank M Chambers https://orcid.org/0000-0002-0998-2093

Supplemental material

Supplemental material for this article is available online.

References

Aaby B (1976) Cyclic climatic variations in climate over the past 5,500 yr reflected in raised bogs. *Nature* 263: 281–284.

Amesbury MJ, Barber KE and Hughes PDM (2012a) Can rapidly accumulating Holocene peat profiles provide sub-decadal resolution proxy climate data? *Journal of Quaternary Science* 27(8): 757–770.

Amesbury MJ, Barber KE and Hughes PD (2012b) The relationship of fine-resolution, multi-proxy palaeoclimate records to meteorological data at Fågelmossen, Värmland, Sweden and the implications for the debate on climate drivers of the peat-based record. *Quaternary International* 268: 77–86.

Anderson DE, Binney HA and Smith MA (1998) Evidence for abrupt climatic change in northern Scotland between 3900 and 3500 calendar years BP. *The Holocene* 8(1): 97–103.

Appleby PG (2001) Chronostratigraphic techniques in recent sediments. In: Last WM and Smol JP (eds) *Tracking Environmental Change Using Lake Sediments Vol. 1: Basin Analysis, Coring, and Chronological Techniques*. Dordrecht: Kluwer, pp. 171–203.

Appleby PG (2008) Three decades of dating recent sediments by fallout radionuclides: a review. *The Holocene* 18: 83–93.

Appleby PG and Oldfield F (1978) The calculation of lead-210 dates assuming a constant rate of supply of unsupported 210Pb to the sediment. *CATENA* 5(1): 1–8.

Archambault S and Bergeron Y (1992) An 802-year tree-ring chronology from the Quebec boreal forest. *Canadian Journal of Forest Research* 22(5): 674–682.

Atherton I, Bosanquet S and Lawley M (2010) *Mosses and Liverworts of Britain and Ireland: A Field Guide*. London: British Bryological Society.

Baker A, Caseldine CJ, Gilmour MA et al. (1999) Stalagmite luminescence and peat humification records of palaeomoisture for the last 2500 years. *Earth and Planetary Science Letters* 165(1): 157–162.

Barber K, Brown A, Langdon P et al. (2013) Comparing and cross-validating lake and bog palaeoclimatic records: A review and a new 5,000 year chironomid-inferred temperature record from northern England. *Journal of Paleolimnology* 49(3): 497–512.

Barber K, Chambers F and Maddy D (2004) Late Holocene climatic history of northern Germany and Denmark: Peat macrofossil investigations at Dosenmoor, Schleswig-Holstein, and Svanemose, Jutland. *Boreas* 33(2): 132–144.

Barber KE (1981) *Peat Stratigraphy and Climatic Change: A Palaeoecological Test of the Theory of Cyclic Bog Regeneration*. Rotterdam: Balkema.

Barber KE, Chambers FM, Maddy D et al. (1994) A sensitive high-resolution record of late holocene climatic change from a raised bog in northern England. *The Holocene* 4(2): 198–205.

Barber KE, Chambers FM and Maddy D (2003) Holocene palaeoclimates from peat stratigraphy: Macrofossil proxy climate records from three oceanic raised bogs in England and Ireland. *Quaternary Science Reviews* 22(5–7): 521–539.

Barber KE and Langdon PG (2007) What drives the peat-based palaeoclimate record? A critical test using multi-proxy climate records from northern Britain. *Quaternary Science Reviews* 26(25–28): 3318–3327.

Barber KE, Maddy D, Rose N et al. (2000) Replicated proxy-climate signals over the last 2000 yr from two distant UK peat bogs: New evidence for regional palaeoclimate teleconnections. *Quaternary Science Reviews* 19(6): 481–487.

Barlow NLM, Shennan I and Long AJ (2012) Relative sea-level response to Little Ice Age ice mass change in south central Alaska: Reconciling model predictions and geological evidence. *Earth and Planetary Science Letters* 315–316: 62–75.

Bégin Y and Payette S (1988) Dendroecological evidence of lake-level changes during the last three centuries in subarctic Québec. *Quaternary Research* 30(2): 210–220.

Bingham EM, McClymont EL, Väliranta M et al. (2010) Conservative composition of n-alkane biomarkers in Sphagnum species: Implications for palaeoclimate reconstruction in ombrotrophic peat bogs. *Organic Geochemistry* 41(2): 214–220.

Blackford J (2000) Palaeoclimatic records from peat bogs. *Trends in Ecology & Evolution* 15(5): 193–198.

Blackford JJ and Chambers FM (1991) Proxy records of climate from blanket mires: Evidence for a Dark Age (1400 BP) climatic deterioration in the British Isles. *The Holocene* 1(1): 63–67.

Blackford JJ and Chambers FM (1995) Proxy climate record for the last 1000 years from Irish blanket peat and a possible link to solar variability. *Earth and Planetary Science Letters* 133(1–2): 145–150.

Blundell A and Barber K (2005) A 2800-year palaeoclimatic record from Tore Hill Moss, Strathspey, Scotland: The need for a multi-proxy approach to peat-based climate reconstructions. *Quaternary Science Reviews* 24(10–11): 1261–1277.

Blundell A and Holden J (2015) Using palaeoecology to support blanket peatland management. *Ecological Indicators* 49: 110–120.

Bond G, Kromer B, Beer J et al. (2001) Persistent solar influence on North Atlantic climate during the Holocene. *Science* 294: 2130–2136.

Bond G, Showers W, Cheseby M et al. (1997) A pervasive millennial-scale cycle in North Atlantic Holocene and glacial climates. *Science* 278: 1257–1266.

Booth RK, Jackson ST, Forman SL et al. (2005) A severe centennial-scale drought in midcontinental North America 4200 years ago and apparent global linkages. *The Holocene* 15: 321–328.

Borgmark A and Schoning K (2006) A comparative study of peat proxies from two eastern central Swedish bogs and their relation to meteorological data. *Journal of Quaternary Science* 21(2): 109–114.

Castro D, Souto M, Garcia-Rodeja E et al. (2015) Climate change records between the mid- and late Holocene in a peat bog from Serra do Xistral (SW Europe) using plant macrofossils and peat humification analyses. *Palaeogeography Palaeoclimatology Palaeoecology* 420: 82–95.

Chambers FM (1984) *Studies on the initiation, growth rate and humification of blanket peats in South Wales*. PhD Thesis, University of Keele, Keele.

Chambers FM (2006) *ACCROTELM: Abrupt Climate Changes Recorded Over the European Land Mass. Final Report to European Commission*, Contract no. EVK2-CT-2002-00166. Cheltenham: CECQR, p.117.

Chambers FM, Beilman DW and Yu Z (2011a) Methods for determining peat humification and for quantifying peat bulk density, organic matter and carbon content for palaeostudies of climate and peatland carbon dynamics. *Mires and Peat* 7(7): 1–10.

Chambers FM, van Geel B and van der Linden M (2011b) Considerations for the preparation of peat samples for palynology, and for the counting of pollen and non-pollen palynomorphs. *Mires and Peat* 7(11): 1–14.

Chambers FM (2016) The ‘Little Ice Age’: The first virtual issue of the Holocene. *The Holocene* 26(3): 335–337.

Chambers FM, Barber KE, Maddy D et al. (1997) A 5500-year proxy-climate and vegetation record from blanket mire at Talla Moss, Borders, Scotland. *The Holocene* 7(4): 391–399.

Chambers FM, Brain SA, Mauquoy D et al. (2014) The ‘Little Ice Age’ in the southern Hemisphere in the context of the last

3000 years: Peat-based proxy-climate data from Tierra del Fuego. *The Holocene* 24(12): 1649–1656.

Charman DJ (2010) Centennial climate variability in the British Isles during the mid–late Holocene. *Quaternary Science Reviews* 29(13–14): 1539–1554.

Charman DJ, Blundell A, Chiverrell RC et al. (2006) Compilation of non-annually resolved Holocene proxy climate records: Stacked Holocene peatland palaeo-water table reconstructions from northern Britain. *Quaternary Science Reviews* 25(3–4): 336–350.

Charman DJ, Hendon D and Packman S (1999) Multiproxy surface wetness records from replicate cores on an ombrotrophic mire: Implications for Holocene palaeoclimate records. *Journal of Quaternary Science* 14(5): 451–463.

Chiverrell RC (2001) A proxy record of late Holocene climate change from May Moss, northeast England. *Journal of Quaternary Science* 16(1): 9–29.

Daley TJ and Barber KE (2012) Multi-proxy Holocene palaeoclimate records from Walton Moss, northern England and Dosenmoor, northern Germany, assessed using three statistical approaches. *Quaternary International* 268: 111–127.

Daniels RE and Eddy A (1985) *Handbook of European Sphagna*. Abbots Ripton, Huntingdon: Institute of Terrestrial Ecology.

Davis SR and Wilkinson DM (2004) The conservation management value of testate amoebae as 'restoration' 'indicators: Speculations based on two damaged raised mires in northwest England. *The Holocene* 14(1): 135–143.

De Vleeschouwer F, Chambers FM and Swindles GT (2010) Coring and sub-sampling of peatlands for palaeoenvironmental research. *Mires and Peat* 7(1): 1–10.

De Vleeschouwer F, Piotrowska N, Sikorski J et al. (2009) Multiproxy evidence of 'Little Ice Age' palaeoenvironmental changes in a peat bog from northern Poland. *The Holocene* 19(4): 625–637.

Drysdale R, Zanchetta G, Hellstrom J et al. (2006) Late Holocene drought responsible for the collapse of Old World civilizations is recorded in an Italian cave flowstone. *Geology* 34: 101–104.

Dupont LM (1986) Temperature and rainfall variation in the Holocene based on comparative palaeoecology and isotope geology of a hummock and a hollow (Bourtangerveen, The Netherlands). *Review of Palaeobotany and Palynology* 48(1–3): 71–159.

Fagan B (2001) *The Little Ice Age: How Climate Made History 1300–1850*. New York, NY: Basic Books.

Fritz SC, Engstrom DR and Haskell BJ (1994) 'Little ice age' aridity in the North American Great Plains: A high-resolution reconstruction of salinity fluctuations from Devils Lake, North Dakota, USA. *The Holocene* 4(1): 69–73.

Grove JM (2001) The initiation of the "Little Ice Age" in regions round the North Atlantic. In: Ogilvie EJ and Jónsson T (eds) *The Iceberg in the Mist: Northern Research in Pursuit of a "Little Ice Age."* New York, NY: Springer, pp.53–82.

Hughes PD, Mallon G, Essex HJ et al. (2012) The use of k-values to examine plant 'species signals' in a peat humification record from Newfoundland. *Quaternary International* 268: 156–165.

Hughes PDM, Mauquoy D, Barber KE et al. (2000) Mire-development pathways and palaeoclimatic records from a full Holocene peat archive at Walton Moss, Cumbria, England. *The Holocene* 10(4): 465–479.

Langdon PG, Barber KE and Hughes PD (2003) A 7500-year peat-based palaeoclimatic reconstruction and evidence for an 1100-year cyclicity in bog surface wetness from Temple Hill Moss, Pentland Hills, southeast Scotland. *Quaternary Science Reviews* 22(2–4): 259–274.

Liu F and Feng Z (2012) A dramatic climatic transition at 4000 cal. yr BP and its cultural responses in Chinese cultural domains. *The Holocene* 22: 1181–1197.

Mann ME (2002) Little Ice Age. In: MacCracken MC and Perry JS (eds) *Encyclopedia of Global Environmental Change Vol. 1*. Chichester: John Wiley, pp. 504–509.

Mann ME, Zhang Z, Rutherford S et al. (2009) Global signatures and dynamical origins of the little ice age and medieval climate anomaly. *Science* 326(5957): 1256–1260.

Matthews JA and Briffa KR (2005) The 'Little Ice Age': Re-evaluation of an evolving concept. *Geografiska Annaler: Series A, Physical Geography* 87(1): 17–36.

Mauquoy D and Barber K (1999) Evidence for climatic deteriorations associated with the decline of *Sphagnum imbricatum* Hornsch. ex Russ. in six ombrotrophic mires from northern England and the Scottish Borders. *The Holocene* 9(4): 423–437.

Mauquoy D, van Geel B, Blaauw M et al. (2002) Evidence from northwest European bogs shows 'Little Ice Age' climatic changes driven by variations in solar activity. *The Holocene* 12(1): 1–6.

Mauquoy D, van Geel B, Blaauw M et al. (2004) Changes in solar activity and holocene climatic shifts derived from 14C wiggle-match dated peat deposits. *The Holocene* 14(1): 45–52.

Mauquoy D, Yeloff D, van Geel B et al. (2008) Two decadally resolved records from north-west European peat bogs show rapid climate changes associated with solar variability during the mid-late Holocene. *Journal of Quaternary Science* 23(8): 745–763.

McCarroll J, Chambers FM, Webb JC et al. (2016a) Informing innovative peatland conservation in light of palaeoecological evidence for the demise of Sphagnum imbricatum: The case of Oxenhope Moor, Yorkshire, UK. *Mires and Peat* 18(8): 1–24.

McCarroll J, Chambers FM, Webb JC et al. (2016b) Using palaeoecology to advise peatland conservation: An example from West Arkengarthdale, Yorkshire, UK. *Journal for Nature Conservation* 30: 90–102.

McCarroll J, Chambers FM, Webb JC et al. (2017) Application of palaeoecology for peatland conservation at Mossdale Moor, UK. *Quaternary International* 432(Part A): 39–47.

McCracken KG and Beer J (2014) Comparison of the extended solar minimum of 2006-2009 with the Spoerer, Maunder, and Dalton Grand Minima in solar activity in the past. *Journal of Geophysical Research Space Physics* 119(4): 2379–2387.

Miller GH, Geirsdóttir Á, Zhong Y et al. (2012) Abrupt onset of the Little Ice Age triggered by volcanism and sustained by sea-ice/ocean feedbacks. *Geophysical Research Letters* 39(2): n/a–n/a.

Moore PD, Webb JA and Collinson ME (1991) *Pollen Analysis*. Oxford: Blackwell.

Petersen KL (1994) A warm and wet little climatic optimum and a cold and dry little ice age in the southern Rocky Mountains, USA. In: Hughes MK and Diaz HF (eds) *The Medieval Warm Period*. New York, NY: Springer, pp.243–269.

Platt Bradbury J (1988) A climatic-limnologic model of diatom succession for paleolimnological interpretation of varved sediments at Elk Lake, Minnesota. *Journal of Paleolimnology* 1(2): 115–131.

Ramsey CB (2009) Bayesian analysis of radiocarbon dates. *Radiocarbon* 51(1): 337–360.

Reimer PJ, Bard E, Bayliss A et al. (2013) IntCal13 and marine13 radiocarbon age calibration curves 0–50,000 years cal BP. *Radiocarbon* 55(4): 1869–1887.

Rodwell JS (1998) *British Plant Communities, 2*. Cambridge: Cambridge University Press.

Roland TP, Caseldine CJ, Charman DJ et al. (2014) Was there a '4.2 ka event 'in Great Britain and Ireland? Evidence from the peatland record. *Quaternary Science Reviews* 83: 11–27.

Ruddiman WF (2003) The Anthropogenic greenhouse era began thousands of years ago. *Climatic Change* 61(3): 261–293.

Sillasoo Mauquoy D, Blundell A et al. (2007) Peat multi-proxy data from Männikjärve bog as indicators of late Holocene climate changes in Estonia. *Boreas* 36(1): 20–37.

Smith AJE (2004) *The Moss Flora of Britain and Ireland*. Cambridge: Cambridge University Press.

Stoneman R (1993) *Holocene palaeoclimates from peat stratigraphy: extending and refining the model*. PhD Thesis, University of Southampton, Southampton.

Swindles GT (2010) Dating recent peat profiles using spheroidal carbonaceous particles (SCPs). *Mires and Peat* 7(3): 1–5.

Swindles GT, Holden J, Raby CL et al. (2015) Testing peatland water-table depth transfer functions using high-resolution hydrological monitoring data. *Quaternary Science Reviews* 120(lement C): 107–117.

Tallis JH (1995) Climate and erosion signals in British blanket peats: The significance of Racomitrium lanuginosum remains. *Journal of Ecology* 83(6): 1021–1030.

Thompson LG, Mosley-Thompson E, Davis ME et al. (2002) Kilimanjaro ice core records: Evidence of Holocene climate change in tropical Africa. *Science* 298: 589–593.

Troels-Smith J (1955) Characterisation of unconsolidated sediments. *Danmarks Geologiske Undersøgelse Series* 4(3): 39–73.

Turner TE, Swindles GT, Charman DJ et al. (2016) Solar cycles or random processes? Evaluating solar variability in Holocene climate records. *Scientific Reports* 6: 23961.

Turner TE, Swindles GT and Roucoux KH (2014) Late Holocene ecohydrological and carbon dynamics of a UK raised bog: Impact of human activity and climate change. *Quaternary Science Reviews* 84: 65–85.

van der Plicht J, Yeloff D, van der Linden M et al. (2013) Dating recent peat accumulation in European ombrotrophic bogs. *Radiocarbon* 55(3): 1763–1778.

van Geel B (1978) A palaeoecological study of Holocene peat bog sections in Germany and the Netherlands, based on the analysis of pollen, spores and macro- and microscopic remains of fungi, algae, cormophytes and animals. *Review of Palaeobotany and Palynology* 25(1): 1–120.

van Geel B and Renssen H (1998) Abrupt climate change around 2,650 BP in North-West Europe: evidence for climatic teleconnections and a tentative explanation. In: Brown N and Issar AS (eds) *Water, Environment and Society in Times of Climatic Change*. Dordrecht: Kluwer, pp. 21–41.

van Geel B, Buurman J and Waterbolk HT (1996) Archaeological and palaeoecological indications of an abrupt climate change in The Netherlands, and evidence for climatalogical teleconnections around 2650 BP. *Journal of Quaternary Science* 11: 451–460.

Winkler S (2000) The 'Little Ice Age' 'maximum in the southern Alps, New Zealand: Preliminary results at Mueller Glacier. *The Holocene* 10(5): 643–647.

Xie S, Nott CJ, Avsejs LA et al. (2004) Molecular and isotopic stratigraphy in an ombrotrophic mire for paleoclimate reconstruction. *Geochimica et Cosmochimica Acta* 68(13): 2849–2862.

Yan H, Wei W, Soon W et al. (2015) Dynamics of the intertropical convergence zone over the western Pacific during the Little Ice Age. *Nature Geoscience* 8(4): 315–320.

Yeloff D and Mauquoy D (2006) The influence of vegetation composition on peat humification: Implications for palaeoclimatic studies. *Boreas* 35(4): 662–673.

Zaborska A, Carroll J, Papucci C et al. (2007) Intercomparison of alpha and gamma spectroscopy techniques used in ^{210}Pb geochronology. *Journal of Environmental Radioactivity* 93: 38–50.

Community Ecology (2021) 22:319–330
https://doi.org/10.1007/s42974-021-00057-4

ORIGINAL ARTICLE

Vegetation community changes in European woodlands amid a changing climate: a palaeoecological modelling perspective

Julia C. Webb[1] · **Anne E. Goodenough**[1]

Received: 29 March 2021 / Accepted: 5 September 2021 / Published online: 22 September 2021

Abstract

Climate has an important role in shaping the composition and structure of woodlands. There is considerable uncertainty regarding how woodlands will respond to climate change over the next hundred years. To better understand likely responses to contemporary climate change, this paper analyses taxonomic richness, evenness and community similarity in palaeoecological data from 31 European woodlands during an abrupt cooling and warming event c. 8200 years ago. Repeated measures ANOVAs demonstrated there was no significant overall difference in richness or community evenness over time. A single significant pairwise contrast was found (richness decreased as climate warmed) but was short-lived, indicating that high-level effects were temporary. However, analysis of species turnover measured using community similarity revealed substantial changes (only 24% of species remained at some sites after climatic change), indicating that the actual species within that community had altered. General(ised) linear models showed variation in the direction and magnitude of community change was not related to the broadscale biogeographical variables of latitude, longitude or altitude. Our research has several important implications for practitioners involved in community ecology research and woodland management. Although site-level richness is largely unaffected, we caution that there will be considerable species turnover in woodlands as the climate warms. As species turnover at individual sites will have a negligible effect if driven by localised random processes, we recommend practitioners to consider long-term gamma diversity wherever possible. This shifts focus towards landscape-scale approaches that span generations rather than the typical 3–5-year span of funding, management plans and political cycles.

Keywords 8.2 ka event · Climate change · Ecological stability · Community similarity · Diversity · Species turnover

Introduction

The influence of humans on Earth's vegetation and climate is becoming increasingly profound. Mean global terrestrial air temperatures have been increased by 0.8 °C since 1900 (Hansen et al., 2006), while the twelve hottest years since records began in 1880 have all occurred since 2000 (NASA/GISS, 2021). Climate change projections for Europe suggest a 4–5 °C temperature increase in Mediterranean and Boreal zones, with an increase of 3 °C likely in Central Europe and 2 °C in the UK and Ireland over the next 80 years (Christensen & Christensen, 2007).

Although higher annual temperatures (driven by an increase in atmospheric CO_2) can increase tree growth rates and promote establishment of woodlands (Anderson et al., 2006; Broadmeadow, 2002), shifting climatic envelopes and increasing frequency of extreme weather events associated with climate change can strongly influence woodland plant communities (Broadmeadow et al., 2009; Cavers & Cottrell, 2015; Hoegh-Guldberg et al., 2018; Ray et al., 2010). The effects can be especially profound at latitudinal extremes and high altitude, where the vegetation is vulnerable to environmental disturbance (Breshears et al., 2008).

It is often assumed that the overall impacts of a warming climate on woodland biodiversity will be substantial and, in many cases, negative (Hooper et al., 2012; McGill et al., 2015; Pimm et al., 2014). However, the impacts of contemporary climate change can be surprisingly challenging to identify in established woodlands, particularly as long-lived arboreal species may be relatively tolerant of wide variations in annual weather conditions. This tolerance is evidenced

✉ Julia C. Webb
jwebb@glos.ac.uk

1 Natural and Social Sciences, Francis Close Hall, University of Gloucestershire, Cheltenham, Gloucestershire GL50 4AZ, England, UK

by ancient trees, some of which established 600 years ago (in the case of the Tortworth Chestnut in Gloucestershire) and survived the cold winters of the Little Ice Age (17th Century) and are currently surviving the recent increase in hot summers.

Woodlands, particularly ancient woodlands and ancient semi-natural woodlands, provide an exceptionally valuable habitat for wildlife (Goldberg et al., 2007; Rotherham, 2011) as well as providing a multitude of ecosystem services (Bullock et al., 2014; Quine et al., 2011). Maintaining rich and diverse woodlands is, therefore, important in conservation contexts, with many practitioners increasingly moving from single-species initiatives to landscape-scale approaches that focus on entire ecological communities (Donaldson et al., 2017; Jones, 2011; Lawton et al., 2010). This often involves considering changes in the richness (number of species), evenness (uniformity of species within the community) and species assemblages. Underpinning research often focuses on understanding spatial patterns or temporal change, including responses to weather and climate, over the short-term (Rull, 2012). It is important that where community and successional processes span centuries, as is common in woodland communities in response to climate, that longer timescales are used to analyse change so that impacts are fully understood (Rull & Vegas-Vilarrúbia, 2011; Willis & Birks, 2006). Thus, present-day ecological patterns are often best understood, and likely future predictions most robust, when consideration of long-term change is undertaken using palaeoecological evidence (Davies and Bunting, 2010; Rull, 2010; Chambers et al., 2017).

Palaeoecological studies quantifying long-term change throughout the Holocene epoch (last c. 11,700 years) demonstrate that, following the termination of the last ice advances, the climate has been relatively stable (Joannin et al., 2012). Climax vegetation communities became established across Europe during the Early Holocene period, following ecological successional processes that occurred without anthropogenic interference, which resulted in a relatively open mosaic wood-pasture landscape (Vera, 2000; Whitehouse & Smith, 2004, 2010). An abrupt climate event occurred 8200 calendar years ago (known as the 8.2 ka event: Fig. 1) broadly characterised by a sudden cooling and warming event culminating in a 1–3 °C air temperature change over the Northern Hemisphere (Matero et al., 2017) (although there is evidence from oceanic records that the pattern might be rather more complex than this: Wiersma and Jongma

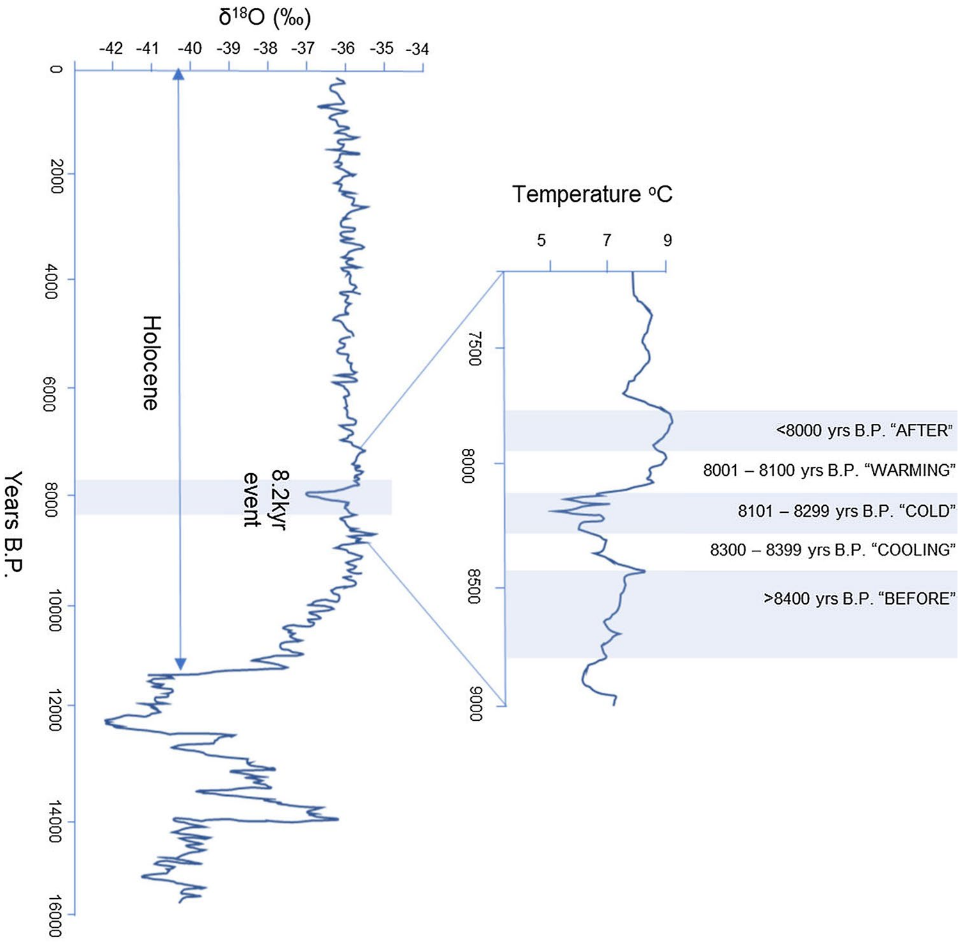


Fig. 1 Left: Holocene 50-year moving average oxygen isotope curve (after: Kobashi et al., 2007) from GISP2 Greenland ice core commonly used to infer temperature in the Northern Hemisphere. Note the relative stability during the Holocene and the abrupt 8.2 ka event. Right: The pollen inferred annual mean temperature record in Europe (after: Veski et al., 2004) including time periods used in this study

(2010)). The 8.2 ka event occurred at a time when the vegetation responses would have been largely uninfluenced by anthropogenic activity (Collins et al., 2012; Olsson et al., 2010). It was widely observed across Europe and although sample resolution and dating accuracy can complicate precise temporal relationships, the event is regarded as synchronous across the continent (Alley et al., 1997). However, there were differences in the climate changes across Europe, with cooler conditions identified in the north compared with cooler and drier conditions in the south (Alley & Ágústsdóttir, 2005; Prasad et al., 2009). Seasonality was also affected with cooler drier winters and longer cooler summers (Prasad et al., 2009). This event, particularly the warming trajectory, provides a unique opportunity to assess the vegetation impacts of past climate changes, which are similar in magnitude to changes forecast over the next 80 years, to further understand the likely responses of woodland vegetation communities to contemporary climate change.

This paper will explore change in woodland vegetation communities using palaeoecological data as a proxy for vegetation change during the 8.2 ka event. We utilise 31 wooded sites from 13 countries across Europe with robustly dated palynological (pollen and spore) records to establish temporal trends in richness, evenness and community similarity over time. We consider both the overall vegetation community and the herbaceous and shrub sub-community, which might be more sensitive to climate change and better able to adapt quickly (shorter-lived, r-strategist species = shorter lag time). We hope that better understanding of widescale changes in vegetation in response to past climatic change will provide useful insights into how woodlands are likely to respond to current and future periods of climatic change.

Method

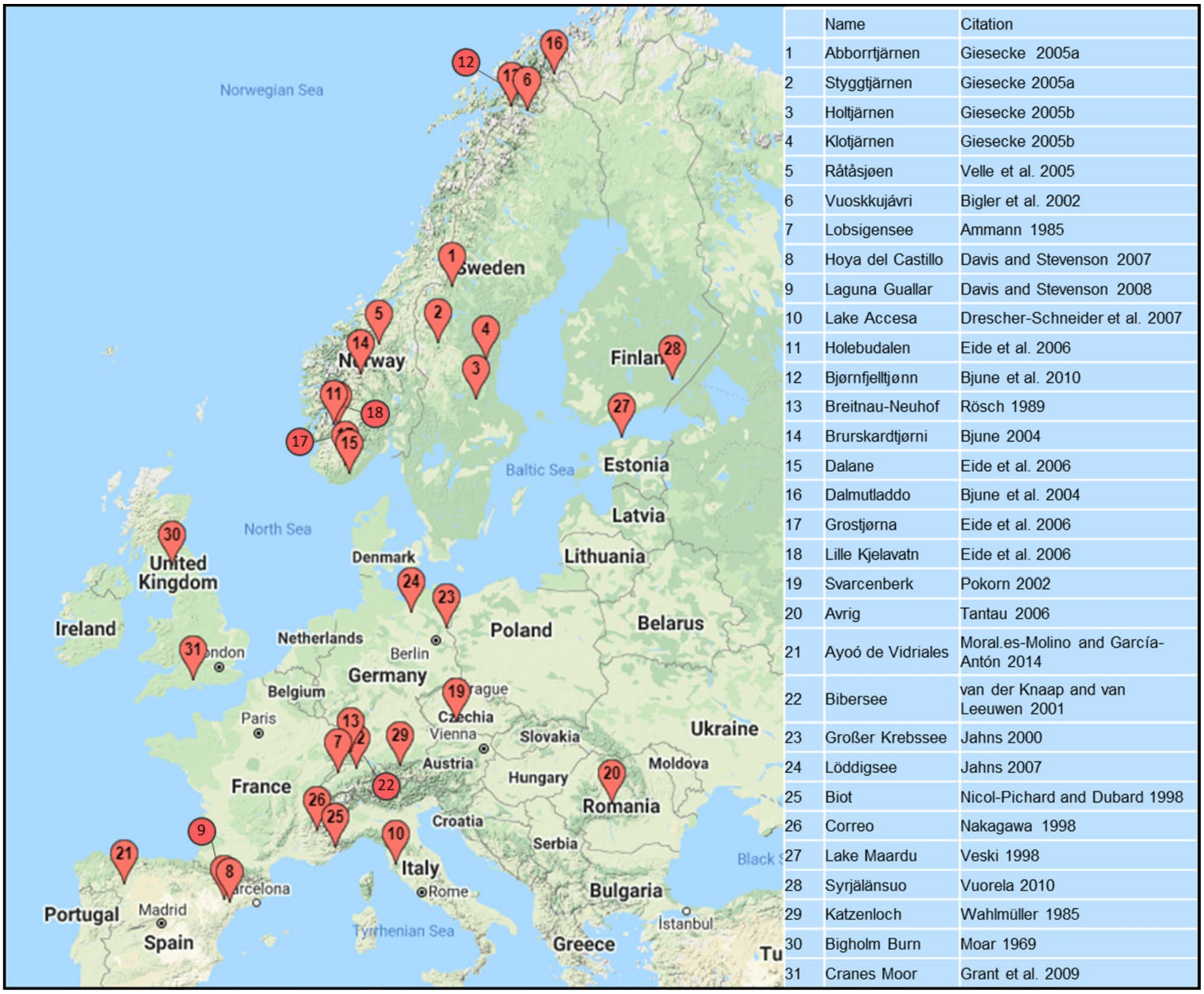
Data acquisition

Initially, the European Pollen Database (EPD) (Fyfe et al., 2009) was mined to extract every site from across Europe that spanned the 8.2 ka event and that was associated with a published output in a peer-reviewed journal. This gave 73 possible sites from 19 countries: this meant that this study adopted a comprehensive approach rather than selecting or choosing particular sites. A rigorous quality control process was then undertaken and only those sites that were considered to be woodland, high-resolution and robustly dated were retained. After this screening step, full palynological data were extracted from the EPD for 31 lacustrine sites across 13 countries (latitude: 41°–69° N; longitude: 6° W–28° E; altitude: 5–1309 m; Fig. 2). Each site had multiple radiocarbon dates underpinning a robust age-depth model to calculate sedimentation rates at that specific site. This information was used to select the appropriate time-sediment horizons and thus the appropriate palynological assemblages. The age-depth models generated by the original authors at the time of publication were used without subsequent recalibration using IntCal20 (the latest calibration curve for terrestrial sites in the Northern Hemisphere) because, as Stuiver et al. (1998) and Reimer (2020) conclude, recalibration in the early Holocene has a negligible effect on the dates generated. Sites with radiocarbon dates taken from bulk samples spanning multiple horizons (i.e. where the sample resolution of radiocarbon dating did not match the sample resolution of palynological analysis) were rejected.

All 31 retained sites had palynological data predating the 8.2 ka event (hereafter termed ‘Before’: > 8400 calibrated years before present), at the peak of the event (‘Cold’: 8101–8299 cal. BP), and postdating the event (‘After’: < 8000 cal. BP); palynological data were also available for the (shorter) transitional periods for some sites (‘Cooling’: 8300–8399 cal. BP, $n = 22$; ‘Warming’: 8001–8100 cal. BP, $n = 23$).

To ensure that we focused exclusively on sites that, at the time of the 8.2 ka event, were vegetated with closed-canopy woodland and thus likely supporting a mature woodland community (Joannin et al., 2012), we only included sites that had ≥ 51% arboreal pollen in each sample horizon (min = 57.1%; max = 98.8%). The rationale was that 51% arboreal pollen was the average for sites supporting ‘relatively open canopy’ in woodlands in West Yorkshire, UK (Bunting, 2002; Tinsley & Smith, 1974), while 50% was reported as the indicative value of woodland presence at or near upland sites (Birks, 1988).

Palynological data were extracted for the time periods of interest using the taxonomic level given by the original author(s). As the taxonomic level to which pollen and spores could be identified varied for different taxa, and at different sites, the overall dataset contained family-level, genus-level and species-level data. This was not problematic because all analysis-related vegetation from one time-sediment horizon to other time-sediment horizons at the same site. Thus, while this study involved multiple sites, change was always quantified site-specifically and the multiple change estimates from each site were further analysed or averaged (see later) rather than raw data from multiple sites being analysed together to produce one overarching vegetation change estimate. Palynological counts for individual sites varied from 229 to 2478 per time period in different studies (mean = 769) but all data analyses used relative or proportional data to account for site-level variation. The total sample size across all time periods and all sites was 105,323 individual data points. Where the original authors included data on bryophytes and ferns, these were excluded, as were data on other non-vascular micro-remains (algae and fungal hyphae), which had not been recorded consistently between studies.



	Name	Citation
1	Abborrtjärnen	Giesecke 2005a
2	Styggtjärnen	Giesecke 2005a
3	Holtjärnen	Giesecke 2005b
4	Klotjärnen	Giesecke 2005b
5	Råtåsjøen	Velle et al. 2005
6	Vuoskkujávri	Bigler et al. 2002
7	Lobsigensee	Ammann 1985
8	Hoya del Castillo	Davis and Stevenson 2007
9	Laguna Guallar	Davis and Stevenson 2008
10	Lake Accesa	Drescher-Schneider et al. 2007
11	Holebudalen	Eide et al. 2006
12	Bjørnfjelltjønn	Bjune et al. 2010
13	Breitnau-Neuhof	Rösch 1989
14	Brurskardtjørni	Bjune 2004
15	Dalane	Eide et al. 2006
16	Dalmutladdo	Bjune et al. 2004
17	Grostjørna	Eide et al. 2006
18	Lille Kjelavatn	Eide et al. 2006
19	Svarcenberk	Pokorn 2002
20	Avrig	Tantau 2006
21	Ayoó de Vidriales	Moral.es-Molino and García-Antón 2014
22	Bibersee	van der Knaap and van Leeuwen 2001
23	Großer Krebssee	Jahns 2000
24	Löddigsee	Jahns 2007
25	Biot	Nicol-Pichard and Dubard 1998
26	Correo	Nakagawa 1998
27	Lake Maardu	Veski 1998
28	Syrjälänsuo	Vuorela 2010
29	Katzenloch	Wahlmüller 1985
30	Bigholm Burn	Moar 1969
31	Cranes Moor	Grant et al. 2009

Fig. 2 European sites selected and included in this study. All palynological data were collated from the European Pollen Database (Fyfe et al., 2009). Original publications for each site are referenced within this figure

Data manipulation

From the palynological data, three metrics were calculated. Taxonomic richness was calculated for each site for each time period as a simple sum of the taxa found. Community evenness, the extent to which the relative abundance of each taxon was similar (uniform distribution) or dissimilar (skewed distribution with some taxa dominating and other taxa being rare), was also calculated for each time period. As noted by Legendre and Legendre (1998) and Peros and Gajewsk (2008), evenness is an important metric in community ecology, but one that remains under-utilised in palaeoecological research. There are numerous evenness indices but here Shannon's evenness (E_{H}) was adopted as this has been used previously for palynological data by Hellman et al. (2009). This was calculated using Shannon's diversity index (H) (Eq. 1) and dividing this value by the natural logarithm (ln) of the taxonomic richness (Eq. 2):

$$\mathrm{H} = -\sum_{i=1}^{s} pi \ln pi \tag{1}$$

$$E_{\mathrm{H}} = \frac{\mathrm{H}}{\ln S} \tag{2}$$

where S is the total number of species (taxa) in the community and pi is the proportion of species i (Shannon, 1948). The resultant metric runs between 0 and 1, with 1 indicating complete evenness (i.e. all taxa uniformly abundant).

The third metric was Jaccard's coefficient of community similarity ($\mathrm{CC_j}$), which is a pairwise value used to summarise the overlap in the actual species within a community between two different sites or, as here, between two different time periods at the same site. This was calculated using Eq. 3.

$$\mathrm{CC}_j = \frac{c}{S} \quad (3)$$

where c is the number of species common to both communities and S is the total number of species found in both communities. The index runs between 0 and 1, with 1 indicating a complete overlap in species. CC_j was calculated pairwise to compare community similarity between the time periods: (1) After *versus* Before; (2) After *versus* Cold; (3) Cold *versus* Before. These time periods were chosen because palynological data were available for each of these periods at all sites. This approach has been used previously to assess homogenisation of fish communities over time (Rahel, 2000).

In all cases, to allow lag effects in the vegetation community to be assessed (i.e. that a change in climatic conditions might take some time to be reflected, especially for arboreal taxa), metrics were calculated both for the overall vegetation community and the herbaceous and shrub sub-community.

Data analysis

To establish whether there were significant differences in taxonomic richness and evenness between the different time periods, a repeated measures ANOVA framework was used. This meant that differences were calculated based on a per-site basis to allow for repeated sampling of the same site (and thus avoid pseudoreplication in analyses). This also accounted for the fact that sites involved different researchers, different sample sizes and data reported at different taxonomic levels. Four repeated measures ANOVAs were calculated: (1) richness for overall community; (2) richness for herbaceous and shrub sub-community; (3) evenness for overall community; and (4) evenness for herbaceous and shrub sub-community. The Greenhouse–Geisser method was used to compensate for a lack of sphericity in all analyses. Post hoc testing was used for pairwise comparisons with a Bonferroni correction applied to allow for multiple comparisons.

To understand any relationship between species turnover, as measured by CC_j, and species richness, we graphically compared CC_j between time periods with richness. Then, to identify whether temporal change in richness and evenness between time periods was related to the spatial variables of latitude, longitude and altitude, General Linear Models were used. Change over time was calculated for both richness and evenness metrics for: (1) After *versus* Before; (2) After *versus* Cold; and (3) Cold *versus* Before. This was undertaken for the overall community and the herbaceous and shrub sub-community, which gave 12 new dependent variables, six describing change in richness and six describing change in evenness. In all cases, change was a simple calculation of the later time period relative to the earlier time period, such that the resultant figure encompassed both the direction of change (positive for an increase, negative for a decrease) and the magnitude of change (deviation from zero). Although each change variable was the mathematical difference between two count variables (richness) or the mathematical difference between two proportional variables (evenness), each of the 12 new variables conformed to a normal distribution, centred on zero, and were thus deemed suitable for analysis within a simple linear framework: residuals were also assessed and found to be homoscedastic and normally distributed in all cases.

To establish whether temporal change in CC_j between time periods was related to the spatial variables of latitude, longitude and altitude, as well as the latitude*altitude interaction term, generalised linear models were performed with a binomial distribution and a logit link function to allow for the fact that CC_j values were effectively proportional data. Six analyses were undertaken in total to model firstly the overall community and secondly the herbaceous and shrub sub-community for: (1) After *versus* Before; (2) After *versus* Cold; and (3) Cold *versus* Before.

Results

For all sites combined, there were 48 arboreal taxa including coniferous trees (e.g. *Abies*, *Picea* and *Pinus*) and deciduous trees (e.g. *Acer, Alnus*, *Betula*, *Fagus, Ilex, Quercus and Salix*). There were also 183 non-arboreal (herbaceous and shrub) taxa spanning flowering plants (including *Artemisia, Calluna, Filipendula, Potentilla, Plantago* and *Rumex*), grasses and cereals (Poaceae and Cerealia), rushes (*Juncus*), sedges (*Carex*), reeds (*Phragmites australis*) and horsetails (*Equisetum*). In total, the combined dataset comprised 231 taxa.

There was no significant overall difference in vegetation richness or evenness over time, either for the overall vegetation community or for the herbaceous and shrub sub-community (repeated measures ANOVA results: Table 1). However, post hoc testing revealed some significant pairwise comparisons between specific time periods. There was a statistically significant decrease in overall richness as the climate warmed between the peak of the 8.2 ka event (Cold mean = 25 taxa; Warming mean = 22 taxa; $P = 0.015$; Fig. 3a), which was driven by this pattern occurring in the herbaceous and shrub sub-community (Cold mean = 15 taxa; Warming mean = 12 taxa; $P = 0.010$; Fig. 3b). For evenness, there was variability between time periods, especially for the herbaceous and shrub sub-community (Fig. 3d), but there were no significant pairwise comparisons.

Although the repeated measures ANOVA indicated that there were no overall significant changes in community richness and evenness, and only minimal short-term changes between Cold and Warming time periods for richness, these analyses do not allow for changes in actual vegetation assemblages due to species turnover. Jaccard's analysis demonstrated that there were substantial changes in communities at the same site over time. When assessing the overall vegetation community, the similarity before the 8.2 ka event and the peak of that event (Before *versus* Cold) ranged between 0.44 and 0.72 (mean = 0.60), while the similarity after the 8.2 ka event compared to the peak of that event (After *versus* Cold) ranged between 0.40 and 0.76 (mean = 0.60) (Fig. 4). A similar but more extreme pattern was seen when comparing the herbaceous and shrub sub-community alone to assess turnover in field and herb layers rather than the overall

Table 1 Repeated measures ANOVA results for taxonomic richness and community evenness comparing five different time phases relative to the 8.2 ka climate event (Before, Cooling, Cold, Warming, After) at 31 sites across Europe [F = Fisher (the ANOVA test statistic), d.f. = degrees of freedom, p = probability]

	Richness			Evenness		
	F	d.f.	*P*	*F*	d.f.	*P*
Overall vegetation community	0.789	1.848	0.455	1.551	2.424	0.223
Herbaceous/shrub sub-community	1.012	1.790	0.369	1.391	1.740	0.265

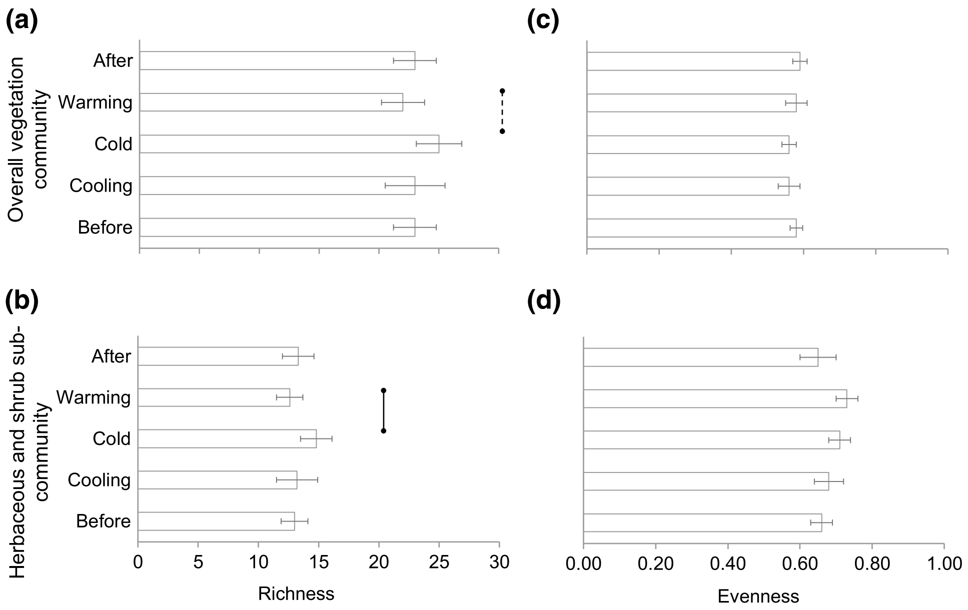


Fig. 3 Vegetation community richness and evenness based on analysis of palynological data from different time periods for: (**a–c**) overall community; and (**b–d**) herbaceous and shrub sub-community. Significant contrasts (post hoc testing with Bonferroni correction after repeated measured ANOVA) are shown, with dashed line indicating $P < 0.05$ and solid line indicating $P < 0.01$

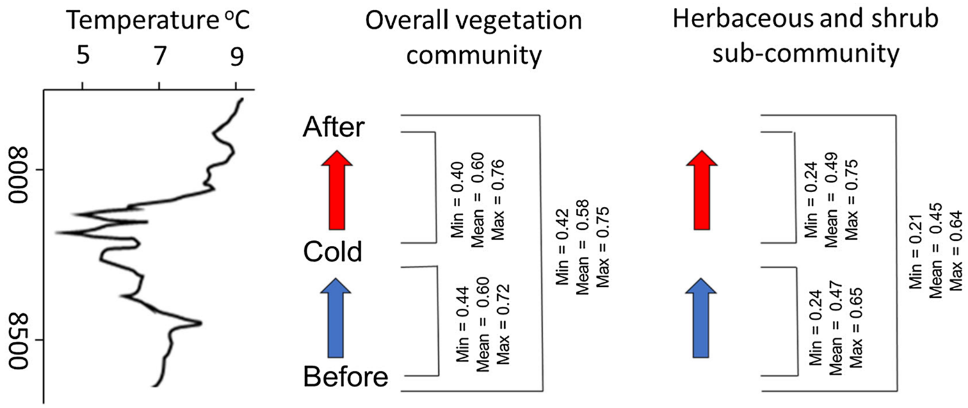


Fig. 4 Jaccard's coefficient of community similarity (CC_j) contrasts to quantify change in vegetation communities due to species turnover at each site (0 = no taxa in common; 1 = all taxa in common). Blue arrows signify cooling; red arrows signify warming. Left: The annual mean temperature curve for the same time periods (after: Veski et al., 2004)

vegetation community: mean similarity dropped to 0.24 for both Before *versus* Cold and After *versus* Cold (Fig. 4).

There was no relationship between CC_j in different time periods and species richness in the earlier time period, suggesting that resilience to climate change in the extent of species turnover was not influenced by species richness (Fig. 5). There was also no clustering of sites in geographical regions, suggesting a lack of broadscale spatial patterns.

The 12 General Linear Models undertaken to identify whether temporal changes in richness and evenness between time periods were related to spatial parameters (latitude, longitude and altitude) were all non-significant (model $P \geq 0.133$; individual spatial variables $P \geq 0.129$) and the amount of variance explained by all models was negligible (adjusted $r^2 \leq 0.094$). The six generalised linear models undertaken to relate changes in vegetation community similarity (CC_j) between time periods in relation to the spatial variables of latitude, longitude and altitude, as well as the latitude*altitude interaction term were also all non-significant (model $P \geq 0.277$; individual spatial variables $P \geq 0.117$) and again the amount of variance explained was negligible (adjusted $r^2 \leq 0.065$). This suggested that while there were site-specific differences in the effect of climatic change on vegetation, this change was not consistently or predictively related to site location at sub-continental scale as also indicated by Fig. 5.

The underpinning dataset with richness, evenness and CC_j for all sites is available on an open-access repository via http://eprints.glos.ac.uk/id/eprint/10077.

Discussion

This paper considers temporal change in vegetation over the 8.2 ka event across Europe at a time when climate changed independently of anthropogenic stressors (Collins et al., 2012; Olsson et al., 2010). We conclude that, overall, there was no significant difference in woodland plant community richness and evenness over a rapid cooling and warming event, demonstrating a surprising resilience to changing climate in these metrics of woodland diversity. Even where there were significant pairwise contrasts found in richness as the climate warmed (a significant decrease between Cold and Warming time periods) these were short-lived, indicating effects were temporary rather than permanent. At this high level, therefore, the frequent assumption that impacts of a warming climate on woodland biodiversity will be substantial and, in many cases, negative (Hooper et al., 2012; McGill et al., 2015; Pimm et al., 2014) is not supported.

This high-level resilience is likely partly due to the long-lived nature of arboreal species relative to the time over which change occurred, coupled with the wide tolerance

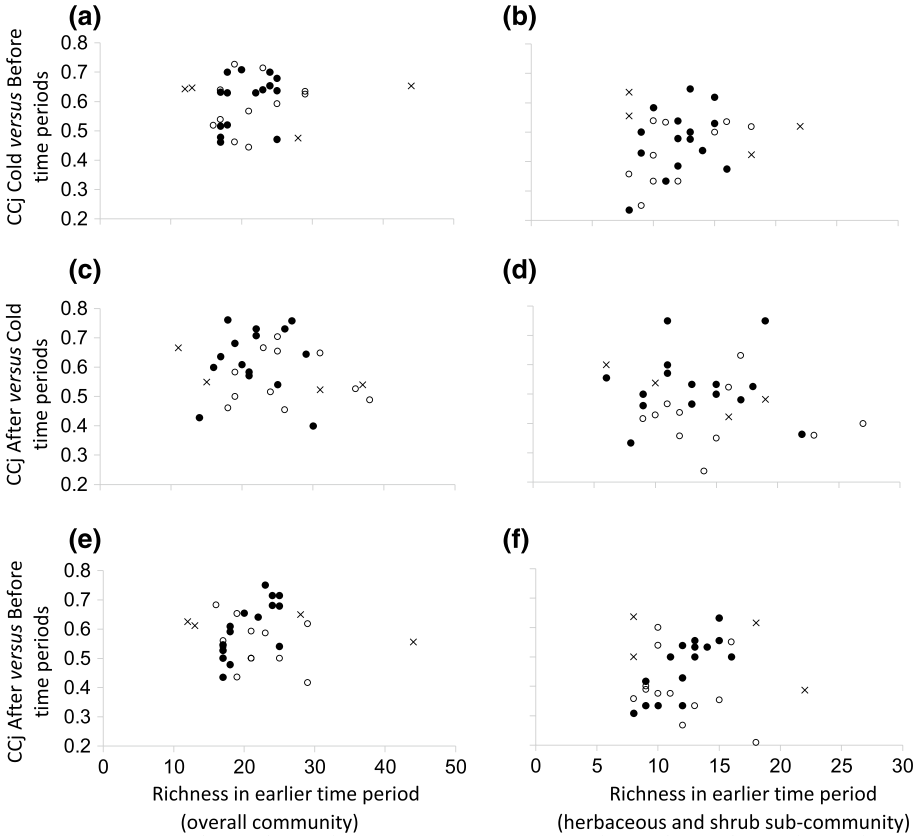


Fig. 5 CC_j at each site in different time periods relative to species richness at that site in the earlier time period for: (**a**–**c**) overall vegetation community and (**d**–**f**) herbaceous and shrub sub-community alone. Sites in Scandinavia are shown using black circles; sites in mainland Europe and the UK are shown using open circles; sites in the Mediterranean are shown using crosses. There is considerable variation but no overall correlation; there is no obvious clustering based on geographical location

ranges of many *K*-strategist species within mature climax ecosystems (Chaideftou et al., 2012; Rull & Vegas-Vilarrúbia, 2011). However, quantifying community similarity using the CC_j metric revealed substantial changes between time periods. This indicates that while the number of species (richness) and the relative abundance of different species within the community (evenness) might not have changed, the actual species within that community had altered. There was an overall mean similarity of 0.59 (59%) across all sites and all time periods when considering the overall vegetation community, which dropped as low as 0.40 (40%) at some sites. When the herbaceous and shrub sub-community was analysed alone, the overall mean similarity was 0.47 (47%) with values as low as 0.21 (21%) being seen at some sites. In other words, species turnover was always considerable, and, in some cases profound especially within the herbaceous and shrub sub-community. This suggests that resilience to climate change was considerably lower when considering species turnover rather than simple diversity metrics, a process discussed by Ewel and Bigelow (1996) as climate-induced ecological recombination. It should also be noted that the level of turnover identified here might be underestimated given that much of the palaeoecological data were restricted to genus or family level (because of the challenges in identifying fossilised pollen to species level), and thus some of the more subtle vegetation changes might not have been detectable.

Interestingly, there was no link between CC_j scores and initial species richness. This is contrary to the diversity-stability concept, which states that higher richness is associated with higher ecological resilience and stability. This has been a central tenet of community ecology since this was originally postulated by Darwin (1859) and extended by MacArthur (1955) and McNaughton (1977). There is considerable experimental evidence for such a link, at least over short timescales, in communities experiencing climate stress or climate change (e.g. Frank & McNaughton, 1991; Tilman et al., 1996). However, this pattern is neither universal nor inevitable (Ives & Carpenter, 2007). For example, Vogel et al. (2012) demonstrated that a positive relationship between richness and stability in grassland communities during climate perturbations only occurred in ecosystems that were highly managed, not those that were dominated by natural processes and interactions, while Isbell et al. (2015) suggest that species richness may be more important for stability under moderate rather than extreme environmental change. Moreover, there is increasing evidence that the link between richness and community-level stability is fundamentally influenced by the actual species present, such that the degree of stability in a community depends idiosyncratically on community composition and biotic interactions (Lawton, 1994; De Boeck et al., 2017), and thus ecological history (Peterson et al., 1998). This also links to the concept of functional diversity: even a species-poor community might be highly resilient to species turnover and ecological tipping point thresholds if each species has a different functional role, such that all ecosystem links are fully operational, albeit with little redundancy (Cadotte et al., 2011; Ives et al., 2000). This has been demonstrated empirically for Argentinian plant communities (Fonseca and Granade, 2001) and simple plant communities compared to tropical rainforests in Costa Rica (Ewel et al., 1991).

At the geographical scale of the present study, spatial analysis showed that the direction and magnitude of vegetation change was unrelated to broadscale spatial variables of latitude, longitude and altitude for richness, evenness and (despite considerable variation between sites) for community similarity too. This suggests that although woodland communities and ecological processes might differ spatially in predictable ways, including latitudinal diversity gradients (Lamanna et al., 2014), and global patterns in plant traits, morphology and productivity (Madani et al., 2018), there is no predictable biogeographical pattern in vegetation response to climate change. This likely reflects community resilience to change being driven by local factors such as woodland size, geology and soil type, topography and exposure, and local weather patterns (especially precipitation). In particular, it is notable that species richness (and in many cases functional diversity and thus stability) increases with woodland area, as does genetic diversity and thus potentially species' adaptability. Indeed, McCarthy et al. (2005) state that 'the mean time to extinction of a single population increases as a power of the habitat area'. Moreover, larger woodlands experience less disturbance from adjacent land uses and fewer edge effects caused by greater exposure to extremes of light, temperature fluctuations and storm damage (Matlack, 1994; Swallow & Goodenough, 2017). In more connected landscapes, colonisation and recolonisation might be higher because the local pool of species from which to draw is greater (Graae, 2000; Honnay et al., 2002). It is also notable that even the broadscale latitudinal diversity gradient that is so well-known today was much less strong in the past: Silvertown (1985) used palynological evidence to show this trend across Europe was considerably shallower 9000–8000 years ago compared to neo-ecological records.

In addition to local abiotic variables affecting the magnitude of vegetation change, variation in local biotic processes might explain part of the variation in vegetation response in the absence of broadscale geographic patterns. In particular, the age of the woodland at each site will likely have a role, as resilience to change usually increases with woodland maturity (Graae, 2000). This is partly driven by plant species having a long history of co-evolution with species in other taxonomic groups, such that ecological interactions, mutualistic relationships, and feedback mechanisms are well-developed (Hubbell & Foster, 1986). In particular, the locally spatially variable role of herbivores has been shown to have a profound effect on plant species turnover (Chase et al., 2000). Linking back to the concept that the degree of community stability depends on species composition (Lawton, 1994; De Boeck et al., 2017), the ratio of generalist to specialist species would have a notable effect on the resilience to climate change (Pálinkás, 2018). Stochastic processes will also have a variable impact, with chance extirpation of a single species potentially having a minimal effect or triggering substantial cascade effects, especially if keystone species are involved.

Looking forward: predictions, recommendations and caveats

A central aim of this study was to use palaeoecological analysis of woodland vegetation during the 8.2 ka event as a vehicle to improve understanding of likely woodland responses to contemporary climate change. We have deliberately focused on a climate change event of similar magnitude to that predicted over the next 80 years to study vegetation response without this being confounded by anthropogenic processes. This paleoecological approach embraces the framework advocated by Davies and Bunting (2010), Rull (2010), and Chambers et al. (2017). However, it is important to note that the connectivity inherent in the natural wood-pasture mosaic during the 8.2 ka event might have enhanced resilience of woodlands, whereas contemporary climate change is occurring concurrently with other environmental pressures, including habitat loss and fragmentation, impacts of non-native species and pollution. These multiple co-stressors might reduce the resilience of ecological communities to climatic change, and mean that we cannot exclude the possibility that an ecological tipping point (Cairns, 2004) might be reached in the future if emissions, and thus climate change trajectory, are not acted upon. This is especially true given that the warming period in the 8.2 ka event was recovery to temperatures experienced before the sudden cooling (i.e. it was returning to the baseline rather than warming from it), and the fact that contemporary climate changes are involving more extreme weather patterns and changes to seasons that were not necessarily seen in the 8.2 ka analogue (Prasad et al, 2009).

Despite these caveats, we predict that if future vegetation responses largely mirror the past changes described here, individual woodland sites are likely to be relatively unaffected in terms of richness and evenness over the next 80 years. It is notable that richness decreases in response to the rapidly changing climate 8200 years ago were all temporary, before richness recovered. When considering broadscale diversity, this demonstrates the need to resist reacting to short-term change and instead to develop monitoring, management and conservation strategies that span generations rather than the typical 3–5-year span of funding, management plans and political cycles.

We suggest that there will be considerable species turnover in woodlands as the climate warms. This is already being seen to some extent (Guerin et al., 2013; Chaideftou et al., 2012). Our retrospective analysis suggests that, for some sites, this could be profound with magnitude of change being driven not by broadscale geography but by local abiotic factors, ecological interactions, initial species composition, functional diversity and stochastic processes. Given that, at a landscape scale, species turnover at individual sites will have a negligible effect if turnover is driven by localised and random processes rather than being systematic (Breshears et al., 2008), we recommend that consideration of gamma diversity—rather than alpha or beta diversity—is of paramount importance. This shifts the focus from single-species and single-site conservation to landscape-scale approaches (Donaldson et al., 2017; Jones, 2011; Lawton et al., 2010). This also avoids 'overmanaging' individual sites with the aim of preventing or buffering natural change, but rather accepts that natural change occurring at multiple sites across the landscape will alter the elements of the landscape mosaic but landscape-level diversity would be less affected. However, extensive and proactive monitoring should be undertaken to identify species that are being systematically lost from multiple sites (Breshears et al., 2008; Guerin et al., 2013). This might include specialist species with narrow tolerance ranges that are more vulnerable to climate change per se, or species that less able to compete in climate-induced ecological recombination. Such species need to be prioritised for conservation action at both landscape and individual site scales. This accords with the 'management of changing nature' and 'innovation in nature' conservation perspectives of Dempsey (2021) and concurrent movement away from paradigms that prioritise species over process, while also realising that *some* single-species initiatives remain valuable.

References

Alley, R., & Ágústsdóttir, A. M. (2005). The 8k event: Cause and consequences of a major Holocene abrupt climate change. *Quaternary Science Reviews, 24*, 1123–1149. https://doi.org/10.1016/j.quascirev.2004.12.004

Alley, R. B., Mayewski, P. A., Sowers, T., Stuiver, M., Taylor, K. C., & Clark, P. U. (1997). Holocene climatic instability: A prominent, widespread event 8200 yr ago. *Geology, 25*(6), 483. https://doi.org/10.1130/0091-7613(1997)025%3C0483:HCIAPW%3E2.3.CO;2

Ammann, B. (1985). Introduction and palynology: Vegetational history and core correlation at Lobsigensee (Swiss Plateau). *In*: Lobsigensee - Late-Glacial and Holocene environments of a lake on the central Swiss Plateau. Dissertationes Botanicae, 87:127–170.

Anderson, K. J., Allen, A. P., Gillooly, J. F., & Brown, J. H. (2006). Temperature-dependence of biomass accumulation rates during secondary succession. *Ecological Letters, 9*, 673–682. https://doi.org/10.1111/j.1461-0248.2006.00914.x

Bigler, C., Larocque, I., Peglar, S. M., Birks, H. J. B., & Hall, R. I. (2002). Quantitative multiproxy assessment of long-term patterns of Holocene environmental change from a small lake near Abisko, northern Sweden. *The Holocene, 12*, 481–496.

Birks, H. J. B. (1988). Long-term ecological change in the British uplands. In M. B. Usher & D. B. A. Thompson (Eds.), *Ecological change in the uplands* (pp. 37–53). Blackwell Scientific Publications.

Bjune, A. E. (2004). Holocene vegetation history and tree-line changes on a north-south transect crossing major climate gradients in southern Norway: Evidence from pollen and plant macrofossils in lake sediment. *Review of Paleobotany and Palynology, 133*, 249–275. https://doi.org/10.1016/j.revpalbo.2004.10.005

Bjune, A. E., Birks, H. J. B., Peglar, S. M., & Odland, A. (2010). Developing a modern pollen-climate calibration data set for Norway. *Boreas, 39*(4), 674–688. https://doi.org/10.1111/j.1502-3885.2010.00158.x

Bjune, A. E., Birks, H. J. B., & Seppä, H. (2004). Holocene vegetation and climate history on a continental-oceanic transect in northern Fennoscandia based on pollen and plant macrofossils. *Boreas, 33*, 211–223. https://doi.org/10.1111/j.1502-3885.2004.tb01142.x

Breshears, D. D., Huxman, T. E., Adams, H. D., Zou, C. B., & Davison, J. E. (2008). Vegetation synchronously leans upslope as climate warms. *PNAS, 105*(33), 11591–11592. https://doi.org/10.1073/pnas.0806579105

Broadmeadow, M. (2002). Climate Change: Impacts on UK forests. *Forestry Commission Bulletin, 125*, 1–198.

Broadmeadow, M. S. J., Webber, J. F., Ray, D., & Berry, P. M. (2009). An assessment of likely future impacts of climate change on UK forests. In D. J. Read, P. H. Freer-Smith, J. I. L. Morison, N. Hanley, C. C. West, & P. Snowdon (Eds.), *Combating climate change - a role for UK forests. An assessment of the potential of the UK's trees and woodlands to mitigate and adapt to climate change.* The Stationery Office.

Bullock, C., Hawe, J., & Little, D. (2014). Realising the ecosystem-service value of native woodland in Ireland. *New Zealand Journal of Forestry Science, 44*(S1), S4. https://doi.org/10.1186/1179-5395-44-S1-S4

Bunting, M. J. (2002). Detecting woodland remnants in cultural landscapes: modern pollen deposition around small woodlands in northwest Scotland. *The Holocene, 12*(3), 291–301.

Cadotte, M. W., Carscadden, K., & Mirotchnick, N. (2011). Beyond species: Functional diversity and the maintenance of ecological processes and services. *Journal of Applied Ecology, 48*, 1079–1087. https://doi.org/10.1111/j.1365-2664.2011.02048.x

Cairns, J., Jr. (2004). Ecological tipping points: A major challenge for experimental sciences. *Asian Journal of Experimental Sciences, 18*(1), 1–16.

Cavers, S., & Cottrell, J. E. (2015). The basis of resilience in forest tree species and its use in adaptive forest management in Britain. *Forestry: an International Journal of Forest Research, 88*(1), 13–26.

Chaideftou, E., Kallimanis, A. S., Bergmeier, E., & Dimopoulos, P. (2012). How does plant species composition change from year to year? A case study from the herbaceous layer of a submediterranean oak woodland. *Community Ecology, 13*(1), 88–96. https://doi.org/10.1556/ComEc.13.2012.1.11

Chambers, F., Crowle, A., Daniell, J., Mauquoy, D., McCarroll, J., Sanderson, N., et al. (2017). Ascertaining the nature and timing of mire degradation: Using palaeoecology to assist future conservation management in Northern England. *AIMS Environmental Science, 4*(1), 54–82. https://doi.org/10.3934/environsci.2017.1.54

Chase, J. M., Leibold, M. A., Downing, A. L., & Shurin, J. B. (2000). The effects of productivity, herbivory, and plant species turnover in grassland food webs. *Ecology, 81*, 2485–2497. https://doi.org/10.1890/0012-9658(2000)081[2485:TEOPHA]2.0.CO;2

Christensen, J. H., & Christensen, O. B. (2007). A summary of the PRUDENCE model projections of changes in European climate by the end of this century. *Climatic Change, 81*, 7–30. https://doi.org/10.1007/s10584-006-9210-7

Collins, P. M., Davis, B. A. S., & Kaplan, J. O. (2012). The mid-Holocene vegetation of the Mediterranean region and southern Europe, and comparison with the present day. *Journal of Biogeography, 39*, 1848–1861. https://doi.org/10.1111/j.1365-2699.2012.02738.x

Darwin, C. (1859). *On the origin of species by means of natural selection or the preservation of favoured races in the struggle for life [reprinted 1964].* Harvard University.

Davies, A. L., & Bunting, M. J. (2010). Applications of palaeoecology in conservation. *The Open Ecology Journal, 3*(1).

Davis, B. A. S., & Stevenson, A. C. (2007). The 8.2 ky event and early-mid Holocene forests, fires and flooding in the Central Ebro Desert, NE Spain. *Quaternary Science Reviews, 26*, 1695–1712. https://doi.org/10.1016/j.quascirev.2007.04.007

De Boek, H. J., Bloor, J. M. G., Kreyling, J., Ransijn, J. C. G., Nijs, I., Jentsch, A., et al. (2017). Patterns and drivers of biodiversity–stability relationships under climate extremes. *Journal of Ecology, 106*(3), 890–902. https://doi.org/10.1111/1365-2745.12897

Dempsey, B. (2021). Understanding conflicting views in conservation: An analysis of England. *Land Use Policy*. https://doi.org/10.1016/j.landusepol.2021.105362

Donaldson, L., Wilson, R. J., & Maclean, I. M. (2017). Old concepts, new challenges: Adapting landscape-scale conservation to the twenty-first century. *Biodiversity and Conservation, 26*(3), 527–552. https://doi.org/10.1007/s10531-016-1257-9

Drescher-Schneider, R. E., de Beaulieu, J. L., Magny, M., Walter-Simonnet, A. V., Bossuet, G., Millet, L., et al. (2007). Vegetation history, climate and human impact over the last 15,000 years at Lago dell'Accesa (Tuscany, Central Italy). *Vegetation History and Archaeobotany, 16*, 279–299. https://doi.org/10.1007/s00334-006-0089-z

Eide, W., Birks, H. H., Bigelow, N. H., Peglar, S. M., & Birks, H. J. B. (2006). Holocene forest development along the Setesdal valley, southern Norway, reconstructed from macrofossil and pollen evidence. *Vegetation History and Archaeobotany, 15*, 65–85. https://doi.org/10.1007/s00334-005-0025-7

Ewel, J. J., & Bigelow, S. W. (1996). Plant life-forms and tropical ecosystem functioning. In G. H. Orians, R. Dirzo, & J. H. Cushman (Eds.), *Biodiversity and ecosystem processes in tropical forests. Ecological studies (Analysis and Synthesis).* (Vol. 122). Springer, Berlin.

Ewel, J., Mazzarino, M. J., & Berishi, C. W. (1991). Tropical soil fertility changes under monocultures and successional communities of different structure. *Ecological Applications, 1*, 289–302. https://doi.org/10.2307/1941758

Fonseca, C. R., & Ganade, G. (2001). Species functional redundancy, random extinctions and the stability of ecosystems. *Journal of Ecology, 89*, 118–125. https://doi.org/10.1046/j.1365-2745.2001.00528.x

Frank, D. A., & McNaughton, S. J. (1991). Stability increases with diversity in plant communities: Empirical evidence from the 1988 Yellowstone drought. *Oikos, 62*, 360–362. https://doi.org/10.2307/3545501

Fyfe, R. M., de Beaulieu, J. L., Binney, H., Brewer, S., Le Flao, A., et al. (2009). The European Pollen Database: Past efforts and current activities. *Vegetation History and Archaeobotany, 18*, 417–424. https://doi.org/10.1007/s00334-009-0215-9

Giesecke, T. (2005a). Holocene forest development in the central Scandes Mountains, Sweden. *Vegetation History and Archaeobotany, 13*, 133–147. https://doi.org/10.1007/s00334-005-0070-2

Giesecke, T. (2005b). Holocene dynamics of the southern boreal forest in Sweden. *The Holocene, 15*, 858–872.

Goldberg, E., Kirby, K., Hall, J., & Latham, J. (2007). The ancient woodland concept as a practical conservation tool in Great Britain. *Journal for Nature Conservation, 15*(2), 109–119. https://doi.org/10.1016/j.jnc.2007.04.001

Graae, B. J. (2000). The effect of landscape fragmentation and forest continuity on forest floor species in two regions of Denmark. *Journal of Vegetation Science, 11*(6), 881–892. https://doi.org/10.2307/3236558

Grant, M. J., Hughes, P. D. M., & Barber, K. E. (2009). Early to mid-Holocene vegetation-fire interactions and responses to climatic change at Cranes Moor, New Forest. In R. M. Briant, M. R. Bates, R. T. Hosfield, & F. F. Wenban-Smith (Eds.), *The quaternary of the Solent Basin and West Sussex raised beaches* (pp. 198–214). Quaternary Research Association.

Guerin, G. R., Biffin, E., & Lowe, A. J. (2013). Spatial modelling of species turnover identifies climate ecotones, climate change tipping points and vulnerable taxonomic groups. *Ecography, 36*(10), 1086–1096. https://doi.org/10.1111/j.1600-0587.2013.00215.x

Hansen, J., Sato, M., Ruedy, R., Lo, K., Lea, D. W., & Medina-Elizade, M. (2006). Global temperature change. *PNAS, 103*, 14288–14293. https://doi.org/10.1073/pnas.0606291103

Hellman, S., Bunting, M. J., & Gaillard, M. J. (2009). Relevant source area of pollen in patchy cultural landscapes and signals of anthropogenic landscape disturbance in the pollen record: A simulation approach. *Review of Palaeobotany and Palynology., 153*(3–4), 245–258. https://doi.org/10.1016/j.revpalbo.2008.08.006

Hoegh-Guldberg, O., Jacob, D., Taylor, M., Bindi, M., Brown, S., Camilloni, I. et al. (2018) Impacts of 1.5ºC global warming on natural and human systems. in Global Warming of 1.5°C: An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. IPCC. Available at: https://www.ipcc.ch/sr15/chapter/chapter-3/

Honnay, O., Verheyen, K., Butaye, J., Jacquemyn, H., Bossuyt, B., & Hermy, M. (2002). Possible effects of habitat fragmentation and climate change on the range of forest plant species. *Ecology Letters, 5*(4), 525–530. https://doi.org/10.1046/j.1461-0248.2002.00346.x

Hooper, D., Adair, E., Cardinale, B., Byrnes, J. E. K., Hungate, B. A., & Matulich, K. L. (2012). A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature, 486*, 105–108.

Hubbell, S. P., & Foster, R. B. (1986). Biology, chance and history and the structure of tropical rain forest tree communities. In J. Diamond & T. J. Case (Eds.), *Community Ecology* (pp. 314–329). Harper and Row.

Isbell, F., Craven, D., Connolly, J., Loreau, M., Schmid, B., Beierkuhnlein, C., et al. (2015). Biodiversity increases the resistance of ecosystem productivity to climate extremes. *Nature, 526*, 574–577. https://doi.org/10.1038/nature15374

Ives, A. R., & Carpenter, S. R. (2007). Stability and diversity of ecosystems. *Science, 317*, 58–62.

Ives, A. R., Klug, J. L., & Gross, K. (2000). Stability and species richness in complex communities. *Ecological Letters, 3*, 399–411. https://doi.org/10.1046/j.1461-0248.2000.00144.x

Jahns, S. (2000). Late-glacial and Holocene woodland dynamics and land-use history of the Lower Oder valley, north-eastern Germany, based on two, AMS 14C-dated, pollen profiles. *Vegetation History and Archaeobotany, 9*, 111–123. https://doi.org/10.1007/BF01300061

Jahns, S. (2007). Palynological investigations into the Late Pleistocene and Holocene history of vegetation and settlement at the Löddigsee, Mecklenburg, Germany. *Vegetation History Archaeobotany, 16*, 157–169. https://doi.org/10.1007/s00334-006-0074-6

Joannin, S., Brugiapaglia, E., de Beaulieu, J.-L., Bernardo, L., Magny, M., Peyron, O., et al. (2012). Pollen-based reconstruction of Holocene vegetation and climate in southern Italy: The case of Lago Trifoglietti. *Climate of the past, 8*, 1973–1996. https://doi.org/10.5194/cp-8-1973-2012

Jones, J. P. (2011). Monitoring species abundance and distribution at the landscape scale. *Journal of Applied Ecology, 48*, 9–13. https://doi.org/10.1111/j.1365-2664.2010.01917.x

van der Knaap, W.O., & van Leeuwen, J.F.N. (2001). Vegetationsgeschichte und menschlicher Einfluss in der Umgebung des Bibersees zwischen 2600 und 50 v. Chr. In: Gnepf Horisberger, U., and Hämmerle, S. (Ed.), Cham- Oberwil, Hof (Kanton Zug - Befunde und Funde aus der Glockenbecherkultur und der Bronzezeit. Antiqua 33 (Veröffentlichung der Schweizerischen Gesellschaft für Ur- und Frühgeschichte) (pp.194–199).

Kobashi, T., Severinghaus, J. P., Brook, E. J., Barnola, J.-M., & Grachev, A. M. (2007). Precise timing and characterization of abrupt climate change 8200 years ago from air trapped in polar ice. *Quaternary Science Reviews, 26*, 9–10. https://doi.org/10.1016/j.quascirev.2007.01.009

Lamanna, C., Blonder, B., Violle, C., Kraft, N. J., Sandel, B., Šímová, I., et al. (2014). Functional trait space and the latitudinal diversity gradient. *PNAS, 111*(38), 13745–13750. https://doi.org/10.1073/pnas.1317722111

Lawton, J. H. (1994). What do species do in ecosystems? *Oikos, 71*, 367–374.

Lawton, J. H., Brotherton, P. N. M., Brown, V. K., Elphick, C., Fitter, A. H., Forshaw, J., et al. (2010). *Making space for nature: A review of England's wildlife sites and ecological network*. Defra.

Legendre, P., & Legendre, L. (1998). *Numerical ecology* (p. 852). Elsevier.

MacArthur, R. H. (1955). Fluctuations of animal populations and a measure of community stability. *Ecology, 36*, 533–536. https://doi.org/10.2307/1929601

Madani, N., Kimball, J. S., Ballantyne, A. P., Affleck, D. L. R., van Bodegom, P. M., Reich, P. B., et al. (2018). Future global productivity will be affected by plant trait response to climate. *Scientific Reports, 8*, 2870. https://doi.org/10.1038/s41598-018-21172-9

Matero, I. S. O., Gregoire, L. J., Ivanovic, R. F., Tindall, J. C., & Haywood, A. M. (2017). The 8.2 ka cooling event caused by Laurentide ice saddle collapse. *Earth and Planetary Science Letters, 473*, 205–214. https://doi.org/10.1016/j.epsl.2017.06.011

Matlack, G. R. (1994). Vegetation dynamics of the forest edge–trends in space and successional time. *Journal of Ecology, 82*, 113–123. https://doi.org/10.2307/2261391

McCarthy, M. A., Thompson, C. J., & Possingham, H. P. (2005). Theory for designing nature reserves for single species. *The American Naturalist, 165*(2), 250–257. https://doi.org/10.1086/427297

McGill, B. J., Dornelas, M., Gotelli, N. J., & Magurran, A. E. (2015). Fifteen forms of biodiversity trend in the Anthropocene. *Trends in Ecology and Evolution, 30*, 2. https://doi.org/10.1016/j.tree.2014.11.006

McNaughton, S. J. (1977). Diversity and stability of ecological communities: A comment on the role of empiricism in ecology. *The American Naturalist, 111*, 515–525. https://doi.org/10.1086/283181

Moar, N. T. (1969). Late Weichselian and Flandrian pollen diagrams from south-west Scotland. *New Phytologist, 68*, 433–467. https://doi.org/10.1111/j.1469-8137.1969.tb06456.x

Morales-Molino, C., & García-Antón, M. (2014). Vegetation and fire history since the last glacial maximum in an inland area of the western Mediterranean Basin (Northern Iberian Plateau, NW Spain). *Quaternary Research, 81*, 63–77. https://doi.org/10.1016/j.yqres.2013.10.010

Nakagawa, T. (1998). Etudes palynologiques dans les Alpes Françaises centrales et méridionales: histoire de la végétation Tardiglaciaire et Holocène [Pollen studies in the central meridional French Alps: Lateglacial and Holocene vegetation history] PhD. Dissertation of Biology and Ecology, University of Marseille III (1998):1-211.

NASA/Goddard Institute for Space Studies (GISS) (2021) Data relating to Global Land-Ocean Temperature Index. Available from: https://data.giss.nasa.gov/gistemp/graphs/graph_data/Global_Mean_Estimates_based_on_Land_and_Ocean_Data/graph.txt Accessed: 04 March 2021

Nicol-Pichard, S., & Dubard, M. (1998). Reconstruction of Late-glacial and Holocene environments in southeast France based on the study of a 66-m long core from Biot, Alpes Maritimes. *Vegetation History and Archaeobotany, 7*, 11–15. https://doi.org/10.1007/BF01393413

Olsson, F., Gaillard, M. J., Lemdahl, G., Greisman, A., Lanos, P., Marguerie, D., et al. (2010). A continuous record of fire covering the last 10,500 calendar years from southern Sweden—the role of climate and human activities. *Palaeogeography Palaeoclimatology Palaeoecology, 291*, 128–141. https://doi.org/10.1016/j.palaeo.2009.07.013

Pálinkás, M., (2018). Ecological responses to climate change at biogeographical boundaries. In Pure and Applied Biogeography (ed Hufnagel L) Croatia: In: Tech, 31–55.

Peros, M. C., & Gajewski, K. (2008). Testing the reliability of pollen-based diversity estimates. *Journal of Paleolimnology, 40*, 357–368. https://doi.org/10.1007/s10933-007-9166-2

Peterson, G., Allen, C. R., & Holling, C. S. (1998). Ecological resilience, biodiversity, and scale. *Ecosystems, 1*, 6–18. https://doi.org/10.1007/s100219900002

Pimm, S., Jenkins, C. N., Brooks, A. R., Gittleman, T. M., Joppa, J. L., Raven, L. N., et al. (2014). The biodiversity of species and their rates of extinction, distribution, and protection. *Science, 344*, 1.

Pokorn, P. (2002). A high-resolution record of Late-Glacial and Early-Holocene climatic and environmental change in the Czech Republic. *Quaternary International, 91*, 101–122. https://doi.org/10.1016/S1040-6182(01)00105-7

Prasad, S., Witt, A., Kienel, U., Dulski, P., Bauer, E., & Yancheva, G. (2009). The 8.2 ka event: Evidence for seasonal differences and the rate of climate change in western Europe. *Global and Planetary Change, 67*(3–4), 218–226.

Quine, C., Cahalan, C., Hester, A., Humphrey, J., Kirby, K., Moffat, A., et al. (2011) Woodlands. UK national ecosystem assessment Cambridge, UK: UNEP-WCMC.

Rahel, F. J. (2000). Homogenization of fish faunas across the United States. *Science, 288*, 854–856. https://doi.org/10.1126/science.288.5467.854

Ray, D., Morison, J. Broadmeadow, M. (2010) Climate change: Impacts and adaptation in England's woodlands England, F.C. (Ed.), p. Research Note FCRN201

Reimer, P. (2020). Composition and consequences of the IntCal20 radiocarbon calibration curve. *Quaternary Research, 96*, 22–27. https://doi.org/10.1017/qua.2020.42

Rösch, M. (1989). Pollenprofil Breitnau-Neuhof: Zum zeitlichen Verlauf der holozanen Vegetationsentwicklung im sudlichen Schwarzwald. *Carolinea, 47*, 15–24.

Rotherham, I. D. (2011). *A landscape history approach to the assessment of ancient woodlands. Woodlands: ecology, management and conservation*. Nova Science Publisher.

Rull, V. (2010). Ecology and palaeoecology, two approaches, one objective. *The Open Ecology Journal, 3*, 1–5. https://doi.org/10.2174/1874213001003020001

Rull, V. (2012). Community ecology: Diversity and dynamics over time. *Community Ecology, 13*, 102–116.

Rull, V., & Vegas-Vilarrúbia, T. (2011). What is long-term in ecology? *Trends in Ecology and Evolution, 26*, 3–4. https://doi.org/10.1016/j.tree.2010.10.002

Shannon, C. E. (1948). A mathematical theory of communication. *The Bell System Technical Journal, 27*(379–423), 623–656. https://doi.org/10.1002/j.1538-7305.1948.tb01338.x

Silvertown, J. (1985). History of a latitudinal diversity gradient: Woody plants in Europe 13,000–1000 years B.P. *Journal of Biogeography, 12*(6), 519–525. https://doi.org/10.2307/2844907

Stuiver, M., Reimer, P. J., & Braziunas, T. F. (1998). High-precision radiocarbon age calibration for terrestrial and marine samples. *Radiocarbon, 40*, 1127–1151.

Swallow, K. A., & Goodenough, A. E. (2017). Double-edged effect? Impact of dual edge proximity on the distribution of ancient woodland indicator plant species in a fragmented habitat. *Community Ecology, 18*(1), 31–36. https://doi.org/10.1556/168.2017.18.1.4

Tantau, I., Reille, M., de Beaulieu, J. L., & Farcas, S. (2006). Late Glacial and Holocene vegetation history in the southern part of Transylvania (Romania): Pollen analysis of two sequences from Avrig. *Journal of Quaternary Science, 21*(1), 49–61. https://doi.org/10.1002/jqs.937

Tilman, D., Wedin, D., & Knops, J. (1996). Productivity and sustainability influenced by biodiversity in grasslands ecosystems. *Nature, 379*, 718–720. https://doi.org/10.1038/379718a0

Tinsley, H. M., & Smith, R. T. (1974). Surface pollen studies across a woodland/heath transition and their application to the interpretation of pollen diagrams. *New Phytologist, 73*, 547–565. https://doi.org/10.1111/j.1469-8137.1974.tb02132.x

Velle, G., Larsen, J., Eide, W., Peglar, S. M., & Birks, H. J. B. (2005). Holocene environmental history and climate of Råtåsj¢en, a low-alpine lake in south-central Norway. *Journal of Paleolimnology, 33*, 129–153. https://doi.org/10.1007/S10933-004-2689-X

Vera, F. W. M. (2000). *Grazing ecology and forest history*. CABI Publishing.

Veski, S. (1998). Vegetation history, human impact and palaeogeography of West Estonia. Pollen analytical studies of lake and bog sediments. Doctoral Dissertation. Striae, 38:1-119.

Veski, S., Seppä, H., & Ojala, A. E. K. (2004). Cold event at 8200 yr B.P. recorded in annually laminated lake sediments in eastern Europe. *Geology, 32*(8), 681–684. https://doi.org/10.1130/G20683.1

Vogel, A., Scherer-Lorenzen, M., & Weigelt, A. (2012). Grassland resistance and resilience after drought depends on management intensity and species richness. *PLoS ONE, 7*, 5. https://doi.org/10.1371/journal.pone.0036992

Vuorela, I. (2010) Pollen profile SYRJALAN, Syrjälänsuo, Finland. European Pollen Database (EPD), PANGAEA

Wahlmüller, N. (1985). Beiträge zur Vegetationsgeschichte Tirols V: Nordtiroler Kalkalpen. Ber. nat.-med. *Verein Innsbruck, 72*, 101–144.

Whitehouse, N. J., & Smith, D. N. (2004). "'Islands'" in Holocene forests: Implications for forest openness, landscape clearance and "'culture-steppe'" species. *Environmental Archaeology, 9*, 203–212. https://doi.org/10.1179/env.2004.9.2.199

Whitehouse, N. J., & Smith, D. (2010). How fragmented was the British Holocene wildwood? Perspectives on the "Vera" grazing debate from the fossil beetle record. *Quaternary Science Reviews, 29*(3–4), 539–553. https://doi.org/10.1016/j.quascirev.2009.10.010

Wiersma, A. P., & Jongma, J. I. (2010). A role for icebergs in the 8.2 ka climate event. *Climate Dynamics, 35*, 535–549. https://doi.org/10.1007/s00382-009-0645-1

Willis, K. J., & Birks, H. J. B. (2006). What is natural? The need for a long-term perspective in biodiversity conservation. *Science, 314*, 1261–1265.

5. Towards contemporary ecology: Lessons from the past

All three papers presented in this chapter are fundamentally ecological papers rather than palaeoecological papers in terms of topic and audience. However, they all take a palaeoecological approach and share the basic concept that ecological research can learn from the past and use palaeoecological data in novel ways to advance ecological understanding, policy and practice. The first paper is a broad review of the many opportunities for using palaeoecological data (not restricted to palynological data) within a contemporary context. Building on this, the second paper uses fossil pollen data and contemporary vegetation data to test the reliability of a commonly-used ecological proxy used in biodiversity audits and site designations, while the third paper uses palaeoecological data to test ecological theory.

Goodenough, A.E. and Webb, J.C. (2022) Learning from the past: opportunities for advancing ecological research and practice using palaeoecological data. *Oecologia:* Online Early*.*

Webb, J.C. and Goodenough, A.E. (2018a) Questioning the reliability of "ancient" woodland indicators: Resilience to interruptions and persistence following deforestation. *Ecological Indicators*, 84, 354-363.

Goodenough, A.E. and Webb, J.C. (submitted) Understanding spatiotemporal variation in taxonomic richness and rate of within-site turnover in vegetation communities across Eurasia over the last 4,000 years. Submitted to *Community Ecology*

5.1 Palaeoecological opportunities

Although palaeoecology is an ecological discipline, as outlined in section 2.2, there are comparatively limited examples of where applied ecologists are reaching back into the past and using palaeoecological approaches, insights and data. Opportunities for further use of palaeoecological data in applied ecological contexts was explored in Goodenough and Webb (2022). Their paper suggests ecologists should look to recent palaeoecology (last 400 years) to add useful additional context to contemporary research and practice, and offers examples to inspire the use of palaeoecology. This includes definitively determining the nativeness of species in a location (for example, *Pinus sylvestris* in Ireland (Roche et al. 2009)); understanding ecological tipping points (Kilgore 1973; Meyer and Safford 2011); assessing hydrological impacts (Bhiry and Filion 1996); and informing rewilding concepts (Jackson and Hobbs 2009; Wingard et al. 2017).

It is notable, however, that while this paper reviews opportunities for the use of palaeoecology in contemporary contexts, the vast majority of their examples are based on single site reconstructions to assist with local questions. As noted in Chapter 3, there is value in these single-site examples in demonstrating the utility of palaeoecology in ecological management for specific sites, but ecologists should also be mindful that palaeoecological data can often be most valuable when multiple sites are used. This precept was used in the other two outputs within this Chapter, as described in section 5.2 for Webb and Goodenough (2018a) and section 5.3 for Goodenough and Webb (submitted).

5.2 Testing a commonly-used ecological proxy: Ancient woodland indicators (AWI)

Indicator species are those in which their presence, prevalence or abundance in a habitat is indicative of the prevailing environmental conditions. For example, aquatic invertebrates to indicate water quality (Paisley et al. 2014) and lichens as air quality indicators (Pinho et al. 2004). In woodlands, disturbance sensitive ground flora are used as indicators of high conservation value ancient (and semi-ancient) woodland. Given the high conservation importance of ancient (undisturbed since 1600 AD in England/Wales) woodland it is essential that there is a robust indicator system to identify such woodlands where historical records are not available. Webb and Goodenough (2018a, presented in this chapter) used recent (400 – 4000 years ago) palaeoecological data to test the resilience and persistence of Ancient Woodland Indicators (AWI) after woodland interruptions. They discovered that both palynologically inferred interrupted woodlands, and clear-felled sites supported extensive AWI communities and so caution the use of the AWI approach in defining woodland status. Surprisingly, given the weight that is given to the AWI system in conservation practice, Webb and Goodenough (2018a) provides the first account of palynology being used to critique the concept of the AWI system across multiple sites. The paper does not attempt to persuade practitioners that there should be a reduction in the number of wooded sites assigned as ancient, nor that the AWI approach is not useful. It is important that practitioners understand the limitations and work towards a more effective approach to allocate woodland status (species weighting; multi-proxy approaches; minimum threshold numbers; reverse indicators or using palynological evidence are suggested). As such, while the paper has to date only a small number of academic citations (see Table 1.1), the research received interest from the Woodland Trust and practitioners within ecological consultancy. The Wood Wise Journal, circulated to Woodland Trust members on a quarterly basis published an article based on the paper that was redrafted for a public audience (Webb and Goodenough 2018b). It also led onto a Woodland Trust Blog, and communications with SLR Consulting Limited, Stafford, UK. Thus, indicating the real impact in applied ecology.

5.3 Testing theory: Diversity patterns

Another example of the utility of fossil pollen in testing ecological theory comes in Goodenough and Webb (Submitted, presented in this chapter). While Webb and Goodenough (2018a) investigate the robustness of theory that is applied in ecological practice, this paper investigates theoretical processes temporally and spatially. Using paleoecologically derived data from 23 Eurasian sites, spatiotemporal change in taxonomic richness and rate of within-site turnover were examined by assessing sequential time horizons. The research found that the widely-recognised theories in community ecology (Latitudinal Diversity Gradient, Altitudinal Diversity Gradient, Diversity Stability Hypothesis) were supported by the fossil data, albeit with complex interactions with time and space. Goodenough and Webb (Submitted) found variation in the strength of the patterns between the overall vegetation community and when the shrub and herb sub-community was assessed in isolation. These variations in strength were not consistent in direction or magnitude between the theoretical patterns. The complex results of the paper highlight the importance of using fossil pollen data, and suggest that more research is needed into understanding the drivers of the patterns reported. This is particularly important given the recent and widespread biodiversity declines within our anthropogenically altered environment.

Both Webb and Goodenough (2018a) and Goodenough and Webb (Submitted) alongside key papers such as Willis et al. (2010) and Birks (2012) demonstrate the importance of testing hypotheses using palaeoecological data. Birks (2012) suggests we are in the honeymoon phase of bringing the two disciplines of ecology and palaeoecology together, and traction must continue in order to answer critical questions in modern ecology. Sutherland et al. (2006, 2009) and Birks (2012) proposed questions of importance in conservation that in 2012 had not been addressed. They highlight in their questions the importance of establishing baseline conditions and understanding natural change, and both Webb and Goodenough (2018a) and Goodenough and Webb (Submitted) have contributed to both the academic and applied fields in this area with their findings. There is much work to do in the next decade to provide answers to Birks (2012) and Sutherland et al.'s (2006, 2009) many questions, but palaeoecological data will play a key role in responding to temporal (particularly in the recent past) perspectives, just as Webb and Goodenough (2018a) and Goodenough and Webb (Submitted) have done.

Oecologia
https://doi.org/10.1007/s00442-022-05190-z

CONCEPTS, REVIEWS AND SYNTHESES

Learning from the past: opportunities for advancing ecological research and practice using palaeoecological data

Anne E. Goodenough[1] · **Julia C. Webb**[1]

Received: 18 October 2021 / Accepted: 17 March 2022

Abstract

Palaeoecology involves analysis of fossil and sub-fossil evidence preserved within sediments to understand past species distributions, habitats and ecosystems. However, while palaeoecological research is sometimes made relevant to contemporary ecology, especially to advance understanding of biogeographical theory or inform habitat-based conservation at specific sites, most ecologists do not routinely incorporate palaeoecological evidence into their work. Thus most cross-discipline links are palaeoecology → ecology rather than ecology → palaeoecology. This is likely due to lack of awareness and/or the misnomer that palaeoecology invariably relates to the "distant past" (thousands of years) rather than being applicable to the "recent past" (last ~ 100–200 years). Here, we highlight opportunities for greater integration of palaeoecology within contemporary ecological research, policy, and practice. We identify situations where palaeoecology has been, or could be, used to (1) quantify recent temporal change (e.g. population dynamics; predator–prey cycles); (2) "rewind" to a particular point in ecological time (e.g. setting restoration/rewilding targets; classifying cryptogenic species); (3) understand current ecological processes that are hard to study real-time (e.g. identifying keystone species; detecting ecological tipping points); (4) complement primary data and historical records to bridge knowledge gaps (e.g. informing reintroductions and bioindicator frameworks); (5) disentangle natural and anthropogenic processes (e.g. climate change); and (6) draw palaeoecological analogues (e.g. impacts of pests). We conclude that the possibilities for better uniting ecology and palaeoecology to form an emerging cross-boundary paradigm are as extensive as they are exciting: we urge ecologists to learn from the past and seek opportunities to extend, improve, and strengthen their work using palaeoecological data.

Keywords Ecological monitoring · Ecological management · Conservation · Spatiotemporal change

Introduction

Palaeoecology is the study of ecological patterns and processes of the past. Palaeoecological studies (and research within the cognate fields of palaeobiology and palaeontology) involve examining fossil and sub-fossil evidence to understand past species distributions, habitats, ecological patterns and ecosystem processes. Such evidence can be preserved within rock, terrestrial sediments (e.g. bog and lacustrine deposits) and marine and estuarine sediments, and can involve many different types of preserved biological material. For plants this includes palynological remains (pollen grains and non-pollen palynomorphs such as spores of ferns) and plant macrofossils such as seeds or preserved vegetative material. For fauna in terrestrial or aquatic environments, evidence includes vertebrate bones, shells from Mollusca and Crustacea, and insect remains (especially of Coleoptera where elytra are often well-preserved). More specialist fossil and sub-fossil evidence includes unicellular testate amoebae and diatoms—both of which can occur within freshwater and marine contexts as well as terrestrial sediments—as well as coral, coralline algae, bryozoans, fungal hyphae/spores, and lichens. It is often possible to date evidence directly (e.g. radiocarbon dating of bones) or indirectly by dating the sediment associated with evidence (e.g. luminescence dating of quartz-rich sediment surrounding preserved pollen). Palaeoecological evidence can also be compared to biogeochemical data to enable inferences to be made about environmental conditions (Roberts 2013; Lowe

---

Communicated by John Loehr.

✉ Anne E. Goodenough
aegoodenough@glos.ac.uk

[1] Natural and Social Sciences, Francis Close Hall, University of Gloucestershire, Cheltenham GL50 4AZ, UK

and Walker 2014). When evidence from several consecutive time periods is pieced together, it can provide insights into temporal transformation of environments.

A classic study demonstrating the utility of palaeoecology to reconstruct past environments was that of Walker (1982), which quantified pollen preserved in terrestrial sediments to identify changing climate after the last ice age in Britain. The 5 m sediment core revealed pollen from pioneer vegetation shortly after the ice retreated 20,000 years ago and the subsequent change in vegetation over thousands of years to a climax community. Similar studies can be undertaken in the marine environment, for example Anderson and Vos (1992) demonstrated how diatoms could be used to study the evolution of the coastline of The Netherlands in relation to changes in sea level caused by natural and anthropogenic processes. More recently, palaeoecology has been employed to study more complex patterns and the use of multiple types of evidence within single studies is now commonplace. Such multi-proxy research can provide a comprehensive understanding of changing climate (e.g. Njagi et al. 2021; Roland et al. 2015), ecological interactions (e.g. Gałka et al. 2018), human occupation and activity (e.g. Kittel et al. 2018; Bishop et al. 2019), or extreme events (e.g. Yao et al. 2021). As the discipline has progressed and methods have advanced, there has been a drive to move away from traditional single-site palaeoecological reconstructions to synthesise data from multiple sites to further understand continental or global-scale ecological patterns (Barber et al. 2003, 2004; Blundell et al. 2008). A good example of this approach is a study by Roland et al. (2015), which documented a major climate event 5200 years ago across multiple sites in Ireland. This study utilised testate amoebae and plant macrofossils, alongside humification analysis and stable isotope analysis, to identify the extent and timing of wetter conditions and revealed the cause to be persistent cyclonic weather patterns across northwest Europe. Such broadscale research can also reveal no-analogue communities. Examples of ecosystems that do not have a modern equivalent in terrestrial environments are reviewed by Jackson and Williams (2004) and Birks (2019), while the work of Novak et al (2013) on the Indonesian coast revealed high biodiversity reef systems in low energy mesophotic environments, which are no longer extant.

Palaeoecology is inherently informed by theory used within contemporary ecology, including use of concepts such as competition, ecological succession patterns, and niche theory to understand and model past dynamics and temporal change (e.g. Birks 2019). It also has an important role in the study of biogeographical patterns and processes, especially linking ecological change to climate. For example, there are single-site studies spanning multiple glacial/interglacial cycles (e.g. temporal change in tree species in the French Massif Central over 430,000 years (de Beaulieu et al. 2006)), cross-continental spatial changes since the end of the last ice age (e.g. range shifts in European flora (Huntley and Birks 1983)) and detailed studies of rapid climate perturbations (e.g. Webb and Goodenough 2021). Understanding of other biogeographical patterns is also informed by palaeoecological investigation, including the Latitudinal Diversity Gradient (e.g. Silvertown 1985; Mannion 2020; Meseguer and Condamine 2020; Song et al. 2020) and the Bergmann and Allen rules of thermal biology for vertebrates (Smith and Betancourt 2003; Boulbes and van Asperen 2019; Davis 2019) and marine protists (Mousing et al. 2017).

To extend reach into more applied ecological contexts, some palaeoecologists have sought to make data and inferences about the past relevant to current challenges, especially in relation to habitat conservation. Such research is often in relation to specific single sites. For example, at Llangorse Lake in South Wales, diatoms, Mollusca, and Crustacea from sediment cores were used alongside sedimentological evidence to demonstrate recent human-accelerated infilling of the lake is ecologically unsustainable (Chambers 1999); Chambers et al. (2007) were also able to utilise palaeoecology to document natural habitat change at upland heath sites in Wales to inform current conservation. However, while some palaeoecological researchers have made considerable effort to make their studies increasingly relevant to contemporary ecological contexts, there are fewer examples of contemporary ecologists routinely reaching back into the past to incorporate palaeoecological evidence in their work. This is despite repeated calls to unite palaeoecology and contemporary ecology (e.g. Kowalewski 2001; Willis and Birks 2006; Willis et al. 2007; Jackson 2007; Davies and Bunting 2010; Rull 2010; Waller et al. 2012; Birks 2019), including specifically for the study of ecosystem change and resilience (Buma et al. 2019), marine protection (Pandolfi 2011), and ecosystem restoration (Jackson and Hobbs 2009; Wingard et al. 2017; Reid et al. 2022). The need to better integrate ecology and palaeoecology is also being recognised in research networks, forums and working groups, such as the US-based Conservation Paleobiology Network and the UK-based Palaeoecology Group within the British Ecological Society.

We suggest that one reason for most links being palaeoecology → ecology rather than being balanced by an equal number of ecology → palaeoecology links might be lack of awareness within the ecological research community of the extensive possibilities for using palaeoecological approaches within current ecological research, as well as applied monitoring, management and conservation initiatives. Possibly more importantly, although ecologists might think that palaeoecological studies invariably relate to the "distant past" (thousands of years ago), actually palaeoecological research can explore change over timescales a short as the last 100–200 years. Examples include studies in terrestrial

New Zealand (Wilmshurst et al. 2013), freshwater ecosystems in the UK (Bennion et al. 2018; Bishop et al. 2019) and marine environments off the west coast of the Iberian Peninsula (Ribeiro et al. 2012) and in the Mediterranean (Agiadi and Albano 2020). These "recent past" timeframes are often vital to understand current patterns and processes in terrestrial and marine environments (Finney et al. 2002; Rull 2010), and palaeoecological approaches are especially valuable where there are incomplete primary survey data or documentary evidence (Davidson et al. 2005; Salgado et al. 2010; Bishop et al. 2019; Seddon et al. 2019). A terrestrial example of this is afforded by Sherriff et al. (2014), who undertook spatially explicit modelling of fire regimes in Colorado over 300 years using both primary survey and palaeoecological data to show that recent fires were larger than those experienced historically and to identify specific high-risk areas outside of historical norms. Pandolfi et al. (2003) took a similar approach in a marine study that combined paleontological, archaeological, historical, and primary ecological data to assess change in coral reefs in relation to human pressures. Where a direct combination of datasets is not possible, use of palaeoecological analogues can sometimes be employed to help understand current ecological changes in ways that have direct implications for ecological policy and practice. For example, Webb and Goodenough (2021) studied biodiversity metrics over a past rapid climate event and showcased how woodland vegetation might respond to contemporary rapid climate change, and related this to the need to focus contemporary monitoring on gamma rather than alpha richness.

In this *Concepts* paper, we review the value of using palaeoecological data in a wide range of contemporary ecological contexts. We are not seeking to provide a fully comprehensive review of any one topic, but rather to highlight—through discussion of themes, questions, and scenarios—the extensive opportunities for greater integration of palaeoecology within ecological disciplines as an emerging cross-boundary paradigm. We discuss examples where palaeoecological data or palaeoecological analogues have been, or could be, used to allow ecologists to better understand the utility of palaeoecology in current ecological research, as well as within in the applied contexts of monitoring, management and conservation. Inevitably there will be some overlap with some previous work—especially Willis and Birks (2006), Willis et al (2007), Davies and Bunting (2010), Rull (2010) and Birks (2019)—but our aim is to build on this important topic and develop new perspectives and insights. We do this in five interconnected ways: (1) writing from an ecological perspective that is accessible to both ecological researchers and practitioners to develop greater ecology → palaeoecology linkages to complement existing palaeoecology → ecology linkages; (2) focusing on applied ecology topics (rather than theoretical ecology or biogeography) that have clear implications for policy and practice; (3) taking a holistic perspective to ensure that studies on a wide range of environments are represented so that, while there may be a slight bias towards terrestrial ecosystems, research using freshwater aquatic and marine palaeoecology is fully embedded; (4) highlighting multiple types of palaeoecological evidence within a single review rather than focusing specifically on palynology; (5) largely, although not exclusively, concentrating on consideration of "recent past" to highlight how palaeoecology can be a useful tool over timespans as recent as the last couple of hundred years. We hope that, by highlighting these possibilities, ecologists will become more aware of opportunities for advancing ecological research and practice using palaeoecological approaches.

Invasion ecology

As noted by Willis and Birks (2006), palaeoecology is inherently concerned with the distribution of past species and thus has obvious relevance to the study of invasion ecology. Although the concept of nativeness—whether a species occurs naturally within a given community—is an intuitive ecological principle, in practice there is often a lack of consensus about the demarcation between "native" and "non-native" (Carlton 1996). The classification of a species depends not only on geographical history, but also how that history fits with a specific date. For example, within Europe, the start of mass trade around the year 1500CE is often used to classify species as either archæophytic (old; native) or neophytic (new; non-native). However, there are many examples of where the history of a species in a particular area is unknown or contentious and these are termed cryptogenic ("secret origin") species. The number of cryptogenic species is, by definition, hard to quantify but a systematic study by Carlton (1996) suggested there were ~ 100 marine cryptogenic species in San Francisco Bay alone.

It is often vital for ecologists to know whether a species is native or non-native: this is not simply an academic debate as there are real implications for legal status and subsequent management (Goodenough 2016). For example, the northern pool frog [*Pelophylax* (formerly *Rana*) *lessonae*] has a cosmopolitan distribution in freshwater aquatic ecosystems across Europe but, until recently, was considered to be an introduced non-native species in the UK. As a result, the species was a candidate for listing under Schedule 9 of the UK's Wildlife and Countryside Act as legally permissible to cull. However, additional research was prompted by historical documents suggesting that *Pelophylax* was already present before the earliest known introductions, and this revealed that the species was, in fact, native (Beebee et al. 2005). Following reclassification, the species moved from being listed as a pest to being considered to be conservation priority: reintroduction and supplementation has now taken place to create self-supporting populations (Buckley and Foster 2005).

In the pool frog example, nativeness was suggested by historical documents, which prompted analysis of museum specimens and genetic profiling, as well as consideration of amphibian bones from radiocarbon dated palaeoecological sites (Gleed-Owen 2000). In many cases though, historical documentation and museum specimens are absent and this is where palaeoecological approaches might be especially helpful. For example, the "non-native" aquatic species water lettuce (*Pistia stratiotes)* in Florida, USA, was shown to be native when palaeoecological records were evaluated (Evans 2013), 800-year-old fossilised pollen in the Galápagos was used to identify which contemporary species are native to the oceanic archipelago (van Leeuwen et al. 2008), and in Ireland palaeoecology was used to resolve a long debate on the nativeness of Scots pine *(Pinus sylvestris)* (Roche 2019). Detailed studies can even reveal complex invasion pathways. This is exemplified by work on the toxic marine phytoplankton species *Gymnodinium catenatum* in the Atlantic Ocean to show spatiotemporal spread along the Iberian Peninsula (Ribeiro et al. 2012) and research on the European eagle owl (*Bubo bubo*), which showed that although the small extant population in the UK is founded upon captive escapees, the palaeoecological record indicates that the species itself is native (Stewart 2007).

It is not just identifying the origins of cryptogenic species where palaeoecology can be useful within invasion ecology research. From an applied perspective, understanding interactions between non-native and native species, and thus wider ecological impacts, is paramount (Goodenough 2010). In some situations, the actual introduction event is known and impacts can be monitored directly from that point. In most cases, however, non-native species become established before any monitoring commences. In such cases, analysing the recent palaeoecological record can be helpful to "rewind" to the initial introduction event and thus study impacts in retrospect. This approach was used on Skomer Island, Wales, to infer the vegetation of the island from pollen records before the introduction of non-native rabbits (*Oryctolagus cuniculus*) (Webb et al. 2017), and in the marine environment to better understand the impacts of the biotic homogenisation in the Mediterranean that occurred after the construction of the Suez Canal in 1869 linked it to the Red Sea (Agiadi and Albano 2020).

Disease ecology

The ability to investigate patterns over many years is useful in the study of ecological pests and diseases where learning lessons from previous disease outbreaks, including vector identification and/or quantifying impacts, can also be helpful. This is especially important as the consequences of pests and diseases are not always immediately apparent (there is a lag time between emergence and impact), but the optimal time to act to prevent damage is at the start of an outbreak, where the chances of eradication or effective suppression are highest (Goodenough and Hart 2017). For example, a widely-recorded decline in elm (*Ulmus*) around 5,500 years ago in northwest Europe and evident in palaeoecological data (review by Parker et al. 2002), was used to inform understanding of the ecological consequences of the more recent Dutch Elm Disease (Flynn and Mitchell 2019). These findings might be useful insights into diseases that are still emerging and increasing in prevalence, such as ash dieback (*Hymenoscyphus fraxineus*). In other situations, the immediate impacts of disease are clear from primary monitoring but the historical context is lacking. For example, starting in the late 1980s, white-band disease nearly eliminated the staghorn coral (*Acropora cervicornis*) from reefs in the coastal waters of Belize, which caused mass species turnover. While this was dramatic, it was not clear whether or not it was atypical until palaeoecological research by Aronson et al. (2002) revealed that staghorn coral had been dominant continuously for the previous 3000 years and no mass turnover had occurred within that timeframe in these uncemented lagoonal reefs. This research highlighted the urgent need for disease alert monitoring in other areas of the Caribbean and reduction of the anthropogenic stressors that facilitate outbreaks of white-band disease.

Community ecology

Ecological communities are intricate networks of co-occurring species that interact with one another and their abiotic environment. Many aspects of community ecology are best studied through direct observational fieldwork or manipulation in the field or laboratory. For example, the effect of herbivores on woodland regeneration can be studied using exclosures (Dauwalter et al. 2018; Bradfield et al. 2021), while lab-based microcosm experiments can be useful for examining species' coexistence (e.g. Luckinbill 1973), competition (e.g. Bonsall and Hassell 1997), and predation (e.g. Morin 1999). However, some aspects of community ecology involve changes over long time periods, or patterns that fluctuate temporally, and it is in these cases that palaeoecology can offer real insights and circumvent the problems inherent in research that uses space-for-time substitution.

Space-for-time (SFT) substitution is an approach to studying longitudinal change, for example in plant succession after glaciation, whereby change is not studied at one fixed location over time but over a spatial gradient, or chronosequence, based on time since deglaciation (Walker et al.

2010). However, SFT has recently been subject to increasing criticism because assumptions are too simplistic for dynamic environments and can lead to inconsistent results (Johnson and Miyanishi 2008; Damgaard 2019). Palaeoecological approaches can be very valuable here, since they allow past changes to be studied directly without SFT assumptions. The approach is currently underutilized (Louys et al. 2021) but examples of where it has been used to good effect can be found in the palaeoecological literature. For example, a detailed study by Payette (1988) demonstrated the complexities in northern Canadian peatland succession, where self-perpetuating vegetation cycles illustrate the dynamic and rapid shifts between autogenic and allogenic succession, while a study in the coastal mangroves of Mexico used palaeoecology to understand the role of natural disturbance (hurricanes) on successional processes and understand modern community ecology patterns (Torrescano-Valle and Islebe 2012).

Palaeoecology can also be helpful when studying aspects of community ecology that, unlike succession, might not be inherently temporal but where examining patterns over many years is advantageous (Rull 2010). Examples of where this approach has been used to good effect include examining species turnover in woodland flora in relation to climate change across Europe (Webb and Goodenough 2021), quantifying variation in predator–prey cycles in Italian mammals (Raia et al. 2007), analyzing the long-term effects of herbivory in the UK (Whitehouse and Smith 2010) and considering long-term synchronous population changes in sockeye salmon (*Oncorhyncus nerka*) in the north Pacific in relation to changes in different freshwater nursery lakes on Kodiak Island, Alaska (Finney et al. 2002).

One area of community ecology that has gained increasing traction as we enter the Anthropocene is the consideration of ecological tipping points. Tipping points occur when an ecological threshold is breached and the system "tips over" into disequilibrium, quickly leading to long-lasting change or ecosystem collapse (Gladwell 2000). As tipping points cannot be studied until they occur, and are notoriously hard to simulate experimentally (Cairns 2004), they are challenging to predict with any accuracy. However, evidence of past tipping points can sometimes be available in the palaeoecological record, which might predict contemporary tipping points and inform appropriate avoidance, mitigation, or buffering strategies. The power of this approach is shown by Sander et al. (2021), who use palaeoecological tools to investigate the collapse of a (fossil) bed of European flat oysters (*Ostrea edulis*) in the North Sea. They conclude that influx of sediment from continental rivers was a stressor but that the tipping point for population collapse was the sudden increase in boring sponge (*Cliona* sp.) c. 700 years ago. This North Sea oyster bed research involved identification of a biotic tipping point but Hájková et al. (2012) demonstrated the approach works for abiotic tipping points too. Hájková studied pollen, plant macrofossils and testate amoeba to reveal that change in moisture levels was the tipping point for abrupt change as calcareous fen replaced original sphagnum bogs in Slovakia c. 10,700 years ago as bog vegetation could no longer be supported. This highlights the importance of contemporary management to reduce the risk of desiccation, for example, by the addition of bunds in drainage channels to change the hydrological regime in similar habitats today.

In rare cases, it might be possible to actually avoid tipping points by studying the fossil record. For example, suppression of the natural fire regime was mandated in several of the early US National Parks, including Yosemite and Sequoia from 1890, because fire was seen as being destructive and thus non-desirable and in 1935 the US Forest Service fire suppression policy was formally adopted by the National Park Service. Although the wisdom of this was questioned—notably by George Wright in the early 1930s (Kilgore 2007)—the Leopold report (Leopold et al. 1963) was the catalyst for slow change towards the current Wildland Fire Use policy. This report highlighted the ecological importance of fire, which was reinforced by later studies showing that giant sequoia (*Sequoiadendron giganteum*) rely on fire to release seeds from cones, expose bare soil for seedlings to root, and to open canopy gaps and reduce competition from shade-loving species (Kilgore 1973; Meyer and Safford 2011). Although this complex series of interactions could probably not have been inferred from pollen data, the presence of charcoal and subsequent high levels of *Sequoiadendron* pollen in the palaeoecological record (potentially after a short lag) would at least have suggested that fire would not be detrimental to this species.

Functional ecology

Keystone species have an especially important role within their ecosystem and, by their very definition, variation in the population of keystone species has a greater impact than variation in the population of non-keystone species. When the identity of a keystone species is known, palaeoecological studies might complement historical or contemporary evidence to enable longer-term study than would otherwise be possible. For example, Slade et al. (2022) studied Mollusca remains over the last 6000 years to indirectly monitor temporal change in the population size of sea otters (*Enhydra lutris*): a keystone predator in British Columbia, Canada. However, it is not always possible to identify a particular species as being keystone until that species declines to a low level or becomes extirpated: in other words, evidence of keystone status often comes from ecosystem consequences of keystone species loss. Indeed, the removal experiment of Paine (1966)—whereby predatory sea stars (*Pisaster ochraceus*) were removed leading to predator release for mussels (*Mytilus californianus*) that then outcompeted other species and ultimately led to the term "keystone species" being coined (Paine 1969)—has become one of most-cited experimental ecological studies (Lafferty and Suchanek 2016). It is, however, not always possible or ethical to undertake removal experiments to identify keystone species, or determine impacts of decline or extirpation, and this is where looking to the palaeoecological record could again be informative. For example, Gilson (2004) undertook research on 1,400 years of vegetation change in Tsavo National Park, Kenya, which demonstrated the ecosystem effects of variation in the population size of African elephant (*Loxodonta africana*). Thus, the currently observable impacts of this species on vegetation through trophic interactions (herbivory) that have caused it to become belatedly classified by many ecologists as a keystone species (Western 1989) were already evident in the palaeoecological record.

One specific type of keystone species is an ecosystem engineer: species that modify (literally "engineer") the environment physically through their activities. Although the impacts of ecosystem engineers can often be studied real-time in field research, being able to establish impacts of ecosystem engineers in the past can be useful, since the role of the ecosystem engineer, and indeed the response of the ecosystem, might not be temporally consistent. For example, American beaver (*Castor canadensis*) fell trees and use these to dam water courses, creating complex riverine ecosystems and transition across terrestrial and freshwater environments. Persico and Meyer (2009) studied sediments in historic beaver ponds and found that beaver presence/absence were associated with different riparian vegetation assemblages, especially in relation to aspen (*Populus tremuloides*) which is preferentially used in beaver dams. This highlights the potential for palaeoecological work to explore causes of the cascade effects of population change in ecosystem engineers at ecosystem level.

Other species that can have a very important role in habitat functioning are dominant species, especially where these are long-lived. Once again, because there is a time dimension here, quantifying the impacts of abundance change in dominant species is not without challenge. Palaeoecological analogues can be helpful, however. For example, in North America the palaeoecological record indicates a sharp decline in hemlock (*Tsuga canadensis*) around 4000–5000 years ago (Bhiry and Filion 1996), which caused long-lasting change to the understory species assemblage, and, in turn, affected local hydrological processes.

Restoration ecology

Rewilding has become one of the most-commonly used (and most-commonly misused) terms in conservation ecology over the last decade. It is often seen as a "magic bullet" in reversing biodiversity decline in the Anthropocene but it remains a controversial concept in conservation (e.g. Rubenstein and Rubenstein 2016; Pettorelli et al. 2019). This is partly because there is no accepted universal definition and the term is thus used for a diverse range of initiatives, and partly because the aims of rewilding are often unclear or unspecified (Nogués-Bravo et al. 2016; Hayward et al. 2019; Schulte to Bühne et al. 2022). One of the key challenges in rewilding, and indeed in ecosystem restoration more generally, is that defining specific ecological targets is often problematic. This is because restoration and rewilding are inherently about recreating previous ecological conditions. Detailed understanding of past habitats, species dynamics and ecological interactions is essential both to allow restoration aims to be clearly articulated and to inform optimal methods to realise those aims (Seddon et al. 2019). For example, it was always assumed that, during the climax communities found in Europe after the last ice age ~ 4000–9000 years ago, land was covered by continuous and relatively-closed wildwood. However, modelling of palaeoecological vegetation and Coleoptera data (Vera 2000; Whitehouse and Smith 2004, 2010; Birks 2005) revealed that actually there was a relatively open mosaic wood-pasture landscape. In an analysis of the more recent past, Hanley et al. (2008, 2009) analysed plant diversity in upland Scotland over the last 400 years using an approach that combined palynological and historical datasets. The authors showed that diversity levels have varied considerably since 1600CE, indicating that static baselines and 'naturalness' are problematic concepts in rewilding (and indeed in conservation and ecology more widely) (Davies and Bunting 2010). Thus, any rewilding schemes should use a palaeoecologically derived baseline to set appropriate habitat targets, or alternatively use the trajectory of natural change derived from the palaeoecological record to determine forward looking goals for restoration (e.g. Jackson and Hobbs 2009; Wingard et al. 2017). In particular, care must be taken when attempting to create an environment that mirrors the palaeoecologically informed past, as the modern species assemblage will inevitably be different (especially in regard to non-native species), which may have important implications.

Within "Pandora's box" of rewilding concepts (Nogués-Bravo et al. 2016) are two specific types of rewilding. Pleistocene rewilding aims to restore ecosystems shaped by populations of megafauna extirpated since the Late Pleistocene where populations of these species are still extant elsewhere and thus could be reintroduced (e.g. European bison (*Bison bonasus*) and musk ox (*Ovibos moschatus*)). Trophic rewilding has a similar aim but uses the mechanism of surrogate species that have a similar ecological role to that of the megaherbivores (e.g. using Heck cattle as a substitute for the extinct aurochs (*Bos primigenius*) (Svenning et al. 2016; Perino et al. 2019)). For such initiatives to work, a detailed understanding of megaherbivore distributions, densities, and ecological impacts is vital. This can sometimes be undertaken using direct palaeoecological study of the species concerned but, in other cases, palaeoecological proxies can be valuable: for example, a recent study (van Asperen et al. 2021) used coprophilous fungal spores that are associated with the dung of megaherbivores as a proxy for spatiotemporal abundance in these species.

Although rewilding is—or at least should be—a large-scale approach, smaller-scale restoration ecology still requires detailed knowledge of past conditions. An excellent example of where palaeoecology has informed contemporary restoration ecology is van de Velde et al. (2019) who used the assemblage of 28 Mollusc species from 2000–2500 years ago found in shelly deposits from Great Turali Lake, Russia, to reveal a baseline for ecological restoration planning that pre-dates the current threats in the region. A similar approach has been used in fen restoration in minerotrophic peatlands in southeastern Canada, where a “reference ecosystem” has been classified based on the complementary analysis of multiple peat cores and characterisation of contemporary natural sites (Bérubé et al. 2017). Palaeoecological reconstructions using species tolerance ranges have also been used to guide restoration targets for abiotic environments at ecosystem level, including those enshrined within environmental policy. For example, in relation to salinity levels in the Everglades in Florida (reviewed by Wingard et al 2017), nitrogen levels in coastal fjords in Norway in relation to the EU Water Framework Directive (Andersen et al. 2004) and modelling natural freshwater flow through the Colorado River to address critical information gaps caused by the lack of direct surveying prior to alteration of natural conditions and to facilitate appropriate restoration (Kowalewski et al. 2000; Rodriquez et al. 2001).

Species reintroduction is undertaken for many different reasons. In some cases, reintroduction is a part of a broader restoration or rewilding project, when the ecological role of a species is the fundamental driver for the reintroduction (Hale and Koprowski 2018). In other cases, reintroduction can be motivated by desire to repair fragmented guilds by reinstating extirpated species, especially where charismatic species are involved or where people feel a moral obligation to replace species lost through direct human activity (Seddon et al. 2005; Hawkins et al. 2020; Lee et al. 2021). There might also be economic arguments, either relating to ecosystem services or ecotourism potential (Stringer and Gaywood 2016; Auster et al. 2020). Reintroduction can also be undertaken as part of species conservation: 43% of the reintroductions analysed by Seddon et al. (2005) involved species listed as threatened by the IUCN, with 21% being endangered or critically endangered. Alternatively, reintroductions might aim to create wild populations for species otherwise only existing in captive collections, to broaden a species’ current range to reduce extinction risk, or to facilitate overall growth in global population if carrying capacity has been reached at current locations (Maunder 1992; Walzer et al. 2012).

Regardless of the motivation for species reintroduction, IUCN (2013) guidelines state that, within the initial feasibility study, it is vital to check: (1) the species was previously present in the target release area (i.e. it is a ***re***introduction); and (2) that, where possible, the target release area is near the core of the historic range or in an area where extirpation was relatively recent. In many cases, these questions can be answered by examining past documentary evidence, considering capture locations of museum specimens (Winker 2004), using instances of where settlement or road names can be used to infer presence of species historically (Williams et al. 2020), and, in some recent cases, through direct ecological records and human memory (e.g. Californian condor (*Gymnogyps californianus*), Przewalski’s horse (*Equus ferus przewalskii*) and common crane (*Grus grus*) (Toone and Wallace 1994; Walzer et al. 2012; Redondo 2017)). However, in some cases, these lines of evidence are missing or ambiguous and this is when palaeoecological investigation can be especially helpful. For example, palaeoecological studies have been helpful in refining historical documentary records of “pelicans” inhabiting aquatic ecosystems in the UK and Denmark to a specific species: the Dalmatian pelican (*Pelecanus crispus*) (Stewart 2004), paving the way for a potential reintroduction to UK wetlands (MacDonald 2020). Reintroductions of extirpated plants are also possible using information from palaeoecological records. Researchers in New Zealand were surprised to find that woodland in Twahiti Rahi now dominated by pohutukawa (*Metrosideros excelsa*) consisted of numerous native trees just 100 years ago and, based on this palaeoecological evidence, programmes have begun to reintroduce native species that were present before inter-tribal disputes caused forest disturbance (Wilmshurst et al. 2013). Similarly, *Pritchardia aylmerrobinsonii* palms were reintroduced on the Hawaiian island of Kaua'i from the adjacent island of Niihau after investigations revealed the species had previously existed on both islands (Burney and Burney 2007).

Another key aspect of a species reintroduction feasibility study is to consider whether or not the habitat conditions currently in existence at a site are similar to the habitat that would have supported the species in that location historically, and thus whether a reintroduction is likely to be successful. A cautionary tale is that of failed attempts to reintroduce a rare aquatic plant, *Najas flexilis*, to the English Lake District. The species was last recorded in the 1980s and was thought to have declined quite rapidly before this time, mainly due to deteriorating water quality. With water quality returning to pre-1980 levels, attempts have been made to reintroduce *N. flexilis* (JNCC 2012) but these ultimately failed. Palaeoecological study incorporating abiotic measurements by Bishop et al. (2019) found that the species actually declined much earlier and that reintroduction attempts had been premature.

Monitoring ecology

The presence/absence and abundance of species can be used by ecologists to infer abiotic conditions (environmental indicators), ecosystem dynamics (biological indictors), or biodiversity patterns (biodiversity indicators). Use of indicator species, and numerical biotic indices such as the Ellenberg system for inferring soil conditions from plants (Ellenberg 1974; Ellenberg et al. 1992) and macroinvertebrate indices for inferring dissolved oxygen in freshwater ecosystems (López-López and Sedeño-Díaz 2015), are common tools within ecology. However, in some cases, additional insights can be gained from considering palaeoecological evidence. For example, Webb and Goodenough (2018) examined UK woodland sites with detailed contemporary vegetation records and palaeoecological data to critique whether "Ancient" Woodland Indicator (AWI) plant species are appropriate for determining whether a wooded site is ancient (> 400 years old) or not. They found that although there are disturbance-sensitive species associated with stable (old) woodland habitats (Peterken and Game 1984; Rackham 2003; Glaves et al. 2009), AWIs can be surprisingly resilient to woodland interruptions or even tree clearance, with rich AWI communities found at sites unwooded for at least 2800 years. They concluded that the use of floristic AWIs alone in defining ancient woodland should be reviewed, and recommended that a weighted AWI index or multi-taxa indicator system should be developed.

While Webb and Goodenough's study involved using palaeoecological data to test the appropriateness of an indictor system widely used in modern ecological contexts, the work of Seddon et al. (2019) provides a nice example of how the same indicator system can be used to enable temporal comparisons when applied to palaeoecological, historical and contemporary data, this time within the aquatic environment. The authors compared data on Gastropoda, Trichoptera and Coleoptera using Biological Monitoring Working Party (BMWP) and Average Score Per Taxon (ASPT) biotic indices (Furse et al. 1981; Armitage et al. 1983) to consider likely oxygen levels, and thus water quality, in a UK chalk river from the ~ 1840s to 2011. This is much longer than would have been possible without incorporation of palaeoecological data. A similar approach was taken using protists to monitor current marine ecological quality along the Norwegian Skagerrak coast in relation to pristine conditions as a way of implementing and auditing pollution legislation compliance (Bouchet et al. 2012). There is also the potential to use the palaeoecological record to highlight how changes within a species community can be used as a form of alert monitoring or Early-Warning Signal of more dramatic change being likely. A good example of this approach is using diatom flickering in Lake Erhai in China as an indicator of a major ecological regime shift being imminent (Wang et al. 2012)—although as Carstensen et al. (2013) highlight, careful statistical processing is vital to ensure observed patterns are not analytical artefacts.

Conclusion and recommendations

Throughout this paper, we have considered the value of using palaeoecological data in contemporary ecological study thematically. We have discussed ecological topics, and provided specific examples, of situations where palaeoecological information can be useful within contemporary ecological research (e.g. quantifying biogeographical patterns, understanding species interactions) or in applied ecological contexts (e.g. informing management of non-native species, creating appropriate restoration or rewilding targets, devising suitable reintroduction schemes). An alternative way to conceptualise this is via consideration of the six main types of opportunity where palaeoecological data can be used within ecological contexts; these are summarised in Fig. 1.

In terms of tangible recommendations for bridging the gap between palaeoecology and ecology, and promoting ways in which some of the opportunities presented in this paper can be acted upon, we suggest that:

1. There needs to be a greater awareness by both research and applied ecologists that palaeoecology is not solely concerned with the "distant past". The opportunities for using palaeoecology to quantify the "recent past" make palaeoecological approaches extremely valuable in many contemporary ecological contexts in terrestrial environments (e.g. Rull 2010; Wilmshurst et al. 2013), freshwater aquatic environments (e.g. Bennion et al. 2018; Bishop et al. 2019) and marine environments (Finney et al. 2002; Agiadi and Albano 2020).
2. There needs to be better appreciation that primary ecological data, historical records, and palaeoecological evidence can be used in complementary ways to form a powerful integrated approach to better understand

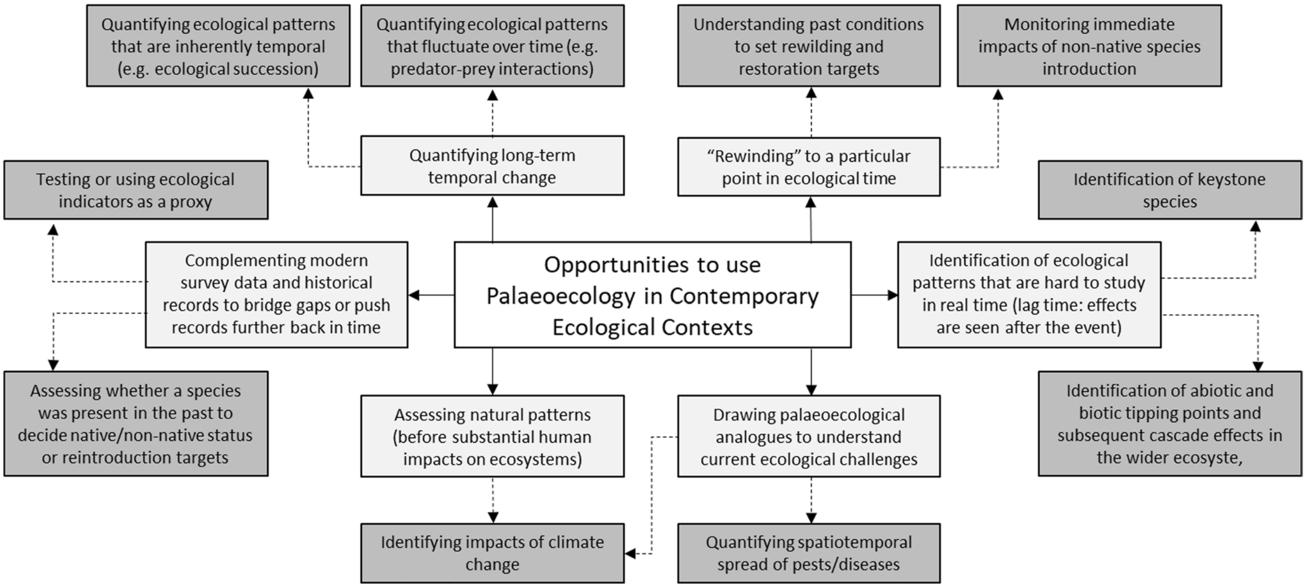


Fig. 1 Overview of the opportunities to use palaeoecology in contemporary ecological contexts

current ecology and solve future ecological challenges. Linking palaeoecological and contemporary ecological approaches also mitigates their inherent disadvantages. Palaeoecology is, by its very nature, observational and correlative and, because it is based on the preservation of material, there can be gaps in the evidence especially where preservation conditions are sub-optimal; there is also the potential for ecological inference to be confounded by taphonomic variables (Lowe and Walker 2014). Palaeoecological studies also have a fundamental limit to the level of detail that is possible, especially as regards temporal resolution (Jackson and Blois 2015), and there can be challenges in disentangling the local pollen signal (and thus local change) from the reginal pollen signal especially for pollen grains with wide dispersal properties (Davis 2000; Hellman et al. 2009). By contrast, contemporary ecology studies can, resources permitting, be much more detailed and there is often greater potential to study causality, including directly via manipulation. However, primary studies—even those that are long-term by the standards of ecological research—actually have comparatively short temporal reach, which might not align with ecologically relevant timespans, and this can make it hard to distinguish natural change from human-induced change (Buma et al. 2019). Combining approaches can be a win–win situation, allowing ecologists to employ detailed, high-resolution, causally based approaches but setting these within a longer temporal context.

3. Despite increases in inter-, multi- and trans-disciplinary working within the natural sciences, many academics and practitioners still work, to a greater or lesser extent, in intellectual silos. Examples of ecological projects that reach into the palaeoecological past are still comparatively limited, especially when compared with the wealth of palaeoecological and ecological research that is undertaken in (relative) isolation. Increasing incorporation of palaeoecological concepts into University ecology modules and mainstream ecological textbooks would, in time, allow basic understanding of palaeoecology to become a routine part of the ecologists' toolkit. In many academic institutions, palaeoecology sits within geographical or earth sciences faculties whereas ecology typically falls within biosciences: greater promotion of cross-faculty working is paramount (Birks 2019).
4. Knowledge of the number and value of data repositories needs to be enhanced. For palaeoecology, key repositories include the European Pollen Database (Fyfe et al. 2009), the North American Pollen Database (accessible via Neotoma) (Williams et al. 2018), and the international Paleobiology Database PBDB (Peters and McClennen 2016)—many other databases are referenced on PAGES http://pastglobalchanges.org. For ecology, key repositories are the Global Biodiversity Information Facility, National Biodiversity Network, and PanTHERIA. Development of data repositories that seamlessly integrate ecological and palaeoecological data would greatly enhance the ability of researchers and practitioners to routinely access both types of data when searching for information on specific taxa, habitats or locations. Users also need better understanding of how to mine resources and better guidance on the constraints, caveats and limitations of the data therein. Such guidance needs to be widely accessible rather than

> assuming subject-specific prior knowledge. For example, ecologists need to understand that palynological data are usually presented at Family level not Genus/Species level because of limitations in the identification of pollen grains, appreciate the impact of different preservation conditions on fossil and sub-fossil evidence, and recognise the importance of dispersal and erosion on drawing inferences from palaeoecological evidence.

Overall, we conclude that the possibilities for using palaeoecology in contemporary ecological contexts are as extensive as they are exciting. We urge ecologists to learn from the past and seek out opportunities to extend, improve, and strengthen ecological research and practice using palaeoecological data.

Author contribution statement AEG and JCW jointly conceived the idea, reviewed the literature, and wrote the paper.

Funding Not applicable.

Availability of data and material Not applicable.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have conflict of interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

References

Agiadi K, Albano PG (2020) Holocene fish assemblages provide baseline data for the rapidly changing eastern Mediterranean. Holocene 30:1438–1450

Andersen JH, Conley DJ, Hedal S (2004) Palaeoecology, reference conditions and classification of ecological status: the EU Water Framework Directive in practice. Mar Poll Bull 49:283–290

Anderson NJ, Vos P (1992) Learning from the past: diatoms as palaeoecological indicators of changes in marine environments. Neth J Aquat Ecol 26:19–30

Armitage PD, Moss D, Wright JF, Furse MT (1983) The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. Water Res 17:333–347

Aronson RB, Macintyre IG, Precht WF, Murdoch TJ, Wapnick CM (2002) The expanding scale of species turnover events on coral reefs in Belize. Ecol Monogr 72:233–249

Auster RE, Barr SW, Brazier RE (2020) Wildlife tourism in reintroduction projects: exploring social and economic benefits of beaver in local settings. J Nat Conserv 58:125920

Barber KE, Chambers FM, Maddy D (2003) Holocene palaeoclimates from peat stratigraphy: macrofossil proxy climate records from three oceanic raised bogs in England and Ireland. Quat Sci Rev 22:521–539

Barber KE, Chambers FM, Maddy D (2004) Late Holocene climatic history of northern Germany and Denmark: peat macrofossil investigations at Dosenmoor, Schleswig-Holstein, and Svanemose, Jutland. Boreas 33:132–144

Beebee TJC, Buckley J, Evans I, Foster JP, Gent AH, Gleed-Owen CP, Kelly G, Rowe G, Snell C, Wycherley JT, Zeisset I (2005) Neglected native or undesirable alien. Resolution of a conservation dilemma concerning the pool frog *Rana lessonae*. Biodivers Conserv 14:1607–1626

Bennion H, Sayer CD, Clarke SJ, Davidson TA, Rose NL, Goldsmith B, Rawcliffe R, Burgess A, Clarke G, Turner S, Wiik E (2018) Sedimentary macrofossil records reveal ecological change in English lakes: implications for conservation. J Paleolimnol 60:329–348

Bérubé V, Rochefort L, Lavoie C (2017) Fen restoration: defining a reference ecosystem using paleoecological stratigraphy and present-day inventories. Botany 95:731–750

Bhiry N, Filion L (1996) Mid-Holocene hemlock decline in eastern North America linked with phytophagous insect activity. Quat Res 45:312–320

Birks HJB (2005) Mind the gap: how open were European primeval forests? Trends Ecol Evol 20:154–156

Birks HJB (2019) Contributions of Quaternary botany to modern ecology and biogeography. Plant Ecol Divers 12:189–385

Bishop IJ, Bennion H, Sayer CD, Patmore IR, Yang H (2019) Filling the "data gap": Using paleoecology to investigate the decline of *Najas flexilis* (a rare aquatic plant). Geo Geogr Environ 6:e00081

Blundell A, Charman DJ, Barber K (2008) Multiproxy late Holocene peat records from Ireland: towards a regional palaeoclimate curve. J Quat Sci 23:59–71

Bonsall MB, Hassell MP (1997) Apparent competition structures ecological assemblages. Nature 388:371–373

Bouchet VM, Alve E, Rygg B, Telford RJ (2012) Benthic foraminifera provide a promising tool for ecological quality assessment of marine waters. Ecol Ind 23:66–75

Boulbes N, van Asperen EN (2019) Biostratigraphy and palaeoecology of European *Equus*. Front Ecol Evol 7:301

Bradfield GE, Cumming WP, Newman RF, Krzic M (2021) Grazing exclosures reveal divergent patterns of change in bunchgrass grasslands of Western Canada. Botany 99:9–22

Buckley J, Foster J (2005) Reintroduction strategy for the pool frog *Rana lessonae* in England. English Nature Research Report 642, Peterborough

Buma B, Harvey BJ, Gavin DG, Kelly R, Loboda T, McNeil BE, Marlon JR, Meddens AJ, Morris JL, Raffa KF, Shuman B (2019) The value of linking paleoecological and neoecological perspectives to understand spatially-explicit ecosystem resilience. Landscape Ecol 34:17–33

Burney DA, Burney LP (2007) Paleoecology and "inter-situ" restoration on Kaua'i. Hawai'i Front Ecol Evol 5:483–490

Cairns J Jr (2004) Ecological tipping points: a major challenge for experimental sciences. Asian J Exp Biol Sci 18:1–16

Carlton JT (1996) Biological invasions and cryptogenic species. Ecology 77:1653–1655

Carstensen J, Telford RJ, Birks HJB (2013) Diatom flickering prior to regime shift. Nature 498:E11–E12

Chambers FM (1999) The Quaternary history of Llangorse Lake: implications for conservation. Aquat Conserv 9:343–359

Chambers FM, Mauquoy D, Gent A, Pearson F, Daniell JR, Jones PS (2007) Palaeoecology of degraded blanket mire in South Wales: data to inform conservation management. Biol Conserv 137:197–209

Damgaard C (2019) A critique of the space-for-time substitution practice in community ecology. Trends Ecol Evol 34:416–421

Dauwalter DC, Fesenmyer KA, Miller SW, Porter T (2018) Response of riparian vegetation, instream habitat, and aquatic biota to riparian grazing exclosures. N Am J Fish Manag 38:1187–1200

Davidson TA, Sayer CD, Bennion H, David C, Rose N, Wade MP (2005) A 250 year comparison of historical, macrofossil and pollen records of aquatic plants in a shallow lake. Freshw Biol 50:1671–1686

Davies AL, Bunting MJ (2010) Applications of palaeoecology in conservation. Open J Ecol 3:1

Davis MB (2000) Palynology after Y2K—understanding the source area of pollen in sediments. Ann Rev Earth Plan Sci 28:1–18

Davis SJ (2019) Rabbits and Bergmann's rule: how cold was Portugal during the last glaciation? Biol J Linn Soc 128:526–549

de Beaulieu J-L, Andrieu-Ponel V, Cheddadi R, Guiter F, Ravazzi C, Reille M, Rossi S (2006) Apport des longues séquences lacustres à la connaissance des variations des climats et des paysages pléistocènes. CR Palevol 5:65–72

Ellenberg H (1974) Indicator values of plants in Central Europe. Scripta Geobot 9:3–122

Ellenberg H, Weber HE, Dull R, Wirth V, Werner W, Paulissen D (1992) Indicator values of plants in Central Europe. Scripta Geobot 18:1–248

Evans JM (2013) *Pistia stratiotes* L. in the Florida peninsula: biogeographic evidence and conservation implications of native tenure for an 'invasive' aquatic plant. Conserv Soc 11:233–246

Finney BP, Gregory-Eaves I, Douglas MS, Smol JP (2002) Fisheries productivity in the northeastern Pacific Ocean over the past 2,200 years. Nature 416:729–733

Flynn LE, Mitchell FJ (2019) Comparison of a recent elm decline with the mid-Holocene Elm Decline. Veg Hist Archaeobot 28:391–398

Furse MT, Wright JF, Armitage PD, Moss D (1981) An appraisal of pond-net samples for biological monitoring of lotic macro-invertebrates. Water Res 15:679–689

Fyfe RM, de Beaulieu JL, Binney H, Bradshaw RH, Brewer S, Le Flao A, Finsinger W, Gaillard MJ, Giesecke T, Gil-Romera G, Grimm EC (2009) The European pollen database: past efforts and current activities. Veg Hist Archaeobot 18:417–424

Gałka M, Feurdean A, Hutchinson S, Milecka K, Tanţău I, Apolinarska K (2018) Response of a spring-fed fen ecosystem in Central Eastern Europe (NW Romania) to climate changes during the last 4000 years: a high resolution multi-proxy reconstruction. Palaeogeogr Palaeoclimatol Palaeoecol 504:170–185

Gilson L (2004) Testing non-equilibrium theories in savannas: 1400 years of vegetation change in Tsavo National Park. Kenya Ecol Complex 1:281–298

Gladwell M (2000) The tipping point: how little things can make a big difference. Little, Brown and Co, New York

Glaves P, Handley C, Birbeck J, Rotherham I, Wright B (2009) A survey of the coverage, use and application of ancient woodland indicator lists in the UK. Hallam Environmental Consultants, Sheffield

Gleed-Owen CP (2000) Subfossil records of *Rana cf. lessonae*, *Rana arvalis* and *Rana cf. dalmatina* from Middle Saxon (c. 600–950 AD) deposits in eastern England: evidence for native status. Amphibia-Reptilia 21:57–65

Goodenough AE (2010) Are the ecological impacts of alien species misrepresented? A review of the "native good, alien bad" philosophy. Community Ecol 11:13–21

Goodenough AE (2016) In the wrong place at the wrong time. The Biologist 63:20–23

Goodenough AE, Hart AG (2017) Applied ecology: monitoring, managing and conserving. Oxford University Press, Oxford

Hájková P, Grootjans AB, Lamentowicz M, Rybníčková E, Madaras M, Opravilová V, Wołejko L (2012) How a Sphagnum fuscum-dominated bog changed into a calcareous fen: the unique Holocene history of a Slovak spring-fed mire. J Quat Sci 27:233–243

Hale SL, Koprowski JL (2018) Ecosystem-level effects of keystone species reintroduction: a literature review. Restor Ecol 26:439–445

Hanley N, Davies A, Angelopoulos K, Hamilton A, Ross A, Tinch D, Watson F (2008) Economic determinants of biodiversity change over a 400 year period in the Scottish uplands. J Appl Ecol 45:1557–1565

Hanley N, Tinch D, Angelopoulos K, Davies A, Barbier EB, Watson F (2009) What drives long-run biodiversity change? New insights from combining economics, palaeoecology and environmental history. J Environ Econ Manag 57:5–20

Hawkins SA, Brady D, Mayhew M, Smith D, Lipscombe S, White C, Eagle A, Convery I (2020) Community perspectives on the reintroduction of Eurasian lynx (*Lynx lynx*) to the UK. Restor Ecol 28:1408–1418

Hayward MW, Scanlon RJ, Callen A, Howell LG, Klop-Toker KL, Di Blanco Y, Balkenhol N, Bugir CK, Campbell L, Caravaggi A, Chalmers AC (2019) Reintroducing rewilding to restoration—rejecting the search for novelty. Biol Conserv 233:255–259

Hellman S, Gaillard MJ, Bunting JM, Mazier F (2009) Estimating the relevant source area of pollen in the past cultural landscapes of southern Sweden—a forward modelling approach. Rev Palaeobot Palynol 153:259–271

Huntley B, Birks HJB (1983) An atlas of past and present pollen maps of Europe, 0–1300 years ago. Cambridge University Press, Cambridge

IUCN (2013) Guidelines for reintroductions and other conservation translocations. IUCN Species Survival Commission, Gland, Switzerland

Jackson ST (2007) Looking forward from the past: history, ecology, and conservation. Front Ecol Environ 5:455

Jackson ST, Blois JL (2015) Community ecology in a changing environment: perspectives from the Quaternary. Proc Natl Acad Sci USA 112:4915–4921

Jackson ST, Hobbs RJ (2009) Ecological restoration in the light of ecological history. Science 325:567–569

Jackson ST, Williams JW (2004) Modern analogs in Quaternary paleoecology: here today, gone yesterday, gone tomorrow? Annu Rev Earth Planet Sci 32:495–537

JNCC (2012) Conservation status assessment for species S1833 Slender Naiad (*Najas flexilis*). Peterborough, UK: JNCC. Retrieved from http://jncc.defra.gov.uk/pdf/Article17Consult_20131010/S1833_ENGLAND.pdf

Johnson EA, Miyanishi K (2008) Testing the assumptions of chronosequences in succession. Ecol Lett 11:419–431

Kilgore BM (1973) The ecological role of fire in Sierran conifer forests: its application to National Park management. J Quat Res 3:496–513

Kilgore BM (2007) Origin and history of wildland fire use in the US national park system. The George Wright Forum 24:92–122

Kittel P, Sikora J, Antczak O, Brooks SJ, Elias S, Krąpiec M, Luoto TP, Borówka RK, Okupny D, Pawłowski D, Płóciennik M (2018) The palaeoecological development of the Late Medieval moat-Multiproxy research at Rozprza, Central Poland. Quatern Int 482:131–156

Kowalewski M (2001) Applied marine paleoecology: an oxymoron or reality? Palaios 16:309–310

Kowalewski M, Serrano GEA, Flessa KW, Goodfriend GA (2000) Dead delta's former productivity: two trillion shells at the mouth of the Colorado River. Geology 28:1059–1062

Lafferty KD, Suchanek TH (2016) Revisiting Paine's 1966 sea star removal experiment, the most-cited empirical article in the American Naturalist. Am Nat 188:365–378

Lee A, Laird AM, Brann L, Coxon C, Hamilton AJ, Lawhon LA, Martin JA, Rehnberg N, Tyrrell BP, Welch Z, Hale B (2021) The ethics of reintroducing large carnivores: the case of the California Grizzly. Conserv Soc 19:80

Leopold AS, Cain SA, Cottam CM, Gabrielson JM, Kimball TL (1963) Study of wildlife problems in national parks: wildlife management in the national parks. Trans North Am Wildlife Nat Resour Conf 28:28–45

López-López E, Sedeño-Díaz JE (2015) Biological indicators of water quality: the role of fish and macroinvertebrates as indicators of water quality. Environmental indicators. Springer, Dordrecht, pp 643–661

Louys J, Price GJ, Travouillon KJ (2021) Space-time equivalence in the fossil record, with a case study from Pleistocene Australia. Quat Sci Rev 253:106764

Lowe JJ, Walker M (2014) Reconstructing quaternary environments. Routledge, Milton Park

Luckinbill LS (1973) Coexistence in laboratory populations of *Paramecium aurelia* and its predator *Didinium nasutum*. Ecology 54:1320–2132

MacDonald B (2020) Rebirding: restoring Britain's wildlife. Pelagic Publishing, London

Mannion PD (2020) A deep-time perspective on the latitudinal diversity gradient. Proc Natl Acad Sci 117:17479–17481

Maunder M (1992) Plant reintroduction: an overview. Biodiv Conserv 1:51–61

Meseguer AS, Condamine FL (2020) Ancient tropical extinctions at high latitudes contributed to the latitudinal diversity gradient. Evolution 74:1966–1987

Meyer MD, Safford HD (2011) Giant sequoia regeneration in groves exposed to wildfire and retention harvest. Fire Ecol 7:2–16

Morin PJ (1999) Productivity, intra-guild predation, and population dynamics in experimental food webs. Ecology 80:752–76019

Mousing EA, Ribeiro S, Chisholm C, Kuijpers A, Moros M, Ellegaard M (2017) Size differences of Arctic marine protists between two climate periods—using the paleoecological record to assess the importance of within-species trait variation. Ecol Evol 7:3–13

Njagi DM, Routh J, Olago D, Gayantha K (2021) A multi-proxy reconstruction of the late Holocene climate evolution in the Kapsabet Swamp, Kenya (East Africa). Palaeogeogr Palaeoclimatol Palaeoecol 574:110475

Nogués-Bravo D, Simberloff D, Rahbek C, Sanders NJ (2016) Rewilding is the new Pandora's box in conservation. Curr Biol 26:R87–R91

Novak V, Santodomingo N, Rösler A, Di Martino E, Braga JC, Taylor PD, Johnson KG, Renema W (2013) Environmental reconstruction of a late Burdigalian (Miocene) patch reef in deltaic deposits (East Kalimantan, Indonesia). Palaeogeogr Palaeoclimatol Palaeoecol 374:110–122

Paine RT (1966) Food web complexity and species diversity. Am Nat 100:65–75

Paine RT (1969) A note on trophic complexity and community stability. Am Nat 103:91–93

Pandolfi JM (2011) The paleoecology of coral reefs. In: Dubinsky Z, Stambler N (eds) Coral reefs: an ecosystem in transition. Springer, Dordrecht, pp 13–24

Pandolfi JM, Bradbury RH, Sala E, Hughes TP, Bjorndal KA, Cooke RG, McArdle D, McClenachan L, Newman MJ, Paredes G, Warner RR (2003) Global trajectories of the long-term decline of coral reef ecosystems. Science 301:955–958

Parker AG, Goudie AS, Anderson DE, Robinson MA, Bonsall C (2002) A review of the mid-Holocene elm decline in the British Isles. Prog Phys Geogr 26:1–45

Payette S (1988) Late-Holocene development of subarctic ombrotrophic peatlands: allogenic and autogenic succession. Ecology 69:516–531

Perino A, Pereira HM, Navarro LM, Fernández N, Bullock JM, Ceaușu S, Cortés-Avizanda A, van Klink R, Kuemmerle T, Lomba A, Pe'er G (2019) Rewilding complex ecosystems. Science 364:6438

Persico L, Meyer G (2009) Holocene beaver damming, fluvial geomorphology, and climate in Yellowstone National Park. Wyoming Quat Res 71:340–353

Peterken GF, Game M (1984) Historical factors affecting the number and distribution of vascular plant species in the woodlands of central Lincolnshire. J Ecol 72:155–182

Peters SE, McClennen M (2016) The paleobiology database application programming interface. Paleobiology 42:1–7

Pettorelli N, Durant SM, Du Toit JT (2019) Rewilding: a captivating, controversial, 21st century concept to address ecological degradation in a changing world. Rewilding. Cambridge University Press, Cambridge

Rackham O (2003) Ancient woodland, its history, vegetation and uses in England. Ancient woodland, its history, vegetation and uses in England (revised edition). Castlepoint Press, Dumfries

Raia P, Meloro C, Barbera C (2007) Inconstancy in predator/prey ratios in Quaternary large mammal communities of Italy, with an appraisal of mechanisms. Quat Res 67:255–263

Redondo AS (2017) Reintroduction ecology of the Eurasian Crane *Grus grus*. PhD thesis. University of Exeter.

Reid REB, McGuire JL, Svenning J-C, Wingard GL, Moreno-Mateos D (2022) Review of ESA SYMP 7: a dynamic perspective on ecosystem restoration–establishing temporal connectivity at the intersection between paleoecology and restoration ecology. Bull Ecol Soc Am 103(1):e01954

Ribeiro S, Amorim A, Andersen TJ, Abrantes F, Ellegaard M (2012) Reconstructing the history of an invasion: the toxic phytoplankton species *Gymnodinium catenatum* in the Northeast Atlantic. Biol Invasions 14:969–985

Roberts N (2013) The holocene: an environmental history. John Wiley and Sons

Roche J (2019) Recent findings on the native status and vegetation ecology of Scots pine in Ireland and their implications for forestry policy and management. Irish Forestry 76:29–54

Rodriquez CA, Flessa KW, Dettman DL (2001) Effects of upstream diversion of Colorado River water on the estuarine bivalve mollusc *Mulinia coloradoensis*. Conserv Biol 15:249–258

Roland TP, Daley TJ, Caseldine CJ, Charman DJ, Turney CSM, Amesbury MJ, Thompson GJ, Woodley EJ (2015) The 5.2 ka climate event: evidence from stable isotope and multi-proxy palaeoecological peatland records in Ireland. Quat Sci Rev 124:209–223

Rubenstein DR, Rubenstein DI (2016) From Pleistocene to trophic rewilding: a wolf in sheep's clothing. Proc Natl Acad Sci USA 113:E1

Rull V (2010) Ecology and palaeoecology, two approaches, one objective. Open J Ecol 3:1–5

Salgado J, Sayer C, Carvalho L, Davidson T, Gunn I (2010) Assessing aquatic macrophyte community change through the integration of paleolimnological and historical data at Loch Leven. Scotland J Paleolimnol 43:191–204

Sander L, Hass HC, Michaelis R, Groß C, Hausen T, Pogoda B (2021) The late Holocene demise of a sublittoral oyster bed in the North Sea. PLoS ONE 16:e0242208

Schulte to Bühne H, Pettorelli N, Hoffmann M (2022) The policy consequences of defining rewilding. Ambio 51:93–102

Seddon PJ, Soorae PS, Launay F (2005) Taxonomic bias in reintroduction projects. Anim Conserv 8:51–58

Seddon E, Hill M, Greenwood MT, Mainstone C, Mathers K, White JC, Wood PJ (2019) The use of palaeoecological and contemporary macroinvertebrate community data to characterize riverine reference conditions. River Res Appl 35:1302–1313

Sherriff RL, Platt RV, Veblen TT, Schoennagel TL, Gartner MH (2014) Historical, observed, and modeled wildfire severity in montane forests of the Colorado front range. PLoS ONE 9:106971

Silvertown J (1985) History of a latitudinal diversity gradient: woody plants in Europe 13,000–1000 years BP. J Biogeogr 12:519–525

Slade E, McKechnie I, Salomon AK (2022) Archaeological and contemporary evidence indicates low sea otter prevalence on the pacific northwest coast during the late Holocene. Ecosystems 25(3):548–566

Smith FA, Betancourt JL (2003) The effect of Holocene fluctuations on the evolution and ecology of *Neotoma* (woodrats) in Idaho and north western Utah. Q Res 59:160–171

Song H, Huang S, Jia E, Dai X, Wignall PB, Dunhill AM (2020) Flat latitudinal diversity gradient caused by the Permian-Triassic mass extinction. Proc Natl Acad Sci USA 117:17578–17583

Stewart JR (2004) Wetland birds in the recent fossil record of Britain and northwest Europe. Br Birds 97:33–43

Stewart JR (2007) The fossil and archaeological record of the eagle owl in Britain. Br Birds 100:481–486

Stringer AP, Gaywood MJ (2016) The impacts of beavers Castor spp. on biodiversity and the ecological basis for their reintroduction to Scotland. UK Mamm Rev 46:270–283

Svenning JC, Pedersen PB, Donlan CJ, Ejrnæs R, Faurby S, Galetti M, Hansen DM, Sandel B, Sandom CJ, Terborgh JW, Vera FW (2016) Science for a wilder Anthropocene: synthesis and future directions for trophic rewilding research. Proc Natl Acad Sci USA 113:898–906

Toone WD, Wallace MP (1994) The extinction in the wild and reintroduction of the California condor (*Gymnogyps californianus*). Creative conservation. Springer, Dordrecht, pp 411–419

Torrescano-Valle N, Islebe GA (2012) Mangroves of Southeastern Mexico: palaeoecology and conservation. Open Geogr J 5:6–15

van Asperen EN, Perrotti A, Baker A (2021) Coprophilous fungal spores: NPPs for the study of past megaherbivores. Geological Society, Special Publications, London, p 511

van Leeuwen JF, Froyd CA, van der Knaap WO, Coffey EE, Tye A, Willis KJ (2008) Fossil pollen as a guide to conservation in the Galápagos. Science 322:1206–1206

van de Velde S, Wesselingh FP, Yanina TA, Anistratenko VV, Neubauer TA, ter Poorten JJ, Vonhof HB, Kroonenberg SB (2019) Mollusc biodiversity in late Holocene nearshore environments of the Caspian Sea: a baseline for the current biodiversity crisis. Palaeogeogr Palaeoclimatol Palaeoecol 535:109364

Vera FWM (2000) Grazing ecology and forest history. CABI Publishing, Oxon

Walker MJC (1982) The late-glacial and early Flandrian deposits at Traeth Mawr, Brecon Beacons. South Wales New Phytol 90:177–194

Walker LR, Wardle DA, Bardgett RD, Clarkson BD (2010) The use of chronosequences in studies of ecological succession and soil development. J Ecol 98:725–736

Waller M, Grant MJ, Bunting MJ (2012) Modern pollen studies from coppiced woodlands and their implications for the detection of woodland management in Holocene pollen records. Rev Palaeobot Palynol 187:11–28

Walzer C, Kaczensky P, Zimmermann W, Stauffer C (2012) Przewalski's horse reintroduction to Mongolia: status and outlook. WAZA 366:3

Wang R, Dearing JA, Langdon PG, Zhang E, Yang X, Dakos V, Scheffer M (2012) Flickering gives early warning signals of a critical transition to a eutrophic lake state. Nature 492:419–422

Webb JC, Goodenough AE (2018) Questioning the reliability of "ancient" woodland indicators: resilience to interruptions and persistence following deforestation. Ecol Indic 84:354–363

Webb JC, Goodenough AE (2021) Vegetation community changes in European woodlands amid a changing climate: a palaeoecological modelling perspective. Comm Ecol 22:319–330. https://doi.org/10.1007/s42974-021-00057-4

Webb JC, McCarroll J, Carpenter WS, Chambers FM, Toms P, Wood MJ (2017) Apparent lack of woodland and abundance of woodland indicator species: the role of humans, birds and rabbits on the changing vegetation of Skomer Island. Wales Archaeol in Wales 56:90–99

Western D (1989) The ecological role of elephants in Africa. Pachyderm 12:43–46

Whitehouse NJ, Smith DN (2004) "'Islands"' in Holocene forests: implications for forest openness, landscape clearance and "'culture-steppe"' species. Environ Archaeol 9:203–212

Whitehouse NJ, Smith D (2010) How fragmented was the British Holocene wildwood? Perspectives on the "Vera" grazing debate from the fossil beetle record. Quat Sci Rev 29:539–553

Williams JW, Grimm EC, Blois JL, Charles DF, Davis EB, Goring SJ, Graham RW, Smith AJ, Anderson M, Arroyo-Cabrales J, Ashworth AC (2018) The neotoma paleoecology database, a multiproxy, international, community-curated data resource. Quat Res 89:156–177

Williams SL, Perkins SE, Dennis R, Byrne JP, Thomas RJ (2020) An evidence-based assessment of the past distribution of golden and white-tailed eagles across wales. Conserv SciPract 2:e240

Willis KJ, Birks HJB (2006) What is natural? The need for a long-term perspective in biodiversity conservation. Science 314:1261–1265

Willis KJ, Araújo MB, Bennett KD, Figueroa-Rangel B, Froyd CA, Myers N (2007) How can a knowledge of the past help to conserve the future? Biodiversity conservation and the relevance of long-term ecological studies. Philos Trans R Soc Lond B Biol Sci 362:175–187

Wilmshurst J, Moar N, Wood J, Bellingham P, Findlater A, Robinson J, Stone C (2013) Use of pollen and ancient DNA as conservation baselines for offshore islands in New Zealand. Conserv Biol 28:208–212

Wingard GL, Bernhardt CE, Wachnicka AH (2017) The role of paleoecology in restoration and resource management—the past as a guide to future decision-making: review and example from the Greater Everglades Ecosystem, USA. Frontiers Ecol Evol 6(5):11

Winker K (2004) Natural history museums in a postbiodiversity era. Bioscience 54:455–459

Yao Q, Liu KB, Wu Y, Aragón-Moreno AA, Rodrigues E, Cohen M, de Souza AV, Farfán LM, Antinao JL (2021) A multi-proxy record of hurricanes, tsunami, and post-disturbance ecosystem changes from coastal southern Baja California. Sci Total Environ 796:149011

Ecological Indicators 84 (2018) 354–363

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Research paper

Questioning the reliability of “ancient” woodland indicators: Resilience to interruptions and persistence following deforestation

Julia C. Webb*, Anne E. Goodenough

School of Natural and Social Sciences, University of Gloucestershire, UK

ARTICLE INFO

Keywords:
Ancient woodland
Old-growth forest
Indicator species
Vascular plants
Conservation

ABSTRACT

Indicator species can provide invaluable insights into environmental conditions but robust empirical testing of their effectiveness is essential. Ancient woodland indicators (AWIs) are plant species considered indicative of sites that have been continuously wooded for a long period by virtue of poor dispersal ability and intolerance of non-woodland habitats. Many countries now utilise AWI species lists to classify ancient woodlands. Here we use a metastudy approach to test resilience of AWIs to interruptions and persistence following deforestation − and thus the robustness of using AWI lists − using a novel approach. We compare current AWI assemblage with woodland history based on pollen evidence at nine sites across the UK with a robustly-dated and spatially-precise palynological profile. Sites were split into: (1) proven continuous woodland; (2) previously interrupted woodland; and (3) previously but not currently wooded. Vegetation history was > 1000 years at most sites. Assessment of ancientness using AWIs agreed with palynologically-proven ancient woodland at two sites, including a species-poor woodland of previously-uncertain age. However, four interrupted woodland sites and three clear-felled sites supported extensive AWI floristic communities. This suggests AWIs are resilient to interruptions, possibly by remaining in the seed bank longer than expected, and persistent following deforestation. Persistence might be due to other species (e.g. heathland plants) acting as pseudo-canopy or because some AWIs are more tolerant of non-woodland locations than previously thought. We conclude that use of floristic AWIs alone in defining ancient woodland should be reviewed, especially where status links to planning policy and conservation prioritisation. We suggest species on AWI lists be reviewed under expert and local guidance and a system of weighting species based on their strict or strong affinity solely with ancient woodland be developed. The use of multi-taxa indicators is recommended to allow stakeholders globally to make informed decisions about ancient woodland status.

1. Introduction

Indicator species are organisms whose presence, prevalence or abundance is indicative of environmental conditions within a particular ecosystem. Use of indicator species is especially helpful when they allow inferences to be made about parameters that are difficult or expensive to measure directly (Landres et al., 1988; McGeoch 1998). While indicator systems can be based on individual species (e.g. using the abundance of a moss, *Hylocomium splendens,* to indicate heavy metal pollution in Alaska: Hasselbach et al., 2005), any system that uses one line of evidence is prone to atypical results. Accordingly, community-based approaches based on multiple species are usually more robust.

In woodlands, species communities can be useful in determining whether a site has been continuously wooded over a pre-determined time period and thus meets the threshold for being considered “ancient” (European terminology) or “old-growth” (American terminology). Ancient woodland is often regarded as having a higher conservation priority, and being worthy of greater protection, than recent woodland (Goldberg et al., 2007). This is because ancient woodlands support highly-specialized or disturbance-sensitive species that are frequently absent from recent woodlands (Peterken and Game, 1984; Rotherham, 2011). Many such species are rare or declining (Kimberley et al., 2013) and ancient woodland itself is often threatened and fragmented (Spencer and Kirby, 1992; Palo et al., 2013; Swallow and Goodenough, 2017). Ancient woodland is thus protected in most countries in planning legislation and land use policies (e.g. National Policy Planning Framework in UK).

Because of the value placed upon ancient woodland, and the way that it is imbedded within planning and conservation practice, it is necessary for ecologists to have an accepted demarcation between ancient and recent sites. The definition of what constitutes “ancient” or “old growth” woodland varies geographically based on climax

---

* Corresponding author at: Francis Close Hall, University of Gloucestershire, Swindon Road, Cheltenham, Gloucestershire, GL50 4AZ, UK.
E-mail address: jwebb@glos.ac.uk (J.C. Webb).

http://dx.doi.org/10.1016/j.ecolind.2017.09.010
Received 9 June 2017; Received in revised form 6 September 2017; Accepted 7 September 2017

community type and natural fire regime. In the Netherlands and coastal Canada, ancient woodlands are regarded as < 1850AD (Grashof-Bokdam, 1997; Wells et al., 1998) whereas ~1770AD is used in Denmark and ~1750AD in Germany (Petersen, 1994; Zacharias, 1994, respectively). In the UK, ancient woodlands are areas greater than 2 ha (Goldberg et al., 2007) that have been wooded continuously and relatively undisturbed since 1600AD in England/Wales and 1750AD in Scotland − i.e. > 400 calendar years before present (cal. BP) in England (Peterken, 1981; Rackham, 2003).

Even where accepted definitions of ancient woodland exist, there is often limited evidence upon which to draw when considering the ancientness status of a specific site (Rackham, 2003). Cartographic information can be useful for the last 150–200 years (occasionally more: good map coverage exists from the late 1700 s for Essex, UK: Chapman and André, 1777). Estate maps can be helpful but coverage is patchy (Rotherham, 2011). Historical records can sometimes to push documentary evidence back further but, even where records exist, information is often vague and locations can be hard to pinpoint accurately (Rackham, 2003). Examining the age of trees, either via dendrochronology (O'Sullivan and Kelly, 2006) or by measuring diameter at breast height (e.g. Rotherham et al., 2008), can be important but these methods are not without problem. Firstly, not all ancient woods support veteran trees due to frequent turn-over due to natural disturbance and regeneration, harvesting management, or because the local conditions do not favour development of over-mature specimens, possibly due to shallow earth or exposure. Secondly, the presence of veteran trees does not axiomatically mean woodland is ancient as it could substantially post-date the veterans.

1.1. Ancient Woodland indicators

In 1898, Buchanan White first noted that old pine forests of Scotland supported plant species that were much less common in recent or disturbed woods (e.g. Twinflower *Linnaea borealis*, Creeping Lady's-tresses *Goodyera repens* and One-flowered Wintergreen *Moneses uniflora*). More systematic study some 76 years later suggested this was part of a more widespread trend with ~30% of woodland plant species seeming to be consistently found at ancient woodland sites (Peterken, 1974). This led to a list of Ancient Woodland Species (AWS) being devised for the UK, with subsequent lists being developed for other countries including in the USA (e.g. Marcot et al., 1998) and countries in mainland Europe (e.g. Honnay et al., 1998; Schmidt et al., 2014; Wulf, 1997). Although those devising the different lists variously used expert opinion, ad-hoc field data, or systematic surveys (with or without statistical testing) the aim remained consistent, viz. identifying species that could be used as Ancient Woodland Indicators (AWIs).

There is an elegant simplicity about using the very species supported by ancient woodland to indicate ancient status (or otherwise) of woodlands. Given the weaknesses inherent in single-species indicator systems, inferences on ancientness are not based on presence of individual species but rather on the presence of multiple species using a community-based approach (Hermy et al., 1999; Kirby et al., 1998; Spencer, 1990). Because of the effects of geographic area, regional species lists have since been developed in many countries (e.g. Glaves et al., 2009). AWIs generally have similar life-history traits: they are disturbance-sensitive (Hermy et al., 1999; Honney et al., 2002), they are slow to colonise and have poor dispersal abilities (Nilsson et al., 2001; Nordén and Appelqvist, 2001; Peterken, 1974), and they are intolerant of non-woodland conditions as they need shelter and consistent humidity (Barkham, 1992). In terms of physical characteristics, many AWIs are short perennial species (Kimberly et al., 2013) and some have niches based on dead wood or other dead organic matter (Hodge and Peterken, 1998). Soil or other physical conditions might also need to be well established; for example, De Keersmaeker et al. (2004) has found the soil on land previously used for farmland is too immature to accommodate AWIs even when it becomes wooded.

Use of AWIs is widespread both in terms of woodland ecology research (e.g. Kimberley et al., 2013; Stefańska-Krzaczek et al., 2016) and practical conservation (e.g. Goldberg et al., 2007; Kirby and Goldberg, 2002). For this to be robust, it is vital that the AWI approach itself is robust. The initial compilation of AWI lists has largely been based on species presence within "known" ancient woodland but this rationale is somewhat circular (Spencer, 1990), especially if non-ancient (and indeed non-woodland) sites are not included for comparison purposes. Moreover, it is often very difficult for woodlands to be definitively known to be ancient and continuously wooded for 400 Cal. BP because of the above-mentioned lack of historical and cartographic evidence. This means that some sites typically regarded as ancient, and used to inform AWI lists, might actually be more recent or have a history of interruptions (Day, 1993).

1.2. Use of palynology in woodland ecosystems

The use of fossilised biological remains to reconstruct past environments is well established. This can involve assessing palynological evidence (pollen grains and spores) to identify environmental change (e.g.Tzedakis et al., 1997) and human influence (e.g. Bryant and Holloway, 1983). Interpreting palynological data requires an awareness of the ecological process that underpin pollen dispersal and preservation. Pollen grains, particularly those that are anemophilous (wind-dispersed), can travel long distances (Fægri and Iverson, 1964), which means that the pollen within natural successional layering of sediment at a site will be influenced not only by immediate vegetation but also the *regional* pollen signal; this is especially true at exposed sites such as peat bogs (e.g McCarroll et al., 2015). In forest hollows or wet ditches, however, the palynological profile is influenced less by pollen morphology (e.g. Xu et al., 2016) and more by the sheltered nature of the area. Only very local vegetation tends to be represented in the profile as the surrounding canopy will prevent pollen dispersing from the site or being introduced from elsewhere via air currents. Although pollen rain from external sources could constitute a very minor part of a palynological profile, pollen fossilised within a peaty woodland hollow largely records *local* vegetation history.

To date, palynological investigation within woodlands has focused upon reconstruction of plant communities to: (1) infer species history (Baker et al., 1978; Bradshaw, 1981); (2) understand the effect of humans on local landscapes (Oldfield et al., 2003); (3) inform restoration management (Brown, 2010; Grant and Edwards, 2008); or (4) explain apparent disconnects between current vegetation and soil type (Dimbleby and Gill, 1955). Palynological profiling has seemingly never been used at a multi-site scale to provide empirical data against which AWIs can be compared. The only study to attempt something similar is Ohlson et al. (1997), which compared epiphytic lichen and saproxylic fungi indicative of old-growth spruce forests with paleoecological records. Intriguingly, this found no relationship between the occurrence of 33 indicator species and the actual time since disturbance (300–2000 years). Rolstad et al. (2002) advocated undertaking a similar study for flora and highlighted comparing AWI communities to known (rather than inferred or supposed) vegetation history as being a research priority. Here, we use a metastudy approach to compare woodland history based on palynological evidence with current AWI species assemblage for sites across the UK to establish the resilience of AWI species to interruptions and their persistence following deforestation.

2. Methods

To inform the metastudy, sites were selected based on: (1) availability of a published, robustly-dated, palynological profile that was spatially precise and thus gave a record of local woodland continuity without being unduly influenced by the regional pollen signal and (2) presence of detailed contemporary vegetation data collected through modern primary surveys. Only sites where data were derived from

Fig. 1. Location of main study sites (black text) and additional sites (grey text).

experienced fieldworkers were included to reduce the chances of misidentifications; data also needed to be reasonably comprehensive to minimise false absences. Current woodland vegetation was ascertained from a variety of sources, including published literature and consultancy research reports, as well as records held by county records centres, county recorders, reserve managers or wardens, or regulatory bodies such as Natural England.

In total, nine main sites fulfilled the above criteria. These were widespread geographically (Fig. 1). Two island sites were represented: Shetland in Scotland and Skomer in Wales. In all cases, the pollen cores were taken from within the woodland (or former woodland) boundaries. Coring sites were damp hollows or ditches or, in two cases (Garbutt Wood and Gunnister), a small closed-system lake fully surrounded by woodland. All cores were complemented by radiocarbon dates, which was essential in order to compare woodland age to established thresholds for ancient woodland and to date any interruptions to woodland continuity. In some cases, radiocarbon dates were cross-calibrated with known events in vegetative history such as planting of *Pinus* (e.g. Derrycunihy Wood in Southwest Ireland; Mitchell, 1988) or elm decline (e.g. Sydlings Copse in Oxfordshire: Day, 1993). Two additional sites, Winding Stonard Wood in Hampshire and Oxborough Wood in Norfolk, were also evaluated but conclusions here were more limited because the cores were not dated (Fig. 1). Details of the sources of palynological information for each study site are given in Table 1.

The current vegetation at each site was compared to the master AWI list compiled by Glaves et al., (2009), which brought together lists in Rackham (2003) and 37 regional lists, as well as additional species listed by Peterken (1974), or that occurred on the Northern Island or Durham AWI lists (Durham County Council, 2008; Woodland Trust, 2007). This master list included trees, shrubs, forbs, graminoids and non-flowering species (bryophytes and ferns). In addition, vegetation was specifically compared to the nearest local AWI list (for the relevant county if possible; if this did not exist the relevant regional list was used) and the strict/strong indicators listed by Rackham (2003). We

Table 1
Details of sites used in this metastudy and the sources of palynological information.

Study sites	County	Latitude and longitude	Study site (ha)	Source of palynological information
Derrycunihy Wood	Kerry	51.9933, −9.5572	136	Mitchell (1988)
Wistman's Wood	Devon	50.5812, −3.9576	3	Bradshaw et al. (2015)
Garbutt Wood	Yorkshire	54.2422, −2.2293	54	Blackham et al. (1981), Oldfield et al. (2003)
Sydlings Copse	Oxfordshire	51.7814, −1.1953	22	Day (1993)
Piles Copse	Devon	50.4441, −3.9094	50	Roberts and Gilbertson (1994), West (1997)
Johnny Wood	Cumbria	54.5199, −3.1611	37	Birks (1993)
Oxborough Wood	Norfolk	52.5857, 0.5462	23	Bradshaw (1981)
Hartlebury Common	Worcestershire	52.3323, −2.2582	90	Brown (1984)
Gunnister	Shetland	60.4515, −1.4124	6	Bennett (1997)
Skomer Island	Pembrokeshire	51.7376, −5.2960	292	Webb et al. (2017)
Winding Stonard Wood	Hampshire	50.8490, −1.4354	2	Dimbleby and Gill (1955)

recognise that using national lists is not ideal, but local lists were unequal in size and so it was impossible to directly compare sites in different regions without using national lists. Glaves et al.,(2009) also notes the robustness of local lists is questionable due to variable attitudes and awareness of those compiling the lists. By using both national and local lists, we avoided relying solely on local lists but also gained the benefit of local knowledge where appropriate. Where possible, we noted temporal fluctuations in specific AWIs within the palynology record but this was only feasible for species that could be identified to species level from pollen remains: *Hyacinthoides non-scripta* (bluebell), *Anemone nemorosa* (wood anemone), and *Mercurialis* (dogs mercury) (Brown 2010; Day, 1993)).

There has been discussion in the literature (Glaves et al., 2009; Honnay, 1998) on the threshold criteria for the minimum number of plant species present to denote an area as ancient woodland. Glaves et al., (2009) notes that the recommendation in parts of South Wales is areas with ≥12 AWIs are classified as ancient, but in Bedfordshire this drops to ≥5 AWIs. Honnay et al. (1998) showed 25–27 plant species were needed to discriminate between ancient and short continuity cover. Any threshold values will axiomatically link to woodland size and thus survey area has been noted in Table 1.

The sites studied in this paper fall into three main groups: (1) sites with a palynologically-proven continuous history of woodland (Fig. 2); (2) sites that are currently wooded but that have also had interruptions to their woodland coverage as revealed by changes in the species represented in the pollen core (Fig. 2); and (3) sites that are not currently wooded, and in some cases have not been for some time according to pollen cores, but that currently support an AWI species community (Fig. 2). These scenarios will be considered in turn to critically examine any difference in AWIs relative to woodland continuity and thus the resilience of AWIs to interruptions and their persistence following deforestation. The number of AWI species found at each site in different vegetation groups is given in Table 2. Full details of the exact species found at each site is given in the supplementary material.

3. Results and discussion

3.1. Palynologically-proven continuous ancient woodland

Derrycunihy Wood in Southwest Ireland lies in the Killarney valley and comprises part of the greatest extent of ancient woodland in Ireland. The current woodland is dominated by *Quercus petrea* with frequent *Betula pubescens* and *Sorbus aucuparia* and an open understory of *Ilex aquifolium* (Kelly, 1981). The woodland also has a rich Atlantic bryophyte flora (Ratcliffe 1968). The pollen history, based on core taken from a wet hollow within the wood by Mitchell (1988), suggests that the site was characterized by stable closed canopy woodland dominated by *Betula*, *Pinus* and *Quercus* between around 4850 Cal. BP (radiocarbon date) and 2400 Cal. BP (interpolated date). Around 2400 Cal. BP, the canopy started to open up considerably with increasing *Plantago*, suggesting disturbed ground, and also *Pteridium*. There was also increased evidence of charcoal, suggesting fire clearance, at this time. The canopy started to close once more around 1290 Cal. BP but the woodland seems to have remained more open than previously for some considerable time before becoming denser towards the present day. There is continued evidence of fire throughout this time as well as, latterly, a decrease in floristic diversity that Mitchell (1988) ascribes to intensive sheep grazing since the 1900s. Palynological evidence,

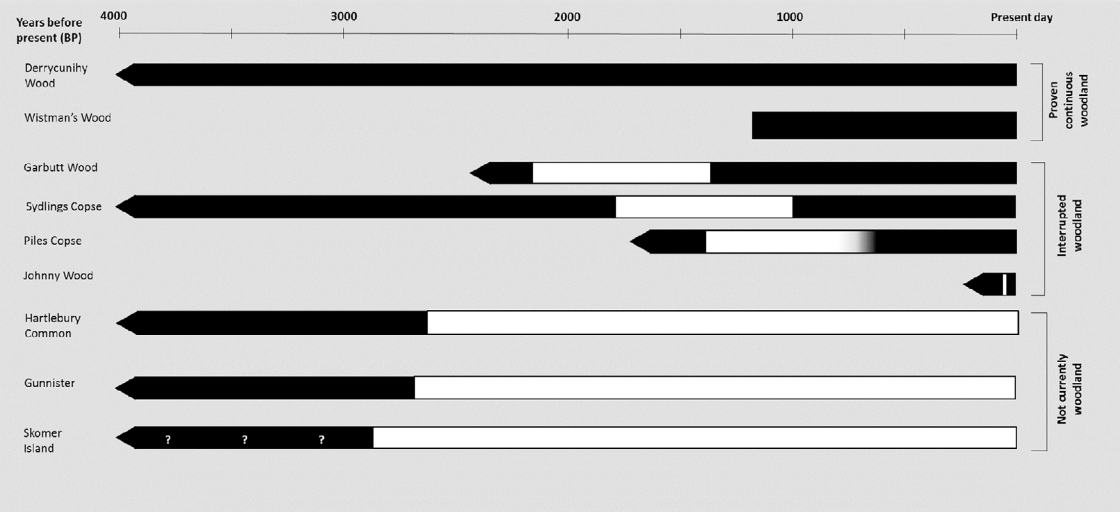


Fig. 2. Timeline of woodland continuity for each study site. Black bars indicates proven woodland coverage, except where overlain by a question mark. White bars indicate periods of woodland interruption or clearance. Instances where the sediment core continues beyond dated horizons are indicated with a left arrow.

Table 2
Ancient woodland indicator species presence at study sites as determined using Glaves et al. (2009), The Woodland Trust (2007), Durham County Council (2008) and Peterken (1974). Species with strict or strong association to ancient woodland as per Rackham (2003).

Plant type	AWI type	Proven continuous woodland		Interrupted woodland					Not currently woodland			Woodland on old heath
		Derrycunihy Wood	Wistman's Wood	Garbutt Wood	Sydlings Copse	Piles Copse	Johnny Wood	Oxborough Wood	Hartlebury Common	Gunnister	Skomer Island	Winding Stonard Wood
Trees	Local	0	0	3	2	0	0	0	0	0	1	0
	Total	3	5	8	3	2	4	2	3	0	2	2
Woody	Local	0	0	1	0	0	0	0	0	0	1	0
	Strict/ strong	0	0	0	0	0	0	0	0	0	0	1
	Total	5	6	9	7	2	5	2	2	2	5	6
Forbs	Local	2	0	10	8	0	2	3	1	0	9	0
	Strict/ strong	2	1	3	7	1	3	0	0	0	0	1
	Total	6	6	50	51	2	23	9	11	6	52	3
Graminoid	Local	2	1	0	9	0	2	0	1	1	1	0
	Strict/ strong	0	0	0	5	0	2	0	0	0	0	0
	Total	3	5	2	14	2	5		4	5	7	2
Non-flowering	Local	1	0	0	0	0	0	0	0	0	1	0
	Total	11	12	3	No data	4	9	No data	1	7	8	No data
Overall	Local	5	1	14	19	0	4	3	2	1	13	0
	Strict/ strong	2	1	3	12	1	5	0	0	0	0	2
	Total	28	34	72	75	12	46	13	21	20	74	13

therefore, suggests that this site has been continually wooded for at least 5000 years albeit with a long history of human influence. Contemporary vegetation records show that the site has a good AWI community with 28 AWIs in total of which 7 are locally-listed. Although there are AWIs in all vegetative groups, the importance of the non-flowering species is striking with 11 species of AWI bryophyte or fern. Two strong AWIs are present: *Oxalis acetosella* and *Lysimachia nemorum*. Derrycunihy thus exemplifies the range and diversity of AWIs present at a site with continuous woodland coverage, which has been proven objectively rather than simply assumed or inferred from documents, and acts as a baseline against which other sites may be compared.

While Derrycunihy is reasonably species-rich, some woodland sites are more homogeneous and thus relatively species-poor. Inferring habitat age based on AWI species could be problematic at such sites for two reasons. The first issue is that ancient woodland sites often have a greater floristic diversity than more recent sites (Dzwonko, 1993; Jacquemyn et al., 2001) and so potential ancient sites could be missed if initial assessment is simply based on plant richness without simultaneous consideration of other evidence. The second issue, somewhat paradoxically, relates to one of the normal strengths of AWI: that is a community-based approach. As noted above, community-based indicator systems are usually more robust than single-species indicators, however, their Achilles heel is that they can be hard to apply if the species community is itself impoverished. It is thus possible that, in some cases, there might be insufficient AWIs to reach the accepted thresholds for declaring a site to be ancient (Glaves, 2009; Honnay et al., 1998).

An example of a species-poor woodland is Wistman's Wood in Devon, a National Nature Reserve that is characterized by stunted *Quercus robur* and exposed granite boulders. There is very little ground flora but there is a diverse bryophyte assemblage (Proctor, 1962; Simmons, 1965). The site has typically been considered to be ancient based upon an assumption of woodland continuity (Coppins and Coppins, 2005) and its landscape context as a remnant patch. However, doubt was cast on the site's woodland age by Coppins and Coppins (2002), who studied the lichen species indicative of old growth woodlands using the Revised Index of Ecological Continuity (RIEC) system (Rose 1976, Rose and Coppins 2002). Wistman's Wood was given a REIC score of 10, which did not support old growth status (Coppins and Coppins, 2002). To provide a definitive answer, Bradshaw et al. (2015) took a pollen core from a pocket of humus accumulation amongst the boulders; this had a basal date of 1200 Cal. BP. The pollen evidence suggests that between 1200 and 900 Cal. BP the woodland was relatively diverse, being dominated by *Quercus* (as currently) but also with *Alnus*, *Betula, Corylus,* and *Fraxinus* represented. The presence of notable amounts of *Calluna* suggests that the woodland was quite open. The diversity of arboreal pollen decreased over time to 170 Cal. BP where the woodland became much more homogenous. Charcoal and *Plantago* were found throughout, suggesting human influence. Based on this palynology study, and despite times when the arboreal pollen concentration dipped to ~25%, the site appears to have been wooded continually for at least the last 1200 years. This interpretation − that such dips were periods of more open woodland rather than such dips being woodland clearance − is strengthened by there being no other woodland in the immediate area, such that there would be little regional arboreal pollen signal. The site now contains 24 AWI species, including 6 non-flowering species and one strong AWI indicator: *O. acetosella*. This suggests that although lichen diversity in the woodland might be insufficient for the site to be deemed old growth, the plant AWI assemblage would meet accepted criteria. This reinforces the value of using multiple taxonomic groups in ecological indicator frameworks, as well as providing a baseline with which other species-poor woodlands can be compared.

3.2. *Interrupted woodland: AWI resilience*

Undertaking palynological work is a powerful way of identifying and dating historical interruptions to woodland coverage. Such interruptions are characterized by changes in the species represented in the pollen core; in particular a sudden and substantial decrease in arboreal pollen and a simultaneous increase in Poaceae and/or Cyperaceae, as well as an increase in species characteristic of disturbance such as *Potentilla*.

Garbutt Wood in the Northeast of England is a Site of Special Scientific Interest (SSSI) and a Yorkshire Wildlife Trust nature reserve. It is dominated by *Quercus* and *Betula* with a *Pteridium* field layer.

Palynological work has focused on cores from Goremire Lake, a water body approximately 150 m wide and 300 m long that is fully encircled by the woodland. The lake has a closed basin hydrological system, thereby minimizing the entry of pollen entry from wider catchment. Although some overland flow is possible, any effect is considered minor (Blackham et al., 1981; Oldfield et al., 2003), such that pollen recruited into lake sediment is primarily from direct lake catchment (i.e. Garbutt Wood). The site was first cored by Blackham et al. (1981). This study showed that, contrary to local belief, the site was unlikely to have been continually wooded but instead had a period of clearance characterized by a reduction in arboreal pollen and a simultaneous increase in non-arboreal pollen. However, this core was undated so the timing and duration of the interruption was unknown. To address this, analysis of a dated core (spanning approximately 3500 years) was undertaken by Oldfield et al. (2003). This showed an interruption to woodland coverage between 2200-1400 Cal. BP. Before this, the site was characterized by closed canopy woodland dominated by *Alnus*, *Colylus*, *Betula*, *Fraxinus*, *Quercus*, *Salix* and *Ulmus*. During the interruption, total arboreal pollen decreased to ~10% and there was a corresponding peak in Poaceae, *Plantago* and *Pteridium*. Following this interruption, the woodland regenerated and has remained continuously wooded since (albeit with a long period of what seems to be more open woodland between 800 and 200 Cal. BP). Today, the site has an extremely rich AWI species assemblage, with 72 species in total distributed across all species groups. Three strong AWIs are present: *A. nemorosa*, *O. acetosella and L. nemorum.*

The comparison of palynology and current AWI floristic community at Garbutt Wood suggests that AWI species assemblages can be resilient to an historical interruption in woodland coverage, possibly because species remain in the seedbank during interruptions or are able to grow in non-wooded conditions during the interruption. However, in the case of Garbutt Wood, the length of time between the interruption and the current day is substantial – around 1400 years – such that AWIs could have recolonised in the intervening period. Analysis of sites with more recent interruptions, including Sydlings Copse in Oxfordshire, Piles Copse in Devon and Johnny Wood in Cumbia, would thus be useful.

Sydlings Copse is a SSSI managed by the Berkshire, Buckinghamshire and Oxfordshire Wildlife Trust. It is one of the most well-documented woodlands in the UK due to its location within the royal mediaeval forest of Stotover and Stowood. Written records and cartographic evidence suggest the site was wooded in 1660 CE (340 Cal. BP) when it was listed as part of a ~240 ha wood (Day, 1993). Before this date, though, vegetation history is less certain, making this site a good candidate for palynological study. Extraction of a pollen core from a woodland ditch by Day (1993) produced a pollen sequence spanning approximately 9500 years, but accurate dates were only possible in the upper section (earliest radiocarbon date = 3820 Cal. BP). The very oldest deposits show, as expected, an initially herb-dominated community with increasing *Corylus* and *Pinus sylvestris*. By the first radiocarbon date of 3820 Cal. BP and up until 1800 Cal. BP, the profile shows evidence of woodland continuity with *Corylus, Tilia, Alnus* all dominant or co-dominant at different times, together with a presence of *Ulmus* and *Quercus*. This period was followed by an abrupt change between 1800 and 1000 Cal. BP when arboreal pollen was replaced almost completely by herbs and *Pteridium* with just a small amount of *Salix* (at maximum 20%) for a period of 800 years. This was followed by a period of woodland regeneration from 1000 Cal. BP with continuous woodland present from this time to the present day. The contemporary woodland records show a plant community with a rich AWI species assemblage. In total, 75 AWIs are present, including 14 locally-listed and several strong indicators: *A.nemorosa*, *Campanula trachelium, Euphorbia amygdaloides, Hypericum hirsutum*, *O. acetosella*, *L. nemorum, Paris quadrifolia*, *Carex pallescens* (identified as a strict AWI by Rackham (2003)*), Carex remota, Luzula pilosa, Melica uniflora* and *Milium effusum.*

Palynological evidence from a different site, Piles Copse in Devon, points to there being an interruption to woodland continuity at a similar date to Sydlings Copse, but it in a very different type of woodland. Similar to Wistman's Wood (see above), Piles Copse has a species-poor community. Many of the trees are stunted and the ground flora is sparse, while the bryophyte community is both rich and abundant (Ratcliffe, 1968). Initial speculation as to the origins of the woodland (Simmons, 1965) highlights the fact the woodland is an isolated patch in an otherwise non-wooded landscape. Early palynologocal work in the 1990s took cores from flush bogs immediately adjacent to the site (Roberts and Gilbertson, 1994). This suggested that there had been a lengthy interruption to woodland within the last 2500 years. However, as the core was undated and taken from outside the woodland, conclusions remained rather non-specific. A second study (West, 1997) provided dated evidence from two cores within the woodland using radiocarbon analysis. This work suggested that < 600 Cal. BP, there was mixed woodland characterized by *Quercus, Corylus, Betula* and *Ulnus*. At around 600 Cal. BP, the arboreal pollen decreased to a trace occurrence (< 1% of the total pollen sum) with a simultaneous decrease in *Polypodium* suggesting complete woodland clearance. At the same time, there was sharp rise in Poaceae, Cyperaceae, and *Potentilla*. Sphagnum moss also disappeared at this point, suggesting drier conditions (McCarroll et al., 2015). The hiatus in woodland spanned a period of 15 cm (from a total 30 cm) in the sediment record younger than 600 Cal. BP, with woodland regeneration only showing within the last 15 cm of sediment accumulation. This suggests that the site could be borderline in terms of whether it is its actually ancient based on traditional criteria (making the very large assumption of sediment accumulation being temporally consistent and not affected by erosion, woodland regeneration would have started at 300 Cal. BP, whereas the ancient woodland threshold is 400 Cal. BP). Despite having a very species-poor community, the site currently supports 12 AWI species, including the strong indicator of *O. acetosella.* This is half the number found at Wistman's Wood, a similar woodland in terms of location, structure, species richness, and species community but which has been wooded continually for the last ~1300 years rather than for the last ~300-500 years.

A site with an even more recent interruption, but high conservation value, is Johnny Wood in Cumbria, Northwest England. This is a SSSI, part of Borrowdale Woods Special Area of Conservation, and lies within the Lake District National Park. The site is covered by broadleaved woodland dominated by *Q. petrea* and is noted for its bryophyte community. Palynological investigation of a short core taken from a woodland hollow by Birks (1993) showed that the site supported mixed deciduous woodland prior to 140 Cal. BP (basal date unknown; 12 cm of sediment accumulation < 140 Cal. BP). This woodland was species-rich, with *Alnus, Betula, Corylus, Fraxinus*, and *Quercus* with low levels of *Salix, Taxus, Tilia,* and *Ulmus* and an understory of Poaceae and Polypodiaceae. Between around 140 and 100 Cal. BP, there is a clear interruption that has been interpreted as localized woodland clearance by Bradshaw et al. (2015) with a residual regional signal. This is characterized by a rapid decline in all arboreal pollen and a substantial increase in Poaceae, *Calluna* and *Potentilla.* Following this period, the woodland recovered but with a different composition to before the interruption, with *Alnus*, *Corylus* and *Fraxinus* all present in trace amounts but other species, including *Acer, Larix* and *Ilex*, appearing for the first time and *Sorbus* having a greater presence than before the clearance, albeit only marginally. The time of this interruption actually means that, by conventional criteria, this site should not be regarded as ancient. The current AWI species community, however, is rich and diverse, with 46 species present including the strong indicators of *C. remota, L. pilosa, L. nemorum* and *O. acetosella,* indicating the ecological value of the site.

The concept of persistence in seed banks is supported by consideration of another site: Oxborough Wood in Norfolk. A 3 m core was taken from a woodland hollow (Bradshaw, 1981) but was undated so interpretation is challenging. The site is currently a plantation: *Populus* was planted around 1930 and then clear felled in all but the wettest areas in 1953, at which time *Pinus* was planted. The site appears to have been continuously wooded from the time of the bottom of the core (313 cm) until 62 cm depth. Within this period, the woodland composition changed with shifts in the dominant species (*Betula* followed by *Pinus/Corylus,* and finally *Quercus, Alnus* and *Tilia*) but continuity remained. After this time, arboreal pollen decreased to ~15% of the total pollen sum, which Bradshaw (1981) interpreted as a period of clearance. At this time, Poaceae and Cyperaceae become dominant, with *Plantago*, Polypodiaceae and *Pteridium* as well as a background regional signal of *Corylus*, *Alnus* and *Quercus*. Based on documentary evidence, it is likely that the plantation was created sometime between 1797, when the site was not marked as woodland on a detailed map of the area, and 1845 when it was listed on a county audit as a plantation (Bradshaw, 1981). The current plantation woodland contains 13 AWI species, including two that are local indicators. Given the comparatively short about of time (~200 years) between the likely timing of woodland planting (following a period of clearance) and the present day, it seems most likely the AWIs currently present at the site remained during the hiatus, either growing or in the seed bank, since recolonization of all 13 AWI species presently found within that time is unlikely.

3.3. Cleared woodland: AWI persistence

The above sites are examples of areas that are currently wooded but have had interruptions to woodland coverage and provide useful insights in AWI *resilience*. A somewhat different scenario is afforded sites that have been wooded in the past but that do not currently support woodland vegetation. Consideration of such sites allows insights into AWI *persistence* in non-wooded environments.

Hartlebury Common, Worcestershire, is dominated by *Calluna vulgaris*, *Erica cinerea* and *Ulex gallii* with patches of *Pteridium*, grassy areas, and occasional *Betula* and *Quercus* seedlings and saplings. The site is a SSSI by virtue of being one of the most important areas of dry dwarf shrub heathland in central England. Analysis of a pollen core taken from a wet depression, however, suggests that the site used to be wooded (Brown, 1984). The pollen record shows that between 9710 and 2610 Cal. BP, the site was dominated by *Pinus, Ulmus, Corylus,* and *Betula*, with some *Quercus* and *Alnus.* The dominance of *Pinus* and *Corylus* decreased in the latter part of this period. After around 2610 Cal. BP, the vegetation changed: arboreal pollen decreased to ~15% and the previously-dominant *Pinus* and *Ulmus* were lost. Instead, Poaceae and Cyperaceae became dominant with *Erica* increasing within the pollen profile over time with lower levels of *Betula* and *Quercus* remaining. This suggests a change from woodland to a grassland/heathland matrix with scattered *Betula* and *Quercus*, much as per the present day. Despite the site not being wooded currently, and seemingly not wooded since 2610 Cal, BP, it still supports 21 AWI species, the majority of which are non-woody.

Gunnister, on the main isle of Shetland, currently supports mixed grassland and wet heathland dominated by Cyperaceae, *Sphagnum* and *C. vulgaris*, with abundant Poaceae (especially *Nardus stricta*). As a whole, Shetland today is almost completely devoid of trees (Johansen, 1975; Scott and Palmer, 1987) although evidence of tree roots, woody debris and palynology suggest that the islands were once extensively wooded (Bennett et al., 1997; Birnie, 1984). Palynological analysis of Gunnister by Bennett et al. (1993) suggests that this particular site was wooded until around 2685 Cal. BP. Before this time, arboreal pollen was abundant and diverse, initially (from 9405 Cal. BP) comprising *Betula*, *Corylus* and *Pinus*, with *Ulmus*, *Quercus, Salix* and *Alnus* entering the pollen record between 9405 and 2685 Cal. BP. Since 2685 Cal. BP, grassy heathland has predominated, with *Calluna* and Cyperaceae increasing over time and Poaceae occurring at a consistent ~20-25% of the total pollen sum. *Sphagnum* and Cyperaceae have increased, suggesting that the area has become wetter over time. Charcoal, which previously only occurred infrequently and at negligible levels, increased from 2685 Cal. BP, suggesting burning and human disturbance after this date. Despite there currently being no trees at the site (or indeed regionally), and palynological evidence suggesting the area was last wooded over 2500 years ago, the site still supports 20 AWI species. This is split approximately equally between forbs, grasses and non-flowering species.

The situation is similar, but even more dramatic, on another island: Skomer, off the Pembrokeshire coast in Southwest Wales. This currently has a maritime vegetation community, which comprises *Crithmum maritimum*, *Spergularia rupicola*, *Festuca rubra*, *Armeria maritima*, *Holcus lanatus*, and *Rumex acetosa*. There are also areas of *Calluna* and *Pteridium*. There is no woodland on the island, which is surrounded by sea and is 8 km from the nearest area of trees on the mainland. However, there is evidence that trees grew between the current west coast of Wales and offshore islands before isostatic rebound in the mid-Holocene when the area was a land bridge (Evans, 1986, Bell, 2007, UKHO, 2011). Moreover, the name of a nearby island, Skokholm, is thought to mean “wooded isle” (Morgan, 1887). The presence of abundant *H. non-scripta* has been previously interpreted as evidence of past woodland on the island (e.g. Evans, 1986). Palynological study from a wet depression on the island by Webb et al. (2017) has provided a detailed profile of the vegetation community between 2840 Cal. BP and the present day. Within this period, the vegetation community has alternated between heath dominated by Ericaceae and grassland dominated by Poaceae, with Cyperaceae and *Rumex* appearing variably throughout and arboreal pollen (*Alnus, Betula, Fraxinous, Pinus, Quercus, Tilia* and *Ulmus*) accounting for ~5% of the total pollen sum. This is likely to be a minor regional signal, especially given the range of species involved and the very small quantities represented. Given the submerged trees between the mainland and island, it is likely that woodland was present before 2840 Cal. BP but it was not possible to establish this definitely due to the Mesolithic period being absent in the core record. Interestingly, though, *Hyacinthoides* was present in the core before 2840 Cal. BP. This insect-pollinated species has been only rarely recorded in sediments in northwest Europe and is generally under-represented (Waller et al., 2012). Modern vegetation surveys have revealed an extremely rich AWI flora, with 74 species represented. This makes Skomer Island second only to Sydlings Copse in total AWI species present, which has been wooded continuously since 1000 Cal BP (75 species), and slightly above Garbutt Wood, which has been wooded continuously since 1400 Cal BP (72 species).

It is notable that all three sites contain heathland plants, especially *Calluna* and *Pteridium*, which are present at three sites and two sites, respectively. It is possible that species such as these act to form a surrogate woodland canopy. This possibility is also supported by analysis of an undated core at Winding Stonard Wood in the New Forest, Hampshire by Dimbleby and Gill (1955), which showed that arboreal pollen was only found in high quantities (> 20%) in the top 5–10 cm of the core; below that Ericaceae was dominant almost to the exclusion of any other taxa (~70%). It seems likely that this site has only fairly recently supported woodland and yet it supports 13 AWI species including two strong species: *Ruscus aculeatus* and *O. acetosella*. This suggests either that these species have colonised unusually rapidly or, as seems more probable, were able to exist on the site before the current woodland was present.

4. Conclusions and recommendations

This study provides the first account of palynology being used to critique the concept and robustness of AWIs across multiple sites. Analysis of vegetation at the species-rich Derrycunihy Wood and the species-poor Wistman’s Wood lends support to the concept that sites *proven* to support ancient woodland based on palynology (rather than being declared ancient based on patchy, inaccurate or imprecise historical records or cartographic evidence), can support good AWI species communities. This remains the case even if the woodland is species-poor, although AWI richness is reduced. However, the high number of AWIs at sites with interrupted woodland – Garbutt Wood, Sydlings Copse, Piles Copse, Johnny Wood and Oxborough Wood – suggests that AWI species: (1) are more resilient to interruptions than often believed; or (2) recolonise more quickly than expected following interruptions. Moreover, analysis of three clear-felled sites – Hartlebury, Gunnister and Skomer – shows that prominent, and sometime rich, AWI communities can occur despite sites not having been wooded for at least 2700 years. This indicates that some, possibly many, AWI species have greater tolerance of non-woodland conditions than is often believed and thus a greater persistence following deforestation.

As regards resilience to interruptions, it is possible that some species are surviving in the seed banks during woodland hiatus. This conjecture is supported by the presence of *Mercurialis perennis* pollen – one of the very few AWI species that can be identified to species level in a core using pollen morphology – in the palynological profile of Sydlings Copse (Day, 1993). The species occurs sporadically after the interpolated date of 3600 Cal. BP whenever arboreal pollen is abundant, is absent during the woodland interruption, and finally reappears at about 600 Cal. BP after an absence of 700 years. For other AWI species, there is evidence to indicate that AWIs can (re)colonise quickly. For example, also at Sydlings Copse, *A. nemorosa* has only occurred within the last 600 years (i.e. after the hiatus), suggesting it might have colonized at this time. However, as the preservation of *A. nemorosa* pollen is unknown we cannot totally discount the possibility that is not present < 600 Cal. BP due to degradation of the fossilised pollen grain rather being truly absent from the core. With any of the interrupted sites, it is important to note that the temporary decreases in the abundance of arboreal pollen could feasibly be driven by a period of active coppice management rather than a genuine woodland interruption. However, the impact of coppicing on pollen records in woodland settings has been considered by Waller et al. (2012) and Bunting et al. (2016) and concluded that coppicing has little or no impact on pollen production. We can thus be confident that periods interpreted as woodland interruptions are likely to be just are clearance events, even those that are very short (e.g. Oxborough and Johnny Wood). This interpretation is strengthened by the fact that all tree species are lost from the pollen record during these times rather than simply the species that would typically be coppiced.

Regardless of past woodland stability, previous interruptions, or contemporary clearance, the number of AWIs in any species community links to its overall species richness. For example, the interrupted species-poor woodland at Piles Copse supported just 12 AWI species in comparison with interrupted species-rich sites, Sydlings Copse and Garbutt Wood, which support 75 and 72 AWIs, respectively. It was also interesting to note that although the area of a site might influence species number (and thus AWI number and likelihood of ancient woodland classification), a simple calculation of total number of AWI species divided by the size for each of our locations ranked Skomer (currently non-wooded) similarly to Derrycunihy Wood (a proven continuously wooded site). This suggests that although Swallow and Goodenough (2017) note that edge sites yield fewer AWI species, and therefore imply that small fragmented ancient woodlands support fewer AWI species, the overall species richness might be a bigger influence.

This research suggests that defining ancient woodland areas needs to be revisited. Points for consideration are:

1) **Utilising palaeoecological evidence**: This study has demonstrated that palynology can provide robust data on woodland history continuity. When coupled with radiocarbon dating, any interruptions can be dated to provide objective empirical evidence upon which to based ancientness classification decisions. However, palynological profiling is by no means a "silver bullet": the amount of time needed to process a core, identify and count pollen within the different layers, and obtain radiocarbon dates is very considerable. The process also requires specialist laboratory facilities and substantial experience. As such, it is unlikely that palynological profiling will become a regular feature of ancientness assessment – except perhaps at the most important or complex sites – and it is thus vital to improve the current AWI system by considering the points below.
2) **Weighting AWI species to create a biotic index**: As many species currently designated as AWIs have been shown in this study to occur in non-woodland habitats, it would be prudent to review lists to weight species typically only found in continuously-wooded areas more highly than those that can be found elsewhere. This concept is similar to highlighting strict/strong AWIs (as per Rackham, 2003), but could be developed by explicitly factoring weighting into AWI lists to create a numerical biotic index. Such an index might be similar, for example, to Chandler's score for invertebrates to assess water quality (Chandler, 1970) and could replace the current AWI biosurvey approach, which is based on simple species lists. In essence, the species rated most highly would be those that occur exclusively in continuous ancient woodland. Based on the data in this study, very few AWIs species occurred exclusively in such sites relative to interrupted woodland or recently-cleared woodland. The two exceptions were *Atrichum undulatum* and *Salix aurita*, but this was probably is more to do with site-specific environmental conditions than the fact that these are true indicator species. However, several of the species occurred only in continuously-wooded and interrupted woodlands. Some of these species had already been identified by Rackham (2003) as strict or strong AWI species and included *E. amygdaloides, H. hirsutum, L. nemorum* and *P. quadrifolia*, but others are new including *Lysimachia vulgaris, O. acetosella, Stellaria holostea* and *Valeriana officinalis*. Such species could be given a high score in a putative AWI biotic index. Species that rely on branched rhizomes for reproduction are thus very slow dispersers such as *A. nemorosa* (Brunet and von Oheimb, 1998), but which appear tolerant of periods of disturbance based on their presence in our interrupted woodland sites, could be given an intermediate score. Finally, species with a low habitat specificity, such as *Galium saxatile* and *Lonicera periclymenum*, would be given a low score, together with species displaying persistence traits noted by Peterken (1974) and Vallet et al. (2010): higher specific leaf area, rosette or semi-rosette form, reliant on seed dispersal (particularly 'light' seeds) rather than underground rhizome regeneration, affinity for base-rich and fertile soils, and short life-spans.
3) **Reverse indicators**: To date, the focus of classifying between ancient and recent woodland has been based upon species that occur in the former. Another option would be to develop lists of species that occur predominantly in recent woodlands (e.g. species that are disturbance specialist) on the basis that, if these were absent from a site, it might indicate that a site was ancient. This could either become a companion list to the AWI list or the concept could be integrated into a numerical biotic index using negative weightings. Such species might include *Chamaenerion angustifolium, Plantago lanceolata* and *Urtica dioica.*
4) **Threshold number of species:** Given the high number of AWIs found at non-ancient sites, we suggest that the number of species present at a site before it is designated ancient should be increased. Consideration should also be given to having a relative threshold (e.g. 10% of species present at a site being AWIs) rather than an absolute threshold. To some extent, this would also address the current inconsistency between local lists and thus the spatial variation in the ease with which a site can be designated as ancient. For example, the Leicestershire and Rutland list contains 18 AWIs whereas Derbyshire has 123 (Glaves, 2009) but the two areas are of similar size (2.2 km^2 and 2.4 km^2, respectively). Although some of this variation will be due to genuine differences, it would appear to be harder for a site to be designated as an AWI in some counties relative to others if only the local list is used. Even if this relative system is adopted, work to rebalance local lists is recommended to reduce spatial differences in ancientness assessment while retaining

the concept that some AWI species are important in a local context only (e.g. *H. non-scripta*, which is a genuine AWI in some areas, but associated with grassland, heath, scrub, hedge banks and sea cliffs in others: Preston et al., 2002).

5) **Moving from within-taxon to between-taxa indicators**: Finally, although within-taxon community indicator approaches are stronger than single-species approaches (diatoms, Rimet et al., 2015; lichens, Llop et al., 2012), we suggest that, in the case of ancient woodland, a between-taxa system should be considered. This might incorporate fungi, lichens and beetles, which have previously been used in isolation as ancient woodland indicators (e.g.Bredesen et al., 1997; Coppins and Coppins 2002; Humphrey et al., 2002; Sroka and Finch, 2006) to create a multi-taxa system, as suggested previously for Italy (Blasi et al., 2010). The value of this approach has already been highlighted in this study in the case of Wistman's Wood (see above). Such expansions would, of course, have implications on the amount of survey effort needed in future surveys, but given that cross-taxa data are already in existence for many sites, adding non-plant taxa into a numerical biotic index might at least be worthy of consideration going forward.

4.1. Final thoughts

Fundamentally, the aim of conservationists globally is to preserve ancient woodland for its unique biodiversity. Many of the vascular species that grow in these old growth forests have slow dispersal mechanisms and/or rely heavily upon the woodland structure for survival. Protection of these woodland settings is therefore essential for the continual success of these diverse and rich environments.

As pointed out by Rotherham (2011), defining exactly what AWIs actually 'indicate' is hugely important but is often overlooked. Many so-called indicators of ancient woodland sites might actually simply be plants of undisturbed ground. Indeed, it is notable that a number of the AWI species present at the three sites that are not currently wooded − Hartlebury, Gunnister and Skomer − have been found previously to be indicative of long-established habitats but not necessarily long-established woodland (Rackham, 2003). For example, *Conopodium majus, Orchis mascula,* and *Primula vulgaris* are all found on Skomer; all of which can be found in old grassland habitats. Moreover, some of species considered to be woodland specialists by virtue of their need for shelter and high humidity seem to be tolerant of non-woodland locations if these can be provided by a surrogate canopy (*Pteridium* on Skomer, for example). From a more philosophical perspective, it could also be argued that a high number of AWIs link to conservation value in two ways: (1) AWI species themselves are often rare and declining; and (2) their presence indicates an area is relatively undisturbed and non-fragmented, two factors that increase ecological value and conservation priority.

By critiquing the method of indicator species in this paper and demonstrating the weakness of the technique we are not attempting to reduce the number of woodlands currently assigned as ancient, nor are we suggesting that the species approach is not useful. However, it is vital that practitioners understand the limitations of the technique and to highlight the need for a standard approach to ancient woodland status allocation.

Acknowledgements

For their help in supplying vegetation data and site records, we thank Martin Longley, Andy Nisbet, Albert Knott, and Matthew Parker (Natural England), Claire Wood (Centre for Ecology and Hydrology), Phillip Whelpdale (Yorkshire Wildlife Trust), Helen Baczkowska and Emily Nobbs (Norfolk Wildlife Trust), Mark Bradfield (Berkshire, Buckinghamshire and Oxfordshire Wildlife Trust), Birgitta Büche and Ed Stubbings (Skomer National Nature Reserve wardens), Peter Jarvis (Birmingham Natural History Society), Gill Smith and Tom Denney (Ryedale Naturalists), Roger Maskew (Hartlebury Common Local Group) and John Howell. We also thank Oliver Moore for checking species and vernacular names.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2017.09.010.

References

Baker, C.A., Moxey, P.A., Oxford, P.M., 1978. Woodland continuity and change in Epping Forest. .Field Stud. 4, 645–669.

Barkham, J.P., 1992. The effects of management on the ground flora of ancient woodland Brigsteer Park Wood, Cumbria, England. Biol. Conserv. 60 (3), 167–187.

Bell, M., 2007. Prehistoric coastal communities: the Mesolithic in western Britain. CBA Research Reports.

Bennett, K.D., Boreham, S., Hill, K., Packman, S., Sharp, M.J., Switsur, V.R., 1993. Holocene Environmental History at Gunnister, North Mainland, Shetland The Quaternary of Shetland: Field Guide. Quaternary Research Association, Cambridge, pp. 83–98.

Bennett, K.D., Bunting, M.J., Fossitt, J.A., 1997. Long-term vegetation change in the Western and Northern Isles, Scotland. Bot. J. Scotland 49 (2), 127–140.

Birks, H.J.B., 1993. Quaternary palaeoecology and vegetation science − current contributions and possible future developments. Rev. Palaeobot. Palynol. 79, 153–177.

Birnie, J., 1984. Trees and shrubs in the shetland islands: evidence for a postglacial climatic optimum? Climatic Changes on a Yearly to Millennial Basis. Springer, Netherlands.

Blackham, A., Davies, C., Flenley, J., 1981. Evidence for late Devensian landslipping and late Flandrian forest regeneration at Gormire Lake, North Yorkshire. The Quaternary in Britain: Essays, Reviews and Original Work on the Quaternary Published in Honour of Lewis Penny on His Retirement. pp. 184.

Blasi, C., Marchetti, M., Chiavetta, U., Aleffi, M., Audisio, P., Azzella, M.M., Brunialti, G., Capotorti, G., Del Vico, E., Lattanzi, E., Persiani, A.M., 2010. Multi-taxon and forest structure sampling for identification of indicators and monitoring of old-growth forest. Plant Biosyst. 144, 160–170.

Bradshaw, R.H., 1981. Quantitative reconstruction of local woodland vegetation using pollen analysis from a small basin in Norfolk, England. J. Ecol. 941–955.

Bradshaw, R.H., Jones, C.S., Edwards, S.J., Hannon, G.E., 2015. Forest continuity and conservation value in Western Europe. The Holocene 25 (1), 194–202.

Bredesen, B., Haugan, R., Aanderaa, R., Lindblad, I., Økland, B., Røsok, Ø., 1997. Wood-inhabiting fungi as indicators of ecological continuity within spruce forests of southeastern Norway. Blyttia (Oslo) 54, 131–140 (in Norwegian with English summary).

Brown, A.G., 1984. The flandrian vegetational history of hartlebury common, worcestershire. Proceedings Birmingham Natural History Society 25 (2), 89–98.

Brown, A.D., 2010. Pollen analysis and planted ancient woodland restoration strategies: a case study from the Wentwood southeast Wales, UK. Veg. Hist. Archaeobot. 19 (2), 79–90.

Brunet, J., von Oheimb, G., 1998. Colonization of secondary woodlands by Anemone nemorosa. Nordic J. Bot. 18, 369–377.

Bryant, V.M., Holloway, R.G., 1983. The role of palynology in archaeology. Adv. Archaeol. Method Theory 6, 191–224.

Buchanan White, F., 1898. The Flora of Perthshire. (Edinburgh).

Bunting, M.J., Grant, M.J., Waller, M., 2016. Pollen signals of ground flora in managed woodlands. Rev. Palaeobot. Palynol. 224 (2), 121–133.

Chandler, J.R., 1970. A biological approach to water quality management. Water Pollut. Control 4, 415–422.

Chapman, J., André, P., 1777. A Map of the County of Essex from an Actual Survey Taken in MDCCLXXII,:LXXIII and MDCCLXXIV.

Coppins, A.M., Coppins, B.J., 2002. Wistman's Wood NNR, cSAC, (VC 3, South Devon): Desk Study − to Discover Relevant Information Relating to Past and Present Site History and Management and the History of Lichen Recording Prior to Carrying Out a Lichen Survey. English Nature, Exeter.

Coppins, B.J., Coppins, A.M., 2005. Lichens −The biodiversity value of western woodlands. Bot. J. Scotland 57 (1–2), 141–153.

Day, S.P., 1993. Woodland origin and 'ancient woodland indicators': a case-study from Sidlings Copse Oxfordshire UK. The Holocene 3 (1), 45–53.

De Keersmaeker, L., Martens, L., Verheyen, K., Hermy, M., De Schrijver, A., Lust, N., 2004. Impact of soil fertility and insolation on diversity of herbaceous woodland species colonizing afforestations in Muizen forest (Belgium). For. Ecol. Manage. 188 (1-3), 291–304.

Dimbleby, G.W., Gill, J.M., 1955. The occurrence of podzols under deciduous woodland in the New Forest. Forestry 28 (2), 95–106.

Durham County Council, 2008. The County Durham Landscape Guidelines: Woodland and Forestry. Durham County Council.

Dzwonko, Z., 1993. Relations between the floristic composition of isolated young woods and their proximity to ancient woodland. J. Veg. Sci. 4, 693–698.

Evans, J.G., 1986. Prehistoric farmers of skomer island: an archaeological guide. Nature Conservation. West Wales Trust, Dyfed, pp. 13–14.

Fægri, K., Iverson, J., 1964. Textbook of Pollen Analysis. Scandinavian University Books, Munksgaard.

Glaves, P., Handley, C., Birbeck, J., Rotherham, I., Wright, B., 2009. A Survey of the Coverage, Use and Application of Ancient Woodland Indicator Lists in the UK. . http://nrl.northumbria.ac.uk/3467/1/Glaves%2C%20Rotherham%2C%20Wright%2C%20Handley%2C%20Birbeck%20-%20A%20survey%20of%20the%20coverage%2C%20use%20and%20application%20of%20ancient%20woodland%20Report.pdf.

Goldberg, E., Kirby, K., Hall, J., Latham, J., 2007. The ancient woodland concept as a practical conservation tool in Great Britain. J. Nature Conserv. 15 (2), 109–119.

Grant, M.J., Edwards, M.E., 2008. Conserving idealized landscapes: past history, public perception and future management in the New Forest (UK). Veg. Hist. Archaeobot. 17 (5), 551–562.

Grashof-Bokdam, C., 1997. Colonization of Forest Plants: the Role of Fragmentation. IBN Scientific Contributions 5, Wageningen.

Hasselbach, L., Ver Hoef, J.M., Ford, J., Neitlich, P., Crecelius, E., Berryman, S., Wolk, B., Bohle, T., 2005. Spatial patterns of cadmium and lead deposition on and adjacent to National Park Service lands near Red Dog Mine Alaska. Sci. Total Environ. 348, 211–238.

Hermy, M., Honnay, O., Firbank, L., Grashof-Bokdam, C., Lawesson, J.E., 1999. An ecological comparison between ancient and other forest plant species of Europe, and the implications for forest conservation. Biol. Conserv. 91 (1), 9–22.

Hodge, S.J., Peterken, G.F., 1998. Deadwood in british forests: priorities and a strategy. Forestry 71 (2), 99–112.

Honnay, O., Degroote, B., Hermy, M., 1998. Ancient-forest plant species in Western Belgium: a species list and possible ecological mechanisms. Belgian J. Bot. 130 (2), 139–154.

Humphrey, J.W., Davey, S., Peace, A.J., Ferris, R., Harding, K., 2002. Lichens and bryophyte communities of planted and semi-natural forests in Britain: the influence of site type, stand structure and deadwood. Biol. Conserv. 107 (2), 165–180.

Jacquemyn, H., Butaye, J., Hermy, M., 2001. Forest plant species richness in small, fragmented mixed deciduous forest patches: the role of area, time and dispersal limitation. J. Biogeogr. 28 (6), 801–812.

Johansen, J., 1975. Pollen diagrams from the shetland and Faroe Islands. New Phytol. 75 (2), 369–387.

Kelly, D.L., 1981. The native forest vegetation of Killarney, south-west Ireland: an ecological account. J. Ecol. 69 (2), 437–472.

Kimberley, A., Blackburn, G.A., Whyatt, J.D., Kirby, K., Smart, S.M., 2013. Identifying the trait syndromes of conservation indicator species: how distinct are British ancient woodland indicator plants from other woodland species? Appl. Veg. Sci. 16 (4), 667.

Kirby, K.J., Goldberg, E., 2002. Ancient Woodland: Guidance Material for Local Authorities. English Nature, Peterborough.

Kirby, K.J., Reid, C.M., Isaac, D., Thomas, R.C., Watkins, C., 1998. The ancient woodland inventory in England and its uses. The Ecological History of European Forests. CAB International, Oxford, pp. 323–336.

Landres, P.B., Verner, J., Thomas, J.W., 1988. Ecological uses of vertebrate indicator species: a critique. Conserv. Biol. 2, 316–328.

Llop, E., Pinho, P., Matos, P., Pereira, M.J., Branquinho, C., 2012. The use of lichen functional groups as indicators of air quality in a Mediterranean urban environment. Ecol. Indic. 13, 215–221.

Marcot, B.G., Croft, L.K., Lehmkuhl, J.F., Naney, R.H., Niwa, C.G., Owen, W.R., Sandquist, R.E., 1998. Macroecology, Paleoecology, and Ecological Integrity of Terrestrial Species and Communities of the Interior Columbia Basin and Northern Portions of the Klamath and Great Basins. Interior Columbia Basin Ecosystem Management Project: Scientific Assessment. Report PNW-GTR-410. U.S. Forest Service, Oregon.

McCarroll, J., Chambers, F.M., Webb, J.C., Thom, T., 2015. Application of palaeoecology for peatland conservation at Mossdale Moor UK. Quat. Int. 432 A, 39–47.

McGeoch, M.A., 1998. The selection: testing and application of terrestrial insects as bioindicators. Biol. Rev. Camb. Philos. Soc. 73, 181–201.

Mitchell, F.J.G., 1988. The vegetational history of the Killarney oakwoods, SW Ireland: evidence from fine spatial resolution pollen analysis. J. Ecol. 76 (2), 415–436.

Morgan, T., 1887. Handbook of the Origin of Place-names in Wales and Monmouthshire. Available online (Accessed 1.8.14). http://llennatur.com/files/u1/on_Place-names_ThosMorgan_Pr_HWSouthey_1887. pdf.

Nilsson, S.G., Hedin, J., Niklasson, M., 2001. Biodiversity and its assessment in boreal and nemoral forests. Scand. J. For. Res. 16 (S3), 10–26.

Nordén, B., Appelqvist, T., 2001. Conceptual problems of ecological continuity and its bioindicators. Biodivers. Conserv. 10 (5), 779–791.

Ohlson, M., Söderström, L., Hörnberg, G., Zackrisson, O., Hermansson, J., 1997. Habitat qualities versus long-term continuity as determinants of biodiversity in boreal old-growth swamp forests. Biol. Conserv. 81, 221–231.

Oldfield, F., Wake, R., Boyle, J., Jones, R., Nolan, S., Gibbs, Z., Appleby, P., Fisher, E., Wolff, G., 2003. The late-Holocene history of Gormire Lake (NE England) and its catchment: a multiproxy reconstruction of past human impact. The Holocene 13 (5), 677–690.

O'Sullivan, A., Kelly, D.L., 2006. A recent history of sessile oak (Quercus petraea (Mattuschka) Liebl.)-dominated woodland in Killarney, SW Ireland, based on tree-ring analysis. Biol. Environ.: Proc. R. Irish Acad. 106B (3), 355–370.

Palo, A., Ivask, M., Liira, J., 2013. Biodiversity composition reflects the history of ancient semi-natural woodland and forest habitats −complication of an indicator complex for restoration practice. Ecol. Indic. 34, 336–344.

Peterken, G.F., Game, M., 1984. Historical factors affecting the number and distribution of vascular plant species in the woodlands of central Lincolnshire. J. Ecol. 72 (1), 155–182.

Peterken, G.F., 1974. A method of assessing woodland flora for conservation using Indicator Species. Biol. Conserv. 6, 239–245.

Peterken, G.F., 1981. Woodland Conservation and Management. Chapman and Hall, London.

Petersen, P.M., 1994. Flora, vegetation, and soil in broadleaved ancient and planted woodland and scrub on Rùsnñs Denmark. Nordic J. Bot. 14, 693–709.

Proctor, M.C.F., 1962. The epiphytic bryophyte communities of the Dartmoor oak woods. Trans. Devon Assoc. Adv. Sci. 94, 531–554.

Rackham, O., 2003. Ancient Woodland, Its History, Vegetation and Uses in England. Ancient Woodland, Its History, Vegetation and Uses in England, revised edition. Castlepoint Press, Dumfries.

Ratcliffe, D.A., 1968. An ecological account of Atlantic bryophytes in the British Isles. New Phytol. 67 (2), 365–439.

Rimet, F., Bouchez, A., Montuelle, B., 2015. Benthic diatoms and phytoplankton to assess nutrients in a large lake: complementarity of their use in Lake Geneva (France–Switzerland). Ecol. Indic. 53, 231–239.

Roberts, C.A., Gilbertson, D.D., 1994. The vegetational history of Pile Copse ancient oak woodland, Dartmoor, and the possible relationships between ancient woodland, clitter, and mining. Proceedings of the Ussher Society 8 (298-298).

Rolstad, J., Gjerde, I., Gundersen, V.S., Sætersdal, M., 2002. Use of indicator species to assess forest continuity: a critique. Conserv. Biol. 16 (1), 253–257.

Rose, F., Coppins, A.M., 2002. Site assessment of epiphytic habitats using lichen indices. In: In: Nimis, P.L., Scheidegger, C., Wolseley, P.A. (Eds.), Monitoring with Lichens – Monitoring Lichens 7. Kluwer Academic Publishers, Dordrecht, pp. 343–348.

Rose, F., 1976. Lichenological indicators of age and environmental continuity in woodlands. In: Brown, D.H., Hawksworth, D.L., Bailey, R.H. (Eds.), Lichenology: Progress and Problems. Academic Press, London, pp. 278–307.

Rotherham, I.D., 2011. A Landscape History Approach to the Assessment of Ancient Woodlands: Ecology, Management and Conservation. Nova Science Publisher, New York.

Rotherham, I.D., Jones, M., Smith, L., Handley, C. (Eds.), 2008. The Woodland Heritage Manual: A Guide to Investigating Wooded Landscapes. Wildtrack Publishing, Sheffield.

Schmidt, M., Mölder, A., Schönfelder, E., Engel, F., Schmiedel, I., Culmsee, H., 2014. Determining ancient woodland indicator plants for practical use: a new approach developed in northwest Germany. For. Ecol. Manage. 330, 228–239.

Scott, W., Palmer, R., 1987. The Flowering Plants and Ferns of the Shetland Islands. Lerwick, Shetland Times Ltd.

Simmons, I.G., 1965. The Dartmoor oak copses: observations and speculations. Fields Stud. 2, 225–235.

Spencer, J.W., Kirby, K.J., 1992. An inventory of ancient woodland for England and Wales. Biol. Conserv. 62 (2), 77–93.

Spencer, J., 1990. Indications of antiquity: some observations of the nature of plants associated with ancient woodland. Br. Wildl. 2, 90–102.

Sroka, K., Finch, O.D., 2006. Ground beetle diversity in ancient woodland remnants in north-western Germany (Coleoptera, Carabidae). J. Insect Conserv. 10 (4), 335–350.

Stefańska-Krzaczek, E., Kacki, Z., Szypula, B., 2016. Coexistence of ancient forest species as indicators of high species richness. For. Ecol. Manage. 365, 12–21.

Swallow, K., Goodenough, A.E., 2017. Double-edged effect? Impact of dual edge proximity on the distribution of ancient woodland indicator plant species in a fragmented habitat. Commun. Ecol (in press.

The Woodland Trust, 2007. Back on the Map: An Inventory of Ancient and Long-established Woodland for Northern Ireland. Available from: (Accessed 26.10.2016). www.backonthemap.co.uk.

Tzedakis, P.C., Andrieu, V., De Beaulieu, J.L., Crowhurst, S.D., Follieri, M., Hooghiemstra, H., Magri, D., Reille, M., Sadori, L., Shackleton, N.J., Wijmstra, T.A., 1997. Comparison of terrestrial and marine records of changing climate of the last 500,000 years. Earth Planet. Sci. Lett. 150 (1), 171–176.

UKHO (2011) INSPIRE Portal and Bathymetry DAC. Available from:http://aws2.caris.com/ukho/mapViewer/map.action (accessed: 31.8.2016).

Vallet, J., Daniel, H., Beaujouan, V., Rozé, F., Pavoine, S., 2010. Using biological traits to assess how urbanization filters plant species of small woodlands. Appl. Veg. Sci. 13 (4), 412–424.

Waller, M., Grant, M.J., Bunting, M.J., 2012. Modern pollen studies from coppiced woodlands and their implications for the detection of woodland management in Holocene pollen records. Rev. Palaeobot. Palynol. 187, 11–28.

Webb, J.C., MCCarroll, J., Carpenter, W., Chambers, F.M., Toms, P.S., Wood, M.J., 2017. Apparent lack of woodland and relative abundance of woodland indicator species: the role of humans, birds and rabbits in the changing vegetation of Skomer Island Wales. Archaeol. Wales (in press).

Wells, R.W., Lertzman, K.P., Saunders, S.C., 1998. Old growth definitions for the forests of british columbia, Canada. Nat. Areas J. 18 (4), 280–294.

West, S., 1997. Geochemical and Palynological Signals for Palaeoenvironmental Change in South West England. PhD. Thesis. University of Plymouth.

Wulf, M., 1997. Plant species as indicators of ancient woodland in northwestern Germany. J. veg. Sci. 8 (5), 635–642.

Xu, Q., Zhang, S., Gaillard, M., Li, M., Cao, X., Tian, F., Li, F., 2016. Studies of modern pollen assemblages for pollen dispersal- deposition- preservation process understanding and for pollen-based reconstructions of past vegetation, climate, and human impact: a review based on case studies in China. Quat. Sci. Rev. 149, 151–166.

Zacharias, D., 1994. Bindung von GefaÈ sspflanzen an WaÈ lder alter Waldstandorte im noÈ rdlichen HarzvorlandNiedersachsens-ein Beispiel fuÈ r die Bedeutung des Alters von Biotopen fuÈ r den Pflanzenart-enschutz. Norddeutsche Naturschutzakademie-Berichte 94 (3), 76–88.

Understanding spatiotemporal variation in taxonomic richness and rate of within-site turnover in vegetation communities across Eurasia over the last 4,000 years

Anne E. Goodenough and Julia C. Webb
School of Natural and Social Sciences, University of Gloucestershire, Cheltenham, UK.
Orchid = 0000-0002-7662-6670 (AEG) and 0000-0002-1652-965X (JCW)

Corresponding author email: aegoodenough@glos.ac.uk

Communities are intricate networks of co-occurring species that vary in complex ways over space and time. However, because of the logistical challenges inherent in collecting primary data, many studies utilise relatively short-term data, often from fairly small geographical areas. In this study, we examine spatiotemporal change in taxonomic richness and turnover over the last 4,000 years at 23 sites from across Eurasia using robustly-dated high-resolution palaeoecological data. We find strong support for the widely-recognised, but not universally-supported, Latitudinal Diversity Gradient and Altitudinal Diversity Gradient, as well as a statistically significant temporal increase in the gradient of both these relationships (something very few previous studies have empirically tested). We also find an increase in (alpha) richness over time despite biodiversity declines at national and international scales, as well as a high but decreasing rate of turnover (throughout the 4,000 year study period. The rate of change in turnover is affected by latitude (steeper negative relationship at higher latitudes) and altitude (steeper negative relationship at lower altitudes). The Diversity-Stability Hypothesis is supported as communities changed from "lower richness, higher turnover" historically to "higher richness, lower turnover" more recently. Causal mechanisms for these complex and interlinked biogeographical patterns remain ambiguous, but likely include strengthening extrinsic changes including climate change, non-native introductions, increasing homogenisation of generalist taxa, landscape simplification and anthropogenic disturbance. Further studies into drivers of the spatiotemporal patterns revealed here is a research priority, which is especially important in the context of biodiversity decline and climate change.

Keywords: altitudinal diversity gradient; latitudinal diversity gradient; diversity-stability hypothesis; European flora; palaeoecology; palynology.

Introduction

Communities are intricate networks of co-occurring species that interact with one another, and their abiotic environment, in complex ways that vary over both time and space. Understanding spatiotemporal patterns is thus integral to the study of community ecology, especially given the pressing contexts of climate change and biodiversity loss (Millien et al. 2006; Ives and Carpenter 2007; Mannion 2020). However, such patterns can be challenging to study over ecologically-relevant timescales because most primary ecological data are relatively recent (Rull 2010, 2012). Several studies have demonstrated the utility of high-resolution palaeoecological data for analysing community dynamics over longer timescales than would have been possible using primary ecological data (e.g. Berglund et al. 2008; Salgado et al. 2010; Bennion et al. 2018; Bishop et al. 2019; Seddon et al. 2019; Agiadi and Albano 2020). In addition to such approaches being helpful in understanding temporal change in ecological communities at specific sites, use of "recent past" palaeoecological data from multiple sites has considerable potential for exploring spatiotemporal patterns in ecological parameters (Buma et al. 2019; Goodenough and Webb 2022). This is a powerful approach since it enables consideration of spatiotemporal patterns in community ecology across timespans that are meaningful for natural shift within the taxa under consideration (Willis et al 2007; Rull and Vegas-Vilarrúbia 2011; Birks et al 2019). This also allows patterns in richness and within-site turnover to be studied throughout the transition from relatively low human environmental impacts to the more intensive pressures of the present day (Jackson 2007; Colombaroli and Tinner 2013).

Richness is influenced by many different geographical and environmental variables. The Latitudinal Diversity Gradient (LDG) suggests richness is typically highest at the equator and decreases towards the poles, while the Altitudinal Diversity Gradient (ADG) suggests that richness is highest at low/mid altitudes and decreases towards higher altitudes. Evidence to support the LDG occurs in most taxonomic groups but is not universal (see meta-analyses by Hillebrand (2004) and Kinlock et al. (2018)). Similarly, numerous studies have confirmed the presence of an ADG, although the exact pattern and specific elevations involved differ depending on location and the taxa studied (review by Fischer et al. (2011)). Temporal patterns in richness have not received as much attention as spatial patterns (Scheiner et al. 2011). Analysis of temporal patterns has, until recently, focused on assessing increases in (alpha) richness due to a net positive in the balance between introductions of non-native species and extirpation of native species (e.g. Sax and Gaines 2003). Two highly-cited studies using primary survey data from constant-effort biodiversity monitoring programmes across the world have, contrary to expectations, concluded that alpha richness is increasing almost universally at local scales (Vellend et al. 2013; Dornelas et al. 2014). However, this research has been criticised (e.g. Gonzalez et al. 2016; Cardinale et al. 2018) for being short-term and data-poor (typically 13-20 years) and using data over different time periods (duration, actual years analysed, and relative position to external stressors such as climatic change). Expanding the time period under consideration using palaeoecological data, Berglund et al. (2008) and Colombaroli and Tinner (2013) have shown similar patterns of richness increase over time but while these studies have a key strength in their duration, their spatial resolution is limited. Multi-site palaeoecological data at inter-continental scale, covering a standardised time period at high resolution, provide a unique opportunity to understand broadscale patterns in community ecology. A dataset that is extensive both spatially and temporally also enables the LDG and ADG to be empirically tested, thereby enabling consideration of potential interactions between spatiotemporal factors. Such interactions are understudied but intriguing results from Silvertown (1985) and Giesecke et al. (2019) showed that the strength of the LDG across Europe increased over the last 13,000 years when studied in 1,000-year and 3,000-year increments, respectively.

Within-site turnover is inherently temporal as it quantifies change in the species that are present in the community at different time periods. However, the *rate* of within-site turnover could vary over time because of factors that vary with time such as climate change (Hillebrand et al. 2010; Ulrich et al. 2014; Gibson-Reinemer 2015; James et al. 2017; Webb and Goodenough 2021), direct human influence (Xu et al. 2012); and non-native introductions (Rahel 2000; Sax and Gaines 2003). Moreover, temporal patterns in the rate of within-site turnover might be mediated by spatial variation. For example, Korhonen et al. (2010) demonstrated that interannual turnover in aquatic ecosystems was greater at higher latitudes than it was at lower latitudes. Advancing understanding of such patterns is increasingly important given the pressing contexts of human-accelerated climate change and other anthropogenic impacts. Most previous research has involved short-term studies of primary data, which is problematic as measured turnover can covary with study duration (Korhonen et al. 2010). Study duration has previously been extended using innovative space-for-time substitution (Hillebrand et al. 2010), but recent studies (Mottl et al. 2021a; Adeleye et al. 2022) have demonstrated that using palaeoecological data to analyse rate of change has untapped potential.

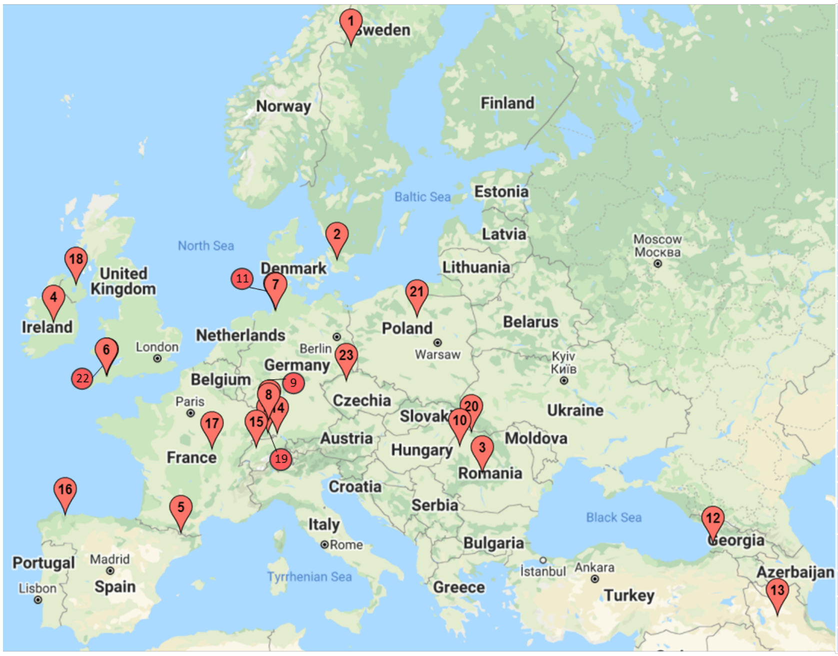
Finally, as well as there being spatiotemporal patterns in richness and the rate of within-site turnover individually, richness and turnover can themselves inter-relate both overall and in ways that covary with time and/or space. The Diversity-Stability Hypothesis (DSH) states that higher richness is associated with higher ecological resilience and thus lower turnover. The DSH was originally postulated by Darwin (1859) before being extended by MacArthur (1955) and McNaughton (1977). The relationship is usually considered to be the result of functional linkages and interactions (especially competition and trophic connections) that are greater when richness is higher. This then decreases invasion and extirpation rates and increases resilience to disturbance, which results in stable ecological states. There is considerable experimental evidence for the DSH, at least over short timescales (e.g. Frank and McNaughton 1991; Tilman et al. 1996). However, the pattern is contentious and complex and an increasing number of studies are finding no significant relationship or a significant opposite trend (see review by Ives and Carpenter (2007)). Quantifying patterns at inter-continental scales over longer timescales would be beneficial.

In this study, we quantify spatiotemporal change in taxonomic richness and the rate of within-site turnover over the last 4,000 years at 23 sites with robustly-dated high-resolution palaeoecological data. Our aims are threefold: (1) to analyse spatiotemporal change in richness across Eurasia to test the latitudinal and altitudinal diversity gradients, quantify temporal patterns, and establish whether there are significant interactions between temporal and spatial variables; (2) to quantify temporal change in the rate of within-site turnover to establish whether, on average, turnover is accelerating, slowing, or static given the increasing human pressures on natural systems, and whether temporal patterns are mediated by spatial variables; and (3) to relate the rate of within-site turnover to taxonomic richness to test the diversity-stability hypothesis on a dataset that is extensive both spatially and temporally.

Methods

Data acquisition

The 2,020 sites listed on the European Pollen Database (Fyfe et al. 2009) at the time of the study were screened to identify all sites where high-resolution palynological data over the last 4,000 years were available electronically. Specifically, sites selected for inclusion needed to have: (1) been used within a published output in a peer-reviewed journal; (2) at least two radiocarbon dates within the last 4,000 years forming a robust age-depth model of sedimentation to allow precise time-horizons to be identified by the original author(s); (3) high-resolution sampling at ≤5 cm intervals; and (4) data from at least one time period every 100 years over the 4,000-year study period (i.e. 0-99 years ago; 100-199 years ago; 200-299 years ago and so on). This last criterion was ultimately relaxed to allow missing data in up to four time periods (10% of the total) to increase the number of eligible sites. This protocol gave 23 sites from 15 countries (latitude: 63°53'0" to 37°39'55" North; longitude: 7°59'35"W to 46°37'55" East; altitude: 1 m to 2,410 m; Fig. 1). Of these sites, 8 had data for all time periods while 15 had missing data up to, but not exceeding, 10% of the total.



	Name	Citation
1	Abborrtjärnen	Giesecke 2005
2	Atteköps Mosse	Nilsson 1964
3	Avrig	Tantau 2003
4	Ballyduff	Stefanini 2008
5	Bosc dels Estanyons	de Beaulieu et al. 2005
6	Broad Down	Fyfe & Woodbridge 2012
7	Flögelner Holz	Rösch 2007
8	Glaswaldsee	Rösch 2011a
9	Huzenbacher See	Rösch & Tserendorj 2011b
10	Iaz	Grindean et al. 2014
11	Immenmoor	Behre & Kucan 1994
12	Ispani	de Klerk et al. 2009
13	Lake Almalou	Djamali et al. 2009
14	Litzelsee	Rösch & Lechterbeck 2016
15	Lobsigensee	Ammann 1985
16	Pedrido	Stefanini 2008
17	Port des Lamberts	Jouffroy-Bapicot 2010
18	Sluggan	Smith & Goddard 1991
19	Steerenmoos	Rösch 2000
20	Steregoiu	Feurdean & Bennike 2004
21	Strazym Lake	Noryskiewicz 1987
22	Winneys Down	Fyfe & Woodbridge 2012
23	Zahájí	Pokorn 2005

Figure 1. Sites included in this study. All palaeoecological data were collated from the European Pollen Database (Fyfe et al. 2009) and original publications for each site are referenced within this figure.

Data manipulation

Palynological data were extracted for the time periods of interest using the taxonomic level given by the original author(s). As the taxonomic level to which pollen and spores could be identified varied for different taxa, the overall dataset contained family-level, genus-level and species-level data (hence we use "taxonomic" and "taxa" rather than "species" throughout this research). It is important to note that there were no systematic differences in data over time (i.e. this was temporally consistent) such that subsequent analysis of temporal patterns was not an artefact of changing taxonomic resolution.

Two biodiversity metrics were calculated. Taxonomic richness calculated for each site for each time period as a simple count of the taxa recorded. Within-site turnover was calculated on presence/absence data for successive sequential time periods to give a series of 39 values for each site describing temporal change in the vegetation community at that site (i.e. 0-99 years compared to 100-199 years, 100-199 years compared to 200-299 years etc). Using presence/absence data ensures that analysis of change in communities is due to species invasion and extirpation rather than compositional change in communities due to change in abundance, which can be skewed by taxa that dominate the palynological data (Mottl et al. 2021b). All within-site turnover calculations were based on Jaccard's Coefficient of Community Similarity (CC_j): this approach has been used previously to assess changes in community ecology in both ecological and palaeoecological contexts (e.g. Jaccard 1912; Rahel 2000; Webb and Goodenough 2021; Hart et al. 2022). Here, we calculated the inverse of CC_j (sometimes called

Jaccard Distance) using equation 1, where c was the number of taxa common to two successive time periods and S was the sum of the total number of taxa found in both time periods. This gave a measure of community *dissimilarity*, running between 0 and 1 where higher numbers were indicative of greater dissimilarity, and thus greater within-site turnover.

Equation 1: $1\text{-}(CC_j = \frac{c}{S})$

To allow better understanding of change over time, taxonomic richness and within-site turnover were calculated for the overall vegetation community and also, separately, for the shrub and herb (hereafter SAH) sub-community. This allowed separate consideration of change in non-arboreal taxa (mainly shorter-lived *r*-strategists) compared to the overall community, which is strongly influenced by arboreal taxa (longer-lived, *K*-strategists).

There is inevitable variability in the number of pollen grains counted by different researchers, which can confound between-site comparisons (Birks and Line 1992; Birks 2007). Here, because taxonomic richness and within-site turnover metrics used presence/absence data rather than abundance data, the variation in total number of pollen grains counted per time period did not bias analysis as counts were above a statistically-derived minimum threshold. All sites included in this study had a high pollen count (number of grains counted per time period per site: average = 2,175; minimum = 336), which was substantially above the 230 grains necessary for 95% reliability (Djamali and Cilleros 2020). This also exceeded the guide that ≥300 pollen grains should be counted for an accurate vegetation profile (Reille 1990; Moore et al. 1991).

Data analysis

Univariate spatial relationships in taxonomic richness were visualised. Then, to analyse the combined effects of space and time a Generalised Linear Model (GLM) was performed. This GLM used taxonomic richness as the dependent variable with latitude, altitude and time period entered as continuous factors: the 2-way interaction between each spatial variable and time were also added (i.e. latitude*time and altitude*time). The model used a Poisson distribution with a log link function, which was appropriate for the count data being analysed and was the optimal model based on comparison of Akaike's Information Criterion scores relative to other statistically-valid model distribution/function combinations such as negative binomial models (Akaike 1973; Hu et al. 2011). A second GLM was created for taxonomic richness for the SAH sub-community only.

Temporal relationships for within-site turnover were plotted graphically. Then, to determine statistically whether the rate of within-site turnover was accelerating, slowing, or static given the increasing human pressures on natural systems, and the possible mediating effects of latitude and altitude on any temporal patterns, two GLMs were performed. These models used within-site turnover data for the overall community (model 1) and within-site turnover data for the SAH sub-community (model 2) as the dependent variable modelled against time, time*latitude, and time*altitude. Scale models were created because, although turnover was technically proportional, each proportion was the result of a complex calculation (Jaccard Distance) rather than arising out of binomial events (such that a binomial distribution was not appropriate); both dependent variables were also normally distributed. A gamma distribution with a log link function was used as this provided a better fit (considerably lower Akaike's Information Criterion scores) relative to a standard linear scale model for both overall community and the SAH sub-community.

Finally, to relate within-site turnover to richness, and thus test the Diversity-Stability Hypothesis, we calculated the relationship between the average turnover scores for each time period and regressed these values against taxonomic richness for the preceding time period. This provided an initial understanding of the relationship between turnover and richness across Eurasia over the last 4,000 years. To explore this further, Principal Components Analysis (PCA) was undertaken to describe the relationship between turnover (y) and richness (x) so that this relationship could itself be related to time (z) to quantify temporal mediating effects. All analyses were conducted in SPSS version 27 (USA: IBM).

Results

Spatiotemporal change in taxonomic richness: univariate patterns

There was a significant negative relationship between taxonomic richness and latitude as predicted by the LDG. This pattern was found for the overall community and the SAH sub-community, but was slightly stronger for the latter (Fig. 2 a-b). At this level of analysis, there was no significant relationship between taxonomic richness and altitude as predicted by the ADG for either overall community or the SAH sub-community (Fig. 2 c-d).

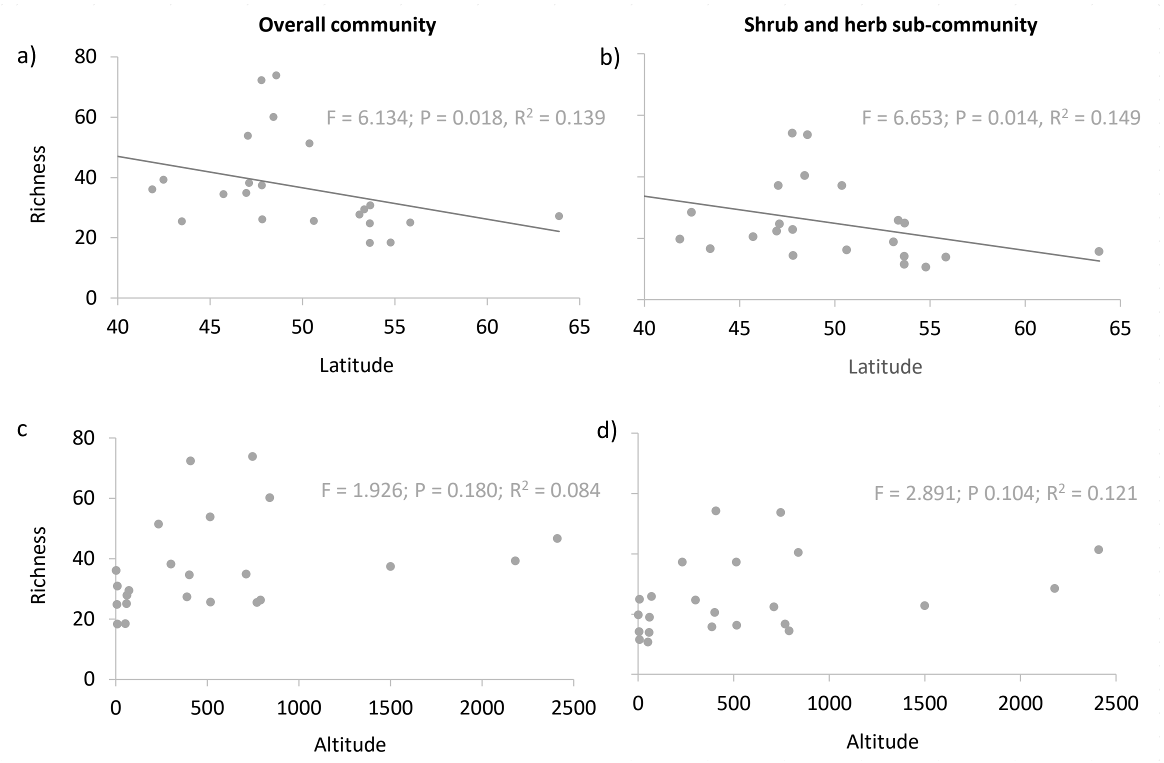


Figure 2: Relationship between spatial variables and taxonomic richness at 23 sites across Eurasia showing: (a-b) latitudinal patterns and (c-d) altitudinal patterns. Univariate regression output is shown for each relationship and regression-derived lines of best fit are shown where patterns are statistically significant.

There was a significant positive relationship between taxonomic richness and time (measured as years before present in 100-year intervals), such that richness was significantly higher in more recent times than it was historically. This trend held for both overall vegetation community (Fig. 3a) and the SAH sub-community (Fig. 3b). The gradient of the line of best fit, which was indicative of the magnitude of change, was almost identical: 0.0028 higher richness per 100-years (11.2 taxa over the entire study period) vs 0.0029 higher richness per 100 years (11.6 taxa over the entire study period), respectively. However, the strength of the relationship differed, being stronger for the SAH sub-community ($R^2 = 0.745$) compared to the overall community ($R^2 = 0.622$). As regards site-specific trends, there was a significant positive relationship between taxonomic richness and time within the overall community (i.e. higher richness in more recent time periods) at 17 of 23 sites (74%); the remaining sites were split evenly between weak positive trends (3 sites; 13%) and weak negative trends (3 sites; 13%), all of which were non-significant. For the SAH sub-community, there were significant positive relationships at 12 of 23 sites (52%), with the remaining 11 sites (48%) being non-significant.

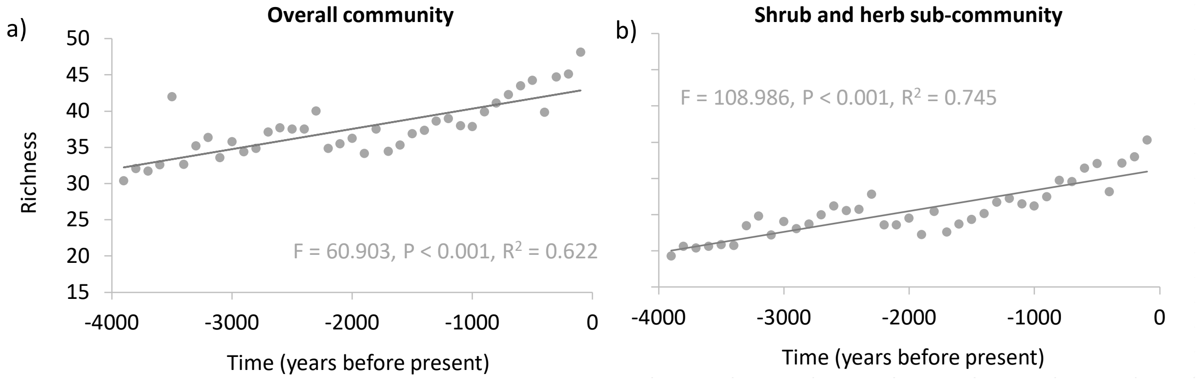


Figure 3: Relationship between taxonomic richness and time for 23 sites across Eurasia. Univariate regression output is shown for each relationship and regression-derived lines of best fit are shown where patterns are statistically significant.

Spatiotemporal change in taxonomic richness: multivariate modelling

A multivariate analysis for taxonomic richness that considered all spatiotemporal variables and the interactions between them revealed complex patterns (Table 1). As expected given the univariate modelling, time was significant as was latitude, both in the expected directions. There was support for the ADG for the overall community but not the SAH sub-community, suggesting that this pattern was driven by changes in arboreal taxa. Interestingly, there was also strong support for spatiotemporal interactions, whereby time mediated (changed) spatial relationships. This meant that both the LDG and ADG increased in strength over time and were thus steeper in more recent times than they had been historically.

Table 1: Generalised Linear Models of taxonomic richness in relation to spatiotemporal variables. Chi square values are likelihood ratio for the overall models and Wald for factors and interaction terms.

	Overall Community		Shrub and Herb Sub-Community		Relationship Direction and Interpretation
	x^2	P	x^2	P	
Overall Model (df = 5)	1144.740	<0.001	1292.695	<0.001	N/A
Time (yr before present; df = 1)	35.232	<0.001	22.207	<0.001	Positive in both models: richness higher in more recent times compared to historically
Latitude (df = 1)	162.069	<0.001	101.668	<0.001	Negative in both models: richness higher at lower latitudes as predicted by Latitudinal Diversity Gradient (LDG)
Altitude (df = 1)	16.375	<0.001	1.891	0.169	Negative for overall community: richness higher at lower altitudes as predicted by Altitudinal Diversity Gradient (ADG). No pattern for sub-community
Latitude*Time (df = 1)	18.208	<0.001	8.058	0.005	Negative in both models: LDG stronger in more recent times compared to historically
Altitude*Time (df = 1)	53.778	<0.001	47.143	<0.001	Negative in both models: ADG stronger in more recent times compared to historically

Spatiotemporal change in rate of within-site turnover

There was no relationship between the rate of within-site turnover in the overall vegetation community relative to time (Fig. 4a). However, there was a statistically significant temporal trend in the rate of within-site turnover within the SAH sub-community (Fig. 4b), such that the rate of turnover was significantly lower in more recent times than it was historically. The gradient of the line of best fit for the SAH sub-community was -0.0012 (i.e. within-site turnover changed by 0.12% for every 100 years or ~5% over the entire study period).

Undertaking analysis on the rate of within-site turnover temporally for each of the 23 sites individually resulted in a mixed picture, with direction and magnitude of change varying considerably between sites. Gradients ranged from -0.0103 to 0.0044 (mean = -0.00004) for the overall community and from -0.0126 to 0.0051 (mean = -0.0012) for the SAH sub-community). Thus, while the *average* rate of turnover was significantly lower in more recent times for the SAH sub-community (Fig. 4b), 2 sites (8%) had a significant opposite trend whereby rate of turnover was higher in more recent times, and 8 sites (35%) had no significant pattern. This suggested that spatial variables might be mediating temporal change. This was confirmed via multivariate modelling that showed significant interactions for time*latitude and time*altitude (Table 2).

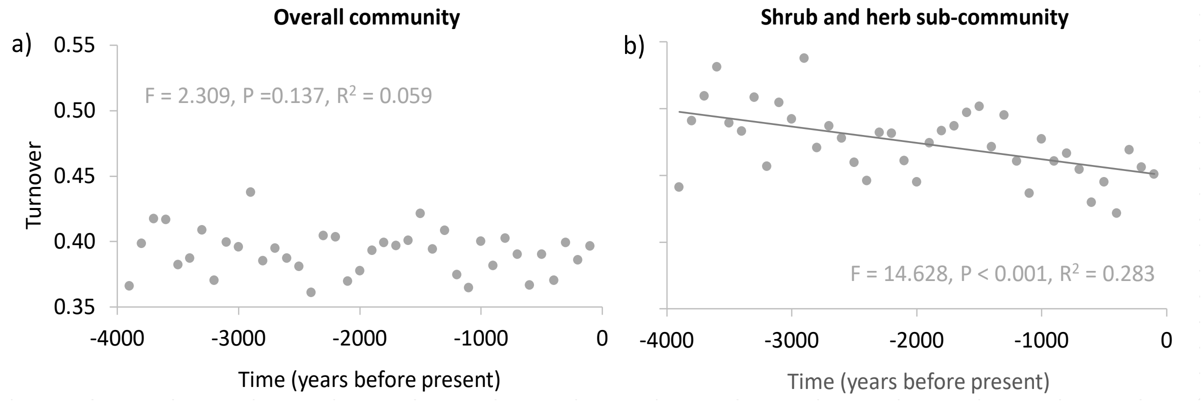


Figure 4: Relationship between the rate of within-site turnover (higher = greater) and time for 23 sites across Eurasia. Univariate regression output is shown for each relationship and regression-derived lines of best fit are shown where patterns are statistically significant.

Table 2: Generalised Linear Models of within-site turnover in relation to time and mediating effects of latitude and altitude. Chi square values are likelihood ratio for the overall models and Wald for factors and interaction terms.

	Overall Community		Shrub and Herb (SAH) Sub-Community		Relationship Direction and Interpretation
	x^2	P	x^2	P	
Overall Model (df = 3)	108.186	<0.001	78.117	<0.001	N/A
Time (yr before present; df = 1)	10.000	0.002	20.716	<0.001	Negative in both models: rate of within-site turnover lower in more recent times compared to historically
Time*Latitude (df = 1)	13.848	<0.001	21.406	<0.001	Positive in both models: general negative effect of time on rate of turnover is greater for sites at higher latitudes (steeper negative relationship) and weaker at lower latitudes (shallower negative relationship).
Time*Altitude (df = 1)	30.266	<0.001	9.643	0.002	Negative in both models: general negative effect of time on rate of turnover was greater for sites at lower altitudes (steeper negative relationship) and weaker for sites at higher altitudes (shallower negative relationship). At very high altitudes, the relationship became positive in some cases.

Relationship between richness and rate of within-site turnover: is the Diversity-Stability Hypothesis supported?

There was a significant negative relationship between taxonomic richness and within-site turnover for both the overall community (Fig. 5a) and the SAH sub-community (Fig. 5b). This meant that turnover was lower when richness was higher, exactly as predicted by the DSH. The relationship was steeper and stronger for the SAH sub-community, with a gradient of -0.0044 (or 0.44% less turnover per additional taxon in the community) and an R^2 of 0.279, compared to the overall vegetation community (gradient = -0.0018; $R^2 = 0.101$).

Based on visual analysis of Fig. 5, the driver for the significant relationship between turnover and richness (and thus the driver for the support found in this study for the DSH) appeared to be time – or, possibly more accurately, other variables that change temporally (see Discussion). The position of the datapoints along the line of best fit strongly linked to time, with older samples being “higher turnover, lower richness” and more recent samples being “lower turnover, higher richness”. This was analysed by estimating the diagonal position of the datapoints using Principal Components Analysis (PC1 passed through the centroid of the data cloud on the longest axis, such that the PC1 score for each datapoint was broadly akin to the position of that datapoint on the lines of best fit shown in Fig. 5a-b). PC1 was statistically negatively related to time expressed as years before present for both overall community ($F_{1,37} = 25.045$, $P < 0.001$, $R^2 = 0.404$) and the SAH sub-community ($F_{1,37} = 64.658$, $P < 0.001$, $R^2 = 0.636$): this meant that the relationship between richness (x) and turnover (y) was causally related to time (z). This result triangulated with the temporal pattern for richness (higher in more recent time periods; Fig 3a and b) and turnover (lower in more recent time periods; Fig 4a and b).

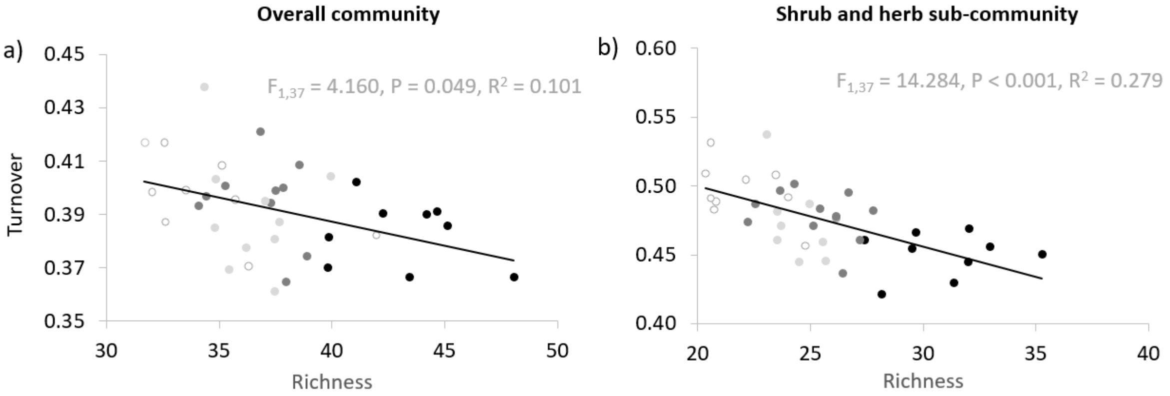


Figure 5: *Relationship between taxonomic turnover (higher = greater) between 39 sequential 100-year time periods and taxonomic richness in the earlier of those time periods for 23 sites across Eurasia for: (a) overall vegetation community; and (b) shrub and herb sub-community. In both cases, data points for sequential 100-year periods falling between present day and 1,000 years ago are shaded black, those 1,000-1,999 years ago are shared dark grey, those 2,000-2,999 are shaded light grey and those 3,000-3,999 are open circles. Note that scales differ between graph panels.*

Discussion

Analysis of paleoecologically-derived data from 23 sites across Eurasia and spanning a 4,000 year period support some of the major spatial relationships (LDG, ADG) and interrelationships (DSH) within community ecology. More importantly, they also demonstrate the integral importance of temporal processes, either directly (taxonomic richness and rate of within-site turnover) or as mediating effects on LDG, ADG and DSH relationships to create the complex spatiotemporal patterns summarised in Figure 6.

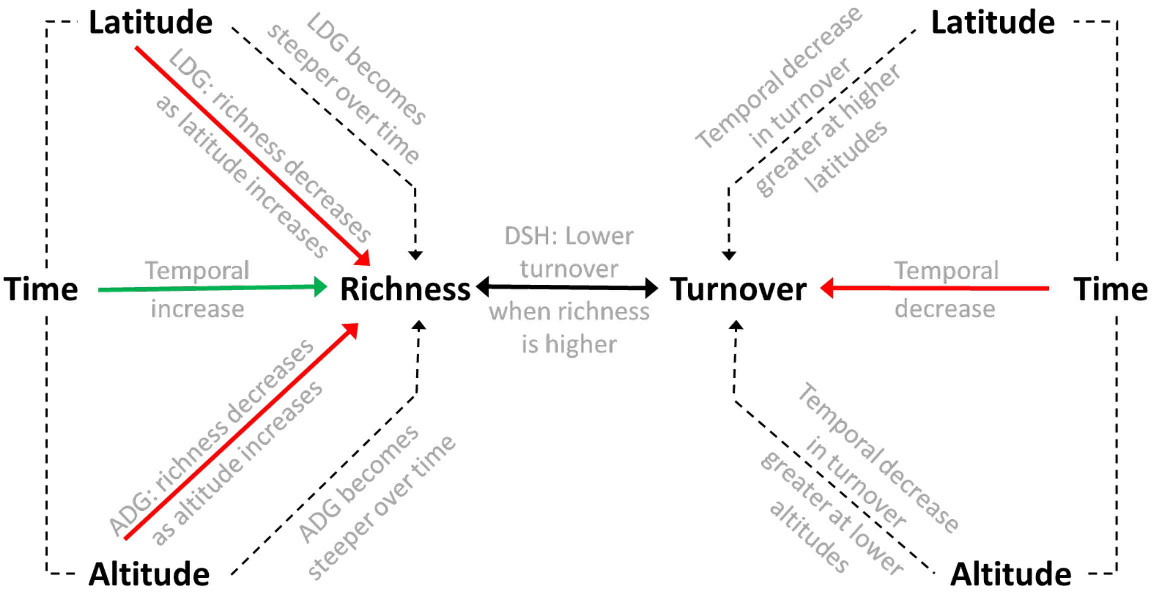


Figure 6: Summary of patterns between taxonomic richness and within-site turnover relative to spatiotemporal variables (time, latitude, altitude). Solid lines denote direct effects, dashed lines denote indirect effects where one factor mediates relationships between other variables. LDG = Latitudinal Diversity Gradient; ADG = Altitudinal Diversity Gradient; DSH = Diversity Stability Hypothesis.

Our data support the LDG and ADG, with taxonomic richness being greater at lower latitudes and altitudes. Such patterns are common, although not universal, as previously shown by Hillebrand (2004), Fischer et al. (2011), and Kinlock et al. (2018) for primary ecological data and palaeoecological data (Reitalu et al. 2019). Where these patterns occur, they can be driven by greater *loss* of taxa at higher latitudes and altitudes and/or greater *gain* of taxa at lower latitudes and altitudes (Mannion 2020). However, our analysis also highlights that time interacts with these relationships, as they have both become steeper over time. The casual mechanisms for this are unclear. In one of the very few previous analyses of temporal change in the LDG, Silvertown (1985) found the latitude-richness gradient became steeper over time for woody taxa across Europe over the last interglacial period when analysed in 1,000-year increments. Silvertown ascribed natural spatiotemporal change in climate to be the causal mechanism and concluded that the LDG steepening was due to flora readjusting after the end of the last ice age. This was tangentially supported by research that demonstrated the lack of any LDG immediately after prehistoric mass extinction events (MEEs), which were followed by strengthening gradients over time (Mannion et al. 2014; Mannion 2020). This includes patterns for North American mammals following the Cretaceous/Palaeocene MEE (Rose et al. 2011) and marine taxa following the Permian/Triassic MEE (Song et al. 2020). However, as our current study spans 4,000 years of relatively stable climate (albeit with human-accelerated climate change effects becoming apparent over the last couple of hundred years), climate change cannot fully explain the observed patterns and combinations of natural and anthropogenic processes are likely to be responsible as discussed below.

There are complex interactions between space and time in relation to richness (Giescke et al. 2019). Here, not only does time mediate the spatial relationships in taxonomic richness, it has an important link to richness itself. The finding that alpha richness has increased over the recent past towards the present day supports the contentious previous studies using primary ecological survey data (Vellend et al. 2013; Dornelas et al. 2014), but using much more robust datasets (longer time, higher resolution, more data rich, and consistent time periods analysed at all sites). This accords with longer-term palaeoecological studies (Berglund et al. 2008; Colombaroli et al. 2012; Colombaroli and Tinner 2013; Schwörer et al. 2015; Giesecke et al. 2019) that have shown similar patterns of richness increase across the Holocene (the last 11,500 years). However, it should be noted that all of these studies using palaeoecological data – and indeed our analysis – are at a temporal resolution that will not allow detection of very recent (>100 years) taxonomic richness declines. Moreover, this baseline temporal increase should emphatically *not* be taken as evidence against the biodiversity crisis since site-specific (alpha richness) trends do not to preclude catastrophic loss of biodiversity over a wider scale (gamma richness) (Gonzalez et al. 2016; Cardinale et al. 2018; Hillebrand et al. 2018). Trends could also be transitory as communities often gain new taxa faster than they lose existing taxa (Jackson and Sax 2009). Moreover, it is possible that a component of the temporal increase in richness is non-desirable biotic homogenisation (Sax and Gaines 2003; Clavel et al. 2011; Le Viol et al. 2012; Giesecke et al. 2019), as well as being complicated by extirpation lag (Jackson and Sax 2009). It is also important to highlight that temporal alpha richness increases are not universal (richness did not increase over time at 26% of sites (overall community) and 48% of sites (SAH sub-community); some non-significant patterns tended towards the negative, especially at higher latitudes and/or altitudes).

The factors that covary with time, and thus might be responsible for the temporal patterns in richness (and the mediating effects of time on the LDG and ADG), are unclear. Thus, while intriguing spatiotemporal patterns have been revealed by this paper, the causal mechanisms are more ambiguous. Speculative possibilities include increases in non-natives (Sax and Gaines 2003) (although if this was the sole explanation an exponential pattern would be expected rather than linear pattern across 4,000 years). A second possibility is change in landscapes caused by human activity is creating habitat mosaics that increase richness, as found by Colombaroli et al. (2012) and Colombaroli and Tinner (2013) in relation to more open landscapes being created in the mid-Holocene. A third possibility is an increase in the number of generalist taxa within communities over time, either with or without loss of more specialist taxa (Clavel et al. 2011; Le Viol et al. 2012): such a pattern could result from increasing landscape simplification (Gámez-Virués et al. 2015) or anthropogenic disturbance (Devictor 2008). Finally, it is likely that this pattern is at least partly driven by human-accelerated climatic change altering fundamental and realised ranges, especially the loss of taxa at high latitudes/altitudes and the poleward/upward expansion of other taxa into Europe at middle latitudes and altitudes) (Goodenough and Hart 2013; Walther et al. 2005). All of the above processes would involve arrival of new taxa at study sites over time and/or slower rates of loss (i.e. giving a net positive balance, but possibly complicated by lag times meaning that, at any one time, a site might be experiencing extinction debt or immigration credit: Jackson and Sax 2009). Although in our study rate of within-site turnover is decreasing temporally, rates still remain at ~40% per hundred years. This means that temporal decrease in within-site turnover is not at odds with processes that involve turnover being responsible for richness increases over time – and indeed it could even be argued that a slowing of turnover would be expected if biotic homogenisation (which, by definition, cannot be infinite) is starting to plateau.

Given that higher richness is thought to drive higher stability (and thus lower turnover) (MacArthur 1955; McNaughton 1977; Frank and McNaughton 1991; Tilman et al. 1996), and that our data support this, it is not surprising that within-turnover has decreased over time while richness has increased over the same time period. Moreover, the main driver for the DSH in our data appears to be time. While natural mechanisms (functional diversity, competition and trophic connections) could be important as they decrease invasion and extirpation risk, human activity might also be a contributory factor, with increased management of ecosystems potentially partly supressing natural turnover. This accords with evidence from Vogel et al. (2012), who demonstrated that a positive relationship between richness and stability in grassland communities only occurred in ecosystems that were highly managed, not those that were dominated by natural processes and interactions. It should be noted that there is also a potential artefact whereby turnover estimates might be higher when richness is lower as change in one or two taxa has a higher relative impact.

Throughout this study, and indeed all studies using palaeoecological data, there is an implicit assumption that vegetation diversity (richness and community composition) is equivalent to palynological diversity, or an appropriate caveat. Where this has been empirically tested, agreement between vegetation community and pollen community varies from there being no significant relationships in tropical environments of Africa and South America (Gosling et al. 2017) significant positive relationships in Europe (Reitalu et al. 2019). Although it recognised that pollen records might not be fully representative of vegetation communities, they are one of the best data sources to evaluate inter-continental changes in community ecology over periods longer than of those covered by primary ecological data.

Overall, our findings support and extend previous work, including widely-recognised biogeographical relationships such as the LDG and ADG as well as more recent (and contentious) work on temporal increases in alpha richness, using a robust dataset that is extensive both spatially and temporally. The casual mechanisms, however, remain ambiguous and although this is indicative of the inherent complexity of ecological systems, we suggest that more research into the drivers of these patterns is undertaken as a matter of priority. This is especially important given the contexts of widespread biodiversity decline and strengthening extrinsic changes including those to climate, non-native introductions, increasing homogenisation of generalist taxa, landscape simplification and anthropogenic disturbance.

Statements and declarations

Competing interests: On behalf of both authors, the corresponding author declares no conflicts of interest

Author contributions: AEG = Conceptualisation, Data Curation, Investigation, Verification, Visualisation, Writing Original Draft; JCW = Data Curation, Verification, Visualisation, Writing Original Draft.

References

Adeleye MA, Connor SE, Herbert A, Haberle SG (2022) Toward the saving of global rainforests. Glob Change Biol In press

Agiadi K, Albano, PG (2020) Holocene fish assemblages provide baseline data for the rapidly changing eastern Mediterranean. The Holocene 30:1438-1450.

Akaike H (1973) Information theory as an extension of the maximum likelihood principle. In: Second International Symposium on Information Theory (ed. By B.V. Petrov and B.F. Csaki). pp 267–281 Academia Kiado; Budapest.

Ammann B (1985) Introduction and Palynology: vegetational history and core correlation at Lobsigensee (Swiss Plateau). In: Lobsigensee - Late-Glacial and Holocene environments of a lake on the central Swiss Plateau. Dissertationes Botanicae 87:127-170.

Behre KE, Kucan D (1994) Die Geschichte der Kulturlandschaft und des Ackerbaus in der Siedlungskammer Flögeln, Niedersachsen, seit der Jungsteinzeit. Probleme der Küstenforschung im südlichen Nordseegebiet 21, Isensee, Oldenburg.

Bennion H, Sayer CD, Clarke SJ et al (2018) Sedimentary macrofossil records reveal ecological change in English lakes: implications for conservation. J. Paleolimnol 60:329-348.

Berglund BE, Gaillard MJ, Björkman L, Persson T (2008) Long-term changes in floristic diversity in southern Sweden: palynological richness, vegetation dynamics and land-use. Veg Hist Archaeobot, 17: 573-583

Birks HJB (2007) Estimating the amount of compositional change in late-Quaternary pollen-stratigraphical data. Veget Hist Archaeobot 16: 197-202

Birks HJB (2019) Contributions of Quaternary botany to modern ecology and biogeography. Plant Ecol Divers 12:189-385

Birks HJB, Line JM (1992) The use of rarefaction analysis for estimating palynological richness from Quaternary pollen-analytical data. The Holocene 2: 1-10

Bishop IJ, Bennion H, Sayer CD, Patmore IR, Yang H (2019) Filling the “data gap”: Using paleoecology to investigate the decline of Najas flexilis (a rare aquatic plant). Geo: Geogr Environ 6:e00081.

Brand A, Allen L, Altman M, Hlava M, Scott J, (2015) Beyond authorship: attribution, contribution, collaboration, and credit. Learn Publ 28:151-155.

Buma B, Harvey BJ, et al. (2019) The value of linking paleoecological and neoecological perspectives to understand spatially-explicit ecosystem resilience. Landsc Ecol 34:17-33.

Cardinale BJ, Gonzalez A, Allington GR, Loreau M (2018) Is local biodiversity declining or not? A summary of the debate over analysis of species richness time trends. Biol Cons 219:175-183.

Clavel J, Julliard R, Devictor V (2011) Worldwide decline of specialist species: toward a global functional homogenization? Front Ecol Environ 9:222-228.

Colombaroli D, Beckmann M, van der Knaap WO, Curdy P, Tinner W (2013) Changes in biodiversity and vegetation composition in the central Swiss Alps during the transition from pristine forest to first farming. Divers Distrib 19: 157-170

Colombaroli D, Tinner W (2013) Determining the long-term changes in biodiversity and provisioning services along a transect from Central Europe to the Mediterranean. The Holocene, 23: 1625-1634

Darwin C (1859) On the origin of species by means of natural selection or the preservation of favoured races in the struggle for life [reprinted 1964]. Cambridge (MA): Harvard University.

de Beaulieu JL, Miras, Andrieu-Ponel V, Guiter F, (2005) Vegetation dynamics in north-western Mediterranean regions: Instability of the Mediterranean bioclimate. Plant Biosyst 139:114-126

de Klerk P, Haberl A, et al (2009). Vegetation history and environmental development since ca 6000 cal yr BP in and around Ispani 2 (Kolkheti lowlands, Georgia). Quat Sci Rev 28:890-910

Devictor V, Julliard R, Clavel J, Jiguet F, Lee A, Couvet D (2008) Functional biotic homogenization of bird communities in disturbed landscapes. Glob Ecol Biogeogr 17:252–261

Djamali M, Cilleros K (2020) Statistically significant minimum pollen count in Quaternary pollen analysis; the case of pollen-rich lake sediments. Rev Palaeobot Palynol 275: 104156

Djamali M, de Beaulieu JL, Andrieu-Ponel V et al. (2009) A late Holocene pollen record from Lake Almalou in NW Iran: evidence for changing land-use in relation to some historical events during the last 3700 years. J Archaeol Sci 36:1363-1375

Dornelas M, Gotelli NJ, et al (2014) Assemblage time series reveal biodiversity change but not systematic loss. Science 344:296-299

Feurdean A, Bennike O (2004) Late Quaternary palaeoecological and palaeoclimatological reconstruction in the Gutaiului Mountains, northwest Romania. J Quat Sci 19:808-827

Fischer A, Blaschke M, Bässler C, (2011) Altitudinal gradients in biodiversity research: the state of the art and future perspectives under climate change aspects. Waldökologie, Landschaftsforschung und Naturschutz, 11:35-47

Frank DA, McNaughton SJ, (1991) Stability increases with diversity in plant communities: empirical evidence from the 1988 Yellowstone drought. Oikos 62:360–62

Fyfe RM, de Beaulieu JL, Binney H et al. (2009) The European Pollen Database: past efforts and current activities. Veg Hist and Archaeobot 18:417–424

Fyfe RM, Woodbridge J (2012) Differences in time and space in upland vegetation patterning: analysis of pollen data from Dartmoor, UK. Landsc Ecol 27:745-760

Gámez-Virués S, Perović DJ, Gossner MM, et al. (2015) Landscape simplification filters species traits and drives biotic homogenization. Nat Commun 6:1-8

Gibson-Reinemer DK, Sheldon KS, Rahel FJ (2015) Climate change creates rapid species turnover in montane communities. Ecol Evol 5:2340-2347

Giesecke T (2005) Holocene forest development in the central Scandes Mountains, Sweden. Veg Hist and Archaeobot 13:133-147

Giesecke T, Wolters S, van Leeuwen JF, van der Knaap PW, Leydet M, Brewer S (2019) Postglacial change of the floristic diversity gradient in Europe. Nature Commun 10: 1-7

Gonzalez A, Cardinale BJ, et al (2016) Estimating local biodiversity change: a critique of papers claiming no net loss of local diversity. Ecol 97:1949-1960.

Goodenough AE Hart AG (2013) Correlates of vulnerability to climate-induced distribution changes in European avifauna: habitat, migration and endemism. Clim Change 118:659-669

Goodenough AE Webb JC (2022) Learning from the past: opportunities for advancing ecological research and practice using palaeoecological data. Oecologia In press

Gosling WD, Julier A et al. (2018) Pollen-vegetation richness and diversity relationships in the tropics. Veg Hist Archaeobot. 27: 411-418

Grindean R, Tantau I, Farcas S, Panait A (2014) Middle to Late Holocene vegetation shifts in the NW Transylvanian lowlands (Romania). Studia UBB Geologia 59:29-37

Hart AG, Dawson M, Fourie R, MacTavish L, Goodenough AE (2022) Comparing the effectiveness of camera trapping, driven transects and ad hoc records for surveying nocturnal mammals against a known species assemblage. Community Ecology In press.

Hillebrand H (2004) On the generality of the latitudinal diversity gradient. Am Nat 163:192-211.

Hillebrand H, Blasius B, Borer ET et al (2018) Biodiversity change is uncoupled from species richness trends: Consequences for conservation and monitoring. J Appl Ecol 55:169-184

Hillebrand H, Soininen J, Snoeijs P (2010) Warming leads to higher species turnover in a coastal ecosystem. Glob Change Biol 16:1181-1193

Hu M, Pavlicova M, Nunes EV (2011) Zero-inflated and hurdle models of count data with extra zeros: examples from an HIV-risk reduction intervention trial. Am J Drug Alcohol Abuse 37:367-375

Ives AR, Carpenter SR (2007) Stability and diversity of ecosystems. Science 317:58– 62

Jaccard P (1912) The distribution of the flora in the alpine zone 1. New Phytol 11:37–50

Jackson ST (2007) Looking forward from the past: history, ecology, and conservation. Front Ecol Environ 5:455-455

Jackson ST, Sax DF (2010) Balancing biodiversity in a changing environment: extinction debt, immigration credit and species turnover. Trends Ecol Evol 25:153-160

James CS, Reside AE et al (2017) Sink or swim? Potential for high faunal turnover in Australian rivers under climate change. J Biogeogr 44:489-501

Jouffroy-Bapicot I (2010) Evolution de la végétation du massif du Morvan (Bourgogne - France) depuis la dernière glaciation à partir de l'analyse pollinique. Variations climatiques et impact des activités anthropiques. PhD Thesis, University of Franche-Comté, 1-2, 1-415

Kinlock NL, Prowant L et al (2018) Explaining global variation in the latitudinal diversity gradient: Meta-analysis confirms known patterns and uncovers new ones. Glob Ecol Biogeogr 27:125-141

Korhonen JJ, Soininen J, Hillebrand H, (2010) A quantitative analysis of temporal turnover in aquatic species assemblages across ecosystems. Ecology 91:508-517

Le Viol I, Jiguet F et al (2012). More and more generalists: two decades of changes in the European avifauna. Biol Lett 8:780-782

MacArthur RH (1955) Fluctuations of animal populations and a measure of community stability. Ecol. 36:533–6

Mannion PD (2020) A deep-time perspective on the latitudinal diversity gradient. Proc. Natl. Acad. Sci. 117:17479-17481

Mannion PD, Upchurch P, Benson RB, Goswami A (2014) The latitudinal biodiversity gradient through deep time. Trends Ecol Evol 29:42-50

McNaughton SJ, (1977) Diversity and stability of ecological communities: A comment on the role of empiricism in ecology. Am Nat 111:515–525

Millien V, Lyons K, Olson L, Smith FA, Wilson AB, Yom-Tov Y (2006) Ecotypic variation in the context of global climate change: revisiting the rules. Ecol Lett 9:853-869

Moore PD, Webb, JA, Collinson, ME (1991) Pollen analysis. Blackwell, Oxford

Mottl O, Flantua SG et al (2021a). Global acceleration in rates of vegetation change over the past 18,000 years. Science 372:860-864.

Mottl O, Grytnes JA et al (2021b). Rate-of-change analysis in paleoecology revisited: A new approach. Rev Palaeobot Palynol 293:104483.

Nilsson T (1964) Standard pollen diagramme und C14 datiengen aus dem Ageröds mosse in mittleren schonen. Lunds Universitets Årsskrift N.F. 59:1-52

Noryskiewicz B (1987) History of vegetation during the Late-Glacial Holocene in the Brodnica Lake District in light of pollen analysis of Lake Strazym deposits. Acta Palaeobot 27:283-304

Pokorn P (2005) Role of man in the development of Holocene vegetation in Central Bohemia. Preslia 77:113-128.

Rahel FJ (2000) Homogenization of fish faunas across the United States. Science 288:854-856

Reille M, (1990) Leçons de palynologie et d'analyse pollinique. Editions du Centre National de la Recherche Scientifique, Paris

Reitalu T, Bjune AE et al. (2019) Patterns of modern pollen and plant richness across northern Europe. J Ecol 107: 1662-1677

Rösch M (2000) Long-term human impact as registered in an upland pollen profile from the southern Black Forest, southwestern Germany. Veg Hist Archaeobot 9:205-218

Rösch M (2007) Botanical evidence for prehistoric and medieval land use in Black Forest. Medieval Rural Settlement in Marginal Landscapes. Ruralia VII, 8th-14th September 2007 Cardiff Wales, U.K., 335-343.

Rösch M, Lechterbeck J (2016) Seven Millennia of human impact as reflected in a high resolution pollen profile from the profundal sediments of Litzelsee, Lake Constance region, Germany. Veg Hist Archaeobot 25:339 -358

Rösch M, Tserendorj G (2011a) Der Nordschwarzwald - früher besiedelt als gedacht? Pollenprofile belegen ausgedehnte vorgeschichtliche Besiedlung und Landnutzung. Denkmalpflege in Baden-Württemberg 40:66-73

Rösch M, Tserendorj G (2011b) Florengeschichtliche Beobachtungen im Nordschwarzwald (Südwestdeutschland). Hercynia N.F. 44:53-71.

Rose PJ, Fox DL, Marcot J Badgley C (2011) Flat latitudinal gradient in Paleocene mammal richness suggests decoupling of climate and biodiversity. Geology 39:163–166

Rull V (2010) Ecology and palaeoecology, two approaches, one objective. Open J Ecol 3:1–5

Rull V (2012) Community ecology: diversity and dynamics over time. Comm Ecol 13:102-116

Rull V Vegas-Vilarrúbia T (2010) What is long-term in ecology? Trends Ecol Evol 26:3-4

Salgado J, Sayer C, Carvalho L, Davidson T, Gunn I (2010) Assessing aquatic macrophyte community change through the integration of paleolimnological and historical data at Loch Leven, Scotland. J Paleolimn 43:191–204

Sax DF, Gaines SD (2003) Species diversity: from global decreases to local increases. Trends Ecol Evol 18:561-566

Scheiner SM, Chiarucci A et al (2011) The underpinnings of the relationship of species richness with space and time. Ecol Monogr 81:195-213

Schwörer C, Colombaroli D, Kaltenrieder P, Rey F, Tinner W. (2015) Early human impact (5000–3000 BC) affects mountain forest dynamics in the Alps. J. Ecology 103: 281-295.

Seddon E, Hill M et al(2019) The use of palaeoecological and contemporary macroinvertebrate community data to characterize riverine reference conditions. River Res Appl 35:1302-1313

Silvertown J (1985) History of a latitudinal diversity gradient: woody plants in Europe 13,000-1000 years BP. J Biogeogr 12:519-525

Smith AG, Goddard IC (1991) A 12500 years record of vegetational history at Sluggan Bog, County Antrim, Northern Ireland (incorporating a pollen zone scheme for the non-specialist). New Phytol 118:167-187

Song H, Huang S et al (2020). Flat latitudinal diversity gradient caused by the Permian–Triassic mass extinction. Proc Natl Acad Sci 117:17578-17583

Stefanini BS (2008) A comparison of climate and vegetation dynamics in central Ireland and NW Spain since the mid-Holocene. Doctoral dissertation. University of Dublin, Trinity College, Dublin, Leinster, Ireland.

Tantau I (2003) Pollen analytic researches in the Eastern Romanian Carpathians: History of vegetation and human impact. Thesis. Aix-Marseille III University and Babes-Bolyai of Cluj-Napoca University.

Tilman D, Wedin D, Knops J (1996) Productivity and sustainability influenced by biodiversity in grasslands ecosystems. Nature 379:718–20

Ulrich W, Soliveres S et al. (2014) Climate and soil attributes determine plant species turnover in global drylands. J Biogeogr 41:2307-2319

Vellend M, Baeten L et al (2013). Global meta-analysis reveals no net change in local-scale plant biodiversity over time. Proc Nat Acad Sci 110:19456-19459

Vogel A, Scherer-Lorenzen M, Weigelt A (2012) Grassland Resistance and Resilience after Drought Depends on Management Intensity and Species Richness. PLoS ONE 7:e36992

Walther GR, Beißner S (2005) Trends in the upward shift of alpine plants. J Veg Sci 16:541-548

Webb JC, Goodenough AG (2021) Vegetation community changes in European woodlands amid a changing climate: a palaeoecological modelling perspective. Comm Ecol 22:319–330

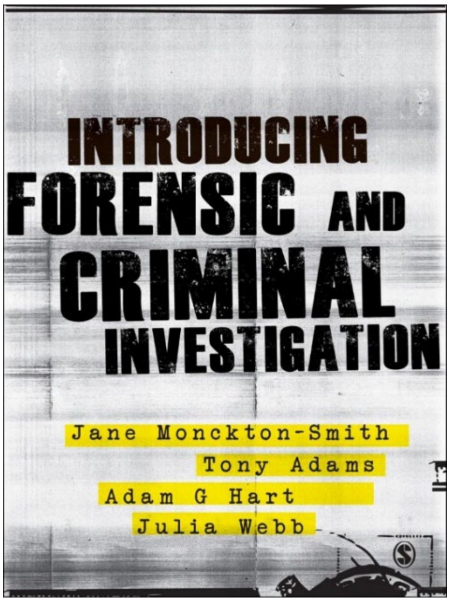
Willis KJ, Araújo MB et al (2007) How can a knowledge of the past help to conserve the future? Biodiversity conservation and the relevance of long-term ecological studies. Philos. Trans. R. Soc. Lond., B, Biol. Sci. 362:175-187

Xu Z, Wan S et al (2012) Effects of water and nitrogen addition on species turnover in temperate grasslands in northern China. PLoS one 7:e39762

6. Pollen as a provenancing tool: from forensic investigations to mapping avian migration pathways

This chapter continues the theme of exploring the utility of pollen in contemporary contexts, but with a particular focus on the use of pollen as a provenancing tool. Tracing the source of an item, person, animal or sediment has many uses, including within forensics and the study of species movement (mapping dispersal patterns and invasion pathways, understanding the spread of disease and pests, identifying foraging locations, and mapping seasonal movements and migration pathways). The first output presented here is a book chapter on the use of biological techniques, including palynology, in forensic studies. (The chapter is from Monckton-Smith et al. (2013), which is co-written by the PhD candidate and covers practical detail on the process of criminal investigations from crime scenes through to court for an undergraduate target audience (Figure 6.1)). The second output is a research paper on forensic palynology (Webb et al. 2018), which considers limitations in commonly-used laboratory procedures for retrieving palynological evidence. The final output, while not forensic in focus, uses similar skills in identifying an origin – in this case mapping migratory routes of Old-World warblers.

Hart A.G and Webb, J.C. Forensic Science Specialisms: palynology, entomology and DNA. *In:* Monckton-Smith, J. Adams, T. Hart, A.G. and Webb, J.C. (2013) Introducing Forensic and Criminal Investigation. Sage. ISBN 978-0857027528




SAGE
Los Angeles | London | New Delhi
Singapore | Washington DC

SAGE Publications Ltd
1 Oliver's Yard
55 City Road
London EC1Y 1SP

SAGE Publications Inc.
2455 Teller Road
Thousand Oaks, California 91320

SAGE Publications India Pvt Ltd
B 1/I 1 Mohan Cooperative Industrial Area
Mathura Road
New Delhi 110 044

SAGE Publications Asia-Pacific Pte Ltd
3 Church Street
#10–04 Samsung Hub
Singapore 049483

Editor: Natalie Aguilera
Editorial assistant: James Piper
Production editor: Sarah Cooke
Copyeditor: Sarah Bury
Proofreader: Anna Gilding
Indexer: Gary Birch
Marketing manager: Sally Ransom
Cover design: Lisa Harper
Typeset by: C&M Digitals (P) Ltd, Chennai, India
Printed and bound by CPI Group (UK) Ltd,
Croydon, CR0 4YY

MIX
Paper from responsible sources
FSC® C013604

First published 2013

Library of Congress Control Number: 2012947813

British Library Cataloguing in Publication data

A catalogue record for this book is available from the British Library

ISBN 978-0-85702-751-1
ISBN 978-0-85702-752-8 (pbk)

Figure 6.1 Undergraduate textbook co-written by the PhD candidate. According to Amazon sales rankings, 4,608 copies have been sold through their platform.

Webb, J.C., Brown, H.A. Toms, H. and Goodenough, A.E. (2018) Differential retention of pollen grains on clothing and the effectiveness of laboratory retrieval methods in forensic settings. *Forensic Science International*, 288. 36-45.

Wood, M.J., Morgan, P.J., Webb, J.C., Goodenough, A.E., Chambers, F.M. and Hart, A.G. (2014) Exploring the prevalence and diversity of pollen carried by four species of migratory Old-World warbler (Sylvioidea) on arrival in the UK. *Bird Study*, 61 (3), 361-370.

6.1 Forensic palynology

Although studies investigating the provenance of pollen are undertaken within palaeoecology to inform vegetation reconstructions (DeBusk 1997; Yang et al. 2016, 2019; Luo et al. 2020) and to assess provenance of archaeological finds (e.g. tracing construction locations of artefacts (Hu et al. 2007; Chester, 2009)), the most well-explored area utilising pollen to locate provenance is within forensic studies (Bryant et al. 1990; Mildenhall 1990; Horrocks and Walsh 1998; Mildenhall et al. 2006; Wiltshire, 2006).

While many criminal cases have been successfully solved (at least in part) by forensic palynology (see Wiltshire (2006a) and Ochando et al. (2018) for some examples), there are a surprising number of unresolved taphonomic considerations that if challenged, could render evidence unusable in court (see Figure 2.4). In the second output in this chapter, Webb et al. (2018) describes the variability of pollen ‘stickiness’ to clothing and finds not only that different pollen morphologies adhere differently to different types of clothing but also that standard procedures for harvesting pollen from exhibits is not as effective as commonly assumed. Expert witnesses can be challenged in court in numerous ways, including facing questioning of continuity of exhibits and the cleanliness of the laboratory, before cross-examination on the actual evidence has begun. It is therefore vitally important to understand the role of taphonomy in relation to forensic cases to enable greater reliability of palynological evidence in court, particularly as this is not simply about academic understanding but something that can change the course of criminal investigations and impact lives by achieving justice for victims, correctly convicting the guilty, and exonerating the innocent. Webb et al. (2018) is an excellent example of this research, and goes further than previous research (Wiltshire et al. 2015), but it is in itself restricted (it only assessed the adherence properties of eight pollen types and three types of material). It would be prudent therefore in every criminal case to investigate the natural decay curve and adhesion efficiency of the pollen on exhibits, and the types of clothing associated with the case before testifying in court. This practice is not commonplace (owing to cost and time pressures) and therefore reliance is placed on a palynologist's knowledge of taphonomic variables, seemingly often without empirical data to support testimonies. See the example given in Wiltshire et al. (2015) whereby she attests that leather uppers on shoes only obtain a rich palynomorph assemblage when they are scuffed through leaf litter, but this is not supported by observations in a research setting. While her experience shows this is probably the case, supporting evidence for this would be beneficial.

There is a huge body of research that could be completed investigating the adhesion of pollen onto clothing, hair, carpets and skin. Several of the papers citing Webb et al. (2018) begin to explore some of the under-investigated areas: Ménard et al. (2021) looks to build a universal protocol for the transfer and persistence of trace evidence (including gunshot residue, DNA, soil, blood etc); Procter et al. (2019) investigates the transfer of soil to clothing; and Hunt and Morawska (2020) examine persistence of pollen on skin after washing. While these papers present results that promote a greater awareness of taphonomic variables in forensic palynology, there remains a huge scope for pollen and fabric specific projects investigating the natural loss of pollen, the retention in weave and the efficiency of washing, as results are not seemingly transferable between pollen types or materials (Webb et al. 2018). It is not inconceivable that transferable skills from landscape modelling in fossil pollen studies (for example POLLSCAPE (Sugita 1993; Gaillard et al. 2008); LOVE (Sugita 2007a); REVEALS (Sugita 2007b)) be adapted and used to model palynological data in forensic settings to overcome some of these taphonomic variables.

In an unexpected development, not only is the work presented in Webb et al. (2018) valuable in forensic disciplines, the paper has also received traction from clinical settings, with findings being used in recommendations for clothing choices for patients with pollen allergies (Roubelat et al. 2020).

6.2 Global transit routes

In the final paper of this thesis, transferable skills from forensic disciplines are used to investigate the pollen diversity in bill encrustations on migrating birds (Wood et al. 2014). Understanding breeding, stopover and migratory routes of birds is particularly important given that habitat loss and climate change is reducing suitable locations for landfall, particularly in regard to rare or declining species. Despite the large numbers of migratory birds that are caught, ringed with unique identifiers and released in the UK, very few are recovered on wintering grounds (Wernham et al. 2002), leaving a poor understanding of migratory routes of migrant birds (Bairlein, 2003). Migratory birds foraging on nectar-rich flowers (or, as is much more typical, insects feeding on flowers) often develop waxy encrustations on the nasofrontal hinge between the base of the bill and forehead. This has been highlighted by Cecere et al. (2011) as an opportunity to retrieve associated pollen from the nectar as a proxy for provenance.

Although both the taxonomic and spatial resolutions reported in Wood et al. (2014) are not sufficient for the authors to answer the initial aims of the paper of fully mapping migratory routes, palynology is able to differentiate species, suggesting differences in foraging habits and/or habitat (and thus migratory route). Thus, whilst Wood et al. (2014) are not able to provide a refined provenance for Old-World warblers while on migration, the results of the study provided the first pollen encrustation records from migratory birds – potentially starting the first database of this type. Subsequently, the method employed in this paper has been adapted by Domínguez-Vázquez et al. (2021) to compare pollen rain with pollen adhering to feathers to successfully assess foraging behaviours of songbirds in Mexico.

Chapter 7
Forensic science specialisms: palynology, entomology and DNA

Introduction

Forensic science seeks to apply scientific principles and techniques to issues that are of interest to the law. It is important to realise that forensic science is not, therefore, solely concerned with high-profile, violent or emotive crimes. Any legal investigation that can be explored though the application of science may need to call on a 'forensic scientist', even though the scientist in question may not be part of a regular forensic team. Thus, an expert on certain chemicals may be called upon to advise an investigation into a suspicious fire, and may even be required to act as an expert witness in court. For the purposes of the fire investigation, such a scientist would be acting as a 'forensic scientist'. However, there are other scientists who specialise on the application of their field to legal issues and it is these scientists that are the focus of this chapter.

We are bombarded with images and stories that combine forensic science-fact with forensic sciencefiction. The result of this is to give an overall impression that science can 'solve' practically any crime through the use of complex equipment, cutting-edge technology and brilliant (and generally glamorous) forensic scientists. As is often the case with television and film, most of what is portrayed is based in fact but the details, timescale and scope of the techniques shown are frequently exaggerated. Forensic science is an extremely important component of the investigation process, but understanding how it can be applied can often be as much about grasping its limitations as getting carried away with its potential. However, with rapidly developing technology supporting a deepening understanding of much of the science underpinning forensics, there is no doubt that the science-fiction observed in popular television series may start to become more of a reality over the coming years.

The basic principle of forensic science was stated by one of the pioneers of the field, the French scientist Edmund Locard (1877–1966). Locard became known as the 'Sherlock Holmes of France' and Locard's Exchange Principle (usually abbreviated to Locard's Principle) can be summed up as 'every contact leaves a trace'. In essence, Locard's Principle distils the idea that 'criminals leave clues' into a scientific principle. By being at a crime scene, by touching objects and by contacting victims, a perpetrator leaves traces. Whether that be DNA shed by blood, semen or skin cells left on door handles, mud from shoes left on the ground, or pollen grains carried in on a trouser hem from the perpetrator's unusually planted garden, it is rarely possible to be involved with a crime scene without leaving traces of your presence. It is the job of the forensic scientist to find and interpret these traces.

Pollen as trace evidence

As we walk along, the soles of our shoes are in contact with the ground surface and there is a simultaneous exchange of traces from the shoes to the ground and from the ground to the shoes. Thus, footprints potentially record a trace of the route taken, while shoes can carry a record of the ground over which they have walked. It is this exchange of traces that is the basis of Locard's Exchange Principle. To a forensic scientist, the exchange of soil, dirt or dust to our soles can be useful in criminal investigations as the traces exchanged can contain a wealth of information (depending on the surface walked upon) allowing us to reconstruct recent movements. Key evidence contained within these traces are pollen grains and spores, which may have originated from vegetation and fungi found along the route taken. To a forensic botanist, or palynologist (someone that studies pollen grains and spores), these microscopic entities can play a pivotal role in piecing together movements of forensically important items.

Pollen grains are generally microscopic particles produced by the male parts of plants and trees. Pollen and spores are generally 20–60 microns in size (there are 1,000 microns in a millimetre), often with a highly ornamented outer shell. They can be identified (that is, the species that produced them can be determined) by this ornamentation, the thickness of outer wall, the surface patterning of the grain and by overall morphology (shape). Many of the plants producing pollen are so-called flowering plants (angiosperms) that typically have conspicuous, brightly coloured flowers to attract insects and other animals to them. These animals transfer pollen from plant to plant thereby fertilising those plants. However, some trees that produce copious quantities of pollen do not have flowers but do produce pollen and seeds. These include pines and other conifers (part of a group of plants called the gymnosperms) and these trees use wind to transfer their pollen. The morphology of pollen tends to vary according to how it is dispersed. Wind-pollinated grains are generally light, large and contain air sacs (to help with buoyancy in their dispersal), whereas insect-pollinated grains tend to be spiky and sculptured to help them to attach to insects and other animals. Spores are produced by a different group of more primitive plants that include ferns and mosses, as well as by organisms that are not plants at all, such as some algae and fungi.

Pollen and spores are produced as part of the reproductive phase of the life cycle of a plant. Once spores are released, most go on to germinate and develop into a new individual. Pollen grains, however, originate from the male reproductive organs of the flower and are released into the environment to seek a suitable female host to complete the fertilisation process. Both spores and pollen are highly variable between species and it is this variation that makes them useful forensic indicators.

The method of release of the pollen varies between species; the two main dispersal mechanisms are by wind and by insects. Those species that pollinate via the wind can produce millions of pollen grains to raise the possibility of reaching a target female for fertilisation. The plant species that rely on insects for pollination produce far fewer pollen grains as insects insure that the pollen will be transferred directly to their destination. Information on pollen abundance of different species is very important to the forensic palynologist as it helps to understand which species can be important in indicating presence at a site. For example, insect-pollinated plant pollen might be transferred to a suspect's jacket only by directly brushing past a specific plant whereas the presence of wind-pollinated pollen on clothing might merely indicate the presence of the suspect in the general vicinity of the site (collecting pollen and spores from abundant wind-pollinated species by the so-called 'pollen rain').

The distance that an individual pollen grain will travel away from the parent plant depends on a variety of factors. It is very important for the forensic scientist to understand these factors as they influence which pollen types can be found at a particular place. Such factors include the method of pollen release (which will impact on the distance the pollen will travel), the height the pollen is released from, any obstructions in the vicinity (buildings, dense vegetation), the prevailing atmospheric conditions (wind, strength and direction, updrafts and downdrafts, humidity and temperature), and also the morphology (shape and structure) of the grain itself, which affects its aerodynamics.

Many pollen grains and spores do not reach their intended destination, but fall near to the source, settling on to natural and man-made surfaces such as pavements, vehicles and buildings. Here they become incorporated into the general dust, and can reside for long periods of time because of their tough outer coating. The fragile reproductive nucleus of pollen and spore grains are lost relatively quickly, but the outer shell of the pollen (made of sporopollenin) can persist for thousands of years given the right conditions (ideally, anaerobic (lacking in oxygen) and/or acidic environments). It is this persistence that makes pollen grains and spores useful in forensic archaeology and the reconstruction of past environments. It is possible to examine archaeological remains, for example, to determine what plants may have been cultivated in that area historically. It is also possible to 'go back in time', by extracting sediments from deep underground and examining them for pollen, which will give an indication of the plants (and therefore the environmental conditions) prevalent at a given point in history.

Ecologically, the environment around us is complex and dynamic, changing seasonally and spatially. It is rare for soils, dust or sediments containing palynological evidence from different areas to be the same, as plants and flowers vary from place to place, often uniquely. In a forensic investigation this can be a valuable way to trace the presence of an item or person to a particular area. By assessing the pollen and spores from objects of interest (clothes, shoes, bags, vehicles, etc.) it may be possible to reconstruct the recent history of that object. It is easier to understand this with a simplified but illustrative example. If a crime has been committed in a garden that contains an unusual assemblage of rare plants, then the presence of pollen from this unusual group of plants on the clothing of a suspect would be highly indicative that they (or at least their clothing) had recently been in the garden associated with the crime scene. However, it is unlikely that the scene of crime was the only area visited by a perpetrator and so a palynologist needs to be able to unpick the mixed array of sites represented by the pollen and spores on an item, and be able to assess the assemblage of vegetation for the crime scene. The presence of rare vegetation types (perhaps present at a crime scene) and grains from insect-pollinated plants (which are far less dispersive than wind-pollinated grains) can be of particular importance in isolating an area.

Palynology has been used in the UK within legal cases since the 1950s with varied success. The success of cases often relies, as with any case, on the strength of the evidence involved. There are a number of issues to take into account when using palynology within criminal investigations and each of these has the potential to overthrow a case.

Fabrics and pollen

The nature of the fabric of items trapping the pollen is an important factor when considering the assemblage of pollen grains contained within and on them. Clothing with an open weave has a tendency to trap more pollen and spores than a tightly woven fine fabric. Static electricity can also be a crucial factor in determining how well a given fabric traps microscopic pollen and spores. Certain fabrics generate a large amount of static electricity and these forces can attract pollen. Consequently, some clothing might yield greater assemblages of pollen than other items. Static forces can also prevent transferral (or loss) of pollen from a surface. For example, once trapped within a static-prone clothing material (such as synthetic fleece fabrics), pollen may be reluctant to be transferred to another fabric. This may be important when assessing whether two people have been in close contact (perhaps exchanging pollen and spores from each other's clothing). In fact, pollen in certain fabrics may be effectively irretrievably bound to that fabric. Extracting the evidence from such materials for analysis is not a problem, as chemicals used in the laboratory to retrieve pollen from fabrics reduce the static and release the pollen and spore grains.

Contamination

Contamination is a recurring theme in forensic science (see the section below on DNA evidence, for example) and it is another hazard when dealing with pollen and spores. Ideally, samples for palynological investigation should be collected as soon as possible after the crime has been committed, reducing the potential contamination or inadvertent destruction of the scene or associated items. Sampling of the crime scene must be done by an experienced Scene of Crimes Officer (SOCO) or palynologist. It is important to remember the context of the crime when sampling soils, dust and sediments to ensure the correct depth of the material is sampled. If a perpetrator has simply walked over a soil surface, then only the very fine surface need be sampled, but if a struggle has taken place on the soil surface, it may be more appropriate to take a range of samples to account for the depths of the soil that may have been disturbed. The tools used in sampling, and the storage vessels for preserving exhibits need to be new, or cleaned thoroughly after each sample is taken to avoid cross-contamination.

Once sampled, the storage of samples and seized exhibits is also important to avoid further contamination. Bacterial and fungal activity in soils and fabrics need to be inhibited (by drying fabrics and freezing soils) to avoid degradation of the pollen and spores or dilution of the palynological evidence from the crime scene with fungal spores that can form after the evidence has been collected. The standard protocols of detailed documentation of the movement of all samples must be undertaken to avoid any suggestion of contamination and to establish a chain of continuity of the possession of the evidence at all times.

Bodies and pollen

It is not only fabrics and objects relating to a crime scene that can be assessed for palynological evidence. Various cavities and areas on bodies can also reveal useful pollen and spore evidence that can be useful in determining the peri-mortem (at or near the time of death) fate of victims. The electrostatic properties of hair (and association with styling products) make it a potential reservoir for pollen and spores. In a murder case, the victim's hair may provide a pollen history of the areas that the victim had visited before death and being able to profile this area based on the pollen and spores trapped within hair can provide important information to investigating teams. Wiltshire (2006) documents a case in 2001 where the hair of a young female victim yielded garden flora that was starkly different from the woodland location where her body was found. The pollen matched a key suspect's garden vegetation and in July 2002 a guilty plea was made and life sentence awarded.

Fingernails and nasal cavities can also yield good palynological evidence. In 1994 a mass grave was discovered in Magdenburg, Germany. The origin of the victims was unknown, but it was thought that either they were killed by the Gestapo in the spring of 1945 or by Soviet secret police during the summer of 1953. Because nasal flushings from several of the victims matched the palynological profile from volunteers' handkerchiefs for mid-summer, it was concluded that the victims were killed by Soviet secret police (Szibor et al. 1998). The application of palynology in criminal investigations is extensive. Not only has it been used to link suspects or objects to scenes of crime, but also to find clandestine graves by profiling the vegetation of the site from a suspect's clothes, footwear or vehicle, and to trace the origin of weapons, drugs and even illegal immigrants in cases of human trafficking.

The forensic palynologist

The forensic palynologist needs to be a botanist as well as having extensive knowledge of pollen and spore identification. Being able to profile an environment from the mixed assemblage of pollen and spores retrieved from key items is a vital skill that stems from extensive knowledge of the taphonomy (dispersal and preservation) of pollen and spores and the nature of the vegetation and its pattern in the environment. There are around 1,500 native species of flora in the UK (not including sub-species, microspecies, forms or hybrids), and also countless exotic species bought in from overseas. This range of species demonstrates the importance of having reference collections (identified samples of pollen from different species) to aid comparisons. Currently there is no reliable computer recognition software to use to identify pollen and spores, so identification relies solely on skilled human interpretation. Unlike DNA, gunshot residues and fingerprints, there is no database available for the palynolopgist to consult for national distributions of vegetation, so it is important to be in contact with local botanists to be able to identify areas that are represented by the pollen found in an investigation. Often contemporary knowledge and vegetation mapping is not enough when dealing with residual pollen in soils. In a murder case in Wales, pollen from *Juglans* (walnut) persisted in the soil for nearly 80 years after the tree that had produced it had been felled (Mildenhall et al. 2006). It highlights the importance of being able to make comparisons from crime scene soil to offending articles, rather than from general vegetation surveys, which might fail to indicate the presence of the single plant that may have produced the pollen of interest.

Looking to the future, there is the potential to form a national database using the DNA extracted from pollen and spores to identify the parent plant, rather than using its morphological characteristics to identify the species. Potentially, the DNA could link to the individual plant, just as human DNA can match to an individual. The current plant DNA database is not large enough to be able to do this, and the task to be able to register all plants is immense, so it is perhaps unlikely that this will be useful in the near future.

The potential of using the Fourier transform infrared (FT-IR) technique has been tested by Gottardini et al. (2007) as an alternative method to the manual, time-consuming method of pollen identification. Presently a library of 11 taxa has been developed using the microspectroscopy FT-IR method, which has produced reliable results on clean, fresh pollen (Dell'Anna 2009). It is unlikely this will replace optical identification as forensic samples are rarely fresh, but rather yield 'dirty' samples, with the pollen on the microscope slides being shared with other debris, and the huge number of taxon would need to be recorded in the library.

Box 7.1 Expert witness: Palynology profiling

Dr Patricia Wiltshire-Hawksworth has been actively engaged in forensic palynology and environmental profiling for 17 years. She and has acted as a consultant for police forces throughout the England, Wales, Ireland, Northern Ireland, Scotland, and abroad on over 200 investigations. Patricia began her career as a botany graduate and laterlectured at King's College principally in microbial ecology, but also in palynology and ecology, with special responsibility for soil science. She was engaged in research in palaeoecology and microbial ecology at King's until, in 1988, With her research then concentrating on environmental reconstruction in archaeology by palynology.

In 1994 Hertfordshire constabulary made contact with Patricia after they discovered a body dumped in a ditch on the edge of an arable field. The police wanted to know if a particular car had been to that field and, realising the vegetation could be useful, contacted Kew Gardens in London. Patricia was put in contact with Hertfordshire police by Kew, and she visited the field and the car. She found that the pollen and other microscopic evidence from the field edge was very similar to that in the car and this evidence helped secure confessions from several individuals and led to their subsequent conviction.

Since then she has worked on cases involving rape, murder, theft and abduction. She has been involved in many high-profile cases, including Operation Maple (the murder of Sarah Payne), Operation Fincham (the Soham murders), Operation Sumac (the murder of five prostitutes in Ipswich) and Operation Ruby (the murder of Amanda Dowler).

She feels that a background in botany, ecology, and particularly soil science, is critically important to a forensic palynologist. Interpretation of palynological data requires a holistic understanding of the environment, as understanding the environment and its ecology is paramount to profiling an area from the assemblage of pollen retrieved from a seized item. 'A career in forensic palynology is difficult to get into, but a background in ecology and knowledge of applied sciences in criminal cases is a useful starting point.'

Forensic entomology

To human beings, a human corpse can be many things: a loved one, a stranger, a victim, or perhaps a potential health hazard that requires disposal. For many organisms, however, a human corpse is a valuable resource and a rich and diverse ecosystem can develop to exploit that resource. In making use of dead bodies, especially for nutrition, these corpse-associated organisms have a valuable role to play in the decomposition process. However, some organisms involved in these post-mortem ecosystems can also play a role in the forensic process. Of particular note here are the insects that are attracted to corpses, and interpreting the insect fauna associated with a dead body to gain information relevant to a criminal investigation forms the basis of the science of forensic entomology. *Post-mortem interval*

The most common use of insects in a forensic investigation is to determine the post-mortem interval (PMI). Although the practical skills and knowledge required to undertake forensic entomological investigations are complex and highly specialised, the ecological and biological principles that allow the forensic entomologist to determine PMI are relatively straightforward.

Succession

The first principle to understand is *ecological succession*. Ecological succession describes the predictable sequence of organisms that colonise a given location. Organisms are frequently highly specialised for particular ecological niches, which is a term that encompasses an organism's place and role in the environment. When a new 'environment' becomes available, perhaps a tree falls down in a forest, opening up a large gap, or, in the same forest, a body is dumped on the ground, this new resource is not equally attractive to all species. Some species are good at colonising the resource, whereas other species can only move in once these initial colonisers have led the way. In the case of forest plants, the initial species are sometimes called 'gap demanders', whereas the species that end up filling the gap are called 'late successional' species, and they form a 'climax community'. With a dead body, the succession pattern of most interest concerns insects and can be more complex, because the insects occupying the body are reducing the resources available from the body by consuming it, and because many of the insects that colonise the body also themselves attract a fauna of insect parasites and predators. However, the general principle, that bodies are colonised by a more or less predictable sequence of organisms over time, plays an important role in forensic entomology.

Insect development

The second principle central to forensic entomology concerns the development of insects from egg to adult. At this stage, it is useful to consider an actual insect, and one of the most important in this context is the blowfly. The insects are divided into a number of different groups called orders. The common names for many of these orders are familiar (for example, the butterflies and moths, or the beetles), although the scientific names are less well known (Lepidoptera and Coleoptera, respectively). Blowflies, along with a number of other flies involved in the colonisation of bodies, belong to an order called the Diptera. Bluebottles, greenbottles, flesh flies, coffin flies and craneflies are well-known members of this order. The Diptera, in common with a number of other insect orders (including the butterflies and moths of the Lepidoptera) undergo a form of development called complete metamorphosis. In the butterflies, this is familiar to most people as the sequence egg–caterpillar–chrysalis–adult. In more general terms, the sequence runs egg–larva–pupa–imago (or adult), although of course in the flies the larva is often called a maggot. The time it takes for an individual to pass through the stages of development varies according to temperature but for any given temperature it is more or less fixed. Since it is possible to identify the different insect stages under a microscope, it is possible to determine the oldest insect stage present on a body and then ‘count backwards’ to work out when the egg must have been laid. This is often called the ‘earliest oviposition date’. Linking this to other information, such as how long through the decomposition process a given species likes to lay eggs on a body, allows an approximation of the post-mortem interval to be made. In fact, as we shall see, insects really only allow for an estimation of the ‘minimum time since death’, but in certain investigations this can be extremely useful.

Box 7.2: The blowfly

Blowfiles are commonly known as bluebottles or greenbottles and belong to the family of flies called the Calliphoridae. Commonly associated with corpses, they are very often the first insects to arrive at a fresh corpse and they have been the insects most commonly used in forensic investigations. However, it is important to realise that not all members of the Calliphoridae are associated with corpses, and that a number of species of flies commonly associated with corpses do not belong to this family. Correctly identifying the species that are present is an essential part of the process of interpreting forensic entomological evidence. It is helpful at this stage to follow a blowfly through its development from egg to adult.

It is the blowfly female that lays eggs on corpses, and a gravid female (one that is carrying fertilised eggs ready to be laid) is attracted to a corpse by odour, especially the smell of blood. Blowfiles are early successional colonisers and are attracted to fresh corpses (in fact many species lay eggs on the wounds of injured animal before they die). Once they have arrived at a body they seek out openings in which to lay eggs. These may be natural openings provided by the mouth, nose, eyes, ear, anus or genitalia, or the flies may use openings created by injury or by scavengers. A body with extensive wounding that is dumped in the open, during daylight and when ambient temperature is high will likely be found within minutes or hours by blowflies, whereas lower temperature or a night-time dumping will mean flies will be slower to colonise. Bodies dumped inside buildings with limited entry points for flies may also experience a slower colonisation, and bodies that are buried will likely never attract blowflies. Having knowledge of the ecology of the corpse site is thus of tremendous importance in interpreting the pattern of insects associated with it.

Typically, females lay eggs in batches of up to 180 eggs, with females able to lay several thousand eggs during their lifetime. If a body is found with eggs but no evidence of larvae, it is likely that the body is fresh. Eggs can be collected and incubated in the laboratory under controlled conditions and, using such a technique, it is possible, in some species, to estimate the time of egg-laying to within two hours. Frequently, though, a mix of eggs and larvae are found together and it is the oldest larvae that can be used to give a minimum time since death. It should be noted that it is all too easy to make simple and incorrect assumptions regarding the insect fauna on a body. For example, the flesh flies (belonging to the family Sarcophagidae) are viviparous, which means that instead of laying eggs on a body, they ‘lay’ larvae. Consequently, it is entirely possible to have the eggs of one species and the larvae of an entirely different species present on a body at the same time. The stages of each species can be used to provide forensically useful information, but only if they are recognised as being of different species. It can be possible to identify species from immature stages, but it can sometimes be easier, and more reliable, to rear up immature stages until the adults emerge, at which point identification becomes much more straightforward.

After a period of time, which depends on temperature, the eggs will hatch into very small and very delicate first instar larvae. The larvae have strongly developed mouth-hooks made from chitin, the same material that forms the tough exoskeleton of hard-bodied insects. Using these mouth-hooks and the backwards pointing bristles that project from the body wall, the larvae begin to burrow into the corpse, feed and grow. By moulting (where the outer skin of the larvae splits to release the next stage) the larvae grows considerably and develops through the second instar and into the third instar. The moult after the third instar sees the larvae develop into pupae, equivalent to the chrysalis of a butterfly caterpillar and the stage when the body is rearranged to make the final stage, the adult or imago. Blowfly larvae leave the body to pupate and so pupae are found on the ground, in crevices and in the soil around a body, rather than on the body itself.

Larvae are growing rapidly and to support this they need to get rid of carbon dioxide and take in oxygen. Larvae are able to perform this gas exchange through spiracles, structures at the end of the larva opposite the mouth that are perhaps best thought of as snorkels. Fortunately for the forensic entomologist, the number and shape of these posterior spiracles allows the identification of the instar stage. Within each stage it is possible to narrow down the age further by measuring the length of the larva, although this is not without problems. For example, the fluids commonly used to preserve insects can cause shrinkage and so it is better to make any measurements on fresh specimens before preservation. It is also possible to determine the age of a pupa, since pupae get progressively darker as they mature and the colouration of a given sample can be compared with pupae of known ages.

Complications

In most situations corpses would be colonised by a number of different female blowflies over a period of time and so multiple broods will be developing. To determine the time since eggs were laid, it is first necessary to find, indentify and age the most developed stages, which of course may include pupae that are no longer present on the corpse. It is also necessary to determine the temperature at the deposition site since temperature has a strong influence on the developmental schedule of the immature stages. It is then possible to use published data on the developmental times of species at different temperatures to count backwards and calculate the minimum time since eggs were laid.

However, although this method is, in principle, very straightforward, a number of factors serve to complicate matters. First, the research on the development of different forensically important insects under different conditions (including temperature, but also degree of burial, presence of chemicals and many other forensically likely scenarios) is by no means complete. Second, although measuring temperature at a given point and a given time is easy, getting a temperature profile for a given site over the time that a body has been there can be very difficult. Local weather stations are often situated far away from the deposition site and, in any case, localised micro-climates can cause large differences in conditions that can have serious knock-on effects for our calculations. Nonetheless, it is still usually possible to make a best-estimate.

As well as variable temperatures, there are a number of other factors that can have a part a play in confounding the forensic entomologist. Bodies can be stored (even frozen) before they are dumped, they can be dumped in places with limited access for flies or during times of the year when flies are less active. Bodies can be treated with insecticides either to confound entomological evidence or as a by-product of other activity. For example, petrol and other accelerants used to burn bodies pre- or post mortem can influence the attractiveness of bodies and the development time of eggs laid there. In some cases, flies may be present before the death (a condition called myiasis) and if this occurs it can cause overestimation of PMI. Even the maggot mass itself can cause issues for the forensic entomologist since their metabolic activity produces heat which can reduce development time of maggots regardless of the ambient temperature recorded outside the body.

Box 7.3

Dr Kate Barnes: Lecturer in Forensic Biology, University of Derby

I got into Forensic Entomology quite late on in my academic life. At school I changed my mind every five minutes about what I wanted to do, but I always enjoyed science. I thought about studying Forensic Science or Biology at university but ended up choosing a broader science degree to keep my options open. I then went on to study an MSc in Bioinformatics, for which I had to complete a six-month research project. I wasn't very enthused by any of the titles offered so went to see a lecturer with an interest in forensic science. He turned out to be an entomologist and I ended up entering the world of forensic entomology! I remember being amazed by how much information a simple blowfly maggot could provide and knowing this was exactly what I wanted to do for the rest of my life.

The experiments I conduct on pig carcasses help me understand what would happen to a human corpse in a similar environment. I am on the local police database so I will be called out when entomological data needs recovering and analysing from a crime scene.

What research are you involved with?

At the moment I am investigating the sequence of carrion-feeding insects that colonise a corpse in woodland. I am also conducting research with students to investigate how drugs affect blowfly development, how insect succession and decomposition rates vary on burned, frozen and wrapped carcasses and if blowflies are active at night.

What's the goriest thing you have to work with?

I've worked with some pretty gory specimens but I think smell-wise the worst thing I have encountered was a waterlogged decomposition site when I was looking at microbial and insect succession on pig carcasses. The first two weeks were lovely, warm, sunshine-filled days; then it rained. The pig carcasses were placed on trays filled with soil, but they were only shallow and became waterlogged quite quickly. The combined result of decomposition plus anaerobic waterlogged soil is not something I want to smell again in a hurry!

I feel very lucky to be working in the field of forensic entomology, especially as openings into this area are few and far between. I think the best thing about my job is having so much variation and no two days are the same. My research is quite varied and uses methods from lots of different disciplines, such as entomology, microbiology, molecular biology and biochemistry. It also means that I work in both laboratory and in the field.

In the UK, most Forensic Entomologists work at universities and museums and conduct consultancy work for the police when requested. There are a few people who work for forensic companies who are specialised in other forensic disciplines, such as blood splatter, but analyse the entomological evidence when it is recovered. For someone who wants to get into this field, I would suggest joining their local entomology and natural history groups. There are also really good national organisations, such as the Amateur Entomological Society (AES). If you go to university, choose an undergraduate degree in something like Forensic Science or Biology/Zoology and make sure it has an entomology module.

DNA evidence

Of all the techniques used by the modern forensic scientist, those involving DNA are perhaps the most commonly identified with criminal investigations. There is something mysterious and, for some people, even disturbing about our ability to analyse the ‘code of life’, yet a chain of developments in biological science since the 1950s have led to increasingly powerful tools that can be used by forensic scientists. Understanding DNA from a molecular perspective and grasping its function within organisms are crucial to understanding how this extremely important molecule is handled in the laboratory and used forensically. By developing a biologically rooted understanding of DNA, it is also possible to explore the limitations and potential abuses of DNA evidence.

DNA

Living things are constructed from cells; microscopic, highly organised ‘packages’ that contain the cellular machinery that power life. In complex organisms made up of many cells, individual cells tend to be specialised for specific roles. Consequently, in the human body there are muscle cells for movement, nerve cells (or neurons) for transmitting nervous impulses, cells in the stomach wall for secreting digestive acid, and so on. Within cells are many different structures that play important roles in the functioning and organisation of that cell, as well as a large number of different types of molecule that are used for different purposes. Some of these molecules, and classes of molecules, are quite familiar to us from our everyday lives, for example water, sugars and fats (also called lipids), whereas others, like phospholipids, polypeptides and DNA, are familiar only within the context of biochemistry. The biochemistry, structure and function of cells are so complex that considerable organisation has to take place within the cell for it to function correctly. Furthermore, many cells produce a number of distinct products, for example enzymes (proteins that act as catalysts, speeding up chemical reactions). DNA is the key to organising the machinery of cells so that the right things are made in the right place at the right time. It is often referred to as the ‘blueprint of life’ but this is misleading. Blueprints are used once, in the building of a structure, whereas the information encoded in DNA is constantly being used by cells. It is perhaps more useful to think of DNA as a trusted recipe book, in near constant use to ensure the end product is correct and consistent.

DNA, or deoxyribonucleic acid, is an informational macro-molecule, which is to say that is it large and that it contains information encoded within its structure. It is that information which is the key to its function in the cell and its role in forensic science. The best way to think about DNA is to imagine a ladder with many rungs. In DNA, the two vertical columns of the ladder are the so-called sugar-phosphate backbones. The sugar we put in our coffee is a molecule called sucrose, which consists of two rings of carbon atoms joined together to form what is called a disaccharide. However, there are many different types of ‘sugar’ (or saccharides), including a monosaccharide (with just one ring of carbon atoms) called deoxyribose, a name that may be familiar from the full name of DNA – deoxyribonucleic acid. Deoxyribose is connected to another molecule, phosphate, and together these alternate up the side of the ladder.

It is the rungs of the ladder where DNA becomes interesting. Connected to each unit of deoxyribose and phosphate is a so-called ‘base’, and together this triple unit forms a new type of molecule called a nucelotide. There are four kinds of base in DNA, called adenine (A), cytosine (C), guanine (G) and thymine (T), and consequently there are four different nucelotides that make up the DNA molecule. Imagine that our DNA ladder has been cut right down the middle, with the cut going through each of the rungs. Each half of the ladder is now like a long chain of connected nucleotides, with the bases sticking out. To join up the two halves of the ladder we need to get the bases to interact with other and make a stable connection. The structure of the different bases is such that the only stable way for them to interact is through specific base pairing, with A always pairing with T and C always pairing with C (and vice versa of course). Pairing up the two halves of the DNA ladder following this so-called complementary base pairing completes the DNA molecule. Properties of the different components of DNA mean that the final shape of the molecule is an elegant double helix, as if our ladder has been twisted to make a spiral.

DNA as an ‘information molecule’

The bases that comprise the ‘rungs’ of the DNA ‘ladder’ are where genetic information is encoded. If we think of DNA as a recipe book, then the end product of the recipe are molecules called proteins. Like DNA, proteins are large molecules that are technically called polymers, since they are made by joining together individual smaller building blocks called monomers. In DNA, the monomers are nucleotides, whereas proteins are polymers made from around 20 different types of amino acid. Combining amino acids in different sequences produces many different types of protein. Proteins have a huge diversity of function within cells and organisms, forming structures (collagen in skin and connective tissue is a protein), controlling biochemical reactions (most enzymes are proteins) and even regulating the use of DNA within the cell. To manufacture a protein within a cell requires a complex interaction between molecules and cell structures, joining the correct amino acid to a growing chain of amino acids that will eventually form a specific protein with a specific role. The protein ‘assembly line’ uses DNA to direct the manufacture of proteins. Along the length of DNA molecules are sequences of base pairs that code for a specific sequence of amino acids that will combine together to form a specific protein; such sequences are called genes. Each amino acid is coded by a sequence of three bases, a triplet which together specify which amino acid goes where in the protein.

It is possible to ‘read’ the genetic code and, by knowing which amino acid corresponds to which triplet, it is possible to determine the structure of proteins. Developing an in-depth knowledge of genetics is incredibly important for many biological applications, including understanding and managing disease. However, it does not provide much of use for the forensic scientist looking to identify individuals from DNA left at crime scene. Being able to identify individuals requires that you analyse properties that differ between people, and the basic genetic code for making proteins tends to be rather similar between people and even between different species in many cases. What the forensic scientist requires are regions of the DNA molecule that differ between individuals. Important coding components of DNA do not offer much variation, but there are regions of DNA that do not seem to code for anything. These non-coding regions may well have an important function in the cell, but for the forensic scientist they are especially useful because of the variation they tend to show between individuals. The variation between people at one part of the molecule may not be great, and it may not be possible to distinguish the DNA from many people at just one site (or locus); it may be that 50 per cent of the population have one type of sequence (called an allele), and 50 per cent another. You can say for sure that a specific sample of DNA did *not* come from someone (if they are in the 50 per cent of the population that do not have a particular type), but you cannot identify specific individuals within the other half of the population. However, if we can examine enough loci (the plural of locus), then we start to narrow things down considerably. To use an analogy, predicting the toss of a single coin correctly is possible 50 per cent of the time, but predicting it correctly ten times would only happen once for every 1,024 times you tried.

DNA at a crime scene

DNA is found within the largest of the structures visible within a cell, the nucleus. Virtually all cells have a nucleus (red blood cells are a common exception) and therefore contain DNA. We are constantly shedding cells and a great deal of everyday activities, like combing our hair, brushing our teeth or lying in bed leave behind large quantities of cells and therefore DNA. Specific activities, including those commonly associated with crimes, may greatly increase the amount of DNA we leave behind through the shedding of fluids and solids such as blood, saliva, semen and faeces (which contain cells from the digestive tract). Modern techniques are able to recover and analyse very small quantities of DNA (even from a single cells in some cases) and so DNA left at a crime scene can be recovered from all manner of physical traces, including (but not limited to): cigarette butts, chewing gum, handkerchiefs, weapons, bite marks, cutlery, plasters, used syringes, blood spots, fingernail scrapings, licked envelope and stamps, and activated airbags in vehicles. The propensity for humans to shed DNA causes one of the biggest issues with forensic DNA analysis; the contamination of samples from a crime scene with DNA from individuals associated with the crime scene but not the crime. Extreme care must be taken to avoid investigator DNA becoming mixed with perpetrator DNA and to prevent the possibility of a perpetrator deliberately contaminating a crime scene with their DNA in order to explain its presence there following subsequent analysis.

DNA analysis

To analyse DNA, it is first necessary to identify and collect suspected DNA evidence at a crime scene. Commercially available FTA® cards produced by Whatman International Ltd are used to collect DNA. These cards contain chemicals that stabilise and preserve DNA, allowing it to be stored for long periods (potentially years) at room temperature. Cards of different shapes and sizes can be pressed against suspected DNA sources (like a blood spot), making DNA collection relatively straightforward and ensuring stable transportation to the laboratory and subsequent storage. It is important to realise that although DNA is used in high-profile crimes like rape and homicide, the relative ease and low cost of modern DNA analysis means that it is also used in lower profile crimes, including burglaries, car crime and malicious letter sending.

DNA fingerprinting

The technique most commonly associated with the forensic use of DNA is DNA fingerprinting, and the most common way of doing that involves using sections of DNA called short tandem repeat markers (STRs). Confusingly, these are also called microsatellites (or microsats) and simple sequence repeats (SSRs). These are short lengths of non-coding sequences (they do not instruct for a sequence of amino acids) that have fewer than 400 base pairs. In this sense, tandem means ‘one after the other’ and within the sequence of base pairs in an STR there will be repetition of a particular sequence of base pairs. For example, at one locus it may be that the sequence CATG is repeated many times; in some people it may have 12 repetitions, while others may have 13, 16 and 18. Many of these STRs are highly variable, and this variation is important. The different number of repeats represents different alleles and each allele may be present unevenly within a population; 75 per cent of people might have 12 repetitions, whereas only 2 per cent might have 18 repetitions. The forensic scientist uses techniques in the laboratory to analyse the lengths of specific pieces of DNA, or, in technical terms, to identify the allele present at a locus. Analysing the alleles present at multiple loci allows the forensic scientist to give a probability that a given DNA sample came from a given person without the need to sequence the entire genetic code. If enough sufficiently variable loci are examined, then the probability of matching a sample to a person becomes greater and greater. Once a profile has been generated, it is very easy to store that profile digitally, in principle forever. The use and storage of DNA profiles by law enforcement and other agencies is a complex, emotive and controversial issue that has both political and scientific dimensions that are beyond the scope of this chapter. However, understanding some of the limitations of DNA can assist in understanding aspects of the controversy that DNA evidence can incite.

Match probability

Clearly, calculating the probability of a DNA sample (perhaps a blood spot from an injury to the perpetrator) matching a particular individual (for example, the suspect) is of extreme importance. To calculate the ‘match probability’ requires that we know the proportion of each allele at each locus found within a population. From this, it is straightforward to calculate the probability that a person selected from that population at random will have the same pattern of alleles as found in the sample. If our DNA profile is based on a single locus, then comparing alleles may give us a fairly high proportion of people in the population that could match. This might be useful in eliminating people that do not match, but there is still a large number of people that do match. Adding in further loci makes a match with any given randomly selected person much less likely. There are more than 2,000 STR markers that can be used for forensic analysis, but in practice only a very small number are actually used to compile a DNA profile, or fingerprint. In the UK, ten markers or loci are used, and the in USA, the FBI used 13 markers. The variation at these loci, and the distribution of alleles within populations, is such that for a random person to match a sample at all loci can have a match probability so low (one in billions) that we can be very confident our suspect produced the DNA in question.

It is easy to get carried away with the reassuring ‘certainty’ of mathematics and numbers. However, interpreting match probabilities requires a careful approach. First, using this technique, we can only ever get a probability that a particular person matches a particular sample of DNA. It might be highly unlikely that the DNA came from someone else, but it is still *possible* that it did. It is highly unlikely that you will win the lottery, but you *could.* The second reason for caution is related to the careful use of ‘population’ in the discussion above. Many of the assumptions underlying the calculation of match probability come from the science of population genetics and involve ‘ideal’ populations where everyone can ‘mate’ (to use the language of population genetics) with everyone, where there is no immigration or emigration, and so on. In most cases these idealised conditions do not apply to humans; we do not live in a homogeneous, uniform population across the world. We have close relatives (who are more likely to share alleles at STR markers than non-relatives), family groups, racial groups and geographical isolation. As a consequence, the frequency of alleles might be higher or lower than expected in certain family, ethnic or geographical populations. These issues do not negate the use of DNA evidence, but they do mean that simple probabilities need to be treated with caution. An excellent example of this is sometimes called the defender’s or defence attorney’s fallacy. Consider a violent rape that occurred in the UK and where DNA evidence seems to connect the crime to the defendant. The match probability might indicate that 200 people in the UK would also match the DNA sample, and the defence attorney asserts that this pool of potential matches is large enough to render the DNA evidence meaningless. However, are there really 200 people in the pool of potential matches? We know the perpetrator is male, so we can already eliminate half the pool (the female matches), making only 100 people potential matches. We also know that the perpetrator was not a child or an elderly man, so even allowing for potential problems of misidentification of age by the victim, we can safely eliminate those males below 16 and over 60. It may also be that the crime occurred in a small town where the suspect actually lives. Suddenly, our pool of 200 matches is starting to look much smaller than the defence would like.

Other techniques

STRs provide a simple, low-cost, high-volume solution to producing DNA profiles that are useful forensically. They also serve to demonstrate the general principles and issues involved in forensic DNA analysis. However, our ability to analyse DNA and related evidence has advanced greatly since the first DNA profiling began and, although STR profiling is the most commonly used technique, other techniques are available. Techniques to analyse sequences on the Y chromosome (carried only by males) can be used in profiling samples of mixed male origin (such as in multiple rape), for example. Base pair sequences can be identified using DNA sequencing techniques and variation in single nucleotides can be revealed by analysing single nucleotide polymorphism (or SNP).

The DNA considered so far has been found in the nucleus, but short lengths of DNA are also found in structures within the cell called mitochondria, involved in respiration. Analysis of the sequences in mitochondrial DNA (mtDNA) has proved useful in the identification of old and badly degraded human (and non-human) remains, including war and disaster victims and the identification of the remains of the Romanov family, killed during the Russian Revolution. Finally, laboratory techniques to analyse very tiny amounts of DNA, even DNA found in a single cell, are now possible. These low copy number DNA profiling

(LCN) techniques can allow for LCN profiling of fingerprints, lip marks, even a single flake of dandruff, although such small samples can result in mixed profiles from more than one person, and are even more susceptible to contamination than conventional profiles.

Case Study 7.1 Colin Pitchfork: Mass DNA screening

As a result of linking two unsolved murders of young girls which occurred in 1983 and 1986 in Narborough, Leicestershire, the police asked for a DNA test to be performed on samples taken from the crime scenes. DNA testing was not standard at this time.

A local man had admitted to one of the murders but denied being involved in the other. Because of the knowledge he had about the murder he claimed to have committed, the police believed him. However, the forensic analysis established that he was not the killer in either case, and he became the first person ever to be exonerated by DNA testing.

The police then decided to perform DNA tests on 5,000 men in the area. After blood grouping, only 10 per cent of the group needed to be tested – those who had the same blood group as the killer. The killer convinced a friend to go and take the test for him, but the friend was heard talking about the switch in a pub, and eventually the real killer, Colin Pitchfork, was charged and convicted of the two murders in 1988.

Forensic Science International 288 (2018) 36–45

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Differential retention of pollen grains on clothing and the effectiveness of laboratory retrieval methods in forensic settings

Julia C. Webb*, Harriet A. Brown, Hannah Toms, Anne E. Goodenough

School of Natural and Social Sciences, University of Gloucestershire, Francis Close Hall, Cheltenham GL50 4AZ, UK

ARTICLE INFO

Article history:
Received 13 December 2017
Received in revised form 23 February 2018
Accepted 7 April 2018
Available online 15 April 2018

Keywords:
Pollen analysis
Forensics
Laboratory protocols
Criminal investigation
Fabric
Clothing

ABSTRACT

Forensic palynology has been important in criminal investigation since the 1950s and often provides evidence that is vital in identifying suspects and securing convictions. However, for such evidence to be used appropriately, it is necessary to understand the factors affecting taphonomic variability (i.e. the variability in the fate of pollen grains before they are found during forensic examination). Here, we test the relative amount of pollen retained on clothing after a period of simulated light or heavy wear based on pollen and fabric characteristics. We also test the efficiency of forensic laboratory protocols for retrieving pollen from fabrics for analysis. There was no statistically significant difference in retention of fresh or dried pollen on any fabric type. There was a substantial difference in pollen retention according to wear intensity, with considerably more pollen being retained after light wear than after heavy wear. Pollen from insect-pollinated species was retained at higher concentrations than pollen from wind-pollinated species. This pattern was consistent regardless of wear intensity but pollination type explained more of the variability in pollen retention after light wear. Fabric type was significantly related to pollen retention, but interacted strongly with plant species such that patterns were both complex and highly species-specific. The efficiency of removing pollen with the standard washing protocol differed substantially according to plant species, fabric type, and the interaction between these factors. The average efficiency was 67.7% but this ranged from 21% to 93%, demonstrating that previous assumptions on the reliability of the technique providing a representative sample for forensic use should be reviewed. This paper highlights the importance of understanding pollen and fabric characteristics when creating a pollen profile in criminal investigations and to ensure that evidence used in testimony is accurate and robust.

1. Introduction

Pollen produced by sexually reproducing plants is both ubiquitous and resilient in the environment. As well as being associated with parent plants, pollen grains from the species growing in a particular area are found in the air, soils and sediments within that locale. Thus, although there is always a regional pollen signal caused by pollen dispersal over a wide area [1], most sites have a localised pollen “fingerprint” superimposed upon the regional signal. The study of pollen grains by palynologists is, therefore, widely used to reconstruct past landscapes and quantify environmental change (e.g. [2,3]. In addition to fossilised pollen being used to understand historical temporal change of specific sites, pollen profiling can also be used to answer contemporary spatial questions. One of the most novel modern-day palynological applications involves analysis of pollen attached to, or embedded within, items connected to a crime.

Since the 1950s, forensic palynology has enabled evidence from pollen and other plant palynomorphs (such as spores associated with plant reproduction) to assist criminal investigation and secure convictions around the world [4–6]. Palynological investigation can involve examining items potentially associated with crimes as diverse as forgery, drug dealing, robbery, terrorism, assault, rape, and homicide [7,8]. Analysis of fabric – including clothing, shoes, and materials such as blankets and carpet – is particularly common in forensic settings. The insights that can be provided by studying fabric include: (1) matching clothing from a suspect to a crime scene; (2) locating proceeds of crime or human remains; (3) analysing clothes of a murder victim to establish peri-mortem fate and to differentiate the scene of a crime from the scene of deposition; and (4) providing evidence to support or refute alibis [9].

In many cases where palynological evidence is used to help solve a crime or secure a conviction, the adhesion of pollen on fabric, and its

---

* Corresponding author.
E-mail addresses: jwebb@glos.ac.uk (J.C. Webb), htoms@glos.ac.uk (H. Toms), aegoodenough@glos.ac.uk (A.E. Goodenough).

https://doi.org/10.1016/j.forsciint.2018.04.010

subsequent retention on that fabric, is critical. In the case of clothing, pollen can be transferred directly (e.g. by brushing up against vegetation: [10] or indirectly (e.g. via contamination by soil or dust: [11]. Following adhesion, pollen can be lost from clothing as a result of everyday activities, including being rubbed or shaken off during wear, generating a natural decay curve. Alternatively pollen can remain on the surface of the fabric or become embedded within the weave [12]. Ensuring that palynological evidence is robust requires an understanding of the factors that influence adhesion and retention of pollen [13]. This is vital to ensure that simplistic assumptions about how pollen (of different species) is transferred to and from clothing (of different fabric types) does not lead to incorrect inferences being made by expert witnesses, which could affect testimony and verdicts in courts of law.

Understanding the complexities of pollen-clothing interactions requires an awareness of the biology of a pollen grain. In general terms, the lack of mobility of individual plants is mitigated by production of small, light, and highly-mobile pollen grains. The mobility of pollen grains *en route* to their intended destination (the stigma of the flower of an adult plant of same species) relies on one of four vectors to transport the individual grains: the wind (anemophily); insects (entomophily); mammals and birds (zoophily) or water (hydrophily) [14]. The latter two pollination vectors are uncommon for angiosperms [15,16]. Following vector transfer, an important factor determining eventual reproductive success is the adhesion of the pollen grains to the recipient stigma. Adhesion efficiency can be increased by the presence of pollinkitt, a viscous liquid coating the exine (outer layer) of pollen grains [17,18]. Pollenkitt is present, in varying quantities, on fresh pollen of all species, but is likely to be most advantageous when an insect vector is involved [18]. From a forensic perspective, it is important to note that pollenkitt degrades with time. Thus it is possible that fresh pollen with abundant pollenkitt may adhere to, and be retained on, clothing or other exhibits to a greater extent than older, dehydrated pollen of the same plant species (even if this is still from the same season).

Pollenkitt is not the only factor that influences the adhesion and retention of pollen grains to various substrates. Firstly, the morphology of the exine is vital. Pollen grains differ markedly in their ornamentation and sculpturing, and this can link to pollination vector mechanism [19–21]. For example, smooth pollen grains tend to be associated with wind-pollinated species while sculptured pollen grains, which might be more likely to adhere to fabric and less susceptible to subsequent removal, tend to be associated with plant species that rely on insect or animal vectors [21–23]. Secondly, the size of the grain can be important, especially in determining how likely a pollen grain is to embed within the fabric [12]. Thirdly, the type of fabric might be important, with the weave or electrostatic forces playing a role in pollen attraction and retention [24,25]. Finally, the intensity of wear after pollen adhesion could affect the proportion retained. This is an important factor when assessing the natural decay curve of pollen from clothing that will be utilised in a forensic investigation.

In addition to understanding the factors that affect pollen adhesion and (non-deliberate) loss of pollen from fabrics before they reach a forensic laboratory, it is also important to quantify the efficacy of standard laboratory protocols to deliberately remove pollen. It is vital that pollen adhering to pertinent objects in a criminal case is removed in a systematic, thorough, efficient and standardised manner. It is not possible to efficiently assess an entire garment to count and identify pollen *in situ*, so pollen must be removed for assessment under a light microscope. Often, due to the destructive method of retrieval, palynology is the last in a line of examinations undertaken by forensics teams [26]. Attempts have been made to retrieve pollen in several different ways, for example by: (1) refluxing small pieces of fabric in potassium hydroxide [26], (2) using sticky-backed tape or roller [27], (3) using a vacuum [28], and (4) via hand washing retrieval techniques [29]. Retrieval rates are variable [30], and despite some methods being quick and easy, such as using tape [27], hand washing retrieval techniques are most efficient [12,31,32,28]. It is vital that forensic retrieval techniques remove a representative sample of the pollen assemblage on the clothing, even if they do not remove all grains. Retrieval techniques need to be thorough enough to retrieve stubbornly attached pollen grains which may be pertinent to a case, particularly as pollen can still be recovered using hand washing techniques after items have been washed in a mechanical washing machine (with household detergent) and after commercial dry cleaning. ([12]; Pers. Obs.)

Although taphonomic variability of pollen (i.e. variability in the fate of pollen between leaving the parent plant and recovery in forensic laboratories) has been studied previously, there are several major gaps in knowledge. Wiltshire [13] highlights the importance of analysing variables rather than assuming knowledge, however, to date, most studies have focussed upon simple description of the relationship between pollen and fabric rather than quantifying the drivers responsible for those trends. Here, we build upon the work done by Bull et al. [12] and Boi [33]. The former studied pollen loss on different fabrics over time and found substantial differences, while the latter examined loss of pollen of different species from different fabrics but without a robust baseline from which to derive accurate retention data. These studies did not investigate the mechanisms driving the patterns they described, such that the potential effects of taphonomic variables including pollen morphology and wear intensity, were not examined. Moreover, neither Bull et al. [12] or Boi [33] examined the efficacy of laboratory retrieval techniques. Seemingly the only study to empirically test laboratory retrieval is that of Jantunen and Saarinen [28] who examined taping and vacuuming protocols, but not hand washing retrieval procedures. There has seemingly been no comprehensive, holistic study of the causal mechanisms behind taphonomic variability of pollen both before clothing arrives at a forensic laboratory and during processing at such a facility. Such research would be helpful in assisting forensic palynologists, who are engaged in the complexities of interpreting palynological data as part of a forensic reconstruction, by providing an insight into multifaceted variability of pollen retention.

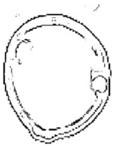
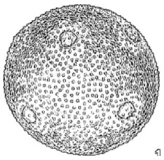
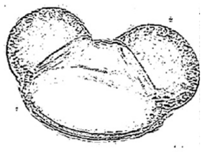
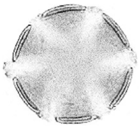
This study seeks to establish relative amount of pollen retained on clothing after a period of simulated light or heavy wear based on characteristics of the pollen (fresh versus dried; pollen morphology; pollen grain size) and on characteristics of the clothes: (fabric type). The specific hypotheses being tested are: (1) fresh pollen will be retained on clothing more readily than dried pollen because of the action of pollenkitt; (2) pollen retention will be lower on fabric with (simulated) heavy wear than (simulated) light wear; (3) pollen from insect-pollinated species will be retained on clothing more readily than pollen from wind-pollinated species because of its surface morphology; (4) small pollen grains will be retained on clothing more readily than large pollen grains as they are likely to embed deeper into the weave of clothing; and (5) pollen grains will be retained at different concentrations on different fabric types based on the weave of the material. We also test whether standard laboratory procedures to retrieve pollen by washing are equally effective regardless of species and fabric. Based on the results, we highlight key limitations of using palynological evidence within forensic settings and make recommendations on the importance of understanding pollen and fabric characteristics.

2. Materials and methods

2.1. Laboratory methods

To explore potential variation in adherence and retention of pollen on fabric, pollen was transferred directly from the flower anthers onto a microscope slide under laboratory conditions. Eight different species were studied: grass (Poaceae), nettle (*Urtica dioca*), ribwort plantain (*Plantago lanceolata*), common lime (*Tilia x europaea*), Scots pine (*Pinus sylvestris*), catnip (*Nepeta cataria),* common daisy (*Bellis perennis*), dandelion (*Taraxacum officinale*). These species were selected to provide a range of grain sizes (18–65 µm), variation in surface structure and ornamentation, and to represent both wind- and insect-pollinated species (Table 1). In all cases, 500 ± 30 grains were precisely counted on the specific microscope slide; any adjustments needed were made. Grains were then transferred from the slide to a piece of test fabric (8.5 cm × 3 cm) by smoothing the slide across a pre-determined

Table 1
Morphological characteristics of pollen types used in this study [34]. Sketched images derived from authors' own photographs.

Species	Size (approximate)	Structure	Surface pattern (ornamentation)	Method of pollination	Illustration (not to scale)
Poaceae Grass	40 µm	Monoporate	Psilate	Wind	
Urtica dioca Common nettle	18 µm	Triporate	Scabrate	Wind	
Plantago lanceolata Ribwort plantain	35 µm	Periporate	Verrucate	Wind	
Tilia x europaea Common lime	45 µm	Triporate	Foveolate	Insect	
Pinus sylvestris Scots pine	65 µm (with sacs separate)	Saccate	Grain body psilate, sacs with irregular reticulum	Wind	
Nepeta cataria Catnip	35 µm	Polyzonocolpate	Eureticulate	Insect	
Bellis perennis Common daisy	35 µm	Inaperturate	Conical echinate	Insect	
Taraxacum officinale Dandelion	45 µm	Inaperturate	Echinate in ridges or crests	Insect	

and pre-marked target area (1 cm^2). The slide was checked under an optical microscope to ensure all grains had been transferred and the transfer process was repeated if necessary. To explore pollen retention on a range of different weave types, three different test fabrics were used: cotton, denim and fleece (photographs from Scanning Electron Microscope (SEM) shown in Fig. 1 for illustrative purposes).

To mimic a person acquiring pollen on their clothes directly by brushing past vegetation, fresh pollen (i.e. that collected from the anthers of freshly-collected plants) was used. This pollen was still influenced by a coating of pollenkitt. Six replicates were undertaken for each species/fabric combination. Then, to mimic a person acquiring pollen indirectly from soil or dust, six further replicates were undertaken for each species/fabric combination using pollen from plants that had been allowed to dry in paper envelopes. In these samples, the pollenkitt had dehydrated. This provided a total sample size of 288 (8 species * 3 fabrics * 12 replicates). Pollen grains were collected from anthers at various stages of development to ensure grains of different maturity were captured. Pollen was not acetelised or dehydrated in alcohol as this causes damage to the pollenkitt, which might affect adherence relative to natural conditions [35].

Once all test fabric samples had been prepared, they were split equally between two wear intensity groups to mimic the natural agitation that clothing undergoes whilst being worn. To simulate light wear following pollen acquisition, 50% of test fabric samples were shaken for one minute on a mechanical shaker (500 oscillations per min). To simulate heavier wear, the test fabric was rubbed 20 times with a clean piece of cotton, mimicking brushing of clothing or contact with upholstered surfaces. Cotton was selected because of its neutral electrostatic charge. In all cases, the number of pollen grains remaining on the test fabric after treatment was counted under the microscope. The number (and percentage) of grains retained relative to the number originally placed on that particular piece of test fabric was then ascertained.

Finally, all fabric pieces were washed in mild surfactant (Multi Purpose Detergent, Teepol, Orpington, UK) to retrieve the remaining pollen from the fabric. As noted above, this process is the standard recommended laboratory protocol for the retrieval of pollen grains from clothing [36,29]. The number of residual pollen grains on the fabric after washing was assessed by counting under the microscope. The efficiency of the washing technique was quantified by dividing the number of pollen grains remaining after washing by the number on the test fabric after simulated wear. The reciprocal was then taken and multiplied by 100 to give washing efficiency expressed as a percentage (e.g 80% washing efficiency indicated that 80% of pollen grains were removed after simulated wear by the washing action). All 288 test fabric pieces were washed in this way. Washing was always done after simulated wear rather than after the pollen transfer step in order to make the timeline of forensic analysis realistic (i.e. clothing delivered to laboratories for processing would typically have been worn after initial pollen transfer).

2.2. Statistical analysis

The first analytical step was to compare pollen retention rates for fresh versus dried pollen both overall and for each individual species using independent samples t-tests. This was done on the basis that this not only tested the first research hypothesis, but would also allow data from fresh and dried samples to be pooled in the event of no statistical differences being found. The same approach was used to test for any differences in pollen retention according to wear intensity.

To analyse the effects of pollen and fabric characteristics on pollen retention after light wear, a range of General Linear Models (GLMs) was constructed. Specifically, univariate models were created to analyse the effects of pollination type (insect-pollinated versus wind-pollinated), pollen grain size, and fabric type (cotton, denim, fleece) in isolation. Ultimately, a fourth univariate model was constructed based on species. Multivariate GLMs were then constructed for combinations of factors (1 = pollination type and fabric; 2 = grain size and fabric; 3 = pollination type, grain size and fabric; 4 = species and fabric). In all cases, all two-way interactions were also entered. To compare between the different competing models, Akaike's Information Criterion (AIC) was calculated using the formula $AIC = -2(\text{log-likelihood}) + 2K$, where K = the number of predictor variables. This allowed identification of the model that best balanced explanatory power (model fit) with parsimony (minimising the number of variables in the model). The model with the smallest AIC value ($\Delta AIC = 0$) was considered the optimum, with any other model within $\Delta AIC \leq 2$ regarded as essentially having the same support as the optimum, models with ΔAIC of 3–4 having strong support, models with ΔAIC having 5–9 considerably less support, and models with $AIC\Delta \geq 10$ having essentially no support [37].

All analysis used raw data (pollen grains retained after simulated wear relative to those initially added), which were normally distributed. Graphical presentation of data used percentage for clarity. All analysis was undertaken in IBM SPSS (v22.0).

3. Results

3.1. Fresh versus dried pollen

Pollen retention after simulated wear was 42.9% on average. However, contrary to prediction, there was no statistically significant difference in retention of fresh or dried pollen either for all species combined (fresh = 44.9% ± 2.4% SEM; dry = 41.0% ± 2.4% SEM; $t = 1.290$, d.f = 286, $p = 0.198$) or for any individual species ($p > 0.05$ in all cases; tests not shown). Accordingly, data from both fresh and dried replicates were pooled on a per-species

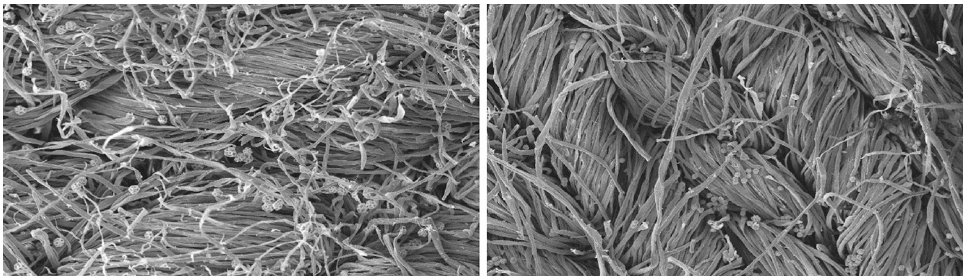


Fig. 1. SEM image at ×200 magnification demonstrating how imbedded individual pollen grains can become in the weave of different fabrics (left: dandelion on denim; right: daisy on cotton).

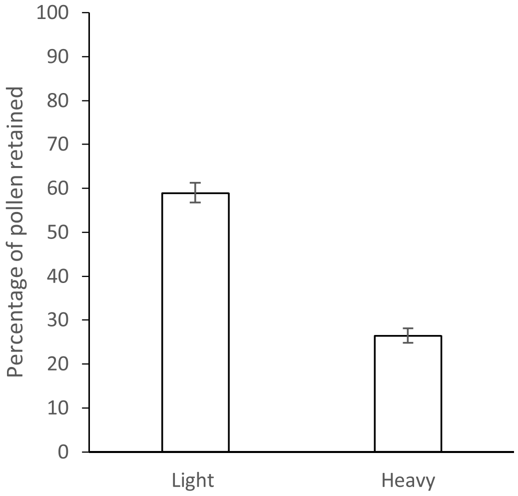


Fig. 2. Pollen retention after light intensity and heavy intensity wear.

basis to double the sample size and thus increase the robustness of subsequent analyses.

3.2. Wear intensity

As expected, there was a substantial difference in pollen retention according to wear intensity, with considerably more pollen being retained after light wear than after heavy wear (59.0% versus 26.5%, respectively, Fig. 2). This was statistically significant ($t = 11.637$, d.f = 286, $p < 0.001$).

3.3. Pollen retention following wear: effect of pollen and fabric characteristics

As predicted, pollen from insect-pollinated species was retained at higher concentrations than pollen from wind-pollinated species (50.4% and 35.0%, respectively). This pattern was consistent regardless of wear intensity (Fig. 3a–b), but statistically pollination type explained more of the variability in pollen retention after light wear ($R^2 = 0.175$) than after heavy wear ($R^2 = 0.035$) (Table 2).

Contrary to expectation, there was no clear linear relationship between pollen size and its retention following wear (Fig. 3c–d). Despite this, retention was higher for some size categories (35 μm and 45 μm) than others, such that size was a significant predictor of pollen retention for both light and heavy wear ($R^2 = 0.188$ and 0.073: Table 2). These non-linear patterns were consistent regardless of wear intensity, which suggested that findings might relate to underlying links to pollen sculpturing rather than size *per se*. This was borne out by echinate pollen occurring in the high retention groups (35 μm = daisy; 45 μm = dandelion), whereas species in the size classes with the lowest retention (18 μm and 40) included nettle and grass, which, although different in their fine sculpturing, have a much smoother surface. Accordingly, a new analysis was undertaken whereby pollen retention was related directly to species. Species identity explained a high proportion of the variation in the retention of pollen for light and heavy wear ($R^2 = 0.343$ and 0.198 respectively), which was substantially higher than the variance explained by either pollination type or grain size (Table 2). The species retained in the highest concentrations were consistent regardless of wear intensity (dandelion and catnip) but species with the lowest retention differed (Fig. 3g–h). Very broadly though, pollen retention increased as the surface morphology became rougher or more echinate (i.e. following the order in which species are presented in Table 1 and Fig. 3g–h).

As expected, fabric type was significantly related to pollen retention regardless of wear intensity but the effect size differed substantially (light wear $R^2 = 0.082$; heavy wear $R^2 = 0.259$: Table 2). Following light wear, fleece retained less pollen than cotton and denim, which were approximately equal (Fig. 3e). Following heavy wear, pollen retention on fleece and cotton were approximately equal but retention on denim was substantially higher (Fig. 3f).

The optimal univariate model for predicting pollen retention after light wear was based on plant species ($\Delta AIC = 0$ and $R^2 = 0.343$; no other models with $\Delta AIC < 10$; Table 2). The model that explained the least variability (and had the highest ΔAIC score) was that based on fabric type. This suggests that: (1) when clothing had only been lightly worn, pollen characteristics were more important than fabric characteristics in determining retention but also that: (2) this was highly species-specific such that pollination type and grain size were too crude to meaningfully predict retention. Conversely, the optimum univariate model for

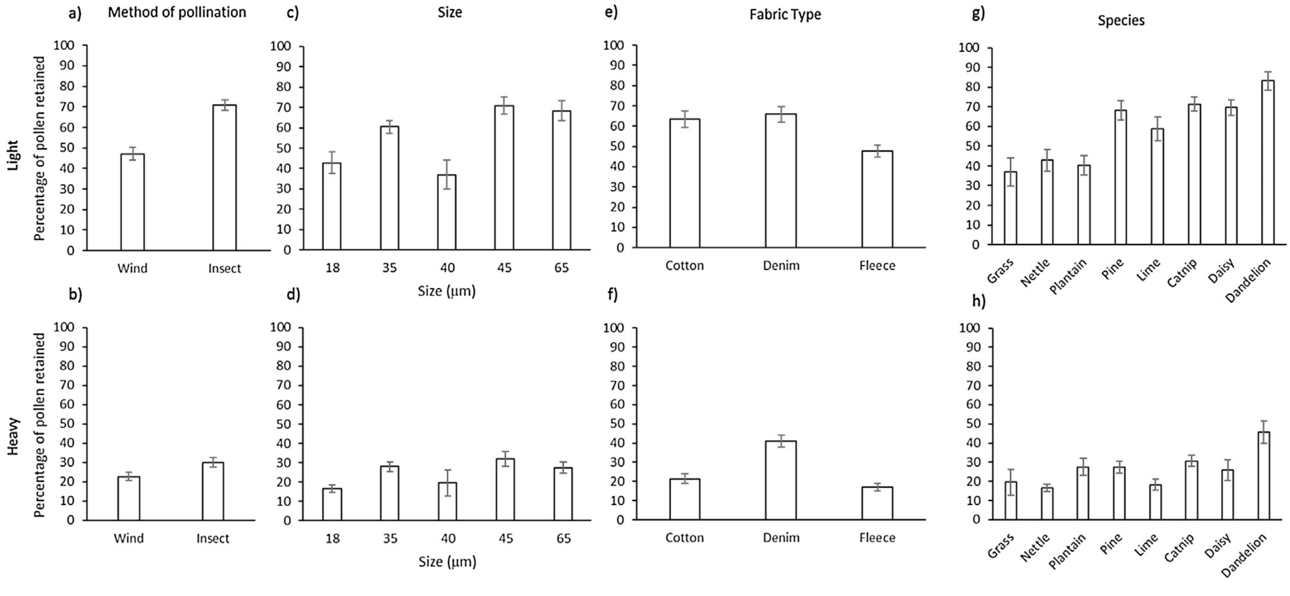


Fig. 3. Pollen retention following wear and the effect of pollen type, size and fabric characteristics.

Table 2
General Linear Models of pollen retention on fabric following light or heavy wear according to pollen and fabric characteristics. Delta Akaike's Information Criterion scores are used to compare all models (ΔAIC^1) and to compare univariate models only (ΔAIC^2) as per the Methods.

		Light wear intensity						Heavy wear intensity					
		F	df	P	R^2	ΔAIC^1	ΔAIC^2	F	df	P	R^2	ΔAIC^1	ΔAIC^2
Univariate models													
1	Pollination Type	27.552	1	<0.001	0.175	49.435	14.263	5.216	1	0.024	0.035	59.418	16.468
2	Size	7.352	4	<0.001	0.188	48.431	13.259	2.753	4	0.031	0.073	56.907	13.957
3	Species	9.254	7	<0.001	0.343	35.172	0	4.753	7	<0.001	0.198	47.9872	5.0372
4	Fabric	5.725	2	0.004	0.082	56.139	20.967	24.608	2	<0.001	0.259	42.95	0
Multivariate models													
1	Pollination Type	31.535	1	<0.001				6.965	1	0.009			
	Fabric	7.615	2	0.001				25.43	2	<0.001			
	Pollination Type* Fabric	3.106	2	0.048				0.371	2	0.691			
	Overall	10.633	5	<0.001	0.297	41.442		11.713	5	<0.001	0.298	41.462	
2	Size	9.913	4	<0.001				3.995	4	0.004			
	Fabric	12.227	2	<0.001				23.048	2	<0.001			
	Size*Fabric	5.428	10	<0.001				2.05	10	0.045			
	Overall model	7.251	17	<0.001	0.465	24.392		6.336	17	<0.001	0.407	30.946	
3	Pollination Type	24.539	1	<0.001				0.08	1	0.777			
	Size	7.539	4	<0.001				2.102	4	0.084			
	Fabric	11.507	2	<0.001				18.944	2	<0.001			
	Pollination Type*Fabric	5.636	2	0.005				1.94	2	0.148			
	Size*Fabric	4.728	10	<0.001				2.447	10	0.017			
	Overall	8.517	20	<0.001	0.559	14.185		5.491	20	<0.001	0.426	31.01	
4	Species	13.179	7	<0.001				9.328	7	<0.001			
	Fabric	12.358	2	<0.001				42.973	2	<0.001			
	Species*Fabric	4.536	14	<0.001				4.352	14	<0.001			
	Overall	8.257	23	<0.001	0.637	0		9.225	23	<0.001	0.639	0	

predicting pollen retention after heavy wear was based on fabric type (ΔAIC = 0 and R^2 = 0.259; no other models with ΔAIC < 5; Table 2). This suggests that following heavy wear, fabric characteristics were more important than pollen characteristics in determining retention.

While it is instructive to examine pollen and fabric characteristics separately, in reality these factors co-occur and interact with one another. The optimal model for both light and heavy wear was that which combined both plant species and fabric type, as well as the interaction between them (ΔAIC = 0; R^2 = 0.640; Table 2). As expected given the univariate results, the relative contribution of these factors to the overall model depends on wear intensity, with species being the larger contributing factor for light wear and fabric being the larger contributing factor for heavy wear. The other multivariate models all had ΔAIC values >10 and substantially lower R^2 values. The complexity of the interaction between species and fabric in determining pollen retention following both light and heavy wear is shown in Fig. 4. For example, following light wear, almost all (>94%) dandelion pollen was retained on cotton and denim versus just 58% on fleece, while 90% of pine pollen was retained on denim versus 60% on cotton and 54% on fleece. Retention of pollen following heavy wear was always lower than following light wear, but interactions between species and fabric were similarly complex. Particularly substantial differences were evident for grass, which ranged from 50% retention on denim to just 7% on cotton and 2% on fleece, and lime, which was retained in approximately equal amounts on denim and fleece (~24%) versus 7% on cotton.

3.4. Effectiveness of laboratory pollen removal techniques

Surprisingly, efficiency of washing was lower than expected: only 67.7% of pollen was removed following standard laboratory protocols, rather than the 100% often assumed. Moreover, this overall mean percentage was subject to considerable variation based on previous wear intensity (78.5% versus 58.9% washing efficiency following light and heavy wear, respectively). Even more concerning, the efficiency of this washing protocol differed very substantially according to plant species, fabric type, and the interaction between these factors (Table 3). These interactions are shown in Fig. 5. Particularly striking are the differences between washing efficiency for dandelion on all fabrics after both light wear (mean = 97%) and heavy wear (87%) versus the very low efficiency for nettle (39% and 45%, respectively). Generally, pollen retention followed a similar species pattern on all fabrics following light wear, although the efficiency of washing for retrieval of lime pollen varied considerably according to fabric type, both in absolute terms and relative to the other species (Fig. 5a–c). The efficiency of washing following heavy wear was much more variable according to both species and fabric; the results of grass (high retrieval on denim, low on cotton and fleece) and plantain (high retrieval on cotton, low on denim and fleece) are particularly notable in this regard (Fig. 5d–f).

4. Discussion

As with most types of forensic evidence, the timing of palynological analysis is critical. Given that almost 60% of the pollen that adheres to clothing is lost through subsequent wear, pertinent objects need to be seized quickly if palynology is likely to be important in forensic reconstruction. This study has also shown that loss versus retention of pollen on clothing varies substantially according to pollen characteristics *and* fabric type. As such, differential loss of pollen of different species during normal activity could mean that the pollen assemblage found during analysis does not fully align with the plant assemblage encountered at the scene. This might be of particular importance in cases where the absence of a specific species in a garment's palynological profile can be explained by taphonomic variables. For example, in a case where an accused assailant flees a scene of crime through an

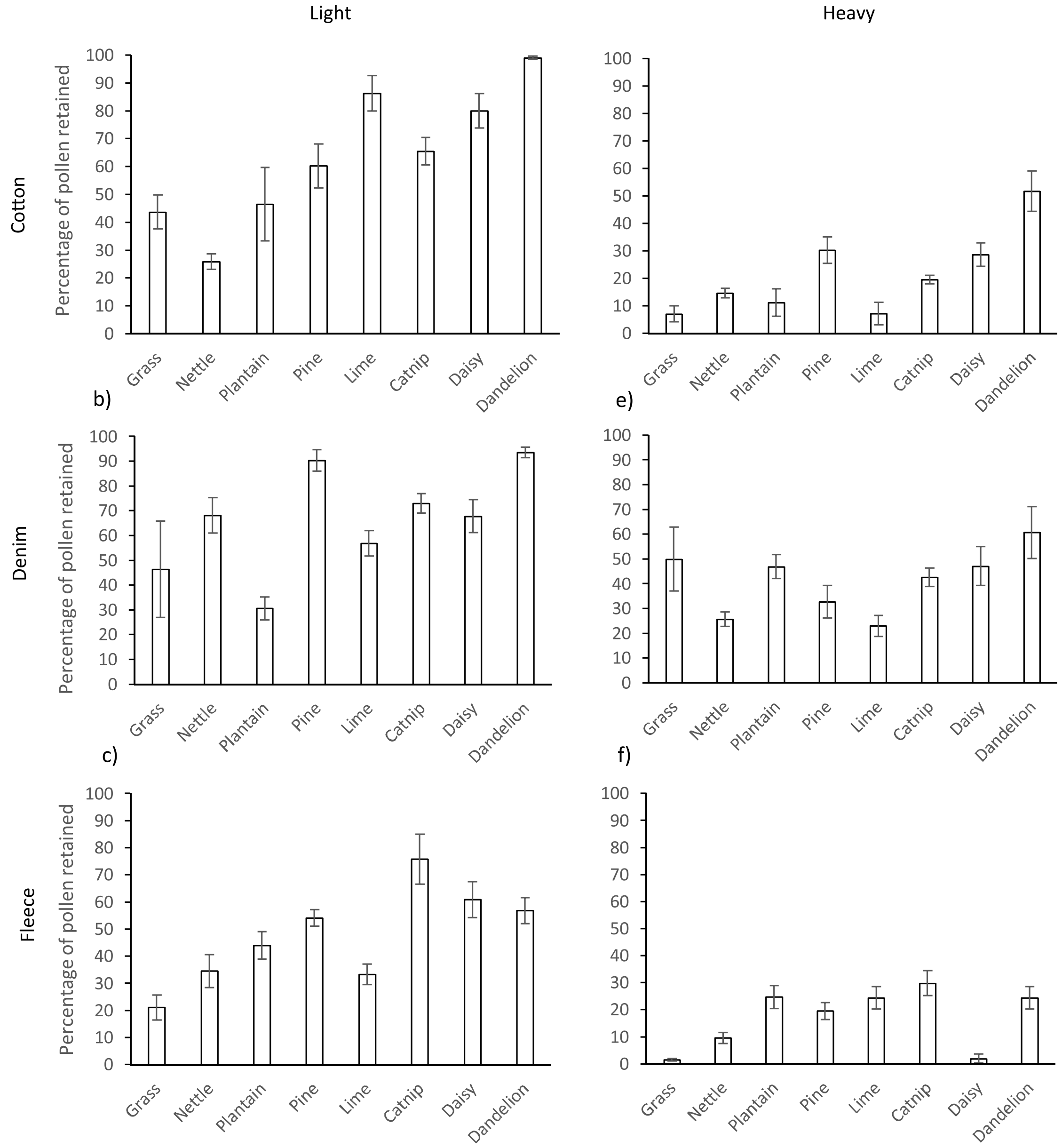


Fig. 4. The complexity of the interaction between species and fabric in determining pollen retention following both light and heavy wear.

area of flowering grasses, it might be assumed that analysis of trouser material below the knee should reveal abundant grass pollen. However, our results show that grass pollen is easily lost from cotton garments during running or walking, or by simply brushing the area that has been in contact with the flowers, such that the absence of grass pollen might not be surprising. It is also vital to understand the effect of wear intensity on pollen retention, since this not only affects the amount of pollen that is retained overall, but also the relative proportion of different species that will be found in forensic analyses.

Table 3
General Linear Models of pollen retention on fabric after hand washing revival according to plant species and fabric typ

	Light wear intensity				Heavy wear intensity			
	F	df	P	R^2	F	df	P	R2
Fabric	9.948	2	<0.001		12.149	2	<0.001	
Species	30.449	7	<0.001		13.54	7	<0.001	
Fabric*Species	6.98	14	<0.001		6.365	14	<0.001	
Overall	14.28	23	<0.001	0.681	9.811	23	<0.001	0.575

4.1. The importance of pollen characteristics

Pollen characteristics affect retention on all fabric types, and at both high and low wear intensities. Surprisingly however, and contrary to our hypothesis, general patterns between pollen retention and simple categories such as pollination type or grain size were comparatively weak. For example, although pollen from insect-pollinated species was retained at higher levels than pollen from wind-pollinated species overall, and this was statistically significant, this finding was too variable to be particularly helpful in palynological interpretation (as demonstrated by the low R^2 values, especially in comparison to models using full species information (Table 2)). Similarly, although grain size was also a significant predictor of pollen retention, this was not a linear trend as suggested by Bull et al. [12]. Thus, there was no generally-applicable pattern that, for example, smaller grains are always

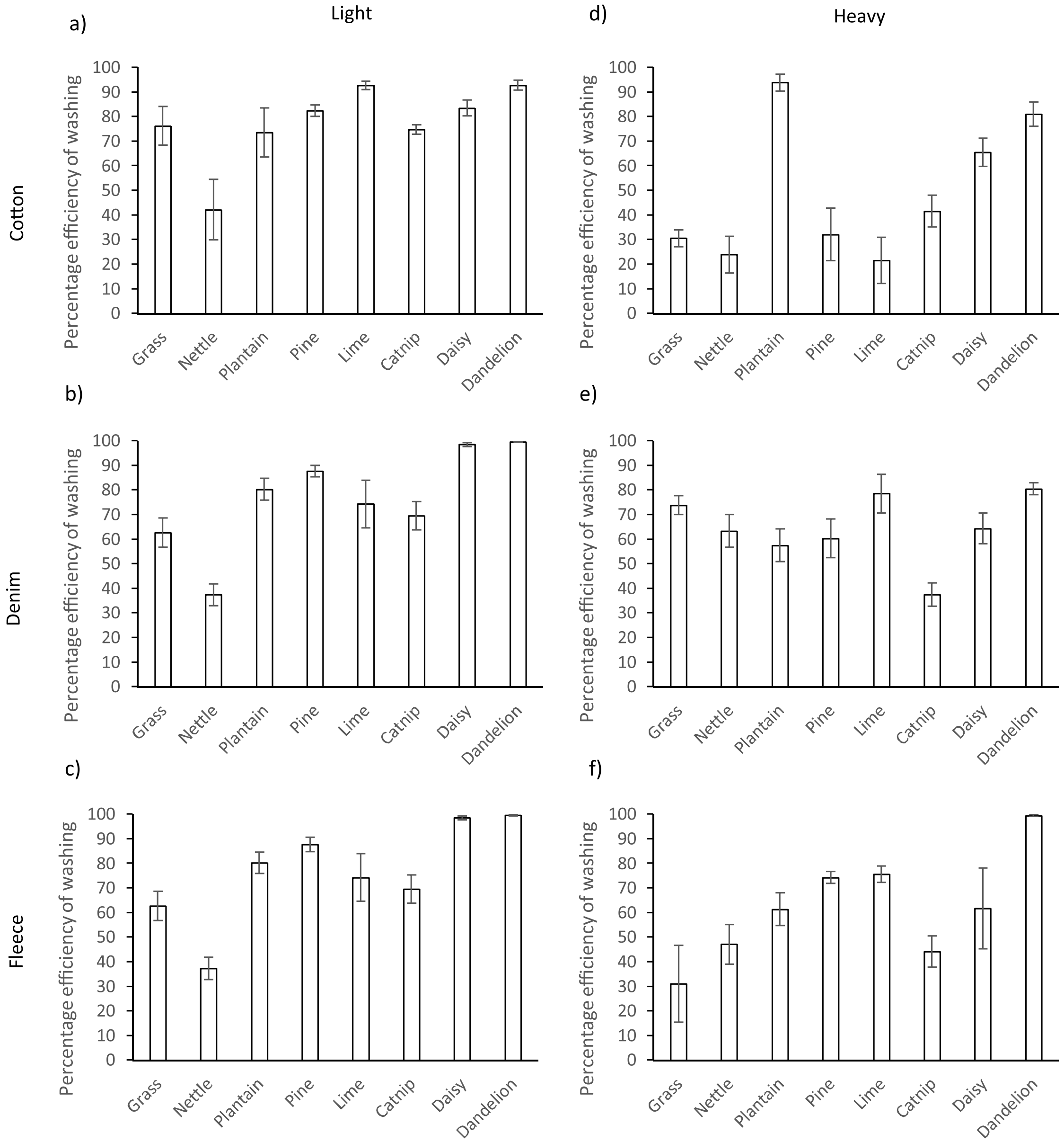


Fig. 5. Efficiency of handwashing using standard laboratory protocols for retrieving forensic palynology evidence. The washing efficiency was quantified by dividing the number of pollen grains remaining post-washing by the number on the test fabric after simulated wear. The reciprocal was then taken and multiplied by 100 to give washing efficiency expressed as a percentage.

likely to be retained more readily, and at higher levels, than larger grains. The lack of the generality of findings can also be applied, to a large extent, to pollen sculpturing. For example, given the high ornamentation of dandelion and daisy grains (Table 1), it is unusual for them not to share more similar retention properties [20].

The models that were most useful in explaining variability in retention all included species identity, rather than general pollen characteristic variables. This shows that pollen retention is highly species-specific and underlines the complexity of pollen:clothing relationships. Accordingly a forensic palynologist should be careful in inferring likely retention of pollen from one specific species based on data from another species — even one pollinated in the same way, of a similar size, and of similar sculpturing. As such, detailed understanding would require species-specific empirical testing rather than application of general principles from other experimental data.

One general finding that does seem to hold, though, is that the age of the pollen does not affect retention and should not confound palynological forensic evidence. This is despite the fact that fresh pollen is characterised by the presence of pollenkitt, a viscous liquid coating the exine, which can affect adhesion [17,18]. Our finding that there is no difference in retention of pollen according to whether it is fresh or dried suggests that pollenkitt is less important than previously thought in terms of adherence to, and retention on, clothing. This means that in a case where an accused assailant has brushed against flowering vegetation and/or come into contact with dried dusty surfaces, the prosecution and defence teams can be assured that there is no differential loss of pollen based on pollen state.

4.2. The importance of fabric characteristics

Alongside the influence of pollen characteristics, fabric type is extremely important in explaining pollen retention, especially following heavy wear. This is in contrast to Zavada et al. [32] who found fabric type did not affect the amount of pollen that adhered to clothing from natural pollen rain. Even more importantly from a forensic evidence perspective, our findings show that there is a significant interaction between species and fabric type, which extends the descriptive findings of Bull et al. [12] and Boi [33]. For example, 86% of lime pollen was retained on cotton fabric versus only 33% on fleece, while 68% of nettle pollen was retained on denim versus 25% on cotton. Such findings mean that, as with pollen characteristics, there are few if any "rules of thumb" for fabric characteristics that can be used by forensic palynologists to predict the likely retention level for any given fabric. As such, the gold standard in palynological evidence would involve simulations like those presented in this paper to model retention for the pollen type in question on the same fabric worn by the accused to provide a baseline for comparison with actual exhibits. However, this would add substantially to the cost of forensic investigations and the time needed to prepare evidence, possibly prohibitively so.

One such case where a simulation of pollen retention might have been useful is a sexual assault case in New Zealand reported by Mildenhall [7], where more pollen was discovered on the thighs of the victim's denim jeans than the knees. This might suggest that weave depth has an influence on the amount of pollen retained on a garment, as suggested in our data by the difference in retention between cotton and denim fabrics where weave depth is different. In Mildenhall's [7] case the weave depth is due to the age and state of the jeans where the knees have become worn down over time. This therefore suggests that, in addition to fabric type being important, the age and condition of the fabric might also have an effect and needs to be factored into any simulations.

In addition to weave, the differential retention across fabric types could be influenced by their electrostatic state, relative to that of pollen grains [24,25]. There has been very little research conducted in this area. Vercoulen et al. [38] assessed the electrostatic charge of birch (*Betula)* and cocksfoot grass (*Dactylis*), and finds that they are weakly negatively charged, but it is possible that different species might have different electrostatic states. Cotton is relatively neutrally charged, so would attract negatively-charged pollen grains [38], whereas polyester fleece is negatively charged and so would repel oppositely charged pollen grains. If pollen grains have different charge states, and given that different fabrics themselves have different charges, this might also partly explain the species:fabric interactions. For example, catnip pollen may be more positively charged and so fleece may have retained more of the pollen than other fabrics due to the electrostatic attraction. [39] have previously highlighted the importance of understanding relative electrostatic forces when interpreting forensic evidence, in relation to negatively charged explosives ions in relation to fleece fabric. Further study on possible differences in the charge of pollen grains of different species, and in relation to different fabrics, is recommended as an area for future research.

4.3. Effectiveness of laboratory pollen removal techniques

Laboratory pollen harvesting from clothing or fabrics is undertaken on the basis that it should be almost 100% efficient (i.e. remove all pollen grains remaining on the fabric upon receipt of the clothing for processing), or at least remove a representative sample. It has previously been reported that handwashing items is the most efficient method of pollen retrieval [12,31,32], but has not been included in previous research on methods of retrieval of pollen from fabrics [28]. However, our study on hand washing retrieval has shown that across the pollen types and fabrics used here there is great variability (range of 21–93% efficiency on cotton fabric between species). Palynological interpretation must only be undertaken with extreme caution as this study shows that the commonly used approach for retrieving pollen from a garment, might not yield a representative assemblage. For example, analysis of clothing from a suspect accused of being at a crime scene characterised by abundant dandelion could be misleading. At face value, if high levels of dandelion were retrieved during forensic investigation, this would be strong circumstantial evidence placing the suspect at the crime scene. However, given that results here show that dandelion is extremely well retained on fabric following wear but also extremely easy to remove via laboratory washing retrieval, a defence barrister might argue that the residual dandelion pollen on the suspect's clothes is present from historical (and innocent) movements, rather than being an indicator that the suspect has been to the crime scene.

4.4. Moving forwards: conclusions and recommendations

One potential way forward here is to utilise Bayesian techniques as palynological evidence is often assessed using Bayes' Theorem. Indeed, [4] discuss the importance of insuring that the pollen profile from a suspect's clothing matches that to a crime scene and outlines a likelihood ratios framework for assessing the extend of that match and its likely significance. However, although this takes into account unequal abundance of different species, it does not account for potential differential retention of the species on the fabrics. One the strengths of a Bayesian approach is the ability to weight different lines of evidence in a model [40,41]. In theory therefore, it would be possible to use general trends relating to pollen or fabric characteristics, as well as important taphonomic variables (such as pollen productivity) to improve the efficiency of the model in characterising the strength of the evidence. In reality, the findings here demonstrate that pollen retention patterns are so complex, and so species specific, it would be difficult to rigidly apply a set of general principles in this way. However, results do suggest that focussing primarily on pollen of insect-pollinated species in forensic palynological investigation may be advantageous.

Forensic palynology is an important field within forensic analysis. However, to ensure that such evidence is maximally useful, it is important to ensure interpretation is based upon empirical simulations rather than being predicated upon simplistic assumptions about how pollen (of different species) is transferred to and from clothing (of different fabric types). This is vital to ensure that incorrect inferences do not confound evidence given by expert witnesses, and thus affect testimony and verdicts in courts of law.

Acknowledgements

The authors would like to thank reviewers for positive and constructive comments, Iain Robertson and Elizabeth Ritchie for sending pine pollen samples down from northern Scotland when we had missed the pollination season in southern England, Connor McGoldrick for his optical photography skills and Elaine Dyer for producing SEM images.

References

[1] K. Fægri, J. Iversen, Textbook of Pollen Analysis, Blackwell Sciecne Publications, Oxford, 1964.
[2] J. McCarroll, F.M. Chambers, J.C. Webb, T. Thom, Application of palaeoecology for peatland conservation at Mossdale Moor, UK, Quat. Int. 432 (A) (2015) 39–47.
[3] J.C. Webb, J. MCCarroll, W. Carpenter, F.M. Chambers, P.S. Toms, M.J. Wood, Apparent lack of woodland and relative abundance of woodland indicator species: the role of humans, birds and rabbits in the changing vegetation of Skomer Island, Wales, Archaeol. Wales 56 (2018) [in press].
[4] M. Horrocks, K.A.J. Walsh, Forensic palynology: assessing the value of the evidence, Rev. Palaeobot. Palynol. 103 (1–2) (1998) 69–74.
[5] P.E. Wiltshire, Hair as a source of forensic evidence, Forensic Sci. Int. 163 (3) (2006) 241–248.

[6] P.A. Bull, A. Parker, R.M. Morgan, The forensic analysis of soils and sediment taken from the cast of a footprint, Forensic Sci. Int. 162 (1–3) (2006) 6–12.
[7] D.C. Mildenhall, Forensic palynology in New Zealand Rev, Palaeobot. Palynol. 64 (1990) (1990) 227–234.
[8] L.A. Milne, V.M. Bryant, D.C. Mildenhall, Forensic palynolog, in: H.M. Coyle (Ed.), Forensic Botany: Principles and Applications to Criminal Casework, CRC Press, Boca Raton, 2004, pp. 217–252.
[9] D. Mildenhall, P.E. Wiltshire, V.M. Bryant, Forensic palynology: why do it and how it works, Forensic Sci. Int. 163 (63) (2006) 163–172.
[10] D. Mildenhall, Hypericum pollen determines the presence of burglars at the scene of a crime: an example of forensic palynology, Forensic Sci. Int. 163 (3) (2006) 231–235.
[11] J. Monckton-Smith, T. Adams, A.G. Hart, J. Webb, Introducing Forensic and Criminal Investigation, Sage, London, 2013.
[12] P. Bull, R. Morgan, A. Sagovsky, G. Hughes, The transfer and persistence of trace particulates: experimental studies using clothing fabrics, Sci. Justice 46 (2006) 185–195.
[13] P.E. Wiltshire, Consideration of some taphonomic variables of relevance to forensic palynological investigation in the United Kingdom, Forensic Sci. Int. 163 (3) (2006) 173–182.
[14] K. Fægri, L. Van Der Pijl, The Principles of Pollination Ecology, Pergamon Press, Oxford, 2013.
[15] C.T. Philbrick, Hydrophily: phylogenetic and evolutionary considerations, Rhodora 93 (873) (1991) 36–350.
[16] E.C. Regan, L. Santini, L. Ingwall-King, M. Hoffmann, C. Rondinini, A. Symes, J. Taylor, S.H.M. Butchart, Global trends in the status of bird and mammal pollinators, Conserv. Lett. 8 (6) (2015) 397–403.
[17] E. Pacini, M. Hesse, Pollenkitt — its composition, forms and functions, Flora 200 (5) (2005) 399–415.
[18] H. Lin, I. Gomez, J. Carson Meredith, Pollenkitt wetting mechanism enables species-specific tunable pollen adhesion, Langmuir 29 (9) (2013) 3012–3023.
[19] M.H. Grayum, Correlations between pollination biology and pollen morphology in the Araceae, with some implications for angiosperm evolution, in: S. Blackmore, I.K. Ferguson (Eds.), Pollen and spores: form and function, Academic Press, NY, London, 1986, pp. 313–327.
[20] H.A. Mizes, Surface roughness and particle adhesion, J. Adhes. 696 (51) (1995) 155.
[21] N. Tanaka, K. Uehara, J. Murata, Correlation between pollen morphology and pollination mechanisms in the Hydrocharitaceae, J. Plant Res. 117 (2004) 265–276.
[22] M.R. Lumaga, S. Cozzolino, A. Kocyan, Exine micromorphology of Orchidinae (Orchidoideae, Orchidaceae): phylogenetic constraints or ecological influences? Ann. Bot. 98 (2006) 237–244.
[23] J. Sannier, W.J. Baker, M. Anstett, S. Nadot, A comparative analysis of pollinator type and pollen ornamentation in the Araceae and the Arecaceae, two unrelated families of the monocots, BMC Res. Notes 2 (2009) 145.
[24] A.J.P. Martin, Tribo-electricity in wool and hair, Proc. Phys. Soc. 53 (1940) 186–189.
[25] H. Chen, Effects of Fiber Electrostatic Cling and Moisture Regain on Timothy Grass. Unpublished Thesis Available from: http://ir.library.oregonstate.edu/concern/graduate_thesis_or_dissertations/4f16c630t. (Accessed 19 November 2017), Oregon State University, 2013.
[26] M. Horrocks, Subsampling and preparing forensic samples for pollen analysis, J. Forensic Sci. 49 (5) (2004) 1024–1027.
[27] L. Flinn, Collection of fiber evidence using a roller device and adhesive lifts, J. Forensic Sci. 37 (1992) 106–112.
[28] J. Jantunen, K. Saarinen, Pollen transport by clothes, Aerobiologia 23 (2011) 339–343.
[29] P.E. Wiltshire, Protocols for forensic palynology, Palynology 40 (1) (2016) 4–24.
[30] B. Adams-Groom, Forensic palynology, in: N. Marquez-Grant, J. Roberts (Eds.), Forensic Ecology Handbook, Wiley-Blackwell, 2012.
[31] C.-L. Wu, C.-H. Yang, T.-C. Huang, S.-H. Chen, Forensic pollen evidence from clothes by tape adhesive method, Taiwania 51 (2006) 123–130.
[32] M.S. Zavada, S.M. McGraw, M.A. Miller, The role of clothing fabrics as passive pollen collectors in the north-eastern United States, Grana 46 (2007) 285–291.
[33] M. Boi, Pollen attachment in common materials, Aerobiologica 31 (2) (2015) 261–270.
[34] P.D. Moore, J.A. Webb, M.E. Collinson, Pollen Analysis, Blackwell Science, Oxford, 1991.
[35] M. Hesse, Pollenkitt and viscin threads: their role in cementing pollen grains, Grana 20 (3) (1989) 145–152.
[36] P.E.J. Wiltshire, D.L. Hawksworth, J.A. Webb, K.J. Edwards, Two sources and two kinds of trace evidence: enhancing the links between clothing, footwear and crime scene, Forensic Sci. Int. 254 (September) (2015) 231–242.
[37] K.P. Burnham, D.R. Anderson, Multimodel avoiding pitfalls when using information-theoretic methods, J. Wildl. Manage. 66 (3) (2002) 912–918.
[38] P.H.W. Vercoulen, R.A. Roos, J.C.M. Marijnissen, B. Scarlett, Measuring electric charge on pollen, J. Aerosol Sci. 23 (1) (1992) 377–380.
[39] N. Talaty, C.C. Mulligan, D.R. Justes, A.U. Jackson, R.J. Noll, G. Cooks, Fabric analysis by ambient mass spectrometry for explosives and drugs, Analyst 133 (2008) 1532–1540.
[40] NRC Report, Strengthening Forensic Science in the United States: A Path Forward, Committee on Identifying the Needs of the Forensic Sciences Community, National Research Council, Document No. 228091, National Academy of Sciences (2009).
[41] S.P. Lund, H. Iyer, Likelihood Ratio as Weight of Forensic Evidence: A Closer Look, J. Res. Natl. Inst. Stand. Technol. 122 (27) (2017).

Bird Study (2014) **61**, 361–370
http://dx.doi.org/10.1080/00063657.2014.938017

Exploring the prevalence and diversity of pollen carried by four species of migratory Old World warbler (Sylvioidea) on arrival in the UK

MATT J. WOOD[1]*, PETER J. MORGAN[2], JULIA C. WEBB[1], ANNE E. GOODENOUGH[1], FRANK M. CHAMBERS[1] and ADAM G. HART[1]

[1]School of Natural and Social Sciences, University of Gloucestershire, Francis Close Hall, Swindon Road, Cheltenham GL50 4AZ, UK; [2]Portland Bird Observatory, The Old Lower Light, Portland Bill, Dorset DT5 2JT, UK

Capsule Pollen encrusted around the bill of migrating warblers can reveal marked differences in foraging ecology between bird species.

Aims To examine patterns of the prevalence and diversity of pollen in four species of warbler, and explore the potential of pollen to act as an indicator of recent foraging behaviour.

Methods By isolating pollen from bill encrustations using laboratory palynological techniques and identification by light microscopy, we examined variation in the prevalence of the five most common pollen taxa, and variation in pollen assemblages in four species of warbler arriving on the south coast of England.

Results All samples contained abundant pollen, with 19 floral taxa identified. *Sylvia* warblers tended to carry *Prunus* and *Citrus* pollen, while *Phylloscopus* warblers mainly carried *Eucalyptus* pollen. Pollen assemblages varied markedly between bird species.

Conclusion Commercial and garden flowering trees are an important resource for migrating warblers. Pollen may be such a valuable resource that flowering plants might be included in the conservation management of stopover sites. The use of pollen to resolve migratory routes may be problematic however, requiring detailed knowledge of both the distribution and flowering phenology of plants *en route*.

Migratory birds have breeding, stopover and wintering sites connected by migratory routes through different climatic zones. This makes them especially vulnerable to habitat and climate change, such that even small changes can have large population-level effects (Newton & Brockie 2008, Carey 2009). Recent declines in many UK migrant passerines, especially trans-Saharan migrants such as Willow Warblers (Morrison *et al.* 2010), emphasize this vulnerability. The geographical complexity of migration and the historical imbalance in the effort devoted to the study of birds that migrate between breeding grounds in northern European countries and southern wintering quarters (an imbalance especially marked in sub-Saharan migratory species) leaves much to be done to improve the patchy understanding of the migratory routes and ecology of migrant birds (Bairlein 2003, Newton & Brockie 2008).

To date, over 36 million birds have been marked with individually numbered rings in the UK (Newton 2010) but, despite the large numbers of migratory passerines ringed, few reliable connections have been made between breeding and wintering sites, although fascinating new insights have recently emerged from studies of species large enough to carry geolocators (McKinnon *et al.* 2013). For example, the Willow Warbler *Phylloscopus trochilus*, a sub-Saharan migrant commonly breeding in North-Western Europe, has more than a million ringing records in the UK, yet only three birds ringed on breeding sites have been recovered on wintering grounds in Central West Africa (Wernham *et al.* 2002). Knowledge of the wintering grounds of migrant passerines is, therefore, limited, particularly for birds wintering in inaccessible or remote habitats such as tropical rainforest. Informed conservation and management interventions for smaller species such as passerines (Goodenough *et al.* 2009) endorse the

*Correspondence author. Email: mjwood@glos.ac.uk

development of innovative empirical approaches (Bairlein 2003, Hobson 2008).

A proportion of migratory passerine birds are seen carrying pollen encrustations around the bill and forehead (Ash 1959, Laursen *et al*. 1997, Cecere *et al*. 2011a) presumably resulting from the exploitation of flowering plants as a fuel source on migration (Cecere *et al*. 2011b). Morphological differences between pollen from different plant taxa mean may, therefore, provide information on the plants the birds have used for foraging. The most comprehensive studies of pollen on migrant birds have focussed on a migration stopover site, the Italian island of Ventotene (Cecere *et al*. 2010, 2011a, 2011b). Behavioural observations showed that warblers foraged for nectar on locally available flowers, confirmed by palynology (the analysis of pollen) which also showed that stopover migrants carried pollen from plants not in flower on Ventotene Island, such as *Citrus* pollen, from foraging activity before arrival (Cecere *et al*. 2011a). A pollen encrustation, therefore, has the potential to act as a log of the previous behaviour of its bird 'vector'. Where a suitably diverse, or distinctive, pollen assemblage is identified, it may also be possible to use plant distribution maps to provide information on potential migratory routes, analogous to the use of pollen to provide information in forensic applications and reconstructing vegetation patterns in climate studies (Prentice & Webb 1998, Mildenhall *et al*. 2006). Thus, palynology has the potential to provide both ecological information on migrant bird foraging and geographical information on wintering grounds, migration routes and stopover, albeit within the potentially broad geographical areas defined by the temporal and spatial distributions of pollen-bearing plants.

Avian palynology studies in migratory species have either examined pollen in the respiratory system, a lethal technique (Tamisier, cited in Laursen *et al*. 1997), or pollen clipped from the feathers around the bird's beak (Ash 1959, Ortega-Olivencia *et al*. 2005), which has the advantage of being non-lethal. This technique has been applied to warblers captured at sites in the UK and France (Ash 1959, Ash *et al*. 1961), Denmark (Laursen *et al*. 1997), in addition to more in-depth studies of opportunistic nectar feeders at stopover sites in Italy (Cecere *et al*. 2010, 2011a) and mechanisms of nectar uptake in Denmark (Holm & Laursen 1982). Bird ringers at Portland Bird Observatory on the south coast of England have become aware of the continued appearance in spring of apparently pollen-bearing migratory birds (P.J. Morgan & M. Cade, pers. obs.), first noted by John Ash at Portland in the 1950s (Ash 1959). We, therefore, screened apparent pollen encrustations using modern palynological techniques to assess the prevalence and diversity of pollen carried by four species of Old World warbler (Sylvioidea). By comparing two pairs of congeneric species with similar foraging behaviour but different migratory strategies (wintering in the Mediterranean versus sub-Saharan Africa), we aimed to examine how (1) the prevalence of pollen and (2) pollen assemblages carried by warblers, varied between bird species, migratory strategy and foraging strategy.

METHODS

Study site and species

Pollen samples (encrustations attached to feathers around the bill) were clipped carefully from birds using clean, fine scissors. Sampled birds were a subset of those trapped in mist-nets and ringed by licensed ringers at Portland Bird Observatory (50° 31′ 10.8″N, 2° 27′ 4.1″W) between April 2006 and June 2008. The site is ideally located for the spring passage of migrant birds. It is situated on the southernmost tip of the Isle of Portland, a conspicuous outcrop of limestone 6 km long by 2.4 km wide jutting lengthwise into the English Channel.

Two congeneric pairs of bird species frequently trapped and ringed at Portland were selected for study. The warbler species in each pair are closely related (Sibley & Ahlquist 1990, Beresford *et al*. 2005, Alström *et al*. 2006), and have similar foraging behaviours and bill and tongue morphology (Holm & Laursen 1982), but have contrasting migratory strategies: one of each pair is a sub-Saharan migrant, the other a Mediterranean migrant (Cramp & Simmons 1983). The *Phylloscopus* pair comprised Willow Warblers *P. trochilus* and Chiffchaffs *Phylloscopus collybita*, and the *Sylvia* pair comprised Blackcaps *Sylvia atricapilla* and Garden Warblers *Sylvia borin*. Willow Warblers are thought to migrate from the UK to tropical West-Central Africa, with Chiffchaffs wintering in the Western Mediterranean (Wernham *et al*. 2002). When on breeding grounds, both *Phylloscopus* species feed on small insects from leaves of trees and shrubs. The *Sylvia* warblers forage in dense forest undergrowth, scrubland and woodland edges when on European breeding grounds, with Blackcaps migrating to the Mediterranean and Iberia and Garden Warblers migrating to sub-Saharan Africa, thought to be Central West Africa (Cramp and Simmons 1983, Wernham *et al.* 2002).

Pollen screening

Samples ($n = 113$) were stored dry in air-tight Eppendorf tubes at 4°C, until processing and screening in the laboratory following standard protocols (Moore *et al.* 1991). Each sample was treated with cold 10% hydrochloric acid to disaggregate particles and to dissolve any carbonates. Excess sporopollenin and residual cellulose within the samples were removed by acetolysis (Erdtman 1960) to aid identification and comparison with type slides. Pollen grains were stained with basic fuchsin and mounted in glycerine jelly for microscopic examination. Ten traverses on each slide were scanned under a light microscope (Nikon Optiphot, ×400 magnification) and the taxa present identified using Moore *et al.* (1991). Taxa present were recorded.

Statistical analysis

The presence or absence of each of the five most common pollen taxa on individual warblers was analysed in relation to the following predictors: (1) the 'host' bird species; (2) sampling date; (3) year; (4) their interactions. A global binary Generalized Linear Model (GLM) was simplified using the information theoretic approach by the backward-stepwise elimination of non-significant predictors, beginning with higher order interactions at each step (i.e. beginning with species:date:year in this case). If the removal of a predictor resulted in a non-significant change in model deviance (i.e. δAIC < 2), the predictor was eliminated from the model (Crawley 2013). Global and final models were broadly similar, and the individual reinsertion of prior deleted predictors into the final model made no difference to the results of analyses. To summarize variation in the pollen assemblages identified among the birds sampled, a Principal Components Analysis (PCA) was carried out on the presence/absence of all 19 pollen taxa. The resulting first two principal components (PCs) were used, in separate analyses, as the dependent variable in generalized linear modelling. GLM analyses were conducted in R v3.01 (R Core Team 2013).

Where bird species was identified as a significant factor, three classes of orthogonal a priori treatment contrasts were introduced (Crawley 2013) to test hypotheses regarding the contributions of (1) bird genus (*Phylloscopus* vs. *Sylvia*), (2) migratory strategy (Mediterranean vs. sub-Saharan) or (3) bird species per se to pollen assemblage variation. These treatment contrasts are a useful analytical approach, because species, genus and migratory strategy could not be incorporated in a single model: in our case of two pairs of species, each with one sub-Saharan migrant and one Mediterranean migrant, 'species' would be equivalent to a 'genus:migratory strategy' interaction, and thus analytically intractable. The first class of treatment contrasts examined genus and migratory strategy (i.e. 'genus' contrast = Blackcap and Garden Warbler vs. Chiffchaff and Willow Warbler; migratory strategy contrast = Blackcap and Chiffchaff vs. Garden Warbler and Willow Warbler); and the second examined treatment contrasts between species. In summary, these orthogonal contrasts relate to the same final models (in which bird 'species' is retained as a factor), and simply vary the structure of the levels (i.e. contrasts) relating to that factor.

To establish whether pollen diversity might be useful to classify birds according to species, genus or migratory strategy, Discriminant Function Analysis (DFA) was undertaken using SPSS version 16.1, on the basis that a high level of classification accuracy was good evidence of substantial interspecific differences. A full DFA was run using presence/absence data for all pollen taxa, with classification power being ascertained using a jackknife cross-validation procedure. The model was calculated repeatedly, each repetition involving the omission of a different individual case that is classified by the model and compared to the known outcome; this means that power was tested using a different data point to those that generated the model (Shaw 2003). This procedure was used because the sample size precluded the use of the preferred split-sample validation process (McGarigal *et al.* 2000). As sample sizes differed between species, classification accuracy was compared with prior probabilities calculated from the data. To determine the pollen genera that were the most important in creating pollen assemblage-level differences, a stepwise DFA was run (criterion to enter $\alpha = 0.05$ and criterion to remove $\alpha = 0.10$). Separate full and stepwise DFAs were also run with bird genus (*Sylvia* or *Phylloscopus*) and migration strategy (Mediterranean or sub-Saharan) as binary classifying variables. The recommended case/variable ratio of 3:1 (Tabachnick & Fidell 2007) was met in all cases. All means are presented ±1 se.

RESULTS

Each of 113 suspected pollen encrustations collected from Willow Warblers, Chiffchaffs, Blackcaps and Garden Warblers contained abundant pollen. Nineteen different taxa were detected, varying considerably in prevalence (Table 1): the majority of samples ($n = 70$) contained *Eucalyptus* pollen, whereas other taxa were found in very few samples (e.g. Umbelliferae, $n = 3$ and *Picea* (spruce) spp., $n = 2$).

Variation in pollen prevalence

We conducted separate analyses of the prevalence of the five most prevalent pollen taxa: *Eucalyptus*, *Citrus*, *Quercus*, *Prunus* and *Pinus* (Fig. 1a–e). Warbler species was the only statistically significant predictor of the presence of the most prevalent pollen taxon, *Eucalyptus* (Fig. 1a: Wald $\chi^2 = 52.9$, df = 3, $P < 0.0001$), with Chiffchaffs carrying significantly more *Eucalyptus* than other warblers ($z = 4.2$, $P < 0.0001$). Migratory strategy and genus were found to be statistically significant species contrasts: Mediterranean migrants carried more *Eucalyptus* pollen than sub-Saharan migrants ($z = 4.1$, $P < 0.0001$) and *Phylloscopus* more than *Sylvia* warblers ($z = 3.4$, $P < 0.001$). The presence of pollen from another commercial tree species, *Citrus* (Fig. 1b), also varied markedly between species (Wald $\chi^2 = 26.6$, df = 3, $P < 0.0001$), but differently in each year (species:year interaction, Wald $\chi^2 = 11.7$, df = 5, $P = 0.039$). Species contrasts indicated that Chiffchaffs ($z = -4.1$, $P < 0.0001$) and, to a lesser extent, Blackcaps ($z = -2.4$, $P = 0.004$) carried more *Citrus* pollen than Garden Warblers and Willow Warblers. Similarly, Mediterranean migrants carried less *Citrus* pollen than sub-Saharan migrants ($z = -4.4$, $P < 0.0001$).

Quercus pollen (Fig. 1c) also varied between warbler species contingent on year (species:year interaction, Wald $\chi^2 = 15.4$, df = 1, $P = 0.009$). Although species was not retained in the model as a main effect, species contrasts revealed that Chiffchaffs had significantly lower levels of *Quercus* pollen than other species ($z = -2.2$, $P = 0.028$). Contrasts of migratory strategy and genus were not supported ($P > 0.05$). *Prunus* pollen (Fig. 1d) varied significantly between warbler species (Wald $\chi^2 = 20.9$, df = 3, $P = 0.0001$), Blackcaps carrying *Prunus* more frequently than other species combined ($z = 2.0$, $P = 0.04$). Genus was the most preferred species contrast ($z = -3.2$, $P = 0.016$): *Sylvia* warblers carried more *Prunus* pollen than *Phylloscopus* warblers. The overall prevalence of *Prunus* pollen also varied between and within years (sample date:year interaction; Wald $\chi^2 = 6.3$, df = 2, $P = 0.044$). Lastly, *Pinus* pollen (Fig. 1e) showed a complex pattern of variation: *Pinus* prevalence varied between species and

Table 1. Prevalence of pollen in four species of Old World warbler (Sylvioidea) on spring passage at Portland Bird Observatory. The 19 pollen taxa collected were found in 113 samples in Spring 2006–2008, they are shown with their PC loadings. Prevalence is shown ± standard error (se), with statistical significance of correlations (Pearson's correlation coefficient, r_p) between the first two PCs and their loading variables (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$). †The five most prevalent pollen taxa were analysed independently (Results; Figs 1a–e).

Pollen taxon		Prevalence	se	PC1	PC2
Eucalyptus†	Gum tree spp.	0.61	0.046	0.78***	−0.11
Citrus†	Orange, lemon, lime, grapefruit spp.	0.26	0.024	−0.60***	−0.10
Quercus†	Oak spp.	0.21	0.020		−0.77***
Prunus†	Cherry, blackthorn, plum, peach, almond spp.	0.16	0.015		0.21**
Pinus†	Pine spp.	0.15	0.014		−0.54***
Salix	Willow spp.	0.062	0.0058		
Poaceae	Grass family	0.053	0.0050		
Acer	Maple spp.	0.044	0.0042		
Alnus	Alder spp.	0.044	0.0042		
Myrtilus-Corylus	Myrtle spp. or Hazel spp.	0.035	0.0033		
Umbelliferae	Carrot family	0.027	0.0025		−0.15***
Betula	Birch spp.	0.018	0.0017		
Chenopodiaceae	Goosefoot family	0.018	0.0017		
Ericaceae	Heather family	0.018	0.0017		
Lychnis	Campion spp.	0.018	0.0017		
Picea	Spruce spp.	0.018	0.0017		
Corydalis	Corydalis spp.	0.0089	0.00083		
Fagus	Beech spp.	0.0089	0.00083		
Fraxinus	Ash spp.	0.0089	0.00083		

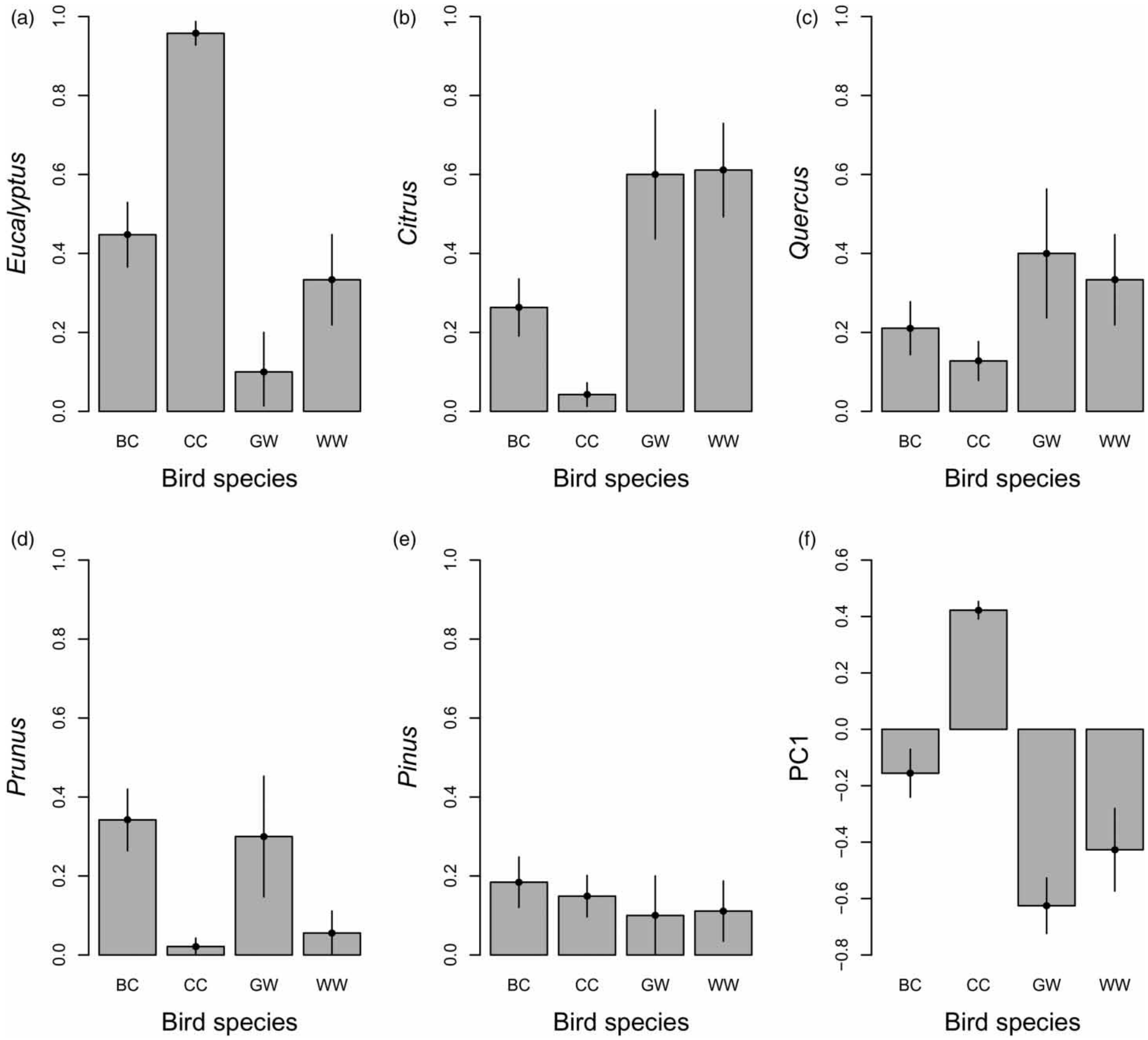


Figure 1. The prevalence of pollen carried by four warbler species sampled at Portland Bird Observatory. Variation in the five most prevalent pollen taxa is shown: (a) *Eucalyptus*, (b) *Citrus*, (c) *Quercus*, (d) *Prunus* and (e) *Pinus* pollen, followed by (f) variation in pollen assemblages captured by PCA (PC1, see main text). Warbler species legend: BC = Blackcap *S. atricapilla*, CC = Chiffchaff *P. collybita*, GW = Garden Warbler *S. borin*, WW = Willow Warbler *P. trochilus*.

years (Wald $\chi^2 = 13.6$, df = 5, $P = 0.019$), and tended to be found earlier in samples from *Sylvia* warblers than *Phylloscopus* warblers (species:date interaction; Wald $\chi^2 = 8.65$, df = 1, $P = 0.034$).

DFA allowed individual cases to be correctly classified to species with 54.1% accuracy using presence/absence of the 19 different pollen taxa found in this study, compared to 37.8% accuracy a priori (i.e. by chance, using prior probabilities based on sample size). This increased to 56.8% when only the presence of *Eucalyptus* pollen was entered into the model, and to 62.2% when only *Eucalyptus* and *Prunus* were entered, reflecting the importance of species in the GLM analysis of these two pollen taxa (Figs 1a & 1d). Misclassifications in the full data set arose mainly because Willow Warblers could not be differentiated from other species on the basis of pollen assemblage, but there was also some confusion between Blackcaps and Chiffchaffs. In the stepwise analyses, classifications of Blackcap and Chiffchaff were excellent (85.6%), but Willow Warblers still could not be distinguished. This was likely because this species had a similar prevalence of *Eucalyptus* to Blackcaps, and although Willow Warblers carried *Prunus* pollen far less frequently than Blackcaps and Garden Warblers, the overall prevalence of *Prunus* was only 16% (Table 1), such that this pollen taxon could not be used to differentiate (classify) all cases. In contrast to the PCA, birds could be classified to the correct genus using DFA with 74.3% accuracy using the complete pollen data set (compared to 51.4% a priori). This increased to 81.8% when only *Eucalyptus* and *Prunus* were entered. There was no bias as to where misclassifications arose in any analysis (ca.18% of *Sylvia* classified as *Phylloscopus* and vice versa).

Variation in pollen assemblages

PCA of pollen assemblages (19 pollen taxa present) resulted in the first two component scores (PC1 and PC2) explaining 43.2% of total variance; if the presence/absence of the 19 pollen taxa were randomly distributed among the PCs, the first two PCs would have accounted for 15.4% of total variance. PC1 and PC2 were most strongly influenced by PC loadings with the five most prevalent pollen taxa (Table 1). The first component score (PC1) was significantly correlated with the two most prevalent pollen taxa: the presence of *Eucalyptus* and the absence of *Citrus* pollen (Table 1). Low values indicate the use of *Eucalyptus*, and high values the use of *Citrus*. PC2 was significantly correlated with the absence of (1) *Quercus* (oak spp.), (2) the presence of *Prunus* pollen and (3) the absence of *Pinus* (pine spp.) pollen, and non-significantly correlated with the absence of three less prevalent pollen taxa (Table 1). The ecological interpretation of PC2's five factor loadings is complex, but a focus on the three highest loadings indicates that PC2 can be thought of as an ordination of the exploitation of forest habitats: low values are associated with use of oak and pine.

Pollen assemblage variation (as described by PC1) varied significantly between warbler species (GLM: Wald $\chi^2 = 88.7$, df = 1, $P < 0.0001$; Fig. 1f). This pattern varied between years (species:year interaction; Wald $\chi^2 = 12.0$, df = 1, $P < 0.0001$). Garden Warblers were distinct from other species in PC1 variation (a priori species treatment contrasts: $z = -2.4$, $P = 0.019$), having less *Eucalyptus* and more *Citrus* than other warbler species. Species treatment contrasts to examine variation by migratory strategy or genus were not found to be statistically significant descriptors of pollen assemblages. PC2 did not vary between warbler species as a simple main effect (GLM: Wald $\chi^2 = 2.0$, df = 1, $P = 0.57$): variation between species was contingent both on year (species:year interaction; $\chi^2 = 20.9$, df = 1, $P = 0.0008$) and sample date (species:date interaction; Wald $\chi^2 = 10.4$, df = 1, $P = 0.015$). The most preferred species contrast was between Chiffchaffs and other species ($z = -2.8$, $P = 0.006$); other contrasts between other warbler species, migratory strategy or genus were not supported ($P > 0.05$). These effects suggest complex variation in the prevalence of pollen taxa contributing to PC2.

Birds could not be classified to migratory strategy using DFA on the basis of their pollen assemblages at rates much above that expected by chance (full data set = 74.3% accuracy; a priori chance = 73.0% accuracy). The stepwise analysis entered *Eucalyptus* and then *Quercus*, but classification accuracy was still low (78.4%). In all cases, the majority of Mediterranean migrants were classified correctly, but classification accuracy for sub-Saharan migrants was low.

DISCUSSION

All 113 putative pollen samples collected from the encrustations at the base of the bills of migratory warblers at Portland Bird Observatory were found to contain abundant pollen. Pollen grains were identified from 19 different taxa, with marked differences in relative abundance across the samples. The phenology of flowering plants was not detected in our analyses, because sampling date did not predict the presence of pollen on warblers (likely due to the importance of warbler species as a predictor). Some of the taxa represented, including *Citrus*, were found in previous studies of pollen in migrant warblers (Ash 1959, Ash *et al.* 1961, Laursen *et al.* 1997, Cecere *et al.* 2010, 2011a) and are consistent with birds passing through a southern European flora *en route* to the UK, while others were not reported in previous studies at Portland Bird Observatory (Ash 1959, Ash *et al.* 1961). We did not detect pollen taxa with sufficiently restricted range distributions to enable the inference of migratory routes. As in a previous study (Cecere *et al.* 2011a), short and long distance migrants varied in the pollen they carried: we found that Mediterranean migrants carried more *Eucalyptus* and less *Citrus* pollen than sub-Saharan migrants.

It is notable that pollen from *Eucalyptus*, a tree genus native to Australia, was especially prevalent in this study, present in 70 (61.9%) of 113 birds sampled.

Eucalyptus species (the current study was not able to differentiate to species level) are now commonly cultivated in Africa and Iberia, where they are used for low-cost furniture and pulpwood (Doughty 2000). Indeed, the replacement of cork oak *Quercus suber* forests with commercial *Eucalyptus* has attracted conservation concerns (Doughty 2000). However, this study suggests that such non-native forests may be important as a foraging resource for some bird species. *Eucalyptus* is wind-pollinated, so to understand the significance of *Eucalyptus* pollen carried by migrant warblers it may be important to distinguish the accumulation of pollen dispersed over long distances, up to 1600 m from an individual tree (Barbour *et al.* 2005), from that accumulated directly by foraging on *Eucalyptus* flowers. Blackcaps and Chiffchaffs winter in the Mediterranean (Wernham *et al.* 2002) and are known to feed on the flowers of *Eucalyptus*, *Citrus* (J. Pérez-Tris, pers. comm.), and exotic garden plants (including *Aloe* spp. at Strait of Gibraltar Bird Observatory; C. Perez, pers. comm). Indeed, Laursen *et al.* (1997) and Cecere *et al.* (2011a) found that both *Eucalyptus* and *Citrus* were most prevalent in their studies, indicating the continued importance of arboriculture to warblers in more easterly Afro-Palaearctic migratory corridors. The *Phylloscopus* warblers carried the most pollen: the relatively light, hanging flowers at tips of branches found in *Eucaplytus* may suit the foraging techniques of these smaller birds.

Bird species was the strongest predictor of pollen prevalence and diversity, in both DFA and GLM approaches, with different bird species carrying different pollen assemblages. For example, *Phylloscopus* warbler pollen assemblages were dominated by *Eucalyptus*, while sub-Saharan migrant assemblages were dominated by *Citrus*. While *Eucalyptus* and *Citrus* are commonly found in spring migrant warblers (Laursen *et al.* 1997, Cecere *et al.* 2011a), we did not find the pollen of *Brassica* spp., Malvaceae and Cyperaceae found by Cecere *et al.* (2011a). Given that variation exists between species in pollen assemblages (as shown by the PCA), it is unsurprising that, overall, species should be an effective predictor in the univariate analyses of the five most common pollen taxa. In the PCA, the PC1 was significantly correlated with the two most prevalent pollen taxa: the presence of *Eucalyptus* and the absence of *Citrus* pollen (Table 1). PC1 might be considered, therefore, as an ordination of the exploitation of Iberian arboriculture by warblers: low values indicate the use of *Eucalyptus*, and high values the use of *Citrus*. PC2 was significantly correlated with the absence of (1) *Quercus* (oak spp.), (2) the presence of *Prunus* pollen and (3) the absence of *Pinus* (pine spp.) pollen, and non-significantly correlated with the absence of three less prevalent pollen taxa (Table 1). The ecological interpretation of PC2's five factor loadings is complex, but a focus on the three highest loadings indicates that PC2 can be thought of as an ordination of the exploitation of forest habitats: low values are associated with use of oak and pine.

DFA was successful in correctly classifying 'unknown cases' to genus, suggesting that pollen assemblage differs substantially on this basis, but the same approach indicated that migratory strategy was not a good predictor of pollen assemblage. Overall, 'species' was a more powerful predictor of pollen assemblages than either genus or migratory strategy. However, it is important to note that 'species' as a factor in this model is, in effect, the factorial of 'genus' and 'migratory strategy'.

A key development of this study would be the consideration of further warbler species, but further species carrying pollen are not seen at Portland Bird Observatory in sufficient numbers to allow comparison (e.g. we have only a handful of pollen samples from Whitethroats *Sylvia communis* and Lesser Whitethroats *Sylvia curruca*). Despite this limitation, DFA was successful in correctly classifying the majority of 'unknown cases' to genus, suggesting that pollen assemblage differs substantially on this basis. The GLM approach suggests that there are particularly strong between-genera differences in the amount of *Pinus* and *Prunus* pollen. Given that the two warbler genera studied here differ in their foraging behaviour (but that both pairs had a representative from each migratory strategy), pollen assemblages may be predicted by foraging behaviour rather than by migratory strategy. As a caveat to this, the ecology of a migrant at one stage in its migratory cycle (e.g. foraging on breeding grounds) is not necessarily an indicator of its ecology at other stages, such as on wintering grounds just before migration or on stopover sites, when the pollen encrustations collected in this study were likely to be formed. According to DFA, migratory strategy was not a good predictor of overall pollen assemblage. However, there were significant differences in the occurrence of *Citrus* and *Eucalyptus* pollen between Mediterranean and trans-Saharan migrants.

The pollen found on different bird species indicates the foraging ecology of birds during their migration, via feeding on the nectar and/or pollen of the flowers of insect-pollinated plants such as *Citrus*, passing through the habitat of anemophilous (wind-pollinated) plants such as oaks and eucalypts, or insect-gleaning at flowers of both pollination strategies. Foraging resources exploited by migratory birds at this crucial stage in their life history are poorly understood, but studies of the importance of nectar feeding in the stopover ecology of migrant warblers (Cecere *et al.* 2011b) indicate that the availability of flowering plants are a potentially important food source for migrant warblers. For example, in an experimental study of the drinking methods of warblers, examination of oral cavity morphology and estimation of fluid speed of a sucrose solution showed that the stomach of a Garden Warbler, with a volume of about 0.2 cm^3 can be filled in about 1 second, illustrating that feeding on nectar is a rapid and effective method of feeding when taking the energetic value of nectar into account (Holm & Laursen 1982). Cecere *et al.* (2011b) found that nectar uptake by warblers was not restricted to birds in poor condition, and may be an important water and energy source: uptake was followed by an increase in plasma glucose levels. The availability on migration of diverse and abundant flowering plants could play a role in the conservation management of long-range migratory warblers by providing strategically located stopover sites *en route*; the identification and support of these sites may be of considerable importance.

Pollen sampled from migrant warblers at the same site in the late 1950s (Ash 1959) found that all 11 putative pollen samples from whitethroats (*S. communis*, *n* = 5), Blackcaps (*n* = 4), a Willow Warbler and a Chiffchaff contained *Citrus* pollen. The small sample sizes preclude statistical comparison with this study, but the absence of *Eucalyptus* is noteworthy. In a later study of warblers sampled in southern France, Ash *et al.* (1961) found that Blackcaps, Willow Warblers, Chiffchaffs and Orphean Warblers *Sylvia hortensis* all carried pollen that contained mostly *Citrus*, with some pine *Pinus*, maple *Acer* and bog myrtle *Myrica*. Ash *et al.*'s (1961) data indicate that between 1.3% and 12% of Blackcaps carried visible pollen, with variation linked to date within a given year. Laursen *et al.* (1997), in a study at five sites in Denmark, found that warbler species differed in the prevalence of pollen (between 0.4% and 4.4%). Notably, samples collected from *Phylloscopus* warblers arriving in Denmark were dominated by *Eucalyptus* pollen (as was the case for *Phylloscopus* warblers sampled in this study). The majority of these previous studies were based on small samples of migratory birds analysed for pollen diversity (*n* = 3, Ash 1959; *n* = 44, Ash *et al.* 1961; *n* = 38, Laursen *et al.* 1997). With these relatively small sample sizes, we can only speculate on the potential role of changing vegetation distribution patterns in the intervening decades, but the detection of *Eucalyptus* pollen in later studies (Laursen *et al.* 1997; Cecere *et al.* 2011a; this study) might reflect an increase in the planting of *Eucalyptus* around the Mediterranean since the 1950s for use in paper production and in drainage projects (Doughty 2000).

At the outset of this study, the aim was to detect pollen from plant taxa that would allow location of wintering grounds. This was not possible, for three reasons. First, the taxonomic specificity of microscopic examination of pollen was not sufficiently detailed to allow use of plant distribution data to indicate bird distribution. More detailed microscopic examination may cast light here. The molecular identification of pollen to reveal hidden taxonomic diversity may also be useful, analogous with the cryptic molecular species diversity of parasites (Bensch *et al.* 2004). Second, the ranges of plants in potential wintering grounds are not mapped at a sufficiently fine geographic scale to be informative: a 'ground-truthing' restriction. Third, it remains unclear whether warblers carrying pollen on arrival in the UK are representative of the wider population. The absence of pollen on the majority of birds ringed at Portland Bird Observatory (~95%, P.J. Morgan & M. Cade, pers. comm.) may indicate that flowering plants are unavailable *en route* (unlikely given the timing of migration) or that most individuals do not need to land and take advantage of the high energy nectar and/or insect resource offered by flowering plants. It may be that those individuals collecting pollen are taking respite during bad weather or are individuals that need to 'top up' reserves, i.e. individuals migrating sub-optimally. Palynologists know that some pollen taxa are more adhesive than others. For example, *Citrus* (F. M. Chambers, pers. comm) and *Eucalyptus* (Girijashankar 2010) are known to be sticky, explaining why they are most commonly found on the birds we sampled (Fig. 2). Little is known about the loss of pollen from subjects, certainly not birds (although Laursen *et al.* 1997 note that pollen encrustations may be retained from previous seasons until feathers are moulted), and so this is an area requiring further research in forensic palynology. Some pollen taxa may, therefore, be more useful than others as geographical markers. The search for African pollen taxa, a hope that tantalized earlier

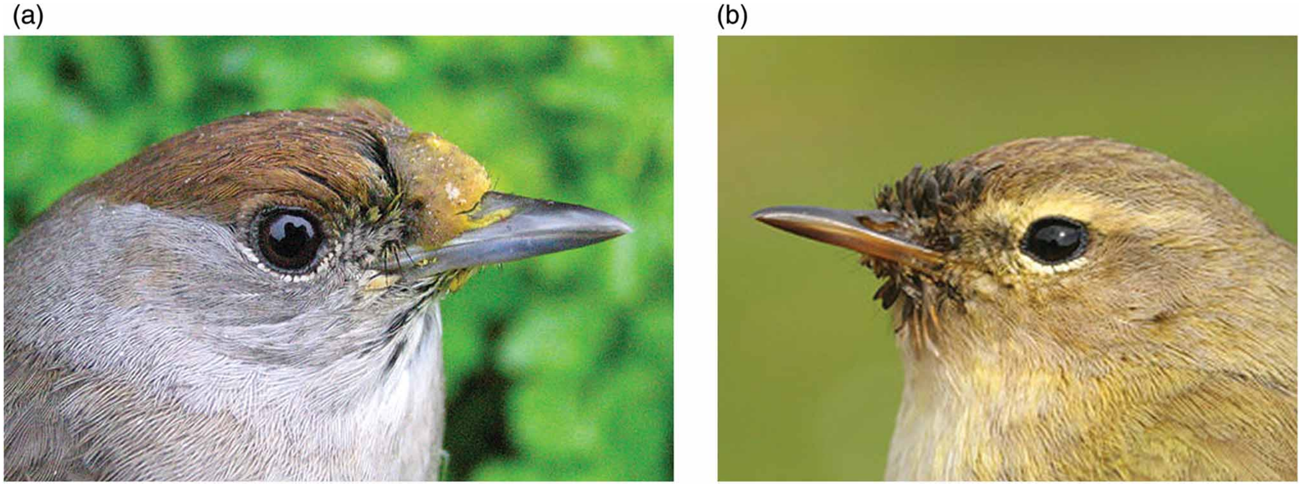


Figure 2. Typical pollen encrustations found on warblers at Portland Bird Observatory. (a) Blackcaps *S. atricapilla* are most commonly found with orange, waxy pollen encrustations (probably from *Citrus*), while (b) Chiffchaffs *P. collybita* typically carry brown-black pollen (probably from *Eucalyptus*).

researchers (Ash 1959, Laursen *et al.* 1997), was unsuccessful in the current study, suggesting that pollen analysis might be useful only as an indicator of migratory behaviour in the short term, and not of migratory wintering quarters. This seems more likely to be a reflection of foraging ecology than a systematic short-coming of the technique, with pollen representing recent flower foraging activity when *en route*, rather than a record of overwintering foraging activity.

Whatever the experience of the minority of birds carrying obvious pollen encrustations, there remains the potential to study the remaining majority of 'clean' birds, by examining small amounts of pollen trapped in feathers. The microscopic structure of feathers appears to trap pollen (J.C. Webb, pers. obs.) potentially enabling the study of birds that may be more representative of the population and so increasing sample size. In order to yield sufficient pollen samples for analysis, this study was limited to two congeneric species pairs of warblers. Wider sampling of 'clean' feathers from a larger number of congeneric species pairs would be feasible, and may provide enough pollen to examine more robustly the contributions of foraging ecology (genus) and migratory strategy to pollen assemblages. Increased sample size may detect more uncommon pollen taxa, which when combined with vegetation distribution mapping, for example, Atlas Florae Europeae (Jalas & Suominen 1988), may enable the identification of wintering grounds in a way similar to that used for forensics and the reconstruction of past vegetation patterns (Huntley & Birks 1983, Delcourt & Delcourt 1991).

Pollen encrustations on migratory warblers act as log of the migratory ecology of their avian carriers, revealing marked differences in foraging ecology and the potential importance of arboriculture as a resource on migration. But inferring migratory routes by the use of pollen studies remains elusive: significant advances in both field and laboratory are required, particularly for small passerines, to combine fieldwork focussed on suspected wintering areas with innovative analytical approaches.

ACKNOWLEDGEMENTS

We thank the warden of Portland Bird Observatory and Field Centre, Martin Cade, and its many visiting bird ringers for their support and assistance with sample collection, in particular Emma Cockburn, Denis and Val Jackson, Hannah and Glenn Thomas, Liz Kerr and Iain Dodd. John R.G. Daniell carried out laboratory pollen identification in the Centre for Environmental Change and Quaternary Research (CECQR) at The University of Gloucestershire.

FUNDING

We thank the British Ecological Society for funding from a Small Ecological Project Grant (ref. 702/815).

REFERENCES

Alström, P., Ericson, P.G.P., Olsson, U. & Sundberg, P. 2006. Phylogeny and classification of the avian superfamily Sylvioidea. *Mol. Phylogenet. Evol.* **38:** 381–397.

Ash, J.S. 1959. Pollen contamination by birds. *Br. Birds* **52:** 421–426.

Ash, J.S., Jone, P.H. & Melville, R. 1961. The contamination of birds by pollen and other substances. *Br. Birds* **54:** 93–100.

Bairlein, F. 2003. The study of bird migrations – some future perspectives. *Bird Study* **50:** 243–253.

Barbour, R.C., Potts, B.M. & Vaillancourt, R.E. 2005. Pollen dispersal from exotic eucalypt plantations. *Conserv. Genet.* **6:** 253–257.

Bensch, S., Pérez-Tris, J., Waldenström, J. & Hellgren, O. 2004. Linkage between nuclear and mitochondrial DNA sequences in avian malaria parasites: multiple cases of cryptic speciation? *Evol.* **58:** 1617–1621.

Beresford, P., Barker, F.K., Ryan, P.G. & Crowe, T.M. 2005. African endemics span the tree of songbirds (Passeri): molecular systematics of several evolutionary 'enigmas'. *Proc. R. Soc. London, Ser. B* **272:** 849–858.

Carey, C. 2009. The impacts of climate change on the annual cycles of birds. *Philos. Trans. R. Soc., B* **364:** 3321–3330.

Cecere, J.G., Matricardi, C., Frank, B., Imperio, S., Spina, F., Gargallo, G., Barboutis, C. & Boitani, L. 2010. Nectar exploitation by songbirds at Mediterranean stopover sites. *Ardeola* **57:** 143–157.

Cecere, J.G., Cornara, L., Mezzetta, S., Ferri, A., Spina, F. & Boitani, L. 2011a. Pollen couriers across the Mediterranean: the case of migrating warblers. *Ardea* **99:** 33–42.

Cecere, J.G., Spina, F., Jenni-Eiermann, S. & Boitani, L. 2011b. Nectar: an energy drink used by European songbirds during spring migration. *J. Ornithol.* **152:** 923–931.

Cramp, S. & Simmons, K.E.L. (eds) 1983. *The Birds of the Western Palearctic*. Oxford University Press, Oxford.

Crawley, M.J. 2013. *The R Book*. John Wiley & Sons, Chichester.

Delcourt, H.R. & Delcourt, P.A. 1991. *Quaternary Ecology: A Paleoecological Perspective*. Springer, New York and London.

Doughty, R.W. 2000. *The Eucalyptus: A Natural and Commercial History of the Gum Tree*. Johns Hopkins University Press, Baltimore.

Erdtman, G. 1960. The acetolysis method, revised description. *Svensk Bot. Tidskr.* **54:** 561–564.

Girijashankar, V. 2010. Effect of *Eucalyptus* pollen isolation methods on pollen viability, debris content, quantity isolated and pollen density per stigma. *J. Plant Breeding Crop Sci.* **2:** 273–279.

Goodenough, A.E., Elliot, S.L. & Hart, A.G. 2009. The challenges of conservation for declining migrants: are reserve-based initiatives during the breeding season appropriate for the Pied Flycatcher *Ficedula hypoleuca*? *Ibis* **151:** 429–439.

Hobson, K.A. 2008. Using endogenous and exogenous markers in bird conservation. *Bird Conserv. Int.* **18:** S174–S199.

Holm, E. & Laursen, K. 1982. Observation and experiments on the oral apparatus and its function in some warbler species (Sylviidae). *Zool. Anz. Jena* **209:** 224–246.

Huntley, B. & Birks, H.J.B. 1983. *An Atlas of Past and Present Pollen Maps for Europe: 0-13000 Years Ago*. Cambridge University Press, Cambridge.

Jalas, J. & Suominen, J. (eds) 1988. *Atlas Florae Europaeae. Distribution of Vascular Plants in Europe*. Cambridge University Press, Cambridge.

Laursen, K., Holm, E. & Sorensen, I. 1997. Pollen as a marker in migratory warblers, Sylviidae. *Ardea* **85:** 223–231.

Mcgarigal, K., Cushman, S. & Stafford, S.G. 2000. *Multivariate Statistics for Wildlife and Ecology Research*. Springer, New York and London.

Mckinnon, E.A., Fraser, K.C. & Stutchbury, B.J.M. 2013. New discoveries in landbird migration using geolocators, and a flight plan for the future. *Auk* **130:** 211–222.

Mildenhall, D.C., Wiltshire, P.E.J. & Bryant, V.M. 2006. Forensic palynology: why do it and how it works. *Forensic Sci. Int.* **163:** 163–172.

Moore, P.D., Webb, J.A. & Collinson, M.E. 1991. *Pollen Analysis*, 2nd edn. Blackwell Scientific Publications, Oxford.

Morrison, C.A., Robinson, R.A., Clark, J.A. & Gill, J.A. 2010. Spatial and temporal variation in population trends in a long-distance migratory bird. *Divers. Distrib.* **16:** 620–627.

Newton, I. 2010. *Bird Migration*. Collins, London.

Newton, I. & Brockie, K. 2008. *The Migration Ecology of Birds*. Academic Press, London.

Ortega-Olivencia, A., Rodriguez-Riano, T., Valtuena, F.J., Lopez, J. & Devesa, J.A. 2005. First confirmation of a native bird-pollinated plant in Europe. *Oikos* **110:** 578–590.

Prentice, I.C. & Webb, T. 1998. BIOME 6000: reconstructing global mid-Holocene vegetation patterns from palaeoecological records. *J. Biogeogr.* **25:** 997–1005.

R Core Team 2013. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Shaw, P.J.A. 2003. *Multivariate Statistics for the Environmental Sciences*, Arnold, London.

Sibley, C.G. & Ahlquist, J.E. 1990. *Phylogeny and Classification of Birds: A Study in Molecular Evolution*. Yale University Press, New Haven, CT.

Tabachnick, B.G. & Fidell, L.S. 2007. *Using Multivariate Statistics*, 5th edn. Allyn & Bacon, London.

Wernham, C.V., Toms, M.P., Marchant, J.H., Clark, J.A., Siriwardena, G.M. & Baillie, S.R. 2002. *The Migration Atlas: Movements of the Birds of Britain and Ireland*. T. & A.D. Poyser, London.

(MS received 22 January 2014; revised MS accepted 16 June 2014)

7. Conclusion

The papers presented in this thesis unite to demonstrate the breadth and scope of palynology. Knowledge is advanced in traditional palaeoecological studies by providing a vegetation history of two exposed islands off the Pembrokeshire coast, Wales (Webb et al. 2017; Webb et al. 2022a). Long-standing questions regarding the natural baseline vegetation on the islands before human occupation have been answered, and the pollen records are used to inform management of the island, and archaeological investigations. Examples of the temporal and spatial scope of palynology is demonstrated through Webb and Goodenough (2021) and Webb et al. (2022b) whereby two short past climate perturbations are reviewed spatially. These unique studies provide further evidence of the importance of spatial data in palaeoecology to explore the role of climate and anthropogenic forced events across wide (multi-site) areas, particularly when understanding regional or global events. Understanding the past is key to understanding future environmental events of similar scales (as predicted to occur in the near future (Christensen and Christensen 2007)) and this theme is carried forward into Webb and Goodenough (2018); Goodenough and Webb (2022); and Goodenough and Webb (submitted) where palaeoecological data is employed to answer contemporary questions or to test ecological theory. The innovative approach in Webb and Goodenough (2018) to evaluate the Ancient Woodland Indicator system demonstrates that this commonly used approach in contemporary ecology has limitations. Importantly, this paper also illustrates that opportunities should be built upon between ecologists and palaeoecologists to further strengthen ecological research over timescales that are not recorded by observations. Finally, the breadth of palynology is supported by Hart and Webb (2013); Wood et al. (2014) and Webb et al. (2018) by using pollen as a provenancing tool. Webb et al. (2018) advances knowledge in forensic palynology by illustrating the importance for empirically testing taphonomic variables before testifying in court, and Wood et al. (2014) investigates the pollen from bird bills to establish trace evidence of migratory foraging stops.

While the papers presented in this thesis are on different topics within palynology, they are united together by their demonstration of the utility of pollen analysis in differing fields. Each paper advances knowledge within the various sub-disciplines, but they also unite in the progression towards sharing data with non-traditional palynologists, whether that is archaeology, contemporary ecology, or forensics.

7.1 Future plans

The culmination of this thesis does not mark the end of my active research. It has been a good exercise to reflect on my experiences so far and to remind myself of the as yet unexplored possibilities for research and collaboration. My research 'niche' remains broad and I continue to span multi-discipline areas encompassing traditional palaeoecology, modern ecology and forensics.

Future plans include a paper exploring another ecological theory (much like Goodenough and Webb (Submitted)) – this time in comparing past Coleopteran richness with pollen richness. Gaston (1996) and Brunbjerg et al. (2018) report that vascular plant species richness is a good indicator of multi-taxon species richness in modern ecology. However, this theory has not been fundamentally tested against palaeoecological data, but it is assumed that the principle of uniformitarianism applies. Data mining the Neotoma database, and calling on data submissions from PalaeoSIG members, Coleopteran and palynological data is being retrieved jointly within robustly dated time horizons. The paper aims to explore whether beetle and pollen richness in the past is as strongly correlated as the present day (of course there are several caveats here to unravel: plant richness and pollen richness are not the same; beetle data is localised and pollen can be regional).

Another project in the pipeline takes advantage of the mutually-beneficial practice of researching with student collaborators. This project, which follows on from Wiltshire (2006b) and investigations I was involved with relating to the Ipswich murders in 2006/7, will consider the natural accumulation of pollen in hair. In Wiltshire (2006b) a case study from a murder investigation reports the palynological profile from a victim's hair. Wiltshire is able to disentangle the assemblage of pollen of an unkempt garden from the background signal of pollen from the victim's burial site. What is unknown throughout this investigation is the 'natural' signal of pollen picked up by hair during routine events, and how much is lost through normal washing procedures. In the reported case Wiltshire considers there is unlikely to be pollen rain on the victim's hair as the victim went missing in November, but if this case was in spring the pollen signal in their hair would be confounded by the natural pollen rain, making successful connections between the victim and pertinent places difficult. The project with student collaborators involves rinsing water from tri-weekly hair washings being collected throughout spring to assess the natural accumulation of pollen in hair during normal daily events (in an urban setting). To further explore the difference made by hair contacting the ground surface (as in many criminal cases), hair washings will also be collected after: (1) contact with (lying on) grassy parkland; and (2) contact with (lying on) a forest floor.

Finally, this autumn and with the spring tide, I plan to return to Pembrokeshire and explore the submerged forest stumps in the sound. Mapping also looks promising for possible coring sites in the wetland heath and slacks beyond Marloes Sands to potentially find the missing Mesolithic sequence. I will be collaborating with a coastal geomorphologist and diatomist, and hoping to supplement palynology with diatom studies to support the reconstructions of the changing coastline throughout the Holocene. Mike Alexander, long-term Skomer Island volunteer (Pers. Comm.) remains very keen to find a longer palynological record on the Island – given the extensive bathymetric survey of the Island that I completed in 2012 to find suitable coring locations, I don’t think that will be the case, but I stand ready to support! The CHERISH project is funded for one more year (unless extended owing to the pandemic hiatus) and may also yield opportunities to collaborate on other coastal locations in a bid to find a longer Holocene sequence and investigate the Mesolithic loss of woodlands in the area.

Regardless of the nature of the research, the projects in my research niche are united by the application of palynology to explore new and understudied areas. While palynology is expensive and time consuming as it is heavily reliant on human power (“a week in the field equals a year in the lab”), palynology has been instrumental in answering so many research questions in the last 100 years (Bryant and Jones 2006; Willis and Birks 2006; Birks and Berglund 2018) and continues to do so.

So, to answer Jacquelyn Gill’s question in her blog (Gill 2013): Is pollen analysis dead? I’d say “no… at least, not yet!”.

Reference list

Aario, L. (1944) Über Die Pollenanalytischen Methoden Zur Untersuchung von Waldgrenzen. Geol Fören i Stockh Förhandl, 66, 337–354.

Alley, R. B., Mayewski, P. A., Sowers, T., Stuiver, M., Taylor, K. C. & Clark, P. U. (1997) Holocene Climatic Instability: A Prominent, Widespread Event 8200 Yr Ago. Geology, 25 (6), 483.

Andersen, S. (1954) A Late-Glacial Pollen Diagram from Southern Michigan, USA. Danm Geol Unders I, 80, 140–155.

Asperen, E. N. van, Kirby, J. R. & Shaw, H. E. (2020) Relating Dung Fungal Spore Influx Rates to Animal Density in a Temperate Environment: Implications for Palaeoecological Studies. The Holocene, 30 (2) 218–232.

Auer, V. (1927) Stratigraphical and Morphological Investigations of Peat Bogs of Southeastern Canada. Communicationes ex Instituto Quaestionum Forestalium Finlandiae Editae, 12, 1–62.

Bairlein, F. (2003) The Study of Bird Migrations – Some Future Perspectives. Bird Study, 50 (3) 243–253.

Bajpai, R. & Kar, R. (2018) Modern Pollen Deposition in Glacial Settings in the Himalaya (India): Abundance of Pinus Pollen and Its Significance. Palynology, 42 (4) 475–482.

Barber, K. E. (1984) An Atlas of Past and Present Pollen Maps for Europe: 0–13000 Years Ago. By Huntley B. and Birks H. J. B. 667 + 34 Overlay Maps. Cambridge University Press, 1983. Price £85.00. Proceedings of the Prehistoric Society, 50, 408–409.

Barker, L., Davis, O., Davies, S., Driver, T., Johnston, B., Carruthers, W. & Webb, J. (2015) Skomer Island: The Excavation of a Prehistoric Field Lynchet Associated with the North Stream Settlement. RCAHMW Publication.

Batten, D. J. & Dutta, R. J. (1997) Ultrastructure of Exine of Gymnospermous Pollen Grains from Jurassic and Basal Cretaceous Deposits in Northwest Europe and Implications for Botanical Relationships. Review of Palaeobotany and Palynology, 99 (1) 25–54.

Bhiry, N. & Filion, L. (1996) Mid-Holocene Hemlock Decline in Eastern North America Linked with Phytophagous Insect Activity. Quaternary Research, 45 (3) 312–320.

Birks, H. J. B. (1993) Quaternary Palaeoecology and Vegetation Science — Current Contributions and Possible Future Developments. Review of Palaeobotany and Palynology, 79 (1–2) 153–177.

Birks, H. J. B. (1996) Contributions of Quaternary Palaeoecology to Nature Conservation. Journal of Vegetation Science, 7 (1) 89–98.

Birks, H. J. B. (2012) Ecological Palaeoecology and Conservation Biology: Controversies, Challenges, and Compromises. International Journal of Biodiversity Science, Ecosystem Services & Management, 8 (4) 292–304.

Birks, H. J. B. (2019) Contributions of Quaternary Botany to Modern Ecology and Biogeography. Plant Ecology & Diversity, 12 (3–4) 189–385.

Birks, H. J. B. & Berglund, B. E. (2018) One Hundred Years of Quaternary Pollen Analysis 1916–2016. Vegetation History and Archaeobotany, 27 (2) 271–309.

Birks, H. J. B., Heiri, O., Seppa, H. & Bjune, A. (2011) Strengths and Weaknesses of Quantitative Climate Reconstructions Based on Late-Quaternary Biological Proxies. The Open Ecology Journal, 3 (1) 68–110.

Birks, H. J. B. & Line, J. M. (1992) The Use of Rarefaction Analysis for Estimating Palynological Richness from Quaternary Pollen-Analytical Data. The Holocene, 2 (1) 1–10.

Bjune, A. E. (2000) Pollen Analysis of Faeces as a Method of Demonstrating Seasonal Variations in the Diet of Svalbard Reindeer (*Rangifer Tarandus Platyrhynchus*). Polar Research, 19 (2) 183–192.

Bond, G., Kromer, B., Beer, J., Muscheler, R., Evans, M. N., Showers, W., Hoffmann, S., Lotti-Bond, R., Hajdas, I. & Bonani, G. (2001) Persistent Solar Influence on North Atlantic Climate During the Holocene. Science, 294 (5549) 2130–2136.

Bradbury, S. (1967) Evolution of the Microscope. New York: Pergamon Press.

Brand, A., Allen, L., Altman, M., Hlava, M. & Scott, J. (2015) Beyond Authorship: Attribution, Contribution, Collaboration, and Credit. Learned Publishing, 28 (2) 151–155.

Brooks, D. & Thomas, K. W. (1967) The Distribution of Pollen Grains on Microscope Slides. The Non-Randomness of the Distibution . Pollen et Spores, 9, 621–629.

Brunbjerg, A. K., Bruun, H. H., Dalby, L., Fløjgaard, C., Frøslev, T. G., Høye, T. T., Goldberg, I., Læssøe, T., Hansen, M. D. D., Brøndum, L., Skipper, L., Fog, K. & Ejrnæs, R. (2018) Vascular Plant Species Richness and Bioindication Predict Multi-taxon Species Richness. Methods in Ecology and Evolution, 9 (12) 2372–2382.

Bryant, V. & Mildenhall, D. (1998) Forensic Palynology: A New Way to Catch Crooks. Contributions Series-American Association of Stratigraphic Palynologists, 33, 145–155.

Bryant, V. M. & Jones, G. D. (2006) Forensic Palynology: Current Status of a Rarely Used Technique in the United States of America. Forensic Science International, 163 (3) 183–197.

Bryant, V. M., Jones, J. G. & Mildenhall, D. C. (1990) Forensic Palynology in the United States of America. Palynology, 14 (1) 193–208.

Bunting, M. J., Armitage, R., Binney, H. A. & Waller, M. (2005) Estimates of ‘Relative Pollen Productivity’ and ‘Relevant Source Area of Pollen’ for Major Tree Taxa in Two Norfolk (UK) Woodlands. The Holocene, 15 (3) 459–465.

Bunting, M. J. & Farrell, M. (2018) Seeing the Wood for the Trees: Recent Advances in the Reconstruction of Woodland in Archaeological Landscapes Using Pollen Data. Environmental Archaeology, 23 (3) 228–239.

Bunting, M. J. & Middleton, D. (2005) Modelling Pollen Dispersal and Deposition Using HUMPOL Software, Including Simulating Windroses and Irregular Lakes. Review of Palaeobotany and Palynology, 134 (3–4) 185–196.

Burge, H. A. (2002) An Update on Pollen and Fungal Spore Aerobiology. Journal of Allergy and Clinical Immunology, 110 (4) 544–552.

Bush, M. B. & Weng, C. (2007) Introducing a New (Freeware) Tool for Palynology. Journal of Biogeography, 34 (3) 377–380.

Calcote, R. (1995) Pollen Source Area and Pollen Productivity: Evidence from Forest Hollows. The Journal of Ecology, 83 (4) 591.

Camuera, J., Jiménez-Moreno, G., Ramos-Román, M. J., García-Alix, A., Toney, J. L., Anderson, R. S., Jiménez-Espejo, F., Bright, J., Webster, C., Yanes, Y. & Carrión, J. S. (2019) Vegetation and Climate Changes during the Last Two Glacial-Interglacial Cycles in the Western Mediterranean: A New Long Pollen Record from Padul (Southern Iberian Peninsula). Quaternary Science Reviews, 205 86–105.

Cecere, J. G., Spina, F., Jenni-Eiermann, S. & Boitani, L. (2011) Nectar: An Energy Drink Used by European Songbirds during Spring Migration. Journal of Ornithology, 152 (4) 923–931.

Chambers, F. M. (2006) ACCROTELM: Abrupt Climate Changes Recorded Over The European Land Mass. Final Report to European Commission, Contract no. EVK2-CT-2002-00166. Cheltenham: CECQR, 117.

Chambers, F. M. (2016) The ‘Little Ice Age’: The First Virtual Issue of The Holocene. The Holocene, 26 (3) 335–337.

Chambers, F. M., Brain, S. A., Mauquoy, D., McCarroll, J. & Daley, T. (2014) The ‘Little Ice Age’ in the Southern Hemisphere in the Context of the Last 3000 Years: Peat-Based Proxy-Climate Data from Tierra Del Fuego. The Holocene, 24 (12) 1649–1656.

Chambers, F. M., Geel, B. van & Linden, M. van der (2010) Considerations for the Preparation of Peat Samples for Palynology, and for the Counting of Pollen and Non-Pollen Palynomorphs [Online]. vol. 7. Available from: <http://www.mires-and-peat.net/,ISSN1819-754X>.

Charman, D., Brown, A., Hendon, D. & Karofeld, E. (2004) Testing the Relationship between Holocene Peatland Palaeoclimate Reconstructions and Instrumental Data at Two European Sites. Quaternary Science Reviews, 23 (1–2) 137–143.

Charman, D. J. & Blundell, A. (2007) A New European Testate Amoebae Transfer Function for Palaeohydrological Reconstruction on Ombrotrophic Peatlands. Journal of Quaternary Science, 22 (3) 209–221.

Charman, D. J., Hohl, V., Blundell, A., Mitchell, F., Newberry, J. & Oksanen, P. (2012) A 1000-Year Reconstruction of Summer Precipitation from Ireland: Calibration of a Peat-Based Palaeoclimate Record. Quaternary International, 268 87–97.

Chevalier, M., Davis, B. A. S., Heiri, O., Seppä, H., Chase, B. M., Gajewski, K., Lacourse, T., Telford, R. J., Finsinger, W., Guiot, J., Kühl, N., Maezumi, S. Y., Tipton, J. R., Carter, V. A., Brussel, T., Phelps, L. N., Dawson, A., Zanon, M., Vallé, F., Nolan, C., Mauri, A., Vernal, A. de, Izumi, K., Holmström, L., Marsicek, J., Goring, S., Sommer, P. S., Chaput, M. & Kupriyanov, D. (2020) Pollen-Based Climate Reconstruction Techniques for Late Quaternary Studies. Earth-Science Reviews, 210 103384.

Christensen, J. H. & Christensen, O. B. (2007) A Summary of the PRUDENCE Model Projections of Changes in European Climate by the End of This Century. Climatic Change, 81 (S1) 7–30.

Clark, C. D., Ely, J. C., Greenwood, S. L., Hughes, A. L. C., Meehan, R., Barr, I. D., Bateman, M. D., Bradwell, T., Doole, J., Evans, D. J. A., Jordan, C. J., Monteys, X., Pellicer, X. M. & Sheehy, M. (2018) BRITICE Glacial Map, Version 2: A Map and GIS Database of Glacial Landforms of the Last British-Irish Ice Sheet. Boreas, 47 (1) 11-e8.

Collins, P. M., Davis, B. A. S. & Kaplan, J. O. (2012) The Mid-Holocene Vegetation of the Mediterranean Region and Southern Europe, and Comparison with the Present Day. Journal of Biogeography, 39 (10) 1848–1861.

Davies, A. L., Harrault, L., Milek, K., McClymont, E. L., Dallimer, M., Hamilton, A. & Warburton, J. (2022) A Multiproxy Approach to Long-Term Herbivore Grazing Dynamics in Peatlands Based on Pollen, Coprophilous Fungi and Faecal Biomarkers. Palaeogeography, Palaeoclimatology, Palaeoecology, 598 p. 111032.

Davies, A. L., Streeter, R., Lawson, I. T., Roucoux, K. H. & Hiles, W. (2018) The Application of Resilience Concepts in Palaeoecology. The Holocene, 28 (9) 1523–1534.

Davis, B. A. S., Brewer, S., Stevenson, A. C. & Guiot, J. (2003) The Temperature of Europe during the Holocene Reconstructed from Pollen Data. Quaternary Science Reviews, 22 (15–17) 1701–1716.

Davis, P. T., Menounos, B. & Osborn, G. (2009) Holocene and Latest Pleistocene Alpine Glacier Fluctuations: A Global Perspective. Quaternary Science Reviews, 28 (21–22) 2021–2033.

Debret, M., Bout-Roumazeilles, V., Grousset, F., Desmet, M., McManus, J. F., Massei, N., Sebag, D., Petit, J.-R., Copard, Y. & Trentesaux, A. (2007) The Origin of the 1500-Year Climate Cycles in Holocene North-Atlantic Records. Climate of the Past, 3 (4) 569–575.

Dietl, G. P., Kidwell, S. M., Brenner, M., Burney, D. A., Flessa, K. W., Jackson, S. T. & Koch, P. L. (2015) Conservation Paleobiology: Leveraging Knowledge of the Past to Inform Conservation and Restoration. Annual Review of Earth and Planetary Sciences, 43 (1) 79–103.

Djamali, M. & Cilleros, K. (2020) Statistically Significant Minimum Pollen Count in Quaternary Pollen Analysis; the Case of Pollen-Rich Lake Sediments. Review of Palaeobotany and Palynology, 275 104156.

Domínguez-Vázquez, G., Raygadas, S., León-Cortés, J. L. & Pérez-Arteaga, A. (2021) Pollen Analysis as an Ecological Proxy for the Assessment of Habitat Use of the Endangered Black-Polled Yellowthroat (*Geothlypis Speciosa*) and Sympatric Common Yellowthroat (*G. Trichas*). Avian Conservation and Ecology, 16 (2)

Driver, T. (2007) Pembrokeshire: Historic Landscapes from the Air. RCAHMW Publication.

Edwards, K. J., Fyfe, R. M., Hunt, C. O., & Schofield, J. E. (2015). Moving forwards? Palynology and the human dimension. Journal of Archaeological Science, 56, 117-132.

Elenga, H., Peyron, O., Bonnefille, R., Jolly, D., Cheddadi, R., Guiot, J., Andrieu, V., Bottema, S., Buchet, G., Beaulieu, J.-L. de, Hamilton, A. C., Maley, J., Marchant, R., Perez-Obiol, R., Reille, M., Riollet, G., Scott, L., Straka, H., Taylor, D., Campo, E. van, Vincens, A., Laarif, F. & Jonson, H. (2000) Pollen-Based Biome Reconstruction for Southern Europe and Africa 18,000 Yr BP. Journal of Biogeography, 27 (3) 621–634.

Erdtman, G. (1928) Studies in the Postarctic History of the Forests of Northwestern Europe. Investigations in the British Isles. Geol Fören i Stockh Förhandl, 50, 123–192.

Evans, J. G. (1990) An Archaeological Survey of Skomer, Dyfed. Proceedings of the Prehistoric Society, 56 247–267.

Faegri, K. & Iversen, J. (1950) Textbook of Modern Pollen Analysis. Copenhagen: Einar Munksgaard.

Faegri, K. & Iverson, J. (1989) Textbook of Pollen Analysis. 4th ed. Chichester: John Wiley.

Fowler, M., Datson, H. & Newberry, J. (2010) Quantitative Assessment of Dust Propagation at a Hazardous Waste Landfill: Directional Monitoring with Elemental Analysis. Journal of Environmental Monitoring, 12 (4), 879.

Froyd, C. A. & Willis, K. J. (2008) Emerging Issues in Biodiversity, Conservation Management: The Need for a Palaeoecological Perspective. Quaternary Science Reviews, 27 (17–18) 1723–1732.

Fyfe, R. M., Beaulieu, J.-L. de, Binney, H., Bradshaw, R. H. W., Brewer, S., Flao, A. le, Finsinger, W., Gaillard, M.-J., Giesecke, T., Gil-Romera, G., Grimm, E. C., Huntley, B., Kunes, P., Kühl, N., Leydet, M., Lotter, A. F., Tarasov, P. E. & Tonkov, S. (2009) The European Pollen Database: Past Efforts and Current Activities. Vegetation History and Archaeobotany, 18 (5) 417–424.

Gaillard, M.-J., Sugita, S., Bunting, M. J., Middleton, R., Broström, A., Caseldine, C., Giesecke, T., Hellman, S. E. v., Hicks, S., Hjelle, K., Langdon, C., Nielsen, A.-B., Poska, A., Stedingk, H. von & Veski, S. (2008) The Use of Modelling and Simulation Approach in Reconstructing Past Landscapes from Fossil Pollen Data: A Review and Results from the POLLANDCAL Network. Vegetation History and Archaeobotany, 17 (5) 419–443.

Gaston, K. J. (1996) Biodiversity - Congruence. Progress in Physical Geography: Earth and Environment, 20 (1) 105–112.

Gauthier, E., Bichet, V., Massa, C., Petit, C., Vannière, B. & Richard, H. (2010) Pollen and Non-Pollen Palynomorph Evidence of Medieval Farming Activities in Southwestern Greenland. Vegetation History and Archaeobotany, 19 (5–6) 427–438.

Ghilardi, B. & O'Connell, M. (2013) Early Holocene Vegetation and Climate Dynamics with Particular Reference to the 8.2 Ka Event: Pollen and Macrofossil Evidence from a Small Lake in Western Ireland. Vegetation History and Archaeobotany, 22 (2) 99–114.

Giesecke, T., Brewer, S., Finsinger, W., Leydet, M. & Bradshaw, R. H. W. (2017) Patterns and Dynamics of European Vegetation Change over the Last 15,000 Years. Journal of Biogeography, 44 (7) 1441–1456.

Gill, J. (2013) Is Pollen Analysis Dead? Paleoecology in the Era of Big Data [Online]. The Contemplative Mammoth. Available from: <https://contemplativemammoth.com/2013/07/10/is-pollen-analysis-dead-paleoecology-in-the-era-of-big-data/> [Accessed 18 May 2022].

Gillson, L. (2015) Biodiversity Conservation and Environmental Change: Using Palaeoecology to Manage Dynamic Landscapes in the Anthropocene. Croydon: CPI Group.

Gillson, L., Whitlock, C., Gell, P., Prader, S., Tinner, W. & Eggleston, S. (2022) Using Paleoecology in Restoration Ecology. Past Global Changes Magazine, 30 (1).

Githumbi, E., Pirzamanbein, B., Lindström, J., Poska, A., Fyfe, R., Mazier, F., Nielsen, A. B., Sugita, S., Trondman, A.-K., Woodbridge, J. & Gaillard, M.-J. (2022) Pollen-Based Maps of Past Regional Vegetation Cover in Europe Over 12 Millennia—Evaluation and Potential. Frontiers in Ecology and Evolution, 10 February.

Goodenough, A. E., Webb, J. C. & Yardley, J. (2019) Environmentally-Realistic Concentrations of Anthelmintic Drugs Affect Survival and Motility in the Cosmopolitan Earthworm *Lumbricus Terrestris* (Linnaeus, 1758). Applied Soil Ecology, 137, 87-95.

Goodwin, H. (1940) Pollen Analysis and Forest History of England and Wales. New Phytologist, 39, 370–400.

Harrison, S., Rowan, A. v, Glasser, N. F., Knight, J., Plummer, M. A. & Mills, S. C. (2014) Little Ice Age Glaciers in Britain: Glacier–Climate Modelling in the Cairngorm Mountains. The Holocene, 24 (2) 135–140.

Hart, A. G. & Webb, J. C. (2013) Forensic Science Specialisms: Palynology, Entomology and DNA. In: Monckton-Smith, J., Adams, T., Hart, A. G. & Webb, J. C. ed., Introducing Forensic and Criminal Investigation. London: Sage, 105–121.

Hellman, S. E. v., Gaillard, M., Broström, A. & Sugita, S. (2008) Effects of the Sampling Design and Selection of Parameter Values on Pollen-Based Quantitative Reconstructions of Regional Vegetation: A Case Study in Southern Sweden Using the REVEALS Model. Vegetation History and Archaeobotany, 17 (5) 445–459.

Hellman, S., Gaillard, M.-J., Broström, A. & Sugita, S. (2008) The REVEALS Model, a New Tool to Estimate Past Regional Plant Abundance from Pollen Data in Large Lakes: Validation in Southern Sweden. Journal of Quaternary Science, 23 (1) 21–42.

Hendrix, W. H. & Showers, W. B. (1992) Tracing Black Cutworm and Armyworm (Lepidoptera: Noctuidae) Northward Migration Using Pithecellobium and Calliandra Pollen. Environmental Entomology, 21 (5) 1092–1096.

Hesse, M., & Waha, M. (1989) A new look at the acetolysis method. Plant Systematics and Evolution, 163(3), 147-152.

Hicks, S., Tinsley, H., Huusko, A., Jensen, C., Hättestrand, M., Gerasimides, A. & Kvavadze, E. (2001) Some Comments on Spatial Variation in Arboreal Pollen Deposition: First Records from the Pollen Monitoring Programme (PMP). Review of Palaeobotany and Palynology, 117 (1–3) 183–194.

Hjelmroos, M. & Franzén, L. G. (1994) Implications of Recent Long-Distance Pollen Transport Events for the Interpretation of Fossil Pollen Records in Fennoscandia. Review of Palaeobotany and Palynology, 82 (1–2) 175–189.

Holt, K. A. & Bennett, K. D. (2014) Principles and Methods for Automated Palynology. New Phytologist, 203 (3) 735–742.

Hooghiemstra, H. & Geel, B. van (1998) World List of Quaternary Pollen and Spore Atlases. Review of Palaeobotany and Palynology, 104 (2) 157–182.

Horrocks, M. & Walsh, K. A. J. (2001) Pollen on Grass Clippings: Putting the Suspect at the Scene of the Crime. Journal of Forensic Sciences, 46 (4) 15074J.

Hultberg, T., Gaillard, M.-J., Grundmann, B. & Lindbladh, M. (2015) Reconstruction of Past Landscape Openness Using the Landscape Reconstruction Algorithm (LRA) Applied on Three Local Pollen Sites in a Southern Swedish Biodiversity Hotspot. Vegetation History and Archaeobotany, 24 (2) 253–266.

Hunt, C. O., Fieller, N. R. J., Gilbertson, D. D., & Ralph, N. G. A. (1985). Recent advances in pollen extraction techniques: a brief review. Palaeobiological Investigations, 266, 181-187.

Hunt, C. O., & Fiacconi, M. (2018). Pollen taphonomy of cave sediments: What does the pollen record in caves tell us about external environments and how do we assess its reliability? Quaternary International, 485, 68-75.

Hunt, C. O. & Morawska, Z. (2020) Are Your Hands Clean? Pollen Retention on the Human Hand after Washing. Review of Palaeobotany and Palynology, 280, 104278.

Hunt, C. O., Rushworth, G., & Dykes, A. P. (2007). UV-fluorescence microscopy and the coherence of pollen assemblages in environmental archaeology and Quaternary geology. Journal of Archaeological Science, 34(4), 562-571.

Huntley, B. & Birks, H. J. B. (1983) An Atlas of Past and Present Pollen Maps for Europe: 0–13000 Years Ago. Cambridge: University Press.

Huntley, B. & Prentice, I. C. (1988) July Temperatures in Europe from Pollen Data, 6000 Years Before Present. Science, 241 (4866) 687–690.

Iversen, J. (1954) The Late-Glacial Flora of Denmark and Its Relation to Climate and Soil. Danm Geol Unders II, 80, 87–119.

Jackson, S. T. & Blois, J. L. (2015) Community Ecology in a Changing Environment: Perspectives from the Quaternary. Proceedings of the National Academy of Sciences, 112 (16) 4915–4921.

Jackson, S. T. & Hobbs, R. J. (2009) Ecological Restoration in the Light of Ecological History. Science, 325 (5940) 567–569.

Jarman, R., Chambers, F. M. & Webb, J. (2019) Landscapes of Sweet Chestnut (*Castanea Sativa*) in Britain — Their Ancient Origins. Landscape History, 40 (2) 5-40.

Jarman, R., Hazell, Z., Campbell, G., Webb, J. & Chambers, F. M. (2019) Sweet Chestnut (*Castanea Sativa* Mill.) in Britain: Re-Assessment of Its Status as a Roman Archaeophyte. Britannia, 50 49-74.

Jarman, R., Mattioni, C., Russell, K., Chambers, F. M., Bartlett, D., Angela Martin, M., Cherubini, M., Villani, F. & Webb, J. (2019) DNA Analysis of *Castanea Sativa* (Sweet Chestnut) in Britain and Ireland: Elucidating European Origins and Genepool Diversity. PLoS ONE, 14 (9).

Jarman, R., Moir, A. K., Webb, J. & Chambers, F. M. (2017) Sweet Chestnut (*Castanea Sativa* Mill.) in Britain: Its Dendrochronological Potential. Arboricultural Journal, 39 (2) 100-124.

Jarman, R., Moir, A. K., Webb, J., Chambers, F. M. & Russell, K. (2018) Dendrochronological Assessment of British Veteran Sweet Chestnut (Castanea Sativa) Trees: Successful Cross-Matching, and Cross-Dating with British and French Oak (Quercus) Chronologies. Dendrochronologia, 51 10–21.

Jones, G. D. (2012) Forensic Pollen Geolocation Techniques Used to Identify the Origin of Boll Weevil Re-Infestation. Grana, 51 (3) 206–214.

Jones, G. D. & Bryant, V. M. (1992) Melissopalynology in the United States: A Review and Critique. Palynology, 16 (1) 63–71.

Jones, G. D. & Bryant, V. M. (2014) Pollen Studies of East Texas Honey. Palynology, 38 (2) 242–258.

Jones, P. D., Briffa, K. R., Osborn, T. J., Lough, J. M., Ommen, T. D. van, Vinther, B. M., Luterbacher, J., Wahl, E. R., Zwiers, F. W., Mann, M. E., Schmidt, G. A., Ammann, C. M., Buckley, B. M., Cobb, K. M., Esper, J., Goosse, H., Graham, N., Jansen, E., Kiefer, T., Kull, C., Küttel, M., Mosley-Thompson, E., Overpeck, J. T., Riedwyl, N., Schulz, M., Tudhope, A. W., Villalba, R., Wanner, H., Wolff, E. & Xoplaki, E. (2009) High-Resolution Palaeoclimatology of the Last Millennium: A Review of Current Status and Future Prospects. The Holocene, 19 (1) 3–49.

Karlsdóttir, L., Hallsdóttir, M., Thórsson, A. Th. & Anamthawat-Jónsson, K. (2008) Characteristics of Pollen from Natural Triploid Betula Hybrids. Grana, 47 (1) 52–59.

Kilgore, B. M. (1973) The Ecological Role of Fire in Sierran Conifer Forests: Its Application to National Park Management. Quaternary Research, 3 (3) 496–513.

Kujawa, E. R., Goring, S., Dawson, A., Calcote, R., Grimm, E. C., Hotchkiss, S. C., Jackson, S. T., Lynch, E. A., McLachlan, J., St-Jacques, J.-M., Umbanhowar Jr., C. & Williams, J. W. (2016) The Effects of Anthropogenic Land Cover Change on Pollen-Vegetation Relationships in the American Midwest. Anthropocene, 15 60–71.

Langgut, D., Almogi-Labin, A., Bar-Matthews, M. & Weinstein-Evron, M. (2011) Vegetation and Climate Changes in the South Eastern Mediterranean during the Last Glacial-Interglacial Cycle (86 Ka): New Marine Pollen Record. Quaternary Science Reviews, 30 (27–28) 3960–3972.

Leach, A. (1918) Flint-Working Sites on the Submerged Land (Submerged Forest) Bordering the Pembrokeshire Coast. Proceedings of the Geologists Association, 29 (2), 46.

Lee-Thorp, J. A., Holmgren, K., Lauritzen, S.-E., Linge, H., Moberg, A., Partridge, T. C., Stevenson, C. & Tyson, P. D. (2001) Rapid Climate Shifts in the Southern African Interior throughout the Mid to Late Holocene. Geophysical Research Letters, 28 (23) 4507–4510.

Lentfer, C. J., & Boyd, W. E. (2000). Simultaneous extraction of phytoliths, pollen and spores from sediments. Journal of Archaeological Science, 27(5), 363-372.

Litt, T., Ohlwein, C., Neumann, F. H., Hense, A. & Stein, M. (2012) Holocene Climate Variability in the Levant from the Dead Sea Pollen Record. Quaternary Science Reviews, 49 95–105.

Lowe, J. & Walker, M. (2015) Reconstructing Quaternary Environments. 3rd ed. Abingdon: Routledge.

Magyari, E. K., Chapman, J., Fairbairn, A. S., Francis, M. & Guzman, M. de (2012) Neolithic Human Impact on the Landscapes of North-East Hungary Inferred from Pollen and Settlement Records. Vegetation History and Archaeobotany, 21 (4–5) 279–302.

Mann, M. E., Zhang, Z., Rutherford, S., Bradley, R. S., Hughes, M. K., Shindell, D., Ammann, C., Faluvegi, G. & Ni, F. (2009) Global Signatures and Dynamical Origins of the Little Ice Age and Medieval Climate Anomaly. Science, 326 (5957) 1256–1260.

Marden, M., Holt, K., Ryan, M., Carrasco, J., Marsaglia, K., Kirby, M. & Palmer, A. (2022) Stratigraphy and Vegetation Signals from an Upland, Landslide-Dammed, Paleolake during the Last Glacial-Interglacial Transition, Waipaoa Sedimentary System, Hikurangi Margin, Eastern North Island, New Zealand. New Zealand Journal of Geology and Geophysics, 65 (1) 220–241.

Martin, A. C. & Harvey, W. J. (2017) The Global Pollen Project: A New Tool for Pollen Identification and the Dissemination of Physical Reference Collections. Methods in Ecology and Evolution, 8 (7) 892–897.

Mauquoy, D., Yeloff, D., Geel, B. van, Charman, D. J. & Blundell, A. (2008) Two Decadally Resolved Records from North-West European Peat Bogs Show Rapid Climate Changes Associated with Solar Variability during the Mid-Late Holocene. Journal of Quaternary Science, 23 (8) 745–763.

Mayer, C., Adler, L., Armbruster, W. S., Dafni, A., Eardley, C., Huang, S.-Q., Kevan, P. G., Ollerton, J., Packer, L., Ssymank, A., Stout, J. C. & Potts, S. G. (2011) Pollination Ecology in the 21st Century: Key Questions for Future Research. Journal of Pollination Ecology, 8–23.

Mayewski, P. A., Rohling, E. E., Curt Stager, J., Karlén, W., Maasch, K. A., Meeker, L. D., Meyerson, E. A., Gasse, F., Kreveld, S. van, Holmgren, K., Lee-Thorp, J., Rosqvist, G., Rack, F., Staubwasser, M., Schneider, R. R. & Steig, E. J. (2004) Holocene Climate Variability. Quaternary Research, 62 (3) 243–255.

McCarroll, J., Chambers, F. M., Webb, J. C. & Thom, T. (2016) Using Palaeoecology to Advise Peatland Conservation: An Example from West Arkengarthdale, Yorkshire, UK. Journal for Nature Conservation, 30 90–102.

McCarroll, J., Chambers, F. M., Webb, J. C. & Thom, T. (2017) Application of Palaeoecology for Peatland Conservation at Mossdale Moor, UK. Quaternary International, 432 39–47.

McCarroll, J., Chambers, F., Webb, J. & Thom, T. (2016) Informing Innovative Peatland Conservation in Light of Palaeoecological Evidence for the Demise of *Sphagnum Imbricatum*: The Case of Oxenhope Moor, Yorkshire, UK. Mires and Peat, 18 (08), 1–24.

Ménard, H., Cole, C., Gray, A., Mudie, R., Klu, J. K. & Nic Daéid, N. (2021) Creation of a Universal Experimental Protocol for the Investigation of Transfer and Persistence of Trace Evidence: Part 1 - From Design to Implementation for Particulate Evidence. Forensic Science International: Synergy, 3, 100165.

Meyer, M. D. & Safford, H. D. (2011) Giant Sequoia Regeneration in Groves Exposed to Wildfire and Retention Harvest. Fire Ecology, 7 (2) 2–16.

Middleton, R. & Bunting, M. J. (2004) Mosaic v1.1: Landscape Scenario Creation Software for Simulation of Pollen Dispersal and Deposition. Review of Palaeobotany and Palynology, 132 (1–2) 61–66.

Mildenhall, D. C. (1990) Forensic Palynology in New Zealand. Review of Palaeobotany and Palynology, 64 (1–4) 227–234.

Mildenhall, D. C. (2006) Hypericum Pollen Determines the Presence of Burglars at the Scene of a Crime: An Example of Forensic Palynology. Forensic Science International, 163 (3) 231–235.

Mildenhall, D. C., Wiltshire, P. E. J. & Bryant, V. M. (2006) Forensic Palynology: Why Do It and How It Works. Forensic Science International, 163 (3) 163–172.

Miller, P. A., Giesecke, T., Hickler, T., Bradshaw, R. H. W., Smith, B., Seppä, H., Valdes, P. J. & Sykes, M. T. (2008) Exploring Climatic and Biotic Controls on Holocene Vegetation Change in Fennoscandia. Journal of Ecology, 96 (2) 247–259.

Miyoshi, N., Fujiki, T. & Morita, Y. (1999) Palynology of a 250-m Core from Lake Biwa: A 430,000-Year Record of Glacial–Interglacial Vegetation Change in Japan. Review of Palaeobotany and Palynology, 104 (3–4) 267–283.

Monckton-Smith, J., Hart, A., Adams, T. & Webb, J. (2013) Introducing Forensic and Criminal Investigation. SAGE.

Moore, P., Webb, J. & Collinson, M. (1991) Pollen Analysis. Oxford: Blackwell Scientific.

More, S. & Bera, S. (2015) Potential of Hairs as a Decisive Tool in Forensic Palynological Investigations: First Experimental Study from India. Research Journal of Forensic Sciences, 3 (6) 1-7

Mottl, O., Grytnes, J.-A., Seddon, A. W. R., Steinbauer, M. J., Bhatta, K. P., Felde, V. A., Flantua, S. G. A. & Birks, H. J. B. (2021) Rate-of-Change Analysis in Paleoecology Revisited: A New Approach. Review of Palaeobotany and Palynology, 293 104483.

Mudie, P. J., & Lelièvre, M. A. (2013) Palynological study of a Mi'kmaw shell midden, Northeast Nova Scotia, Canada. Journal of Archaeological Science, 40(4), 2161-2175.

Munsterman, D. & Kerstholt, S. (1996) Sodium Polytungstate, a New Non-Toxic Alternative to Bromoform in Heavy Liquid Separation. Review of Palaeobotany and Palynology, 91 (1–4) 417–422.

Nogué, S., Nascimento, L. de, Froyd, C. A., Wilmshurst, J. M., Boer, E. J. de, Coffey, E. E. D., Whittaker, R. J., Fernández-Palacios, J. M. & Willis, K. J. (2017) Island Biodiversity Conservation Needs Palaeoecology. Nature Ecology & Evolution, 1 (7) 0181.

Ochando, J., Munuera, M., Carrión, J. S., Fernández, S., Amorós, G. & Recalde, J. (2018) Forensic Palynology Revisited: Case Studies from Semi-Arid Spain. Review of Palaeobotany and Palynology, 259 29–38.

Olsson, F., Gaillard, M.-J., Lemdahl, G., Greisman, A., Lanos, P., Marguerie, D., Marcoux, N., Skoglund, P. & Wäglind, J. (2010) A Continuous Record of Fire Covering the Last 10,500 Calendar Years from Southern Sweden — The Role of Climate and Human Activities. Palaeogeography, Palaeoclimatology, Palaeoecology, 291 (1–2) 128–141.

Paisley, M. F., Trigg, D. J. & Walley, W. J. (2014) revision of the biological monitoring working party (BMWP) score system: derivation of present-only and abundance-related scores from field data. River Research and Applications, 30 (7) 887–904.

Peglar, Sylvia M. & Birks, H. J. B. (1993) The Mid-Holocene *Ulmus* Fall at Diss Mere, South-East England: Disease and Human Impact. Vegetation History and Archaeobotany, 2 (2) 61-68.

Pinho, P., Augusto, S., Branquinho, C., Bio, A., Pereira, M. J., Soares, A. & Catarino, F. (2004) Mapping Lichen Diversity as a First Step for Air Quality Assessment. Journal of Atmospheric Chemistry, 49 (1–3) 377–389.

Pirrie, D., Power, M. R., Rollinson, G. K., Wiltshire, P. E. J., Newberry, J. & Campbell, H. E. (2009) Automated SEM-EDS (QEMSCAN®) Mineral Analysis in Forensic Soil Investigations: Testing Instrumental Reproducibility. In: Criminal and Environmental Soil Forensics. Dordrecht: Springer Netherlands, 411–430.

Pirrie, D., Rollinson, G. K., Power, M. R. & Webb, J. (2013) Automated Forensic Soil Mineral Analysis; Testing the Potential of Lithotyping. Geological Society, London, Special Publications, 384(1), 47-64

Plicht, J. van der, Yeloff, D., Linden, M. van der, Geel, B. van, Brain, S., Chambers, F. M., Webb, J. & Toms, P. (2013) Dating Recent Peat Accumulation in European Ombrotrophic Bogs. Radiocarbon, 55 (2–3).

Post, L. von & Granlund, E. (1926) Södra Sveriges Torvtillgångar. Sver Geol Unders Series C, 335 (1), 1–127.

Prentice, I. C. (1985) Pollen Representation, Source Area, and Basin Size: Toward a Unified Theory of Pollen Analysis. Quaternary Research, 23 (1) 76–86.

Procter, F. A., Swindles, G. T. & Barlow, N. L. M. (2019) Examining the Transfer of Soils to Clothing Materials: Implications for Forensic Investigations. Forensic Science International, 305 110030.

Quamar, Md. F., Tiwari, P. & Thakur, B. (2021) The Modern Pollen–Vegetation Relationship in Jammu, India: A Comparative Appraisal. Acta Palaeobotanica, 61 (1) 1–19.

Reiter, P. (2000) From Shakespeare to Defoe: Malaria in England in the Little Ice Age. Emerging Infectious Diseases, 6 (1) 1–11.

Rhodes, R. H., Bertler, N. A. N., Baker, J. A., Steen-Larsen, H. C., Sneed, S. B., Morgenstern, U. & Johnsen, S. J. (2012) Little Ice Age Climate and Oceanic Conditions of the Ross Sea, Antarctica from a Coastal Ice Core Record. Climate of the Past, 8 (4) 1223–1238.

Riding, J. B., Rawlins, B. G. & Coley, K. H. (2007) Changes in Soil Pollen Assemblages on Footwear Worn at Different Sites. Palynology, 31 (1) 135–151.

Roberts, N. (2015) The Holocene: An Environmental History. 3rd ed. Oxford: Wiley-Blackwell.

Roche, J. R., Mitchell, F. J. G. & Waldren, S. (2009) Plant Community Ecology of Pinus Sylvestris, an Extirpated Species Reintroduced to Ireland. Biodiversity and Conservation, 18 (8) 2185–2203.

Rodrigues, M. O., Gonçalves, A. M. M., Gonçalves, F. J. M. & Abrantes, N. (2020) Improving Cost-Efficiency for MPs Density Separation by Zinc Chloride Reuse. MethodsX, 7, 100785.

Roubelat, S., Besancenot, J.-P., Bley, D., Thibaudon, M. & Charpin, D. (2020) Inventory of the Recommendations for Patients with Pollen Allergies and Evaluation of Their Scientific Relevance. International Archives of Allergy and Immunology, 181 (11), 839–852.

Rull, V. (2012) Community Ecology: Diversity and Dynamics over Time. Community Ecology, 13 (1) 102–116.

Sawyer, R. (1985) Misrepresentation - a Simplified Procedure. Bee Craft, 67, 167–169.

Seddon, A. W. R., Mackay, A. W., Baker, A. G., Birks, H. J. B., Breman, E., Buck, C. E., Ellis, E. C., Froyd, C. A., Gill, J. L., Gillson, L., Johnson, E. A., Jones, V. J., Juggins, S., Macias-Fauria, M., Mills, K., Morris, J. L., Nogués-Bravo, D., Punyasena, S. W., Roland, T. P., Tanentzap, A. J., Willis, K. J., Aberhan, M., Asperen, E. N., Austin, W. E. N., Battarbee, R. W., Bhagwat, S., Belanger, C. L., Bennett, K. D., Birks, H. H., Bronk Ramsey, C., Brooks, S. J., Bruyn, M., Butler, P. G., Chambers, F. M., Clarke, S. J., Davies, A. L., Dearing, J. A., Ezard, T. H. G., Feurdean, A., Flower, R. J., Gell, P., Hausmann, S., Hogan, E. J., Hopkins, M. J., Jeffers, E. S., Korhola, A. A., Marchant, R., Kiefer, T., Lamentowicz, M., Larocque-Tobler, I., López-Merino, L., Liow, L. H., McGowan, S., Miller, J. H., Montoya, E., Morton, O., Nogué, S., Onoufriou, C., Boush, L. P., Rodriguez-Sanchez, F., Rose, N. L., Sayer, C. D., Shaw, H. E., Payne, R., Simpson, G., Sohar, K., Whitehouse, N. J., Williams, J. W. & Witkowski, A. (2014) Looking Forward through the Past: Identification of 50 Priority Research Questions in Palaeoecology. Journal of Ecology, 102 (1) 256–267.

Seppä, H., Alenius, T., Bradshaw, R. H. W., Giesecke, T., Heikkilä, M. & Muukkonen, P. (2009) Invasion of Norway Spruce (Picea Abies) and the Rise of the Boreal Ecosystem in Fennoscandia. Journal of Ecology, 97 (4) 629–640.

Sillasso, U., Mauquoy, D., Blundell, A., Charman, D., Blaauw, M., Daniell, J. R. G., Toms, P., Newberry, J., Chambers, F. M. & Karofeld, E. (2007) Peat Multi-Proxy Data from Männikjärve Bog as Indicators of Late Holocene Climate Changes in Estonia. Boreas, 36 (1) 20–37.

Siska, P. P., Bryant, V. M. & Jones, J. G. (2001) The Spatial Analysis of Modern Pollen Rain in Big Bend National Park. Palynology, 25 (1) 199–216.

Sniderman, J. M. K., Matley, K. A., Haberle, S. G. & Cantrill, D. J. (2018) Pollen Analysis of Australian Honey. PLOS ONE, 13 (5) e0197545.

Stefaniak, K., Stachowicz-Rybka, R., Borówka, R. K., Hrynowiecka, A., Sobczyk, A., Moskal-del Hoyo, M., Kotowski, A., Nowakowski, D., Krajcarz, M. T., Billia, E. M. E., Persico, D., Burkanova, E. M., Leshchinskiy, S. v., Asperen, E. van, Ratajczak, U., Shpansky, A. v., Lempart, M., Wach, B., Niska, M., Made, J. van der, Stachowicz, K., Lenarczyk, J., Piątek, J. & Kovalchuk, O. (2021) Browsers, Grazers or Mix-Feeders? Study of the Diet of Extinct Pleistocene Eurasian Forest Rhinoceros Stephanorhinus Kirchbergensis (Jäger, 1839) and Woolly Rhinoceros Coelodonta Antiquitatis (Blumenbach, 1799). Quaternary International, 605–606 192–212.

Sugita, S. (1993) A Model of Pollen Source Area for an Entire Lake Surface. Quaternary Research, 39 (2) 239–244.

Sugita, S. (2007a) Theory of Quantitative Reconstruction of Vegetation II: All You Need Is LOVE. The Holocene, 17 (2) 243–257.

Sugita, S. (2007b) Theory of Quantitative Reconstruction of Vegetation I: Pollen from Large Sites REVEALS Regional Vegetation Composition. The Holocene, 17 (2) 229–241.

Sutherland, W. J., Adams, W. M., Aronson, R. B., Aveling, R., Blackburn, T. M., Broad, S., Ceballos, G., Cote, I. M., Cowling, R. M., Fonesca, G. A. B. da, Dinerstein, E., Ferraro, P. J., Fleishman, E., Gascon, C., Hunter Jr., M., Hutton, J., Kareiva, P., Kuria, A., MacDonald, D. W., MacKinnon, K., Madgwick, F. J., Mascia, M. B., McNeely, J., Milner-Gulland, E. J., Moon, S., Morely, C. G., Nelson, S., Osborn, D., Pai, M., Parsons, E. C. M., Peck, L. S., Possingham, H., Prior, S. v., Pullin, A. S., Rands, M. R. W., Ranganathan, J., Redford, K. H., Rogriguez, J. P., Seymour, F., Sobel, J., Sodhi, N. S., Stott, A., Vance-Borland, K. & Watkinson, A. R. (2009) One Hundred Questions of Importance to the Conservation of Global Biological Diversity. Conservation Biology, 23 (3) 557–567.

Sutherland, W. J., Armstrong-Brown, S., Armsworth, P. R., Tom, B., Brickland, J., Campbell, C. D., Chamberlain, D. E., Cooke, A. I., Dulvy, N. K., Dusic, N. R., Fitton, M., Freckleton, R. P., Godfray, H. C. J., Grout, N., Harvey, H. J., Hedley, C., Hopkins, J. J., Kift, N. B., Kirby, J., Kunin, W. E., MacDonald, D. W., Marker, B., Naura, M., Neale, A. R., Oliver, T., Osborn, D., Pullin, A. S., Shardlow, M. E. A., Showler, D. A., Smith, P. L., Smithers, R. J., Solandt, J., Spencer, J., Spray, C. J., Thomas, C. D., Thompson, J., Webb, S. E., Yalden, D. W. & Watkinson, A. R. (2006) The Identification of 100 Ecological Questions of High Policy Relevance in the UK. Journal of Applied Ecology, 43 (4) 617–627.

Szibor, R., Schubert, C., Schöning, R., Krause, D. & Wendt, U. (1998) Pollen Analysis Reveals Murder Season. Nature, 395 (6701) 449–450.

Theuerkauf, M. & Couwenberg, J. (2021) Pollen Productivity Estimates Strongly Depend on Assumed Pollen Dispersal II: Extending the ERV Model. The Holocene, 095968362110417.

Theuerkauf, M., Kuparinen, A. & Joosten, H. (2013) Pollen Productivity Estimates Strongly Depend on Assumed Pollen Dispersal. The Holocene, 23 (1) 14–24.

The Wildlife Trust of South and West Wales (2021) Lucky Finds from Rabbit Hole Re-Write Prehistory of Skokholm Island! re-write prehistory of Skokholm Island! Available from https://www.welshwildlife.org/pressreleases/lucky-finds-from-rabbit-hole-re-write-prehistory-of-skokholm-island/.

Tipping, R. (2000) Pollen Preservation Analysis as a Necessity in Holocene Pollen Analysis. In: Huntley, J. & Stallibrass, S. ed., Taphonomy and Interpretation. Symposia of the Association for Environmental Archaeology. vol. 14. Oxford: Oxbow Books, 23–34.

Tipping, R. M. (2008) Long-Distance Transported Pinus Pollen as a Possible Chronostratigraphic Marker in the Scottish Early Postglacial. Boreas, 18 (4) 333–341.

Trondman, A. -K., Gaillard, M. -J., Mazier, F., Sugita, S., Fyfe, R., Nielsen, A. B., Twiddle, C., Barratt, P., Birks, H. J. B., Bjune, A. E., Björkman, L., Broström, A., Caseldine, C., David, R., Dodson, J., Dörfler, W., Fischer, E., Geel, B., Giesecke, T., Hultberg, T., Kalnina, L., Kangur, M., Knaap, P., Koff, T., Kuneš, P., Lagerås, P., Latałowa, M., Lechterbeck, J., Leroyer, C., Leydet, M., Lindbladh, M., Marquer, L., Mitchell, F. J. G., Odgaard, B. v., Peglar, S. M., Persson, T., Poska, A., Rösch, M., Seppä, H., Veski, S. & Wick, L. (2015) Pollen-based Quantitative Reconstructions of Holocene Regional Vegetation Cover (Plant-functional Types and Land-cover Types) in Europe Suitable for Climate Modelling. Global Change Biology, 21 (2) 676–697.

Twiddle, C. L. & Bunting, M. J. (2010) Experimental Investigations into the Preservation of Pollen Grains: A Pilot Study of Four Pollen Types. Review of Palaeobotany and Palynology, 162 (4) 621–630.

Tzedakis, P. C. (1994) Vegetation Change through Glacial—Interglacial Cycles: A Long Pollen Sequence Perspective. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 345 (1314) 403–432.

Vegas-Vilarrúbia, T., Rull, V., Montoya, E. & Safont, E. (2011) Quaternary Palaeoecology and Nature Conservation: A General Review with Examples from the Neotropics. Quaternary Science Reviews, 30 (19–20) 2361–2388.

Vincens, A., Garcin, Y. & Buchet, G. (2007) Influence of Rainfall Seasonality on African Lowland Vegetation during the Late Quaternary: Pollen Evidence from Lake Masoko, Tanzania. Journal of Biogeography, 34 (7) 1274–1288.

Walter, O., Adekanmbi, O. & Olowokudejo (2019) Palynological and Lithological Investigation of Forensic Materials at the University of Lagos, Nigeria: First Experimental Palynological Approach in Nigeria. Journal of Forensic Science and Criminology, 7 (1), 104.

Wanner, H., Beer, J., Bütikofer, J., Crowley, T. J., Cubasch, U., Flückiger, J., Goosse, H., Grosjean, M., Joos, F., Kaplan, J. O., Küttel, M., Müller, S. A., Prentice, I. C., Solomina, O., Stocker, T. F., Tarasov, P., Wagner, M. & Widmann, M. (2008) Mid- to Late Holocene Climate Change: An Overview. Quaternary Science Reviews, 27 (19–20) 1791–1828.

Wanner, H., Solomina, O., Grosjean, M., Ritz, S. P. & Jetel, M. (2011) Structure and Origin of Holocene Cold Events. Quaternary Science Reviews, 30 (21–22) 3109–3123.

Wan, Q., Huang, K., Zhang, X., Yue, Y., Peng, H., Ma, T., Yang, X. & Zheng, Z. (2022) Regional Land Cover Changes of the Last 6,500 Years in Middle and Southern Subtropical China. Quaternary International. Online first.

Webb, J. C., Brown, H. A., Toms, H. & Goodenough, A. E. (2018) Differential Retention of Pollen Grains on Clothing and the Effectiveness of Laboratory Retrieval Methods in Forensic Settings. Forensic Science International, 288, 36-45

Webb, J. C. & Goodenough, A. E. (2018a) Questioning Ancient Woodland Indicators. Wood Wise - Woodland Conservation News [Online], 4–7. Available from: <https://www.woodlandtrust.org.uk/media/1710/wood-wise-forest-flora.pdf> [Accessed 10 May 2022].

Webb, J. C. & Goodenough, A. E. (2018b) Questioning the Reliability of “Ancient” Woodland Indicators: Resilience to Interruptions and Persistence Following Deforestation. Ecological Indicators, 84.

Webb, J. C. & Goodenough, A. E. (2021) Vegetation Community Changes in European Woodlands amid a Changing Climate: A Palaeoecological Modelling Perspective. Community Ecology, 22 (3) 319–330.

Webb, J. C., McCarroll, J., Chambers, F. M. & Thom, T. (2022b) Evidence for the Little Ice Age in Upland Northwestern Europe: Multiproxy Climate Data from Three Blanket Mires in Northern England. The Holocene, 32 (5) 451–467.

Webb, J., McCarroll, J., Carpenter, W., Chambers, F., Toms, P. & Wood, M. (2017) Apparent Lack of Woodland and Abundance of Woodland Indicator Species: The Role of Humans, Birds and Rabbits on the Changing Vegetation of Skomer Island, Wales. Archaeology in Wales, 56, 90–99.

Webb, J., McCarroll, J. & Morrow, D. (2022a) Palynological Contributions towards the Archaeology of Skokholm Island, South West Wales. Archaeology in Wales, 60, 45–48.

Weng, C., Hooghiemstra, H. & Duivenvoorden, J. F. (2006) Challenges in Estimating Past Plant Diversity from Fossil Pollen Data: Statistical Assessment, Problems, and Possible Solutions. Diversity & Distributions, 12 (3) 310–318.

Wernham, C. V., Toms, M. P., Marchant, J. H., Clark, J. A., Siriwardena, G. M. & Baillie, S. R. (2002) The Migration Atlas: Movements of the Birds of Britain and Ireland. London: T. & A.D. Poyser.

Williams, J. W., Grimm, E. C., Blois, J. L., Charles, D. F., Davis, E. B., Goring, S. J., Graham, R. W., Smith, A. J., Anderson, M., Arroyo-Cabrales, J., Ashworth, A. C., Betancourt, J. L., Bills, B. W., Booth, R. K., Buckland, P. I., Curry, B. B., Giesecke, T., Jackson, S. T., Latorre, C., Nichols, J., Purdum, T., Roth, R. E., Stryker, M. & Takahara, H. (2018) The Neotoma Paleoecology Database, a Multiproxy, International, Community-Curated Data Resource. Quaternary Research, 89 (1) 156–177.

Williams, J. W., Shuman, B. N., Webb, T., Bartlein, P. J. & Leduc, P. L. (2004) Late-Quaternary vegetation dynamics in North America: scaling from taxa to biomes. Ecological Monographs, 74 (2) 309–334.

Willis, K. J., Araújo, M. B., Bennett, K. D., Figueroa-Rangel, B., Froyd, C. A. & Myers, N. (2007) How Can a Knowledge of the Past Help to Conserve the Future? Biodiversity Conservation and the Relevance of Long-Term Ecological Studies. Philosophical Transactions of the Royal Society B: Biological Sciences, 362 (1478) 175–187.

Willis, K. J., Bailey, R. M., Bhagwat, S. A. & Birks, H. J. B. (2010) Biodiversity Baselines, Thresholds and Resilience: Testing Predictions and Assumptions Using Palaeoecological Data. Trends in Ecology & Evolution, 25 (10) 583–591.

Willis, K. J. & Birks, H. J. B. (2006) What Is Natural? The Need for a Long-Term Perspective in Biodiversity Conservation. Science, 314 (5803) 1261–1265.

Wiltshire, P. E. J. (2006a) Consideration of Some Taphonomic Variables of Relevance to Forensic Palynological Investigation in the United Kingdom. Forensic Science International, 163 (3) 173–182.

Wiltshire, P. E. J. (2006b) Hair as a Source of Forensic Evidence in Murder Investigations. Forensic Science International, 163 (3) 241–248.

Wiltshire, P. E. J. (2016) Protocols for Forensic Palynology. Palynology, 40 (1) 4–24.

Wiltshire, P. E. J., Hawksworth, D. L. & Edwards, K. J. (2015) A Rapid and Efficient Method for Evaluation of Suspect Testimony: Palynological Scanning. Journal of Forensic Sciences, 60 (6) 1441–1450.

Wiltshire, P. E. J., Hawksworth, D. L., Webb, J. A. & Edwards, K. J. (2015) Two Sources and Two Kinds of Trace Evidence: Enhancing the Links between Clothing, Footwear and Crime Scene. Forensic Science International, 254 231–242.

Wingard, G. L., Bernhardt, C. E. & Wachnicka, A. H. (2017) The Role of Paleoecology in Restoration and Resource Management—The Past as a Guide to Future Decision-Making: Review and Example from the Greater Everglades Ecosystem, U.S.A. Frontiers in Ecology and Evolution, 5, 11.

Wood, M. J., Morgan, P. J., Webb, J. C., Goodenough, A. E., Chambers, F. M. & Hart, A. G. (2014) Exploring the Prevalence and Diversity of Pollen Carried by Four Species of Migratory Old-World Warbler (Sylvioidea) on Arrival in the UK. Bird Study, 61 (3).

Yamada, K., Omori, T., Kitaba, I., Hori, T. & Nakagawa, T. (2021) Extraction Method for Fossil Pollen Grains Using a Cell Sorter Suitable for Routine 14C Dating. Quaternary Science Reviews, 272 107236.

Yeloff, D. & Hunt, C. (2005) Fluorescence Microscopy of Pollen and Spores: A Tool for Investigating Environmental Change. Review of Palaeobotany and Palynology, 133 (3–4) 203–219.