ANCIENT WOODLAND VEGETATION: DISTINCTIVENESS AND COMMUNITY ECOLOGY

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Abstract

The high floristic biodiversity value of ancient woodland is widely acknowledged, as is its status as a fragmented habitat of limited spatial extent. The distinctive vegetation of ancient woodland is an important factor in its conservation. Specifically, Ancient Woodland Indicator (AWI) species have been shown to be poor dispersers and incompatible with a fragmented habitat that is subject to environmental change. In recognition of their ecological importance, both Ancient Semi-Natural Woodland (ASNW) and Ancient Replanted Woodland (ARW) are protected by legislation.

This thesis took the novel approach of examining the distinctiveness and community ecology of vegetation communities in all three woodland types of ASNW, ARW, and recent woodland. Importantly, analyses were based on new high-granularity primary vegetation and soil data.

To address questions raised in the literature regarding the accuracy of ancient woodland and AWI identification, this research examined the metrics used to distinguish these habitats and species. Increasingly, the literature calls for further understanding of the ecological drivers of ancient woodland vegetation distinctiveness. In response, this research tested for differences in species composition of canopy, shrub, herb layer, AWI, and moss communities across all three woodland types. For AWI species, biotic, abiotic, and biogeographical variables were analysed for their contribution to community distinctiveness.

Results highlighted the importance of consistency in metric selection when assessing the distinctiveness of ancient woodland and determining indicator species. In addition to the usual alpha scale measure of distinctiveness, assessing richness and community composition at the beta and gamma scales is recommended to inform conservation. Life traits and dispersal mechanisms were important differentiators for herb layer community composition among the woodland types. AWI richness was equally strongly explained by biogeographical variables as by ASNW, ARW, and recent status. Overall, this thesis supported ecological and biogeographical explanations for the distinctiveness of ancient woodland vegetation.

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. Chapter 6 is a co-authored paper, for which a statement of authors' contributions is included in Appendix I.

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

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Table of contents

List of figures

List of tables

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Glossary of key terms

Ancient semi-natural woodland: 'Ancient woodland composed of predominantly site native tree species, usually derived from coppice or natural regeneration; the composition may be modified by past management and more recent planting of native species' (Forestry Commission, 2010:48)

Ancient woodland: 'A classification for woodland which has been in continuous existence from before AD 1600 in England, Wales and Northern Ireland and from before AD 1750 in Scotland' (Forestry Commission, 2010:48).

Ancient woodland indicator species: Vascular plants that are strongly associated with, but not exclusive to, ancient woodland (Hermy *et al*., 1999; Rose, 1999). Usually referring to shrub, field and herb layer species, although could refer to non-vascular plants.

Ancient woodland species: Plant species with a strong affinity for ancient woodland, slow-colonisers, that are indicative of woodland interiors, habitat continuity and potentially original woodland conditions (Hermy *et al*., 1999). Usually referring to shrub, field and herb layer species. Sometimes used interchangeably with **ancient woodland indicator species**.

Ancient woodland vascular plant species: - another term for **ancient woodland indicator species**, although less commonly used. Usually referring to shrub, field and herb layer species (Rose, 1999).

Continuity indicator: Similar in scope to **ancient woodland indicator species**, but this term tends to be used for lichen studies (Whittet and Ellis, 2013).

Old-growth forest: Defined as 'stands in primary or secondary forests that have developed the structures and species normally associated with old primary woodland of that type that have sufficiently accumulated to act as a woodland ecosystem distinct from any younger age class', which contain veteran trees over 200 years old (Forestry Commission, 2010: 50). Less commonly used in the UK than the similar term **ancient semi-natural woodland**.

Primary woodland: A continuation of primaeval woodland which has never been cleared (Peterken, 1993).

Recent woodland Species: Plant species with a strong affinity for recent woodland, tending to be ruderal or competitive, with a persistent seed bank and good dispersal mechanisms (Graae *et al*., 2004). Usually referring to shrub, field and herb layer species.

Secondary woodland: 'Secondary woodland grows on land that was formerly used pasture, meadow, arable, grouse moors, deer forest, habitation, quarries, etc.' (Peterken, 2013: 79).

Woodland generalists/ woodland species: Plant species that prefer woodland but can occur in more open habitats (Brunet *et al*., 2011). Usually referring to shrub, field and herb layer species.

Woodland specialists/ true woodland species/ core woodland species/ strict woodland species: A range of terms used to refer to plant species occurring in closed woodland (Whigham, 2004; Bierzychudek, 1982). Usually referring to shrub, field and herb layer species. Sometimes used interchangeably in the literature with **ancient woodland species** or **ancient woodland indicator species**.

Woodland: The National Forest Inventory defines woodland in Britain as an area with minimum 20% canopy cover, of least 0.5 hectares, and width of 20 metres (Forestry Commission, 2011a).

Acronyms and abbreviations

AONB – Area of Outstanding Natural Beauty

ASNW – Ancient Semi-Natural Woodland

ARW – Ancient Replanted Woodland

AWI – Ancient Woodland Indicator

AWS – Ancient Woodland Species

Cal. BP – Calendar Years Before Present

DEFRA – Department for Environment, Food and Rural Affairs

d.f. – degrees of freedom

GIS – Geographical Information System

GPS – Geographical Positioning System

ha – hectares

km – kilometre

MAGIC map – Multi-Agency Geographic Information for the Countryside

m.a.s.l. – metres above sea level

OS – Ordnance Survey

RAWS – Restored Ancient Woodland Sites

s.d. – standard deviation

s.e. – standard error

SNA – Strategic Nature Area

Terminology

Woodland/ forest: ‘Woodland’ is the term used in UK policy and legislation, and so is used in place of ‘forest’ for consistency when referring to international studies, except where forest is an accepted part of the nomenclature for instance of a place, organisation or title.

Ancient woodland/ Ancient Semi-Natural Woodland (ASNW): Many studies refer to ancient woodland as contrasted with recent woodland. However, in Britain ancient woodland comprises two categories ASNW and ARW. The majority of studies use the term ‘ancient woodland’ to mean ASNW but not ARW. Therefore, throughout this thesis ASNW is used in place of ‘ancient’ where it refers to ASNW only.

Ancient Replanted Woodland/ Plantation on Ancient Woodland Site. ‘Ancient Replanted Woodland’ (ARW) is used throughout instead of ‘Plantation on Ancient Woodland Site (PAWS), as the former terminology is becoming more common in recent literature.

Ancient Woodland Indicator species. Throughout this thesis the term ‘Ancient Woodland Indicator’ (AWI) will encompass other terminology such as Ancient Woodland Species, woodland specialists, true woodland species. The terminology for woodland flora varies amongst studies and some terms are used interchangeably or conflated. The major terms are defined in the glossary. AWI is predominantly used in the UK. However, AWI and AWS terms are often used interchangeably in some European studies. Ancient Woodland Indicator (AWI) species are more correctly a subset of AWS (Hermy *et al*. 1999; Schmidt *et al*., 2014) and are not only strongly ecologically associated with ancient woodland, but are also used to identify it. The majority of studies distinguish between ‘Ancient Woodland Species’ (AWS) or ‘Ancient Woodland Indicators’ (AWI), ‘recent woodland species’ and ‘other woodland species’ (De Keersmaeker *et al*., 2014; Kelemen *et al*., 2014; Schmidt *et al*., 2014; Kirby and Morecroft, 2011; Orczewska, 2009; Sciama *et al*., 2009; Hermy *et al*., 1999; Bossuyt and Hermy, 2000; Wulf, 1997). Other studies refer to ‘true woodland species’, ‘woodland core species’, ‘woodland specialists’, as opposed to ‘woodland generalists’, to distinguish those associated with good quality, established (often ancient) semi-natural woodland (Brunet *et al*., 2011; Vellend *et al*., 2007; Graae *et al*., 2004).

Referencing

This thesis employs the University of Gloucestershire Harvard system.

Taxonomic nomenclature

This thesis follows The Plant List http://www.theplantlist.org/ (Royal Botanic Gardens Kew and Missouri Botanical Garden, 2013).

1. Introduction and literature Review

Hyacinthoides non-scripta. Francombe Wood, Sapperton Strategic Nature Area 04/05/14. Grid ref. SO 95469 05612.

Scope of Chapter

This combined introduction and literature review explores the ancient woodland concept in global, European and British contexts. Woodland history in Britain is outlined to set the context of current conservation policy and priorities. The identification of ancient woodland and ancient woodland indicator species are described and critiqued. The vegetation ecology of ancient woodland relative to recent woodland is discussed in the context of British and European literature. The structure of the thesis is outlined. This chapter closes with the rationale for the research and statement of research aim and objectives.

1.1 Woodland definition, cover and context

This section presents a broad introduction to woodland internationally, and a detailed description of woodland types and classifications in Britain, with specific focus on the concept of ancient woodland.

1.1.1 Global and European context

Woodland and forests globally are firmly established as a high conservation priority (CBD, 2016; IUCN, 2016; UNEP, 2016). Research-informed sustainable management of woodland and forest is necessary for the conservation of biodiversity, the regulation of environmental processes, and resource provision for human use (FAO, 2015).

Long-established and continuously wooded areas are internationally recognised as having the highest conservation value of all types of woodland (Wirth *et al*., 2009a; Rackham, 2008, Rackham, 2003; Peterken, 1993). These Ancient Semi-Natural Woodlands (ASNW) or old-growth forests are acknowledged not only for their high biodiversity value in comparison to recent woodland (Peterken, 1993), but also because they are considered to be the closest habitat to natural climatic climax vegetation due to their long-term continuously wooded state (Corney *et al*., 2008b). This is particularly important for countries with a history of extensive land use change and population growth, meaning that little primary woodland remains. As such they provide a semi-natural baseline or reference point for research which informs sustainable management of all woodland types (Wirth *et al*., 2009a; Corney *et al*., 2008b; Goldberg *et al*., 2007).

The distinction between ASNW and recent woodland originated in Britain (Peterken, 1977; Rackham, 1976) and has been adopted internationally, with the term 'ancient' being used within Europe and 'old-growth' in other continents. Although the two terms are not precisely synonymous (Wirth *et al*., 2009b), the principle is embedded in conservation and ecology research in North America (e.g. Vellend *et al*., 2007; Vosick *et al*., 2007), South America (e.g. Barlow *et al*., 2007; Clapp, 1998); Asia (e.g. Miura *et al*., 2007; Zhang *et al*., 2000); Africa (e.g. Lawton *et al*., 1998); Australasia (e.g. Rudel *et al*., 2005; Keenan and Ryan 2004; Woodgate *et al*., 1996); and Europe (e.g. Kirby and Morecroft, 2011; Hermy *et al*., 1999). Despite the apparent value of such woodland, it is at risk from a multiplicity of threats, such as fragmentation, habitat loss, non-native species, pests and pathogens, disturbance, pollution and climate change (Corney *et al*., 2008b; Rackham, 2008).

To assist with identification of ancient woodland for conservation purposes, many European countries have developed lists of indicator species that exhibit an affinity for long-established woodland (e.g. Schmidt *et al*., 2014; Blasi *et al*., 2010; Kirby, 2004; Rose, 1999; Honnay *et al*., 1998; Wulf, 1997; Peterken, 1974). Such indicators may include bryophytes, lichens, herbaceous and woody plants, molluscs, birds, invertebrates, and fungi (Blasi *et al*., 2010; Glaves *et al*., 2009), although most commonly used are herb and shrub layer plants. The importance of Ancient Woodland Indicator (AWI) species research is two-fold: (a) the high status of ancient woodland in policy and legislation means robust and up-to-date lists for the identification of ancient woodland are required; and (b) the species are often of conservation value in their own right due to their restricted range within a fragmented and depleted habitat, combined with poor dispersal abilities (Hermy *et al*., 1999).

1.1.2 Woodland cover in Britain

The last truly natural woodland extant in Britain was the Atlantic forest of 5000-7000 Calendar years Before Present (Cal. BP), the climatic climax vegetation that developed after the last glaciation (Table 1.1) (Peterken, 1981). Both Peterken and Rackham (1990) refer to this early woodland as ‘wildwood’ (although the exact nature of this is contested (Vera, 2000)), and conclude it is unlikely that any of this woodland has persisted until the present. The vegetation of woodland in Britain is partly explained by historical biogeographical factors. As the last glaciation ended, biomes that had shifted South, moved North again, facilitated by the low sea levels and land bridges to Europe (Rackham, 2000). Pollen records show that pine and birch woodland developed rapidly in the early post-glacial period, possibly having already been present in the non-glaciated south of the British Isles (Pennington, 1969). Today, the flora of mainland Europe is much richer than Britain’s, caused by rising sea levels around 7500 Cal. BP which disconnected Britain from Europe and reduced further migration of species.

Table 1.1. Changes in composition and human influence on woodland in Britain during the Flandrian period (after Peterken (1981:6) and Godwin (1975).

Calendar years before present (Cal. BP)	Blytt and Sernander periods	Forest Cover
0-2700	Sub-Atlantic	Substantial clearance by Iron Age cultures onwards. Severe reduction in forest cover. Extensive cultivation and soil modification. Alder, birch, oak, beech woodland.
2700-5000	Sub-boreal	Some forest clearance by Neolithic and Bronze Age cultures, but forest remaining widespread. Alder, oak, lime woodland.
5000-7500	Atlantic	Forest cover complete. Alder, oak, elm, lime woodland with pine, birch in North.
7500-9500	Boreal	Forest cover complete. Hazel, birch, pine woodland, becoming pine, hazel, elm, oak.
9500-10,500	Pre-boreal	Some open vegetation remaining from the last glaciation but forest cover increasing and becoming almost complete. Birch, pine woodland.

In terms of early human influence, Rackham (1990) acknowledges the impacts of small-scale woodland clearances 9500-6000 Cal. BP, but agrees with Peterken (1981) that the first large-scale human influence on woodland began with agricultural expansion 6000-4000 Cal. BP. Woodland clearance and coppice management continued between 4000-2000 Cal. BP. Rackham (2000) depicts highly managed woodlands during 2000-1500 Cal. BP with little change occurring in wooded areas during 1400-1000 Cal. BP when charters began to provide more accurate written records. The Domesday book of 1086 shows England to be very unevenly and sparsely wooded, with an estimated 15% cover (Rackham, 2000); Forestry Commission, 2011a) (Fig. 1.1).

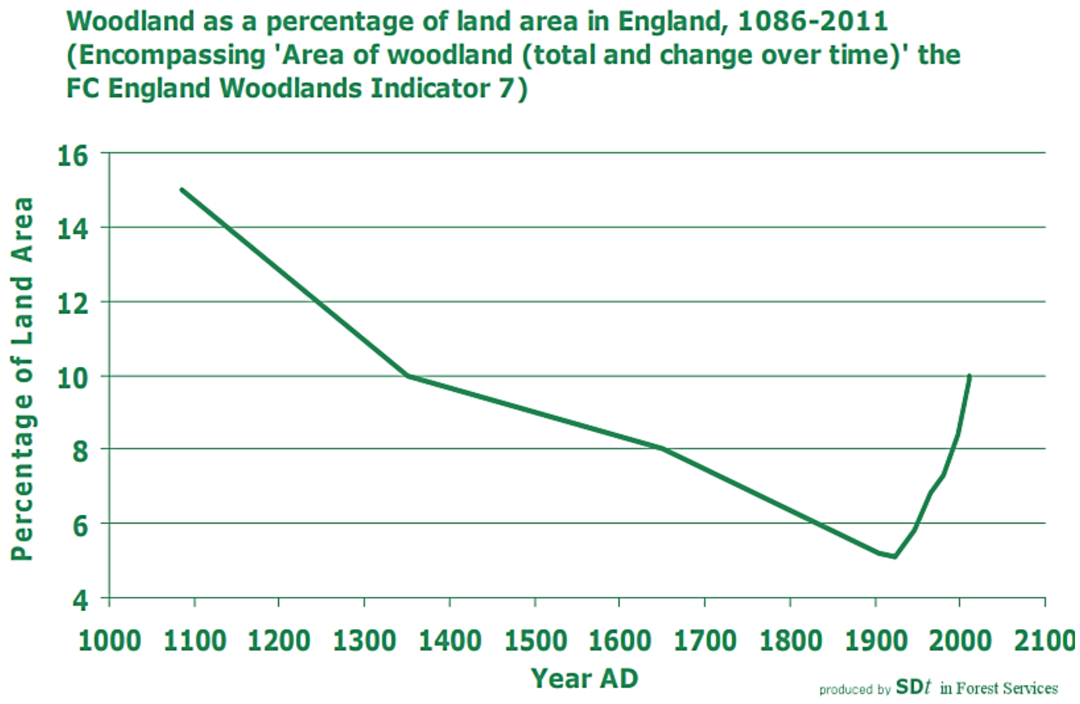

Figure 1.1. Woodland as a percentage of land area in England 1086-2011. Forestry Commission, 2011a)

Woodland planting was not common before c1600 (Goldberg *et al.*, 2007). Despite woodland planting, net woodland cover decreased through the 1700s and 1800s (Fig. 1.1) (Forestry Commission, 2011a). By 1900 woodland cover was only 5%, necessitating the establishment of the Forestry Commission in 1919 to manage timber supplies (Forestry Commission, 2012). As a result, fast-growing species were needed and approximately 38% of ASNW was felled during the 19th century mainly to grow conifer plantations, creating Ancient Replanted Woodland (ARW). A further 7% was felled for conversion to agriculture (Atkinson and Townsend, 2011).

The most rapid period of woodland cover change was an increase of 696,000ha in 1947 to 904,000ha in 2002 (Hopkins and Kirby, 2007). Net woodland cover increased through afforestation of non-wooded habitat, but initially of mainly non-native coniferous species, then later with increased planting of broadleaf species due to the Broadleaves Policy (1985) (Hopkins and Kirby, 2007). ASNW gained stronger protection through the UK Biodiversity Action Plan initiated in 1994, as well as restoration of ARW (Pryor *et al.*, 2002). Consequently, in the late 1900s, conservation issues became more prominent and planting shifted from conifers to broadleaves (Forestry Commission, 2012). Today 10% of England's land use is woodland cover, and 13% of the UK is wooded (Forestry Commission, 2017) in comparison to a European average of 37% (Atkinson and Townsend, 2011).

1.1.3 Woodland classification in Britain

Ecological woodland classification systems evolved through the 20th century, including: The Woodland Associations of Great Britain system (Moss *et al.*, 1910); Tansley's classification (1939); Peterken's (1981, 1980) Stand Theory; Rackham's (1980) classification; the Merlewood System (Bunce, 1982); and National Vegetation Classification (Rodwell, 1991). Each system is based on a different paradigm, and different sources and scales of primary data.

Woodland classifications must keep pace with woodland changes in, for example, management, canopy cover, external factors or stochastic events (Hopkins and Kirby, 2007). Since the development of early classifications, many oak woodlands have been felled and replaced with other broadleaves (Peterken, 1993), diseases, such as elm disease (*Ophiostoma ulmi*), have altered canopy composition, and coppice abandonment has altered woodland structure (Hopkins and Kirby, 2007). Additionally, several shifts in policy have occurred, particularly the Broadleaves Policy (1985) which aimed to increase and maintain broadleaf woodland, particularly ASNW. Research continues to inform the development of classification systems, particularly the debate over the phytosociological approach that treats canopy and understorey strata as one community despite functioning at very different temporal and spatial scales (Rackham, 2003; Peterken, 1993).

National Vegetation Classification (NVC) is the current standard Phase 2 habitat classification system applied to woodland and other habitats in Britain. It has been fully adopted by governmental and non-governmental organisations, ecological consultancies, and educational institutions (Rodwell, 2006; Kirby, 2003). NVC takes a phytosociological approach through the study of plant community composition and abundance and frequency occurrence of species in all woodland layers. Phytosociology is defined by Rodwell (2006:58) as ‘the science of characterising and understanding plant associations through the collection and tabling of relevés’. NVC is not solely a woodland classification; it covers all semi-natural and major artificial habitat types in Britain, enabling standardisation and a continuity in the classification of habitat types.

NVC recognises 19 different upland and lowland woodland types (W1-19), divided into 73 sub-categories, plus six scrub communities (W20-25) (Table 1.2). The classification was based on 2800 sample plots representative of ASNW and recent woodlands throughout Great Britain (Kirby, 2003), whereby stands were surveyed via five to ten nested quadrats of 50x50m, 10x10m and 4x4m. Species abundance was recorded using the DOMIN scale, and frequency occurrence in plots within a sample stand was allocated to classes 1-5.

Table 1.2. NVC classification for woodland and scrub (Rodwell, 1991).

W1	*Salix cinerea - Galium palustre* woodland
W2	*Salix cinerea - Betula pubescens - Phragmites australis* woodland
W3	*Salix pentandra - Carex rostrata* woodland
W4	*Betula pubescens - Molinia caerulea* woodland
W5	*Alnus glutinosa - Carex paniculata* woodland
W6	*Alnus glutinosa - Urtica dioica* woodland
W7	*Alnus glutinosa - Fraxinus excelsior - Lysimachia nemorum* woodland
W8	*Fraxinus excelsior - Acer campestre - Mercurialis perennis* woodland
W9	*Fraxinus excelsior - Sorbus aucuparia - Mercurialis perennis* woodland
W10	*Quercus robur - Pteridium aquilinum - Rubus fruticosus* woodland
W11	*Quercus petraea - Betula pubescens - Oxalis acetosella* woodland
W12	*Fagus sylvatica - Mercurialis perennis* woodland
W13	*Taxus baccata* woodland
W14	*Fagus sylvatica - Rubus fruticosus* woodland
W15	*Fagus sylvatica - Deschampsia flexuosa* woodland
W16	*Quercus* spp. - *Betula* spp. - *Deschampsia flexuosa* woodland
W17	*Quercus petraea - Betula pubescens - Dicranium majus* woodland
W18	*Pinus sylvestris - Hylocomium splendens* woodland
W19	*Juniperus communis* ssp. *communis - Oxalis acetosella* woodland
W20	*Salix lapponum - Luzula sylvatica* scrub
W21	*Crataegus monogyna - Hedera helix* scrub
W22	*Prunus spinosa - Rubus fruticosus* scrub
W23	*Ulex europaeus - Rubus fruticosus* scrub
W24	*Rubus fruticosus - Holcus lanatus* underscrub
W25	*Pteridium aquilinum - Rubus fruticosus* underscrub

NVC was greatly informed by earlier classification systems, and addressed some of their weaknesses. Tansley's (1939) system classified woodland by the dominant species in the tallest layer, for example oakwoods, ashwoods, and scrub. Therefore mixed stands were not distinctly represented (Peterken, 1993). This criticism was addressed by NVC via a more comprehensive phytosociological approach. Peterken's (1981, 1980) Stand Theory was the accepted classification system used by conservation agencies until 1986 (Hall and Kirby, 1998). It included only the tree and shrub species, in conjunction with edaphic, historical and geographical characteristics. However, unlike Tansley's system, mixed stands were more distinctly recognised in the Stand Theory (Peterken, 1993). Like NVC, stand species cover was recorded within each plot via a modified DOMIN scale and a constancy class was allocated for the proportion of plots within a woodland in which each species occurs (Peterken, 1993). NVC also adopted the floristic approach of the Merlewood classification system (Bunce, 1982), which considered canopy, shrub, field and ground strata, alongside abiotic data, to classify woodlands into 32 plot types. NVC records all vascular plant species, and moss species, whereas the Merlewood system groups species and focuses on the more easily identifiable (Peterken, 1981).

The phytosociological paradigm of NVC is perhaps its most debated aspect. The criticisms of the phytosociological classification originate in the extent to which the plant communities at the canopy, shrub understorey, field and ground layers should be considered as separate communities. Rackham (2003) and Peterken (1993) argue against the use of phytosociological classifications, emphasising instead the inclusion of abiotic factors, history, time, and management. For this reason, NVC emphasises the understorey species, which are unlikely to have been deliberately altered, making the classification suitable for recent woodlands and plantations but, according to Rackham (2003), less suitable for ancient woodland.

NVC is accepted as the standard methodology yet devised for woodland classification. It has a number of potential research applications beyond basic classification. Whilst NVC alone is not recommended for long-term monitoring, an initial survey can be followed by percentage cover or count data from quadrat or transect surveys (Birnie *et al*., 2005). NVC analytical tools, such as the similarity coefficients produced by classification software, can be used in conjunction with quadrat data to monitor and predict changes in vegetation classification, for example post-management intervention (Birnie *et al*., 2005). There is potential for NVC to be used as a predictive proxy measure of environmental conditions of a site, although this type of study has only been carried out for grassland and mire communities (Sanderson *et al*., 1995).

1.2 The ancient woodland concept

This section sets the context of ancient woodland in England within the concepts of continuity and naturalness. The policy context is explained and the methods by which ancient woodland is identified are critiqued.

1.2.1 Woodland age, continuity, and naturalness

Although woodland in England covers only 10% of the land area (Forestry Commission, 2017), it comprises significant variation in age, origin, structure, geographic configuration, and vegetation. Woodland can be assessed for ecological or conservation purposes by age, naturalness, and broad management structure (Fig. 1.2).

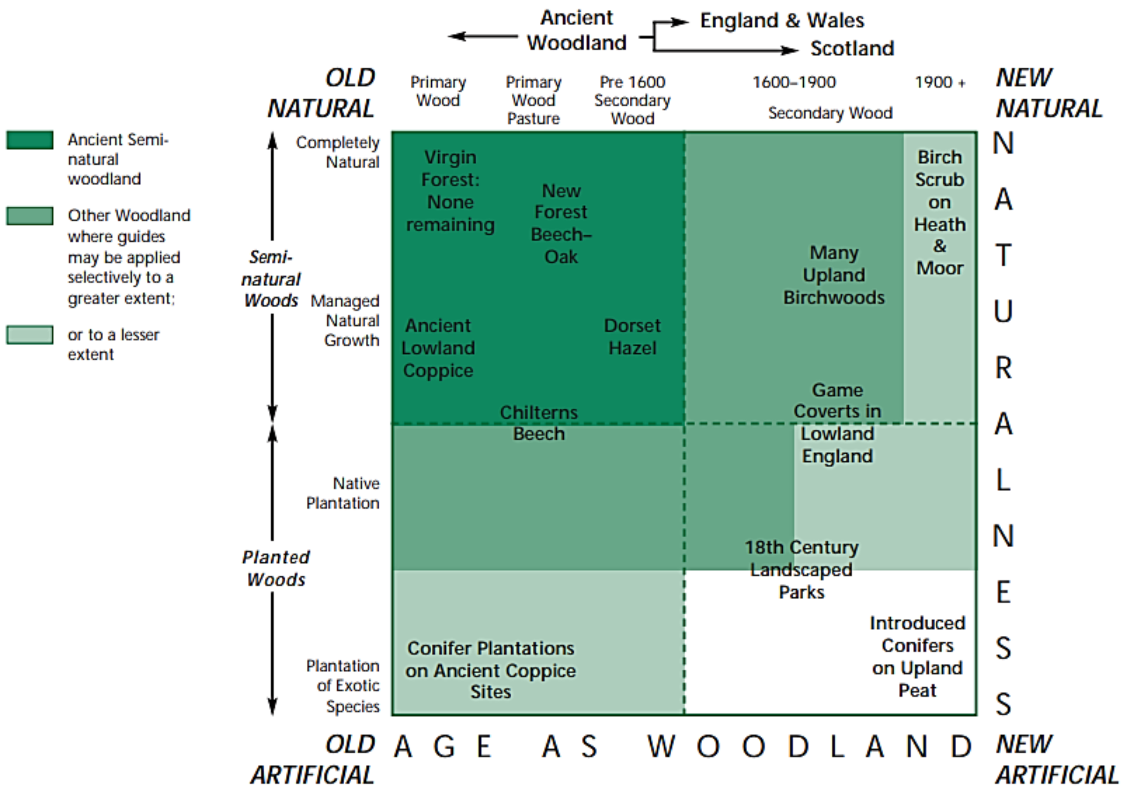

Figure 1.2. Classification of woodland according to age and naturalness (Forestry Commission, 2003)

Woodland age and continuity have become important distinctions in conservation and land use policy (Goldberg *et al*., 2007). ASNW in England is defined as having been continuously wooded since at least 1600 and containing predominantly naturally regenerated native species (Kirby and Goldberg, 2002). ARW has also been continuously wooded since 1600, but felled and replanted with predominantly non-native species, usually conifers (Kirby and Goldberg, 2002). The term ‘ancient woodland’ comprises both ASNW and ARW. Of the 1,294,000ha of woodland in England approximately 340,000ha are ancient (200,000ha ASNW and 140,000ha ARW) (Forestry Commission, 2010). A new category for Restored Ancient Woodland sites (RAWS) is increasingly used, although no land use cover data is yet available (Forestry Commission Wales, 2012). Woodland that is not classified as ‘ancient’ is generally termed ‘recent’, although theoretically it could have been established in 1601. Recent woodlands can be subcategorised into naturally regenerated or plantation.

Degree of naturalness is often used to classify woodlands and as a proxy measure for biodiversity value. The Forestry Commission (2003:3) defines semi-natural woodland as being ‘composed of locally native trees and shrubs which derive from natural regeneration or coppicing rather than planting’. By contrast, Peterken (1993) allows that plantations of native species could also be considered as semi-natural, provided they are native to that site. Using the Forestry Commission definition, in England 416,000ha of woodland are semi-natural, comprising 206,000ha ASNW and 210,000ha recent semi-natural woodland (Atkinson and Townsend, 2011). Semi-natural, rather than absolutely natural, is the realistic standard for ecologically valuable woodland; the term recognises traditional management and sustainable anthropogenic uses.

Methods of establishment and regeneration are important factors when considering naturalness. The terms ‘primary’ and ‘secondary’ woodland were established by Peterken (1977). Primary woodland is defined as being a continuation of primaeval woodland which has never been cleared, whereas secondary woodland has established on land that was unwooded for a period of time. It is likely that very little, if any, truly primary woodland remains in Britain (Rackham, 2003; Peterken, 1993). Primary and secondary are not interchangeable with the terms ancient and recent, as ancient woodland could be of secondary development before 1600 (Peterken, 1993). Palynological studies have also demonstrated that ancient woodland may be secondary woodland (such as Sidlings Copse, Oxfordshire, established c1000 years ago (Day, 1993)), or may have experienced interruptions to continuous cover several hundred years ago (Webb and Goodenough, 2018).

Regeneration of trees through natural succession, coppicing or planting is another determining factor of naturalness, and consequently biodiversity value. Coote *et al*. (2012) concluded that native *Fraxinus excelsior* plantations supported fewer woodland specialist species than succession-regenerated *Fraxinus excelsior* woodland. Coppicing is valued as a traditional regeneration technique that enables stability and continuity, as well as sustainable production, whilst the light-shade cycle provides temporal niche differentiation for a greater diversity of woodland specialist species within a given area (Rackham, 2001).

The 'nativeness' of the dominant tree cover factors into the judgement of 'naturalness'. At the simplest level, woodland can be classified by the taxonomy of the dominant tree cover as broadleaf or coniferous. Of the 1,306,000ha of woodland in England 74% is broadleaf and 26% coniferous, (UK 49% and 41% respectively) (Forestry Commission, 2017). The most common native broadleaf canopy species listed in the National Forest Inventory of England are *Quercus* sp*., Fagus sylvatica, Fraxinus excelsior, Betula* sp., *Salix* sp*.,* and *Alnus glutinosa* (Forestry Commission, 2013). Whilst these are native nationally, their native range may not extend into every locality, and therefore could be considered less desirable than locally-native trees. *Acer pseudoplatanus* is a naturalised canopy species (Rackham, 2003). Native British canopy conifers are *Pinus sylvestris*, and occasionally *Taxus baccata* (Forestry Commission, 2017).

1.2.2 Ancient woodland

The ancient woodland paradigm is a powerful conservation tool (Rotherham, 2011; Goldberg *et al*., 2007), which has become firmly integrated into ecological research and conservation policy (Sutherland *et al*., 2006) since it was first widely promulgated by

Rackham (1976) and Peterken (1977). The term ‘ancient’ was implemented to encompass all long-established woodland, whether of primary or secondary origin (Rackham, 2003).

Ancient woodland in England is defined as an area that has been wooded continuously since at least the year 1600 (Kirby and Goldberg (2002:11). Three prominent reasons exist for the wide recognition of the ancient woodland classification. Firstly, the biodiversity value of ancient woodland is higher than recent woodland (Honnay *et al.*, 1999a; Wulf, 1997; Peterken, 1974), and it has the dubious status of containing a higher number of rare and threatened species than any other UK habitat (Corney *et al.*, 2008b; Peterken, 1983). Secondly, ancient woodland is valued as the ‘terrestrial habitat most representative of original, natural, stable conditions’ (Corney *et al.*, 2008b:10), providing a baseline against which to measure the impacts of land use and climate change. Thirdly, the extent of ancient woodland is limited: it covers just 2.3% of the UK’s land area in fragmented stands (Atkinson and Townsend, 2011:11). The definition of ancient woodland as irreplaceable (except in the very long-term) combined with threats to its geographical, ecological and historical integrity, readily justifies research.

Accurate identification and mapping of ancient woodland is necessary not only for conservation purposes, but also due to its prominent legislative standing. A specific ancient woodland inventory, initiated by the Nature Conservancy Council in the 1980s (Spencer and Kirby, 1992), is now co-ordinated by Natural England. The original survey included only woodlands of over 2ha. Ancient woodland is identified through an aggregate of historic and biotic methods including: historic maps such as tithe maps; current maps, aerial photographs and satellite images; documentary evidence such as estate records or census information; archaeological evidence such as earthworks; location; name etymology e.g.

copse; biotic evidence such as the richness of AWI species; and presence of veteran trees (Glaves *et al*., 2009; Rackham, 2003).

Updates to the initial survey have been made on a county basis (Goldberg *et al*., 2007). The current definitive map for ancient woodland in England is the MAGIC map directed by Natural England and DEFRA. Since the original ancient woodland inventory (Spencer and Kirby, 1992), further guidance for defining ancient woodland has been published (Kirby and Goldberg, 2002). Ongoing work by Rotherham (2011) seeks to establish a legally robust methodology for proving woodland is ancient, which will be used for conservation as well as informing planning applications. Ancient woodland inventories are maintained by the National Forest Inventory at the county level, and many local authorities have revised their lists using recent methodologies and have included woodland less than 2ha (Natural England, 2012).

The recently published 25 year Environment Plan, highlights a commitment to greater protection for 'irreplaceable' ancient woodland (HM Government, 2018:47). Ancient woodland is currently highly, but not absolutely, protected by planning and biodiversity legislation, which recognise its historic and ecological values. The National Planning Policy Framework (Communities and Local Government, 2012:118) states that:

> 'planning permission should be refused for development resulting in the loss or deterioration of irreplaceable habitats, including ancient woodland and the loss of aged or veteran trees found outside ancient woodland, unless the need for, and benefits of, the development in that location clearly outweigh the loss'.

The later National Planning Policy Guidance accords equal status to ASNW and ARW (Forestry Commission and Natural England, 2014). Additionally, the Government Forestry and Woodlands Policy Statement (DEFRA, 2013) places strong emphasis on ancient woodland protection, and confirms its commitment to the Keepers of Time policy statement on England's ancient and native woodland, which includes the maintenance of the existing area of ancient woodland. In addition to the above, Natural England and the Forestry Commission commit to restoration of ancient woodland (Forestry Commission and Natural England, 2014). A government white paper called for greater ancient woodland protection under the National Planning Policy Framework (House of Commons Environment, Food and Rural Affairs Committee, 2017).

Despite acceptance of the ancient woodland concept in practice and policy, the 1600 threshold has come under scrutiny. It has sound justification in being before widespread woodland planting (as promoted by the publication of 'Sylva: a discourse on the propagation of timber' (Evelyn, 1670)), but few accurate maps of this date exist (Goldberg *et al*., 2007). Interestingly, Rackham (2003:6), the proponent of the concept, suggests '1700' as being potentially suitable in Britain. Indeed, most European countries have defined 'ancient' by the earliest reliable maps, usually between 1700 and 1800 (Fig. 1.3; Hermy and Verheyen, 2007), including Scotland at 1750 (Goldberg *et al*., 2007). France uses later thresholds of 1800 to 1850 and biodiversity studies using this date have shown significant vegetation differences in comparison to younger woodland (Cateau *et al.,* 2015; Sciama *et al*., 2009). The old-growth forest concept as used in North America has no specific age threshold, although 150 years is stipulated in some definitions (Wirth *et al*., 2009b). The variation in thresholds among countries should be acknowledged when reviewing and comparing prior studies.

Table 1.3. Ancient woodland/ long-established woodland date thresholds in northern and central Europe. After Hermy and Verheyen, 2007:363), modified with some threshold dates used in more recent studies referred to in this thesis.

<table>
<tr><td colspan="3">Decreasing age of woodland
→</td></tr>
<tr><td colspan="2">Ancient</td><td>Recent</td></tr>
<tr><td>Primary</td><td colspan="2">Secondary</td></tr>
</table>

Threshold date	Country	Study
1600	GB	Peterken (1974)
1700	GB	Rackham (1980)
1750	Scotland	Goldberg *et al*. (2007)
1765-1780	Poland	Orczewska (2009)
1770-1800	Belgium	Hermy (1985)
1775	Belgium	De Keersmaeker *et al*. (2004)
1782-1785	Hungary	Kelemen *et al*. (2014)
1800-1830	France	Sciama *et al*. (2009)
1850	France	Cateau *et al.* (2015)

Recent publications challenge the accuracy of ancient woodland designation. Palynological evidence showed AWI communities present in areas that had been unwooded for at least 2700 years, and in sites that had experienced canopy interruptions (Webb and Goodenough, 2018). Stone and Williamson (2013) argue the case for ‘pseudo-ancient woodlands’: those that when surveyed in the field exhibit both the archaeological and ecological characteristics of ancient woodland, but are not present on 18th century maps. Barnes and Williamson (2015) re-evaluate the ancient woodland concept in the light of archaeological field evidence in Norfolk, concluding that many ancient woodlands are largely man-made environments, including those dating to the late medieval period (before the 1600 threshold), and others dating only to the 19th century.

Ancient woodland does not have to be as extensively old as the word ‘ancient’ implies. The terminology is perhaps misleading – ancient woodland can include primary relict woodland or virgin forest (Wirth *et al*., 2009b; Rackham, 2008), but equally woodlands only 170 years old under the threshold dates used in France or North America. Moreover, ancient woodlands are not an ‘untouched’ environment: the ancient woodland concept clearly recognises that human input, such as coppicing, is an important and accepted aspect of ancient woodland ecology (Forestry Commission, 2010; Rackham, 2008). Expectations of degree of naturalness, successional phase, and representativeness of primary woodland naturally vary considerably depending on the definition used. Arguably the ancient woodland concept should be treated as just that – a concept – rather than a rigid definition. In a comprehensive literature review of definitions of old-growth forest and related terminology, Wirth *et al*. (2009b) state that they will not attempt to define ‘old-growth forest’, instead taking the view that a single definition is not desirable (Wells *et al*., 1998).

Cateau *et al*. (2015) question the binary classification of ancient/ recent, as ancientness only encompasses the length of time an area of land has been wooded without acknowledgement of the age of the trees or successional stage of the ecosystem. A number of researchers also favour the continuity or ecological approach to identifying woodland of value (Stone and Williamson, 2013; Rotherham, 2011; Spencer, 1990). For conservation purposes, Cateau *et al*. (2015) propose a multi-category classification system (Table 1.4) encompassing age and maturity. The maturity classification also considers aspects of habitat quality and diversity. A classification focusing on ecology as well as continuity addresses the research impediment of a single age threshold: the biotic community of a wood established in 1601 may not be comparable with one established 200 or 400 years later. A more holistic classification obviates the problem of variation in thresholds among countries, and enables easier comparison of research findings.

Table 1.4. A woodland maturity classification (after Cateau *et al*. (2015).

Ancientness	Over 8000 years old Over 2000 years old Over 600 years old Over 150 years old
Maturity	A scale of maturity based on: • Average age of the stand • Number of very large trees • Amount of deadwood • Abundance of dendro-microhabitats

The concept of continuity avoids the conflation of land-use duration and habitat stability. Many woodland specialist species are dependent on continuity (Rolstad *et al*., 2002) rather than age *per se*. The term ‘ecological continuity’ was introduced by Rose (1974) and is used in the literature with reference to study of lichens rather than vascular plants where ‘ancientness’ is more commonly used. Continuity is explained by Whittet and Ellis (2013) as comprising both habitat quality and extended time for colonisation. Habitat quality includes old-growth structural elements such as microhabitats and deadwood (Coppins and Coppins, 2002). Nordén and Appelqvist (2001) examine the concept of continuity in respect of theories of natural equilibrium and disturbance ecology, with continuity representing persistence through time without major disturbance. The phrase ‘continuity forest’ is used by Røsok (1998:154) rather than ancient forest. Ohlson *et al*. (1997:221) refer to ‘continuity indicator species’, rather than ancient woodland indicator species, and promote a more careful and precise use of the term continuity to distinguish it from age alone.

Despite debate around the ancient woodland concept, there is strong justification for conducting research under the existing ancient woodland paradigm, due to: (a) its full integration into policy; and (b) agreement among scientific publications that long-established, continuously-wooded sites commonly have distinct vegetation communities and greater ecological value than recent sites, regardless of what the terminology for such sites should be (e.g. Barnes and Williamson, 2015; Wirth *et al*., 2009b; Peterken and Game, 1984). Further research aiming to identify and understand the ecology of long-established woodland will certainly assist in enabling a more robust classification.

1.3 Ancient Woodland Indicator (AWI) species

This section discusses and critiques the use of Ancient Woodland Indicator (AWI) vascular plant species in the identification of ancient woodland and examines the autecology and synecology of AWI species.

1.3.1 Defining AWI species.

Ancient Woodland Indicator (AWI) species are defined as vascular plants particularly, but not exclusively, associated with ancient woodland (Glaves *et al*., 2009). Numerous studies have evidenced a strong affinity between some woodland species and long-established, continuously wooded habitat (e.g. Kelemen *et al*., 2014; Schmidt *et al*., 2014; Hofmeister *et al*., 2013; Orczewska, 2009; Verheyen and Hermy, 2004; Graae *et al*., 2003; Honnay *et al*., 1999a; Hermy *et al*., 1999; Wulf, 1997). Such species are termed Ancient Woodland Indicators. AWI species often act as such in only parts of their geographical range, necessitating separate identification at the local or regional scale (Kirby, 2004; Rackham, 2003; Hermy *et al*., 1999). The association of such species with ancient woodland holds true outside of Europe, for example in North America (Thomas and Packham, 2007; Gerhardt and Foster, 2002).

The identification of AWI species is of two-fold importance: (a) the contribution of indicator species to ancient woodland identification for conservation and legislative purposes; and (b) the intrinsic value of some AWI species such as *Hyacinthoides non-scripta* and *Lamiastrum galeobdolon* that hold protected status (JNCC, 2017), whilst others, such as *Paris quadrifolia* have low prevalence in England in comparison to woodland generalist species (Hill *et al*., 2004). AWI species are of notable value due to their restricted range and affiliation with ancient woodlands. The relative scarcity of AWI species is considered to be due to poor dispersal and colonisation abilities (Baeten *et al*., 2009; Hermy and Verheyen, 2007; Verheyen and Hermy, 2004; Hermy *et al*., 1999).

The first widely-recognised study of plants associated with ancient woodland, particularly ASNW, was carried out by Peterken (1974). AWI species are extensively researched as a functional group of high conservation value due to being range-restricted and in some cases scarce (Rose, 1999; Peterken, 1974). AWI lists were originally intended as tools for the identification of ancient woodlands (Kirby, 2004; Rose, 1999; Peterken, 2000; Peterken, 1974) and are still valued for determining these protected habitats (Mölder *et al*., 2015; Schmidt *et al*., 2014), but are additionally used to assess the biodiversity value of woodland (Glaves *et al*., 2009).

The majority of AWI research has taken place within the fields of: landscape ecology (Bailey *et al*., 2002); dispersal and colonisation (Brunet and Von Oheimb, 1998); habitat ecology and change (Thomaes *et al*., 2014; Brunet *et al*., 2011; Van Couwenberghe *et al*., 2011); life traits (Kimberley *et al*., 2013; Verheyen *et al.*, 2003a); restoration and management (Palo *et al*., 2013; Godefroid *et al*., 2005); population ecology and genetics (Jacquemen *et al*., 2006; Jacquemen *et al*., 2003b; Brys *et al*., 2004). In the UK, extensive long-term studies of AWI species ecology have been carried out at Wytham Woods, including research on the influence of woodland size and continuity on AWI presence and prevalence (Kirby and Morecroft, 2011). Recent work by Kimberley *et al*. (2016; 2014; 2013) has contributed strongly to understanding AWI life trait ecology at the national scale.

1.3.2 Identification of AWI species

Methods used to distinguish AWI species are far from standardised temporally, spatially, or among researchers. Initially AWI species were identified via a combination of field survey and anecdotal evidence. The AWI species noted by Peterken (1974) were derived from field research with 85 primary and over 150 secondary woodlands surveyed in Lincolnshire. Although AWI species were selected systematically, seemingly no statistical technique was used to establish the degree of species' associations. Rose's (1999) lists of AWI species for the four southern regions of England were based on published literature (Horby and Rose, 1986) and expert opinion. Rose states clearly that no strictly objective methods were used in compilation of the list. Kirby's (2004) AWI lists for 13 regions were based on historical information and opinion of experienced field surveyors; these are the standard lists used in prominent studies (e.g. Kimberley *et al*., 2013).

Concern over the use of anecdotal evidence to distinguish AWI species from other woodland species (Kimberley *et al*., 2013) has been addressed through more statistically robust studies. Several studies have quantified and tested the suitability of woodland plants as indicators of woodland continuity, in order to either create new AWI lists or to test them. An early study by Wulf (1997:636) identified 21 herbaceous and woody species as 'very good indicators' of ASNW beechwood in northwestern Germany, using chi-squared and Fisher's exact test to analyse association. Honnay *et al*. (1998) used a chi-squared statistic to indicate the preference of species for ASNW in Belgium. Verheyen *et al*. (2003b) applied Pearson chi-squared test and found significant associations between six species and *Quercus*-dominated ASNW, and nine species and *Populus*-dominated ASNW in Europe and North America. Kelemen *et al*. (2014) applied Dufrene and Legendre's (1997) Indicator Species Analysis technique, a specific mathematical model for indicator identification.

In Britain, AWI suitability has been re-analysed and updated usually on a small-scale (county) basis. A revision of the ancient woodland inventory and AWI list for Somerset (Thompson *et al*., 2003a) applied an index of faithfulness to ancient woodland (number of sites in which the species occurs/total number of occurrences of the species), as well as chi-squared to test if the distribution of each species was independent of woodland type. However, the species tested were selected from the original list of 100 species produced by qualitative means by Hornby and Rose (1986). It is possible that species not included on the original list may also have been worthy of investigation, or significant changes in woodland ecology over the last 30 years may have altered the suitability of some species as indicators.

By contrast, some studies have identified AWI species over a much larger area by synthesising published findings. Hermy *et al*. (1999) identified 132 AWI species from 22 studies of deciduous and coniferous woodlands in north-western and central Europe (Table 1.5). Wulf (1997) investigated the association of AWI species found in north-western Germany with ancient woodlands in other northern European countries based on the literature. The results showed five species strongly related to ancient woodland across northern Europe (Table 1.5). Schmidt *et al.* (2014) devised a supra-regional list of AWI species for north-western Germany based on survey data collected between 1961 and 2012. However, as acknowledged by Hermy *et al*. (1999), literature-informed lists depend on studies with varying methodologies, study size and a combination of anecdotal and statistical studies.

Table 1.5 Findings of supra-regional AWI studies.

AWI species cited in 9 or more of 22 studies over north-western and central Europe (Hermy *et al*., 1999)	Five species showing strong relation to ancient woodland across northern Europe (Wulf, 1997)
Anemone nemorosa	*Carex sylvatica*
Convallaria majalis	*Paris quadrifolia*
Lamiastrum galeobdolon	*Chryosplenium alternifolium*
Luzula pilosa	*Melica uniflora*
Melica uniflora	*Lamiastrum galeobdolon*
Mercurialis perennis	
Oxalis acetosella	
Paris quadrifolia	
Polygonatum multiflorum	
Sanicula europaea	

The association of species with ancient woodland could more appropriately be regarded as a continuum between older-recent woodlands. Peterken and Game (1984) state that the bias of some species for ancient or recent woods varied even within their Lincolnshire study area. AWI species have occasionally been shown to be associated with recent woodland. Kelemen *et al*. (2014) demonstrated a significant link between the presence of both *O. acetosella* and *G. odoratum* and recent woodland in Hungary. Kirby and Morecroft (2011) found that *Adoxa moschatellina*, *Potentilla sterilis* and *Tamus communis* occurred exclusively in recent woodland plots in Wytham Woods, Oxfordshire.

A difficulty arises in statistical testing for rare species association with ancient woodland, usually ASNW. Many studies test for habitat association when species occur with frequency over a given threshold, for example: Wulf (1997) tested species occurring in >30 localities; Palo *et al*. (2013) excluded those with <3 observations; Graae *et al*. (2003) included only species with >10 observations. Some rare species are considered to have affinity to ancient woodland (Rose, 1999) but their infrequent occurrence makes it difficult to statistically test them, and their use as indicators is limited due to the low probability of sampling them during woodland surveys. However, Sciama *et al*. (2009) applied Fisher's exact test to assess association of infrequently occurring species (n ≤ 5) with woodland age. In the case of rare species, expert observation could be supplementary to statistical testing of very infrequently occurring plants, as used for the Rare Ancient Woodland Vascular Plant species list (Rose, 1999).

Testing for recent woodland indicators or reverse ancient woodland indicators is an alternative way of demonstrating the distinctiveness of ancient woodland (Schmidt *et al*., 2014; Kirby and Morecroft, 2011; Sciama *et al.,* 2009; Graae *et al*., 2003). Bossuyt and Hermy (2000) not only tested for difference in abundance of AWI species, but also for general woodland species and recent woodland species across four age categories. Results showed higher abundance of recent woodland species in younger in comparison to older woodlands and vice-versa for ancient woodland species. General woodland species had significantly higher abundance in ASNW compared to all younger woodland categories. Studies by Sciama *et al*. (2009) and Verheyen *et al.* (2003a) also developed lists of species associated with recent woodland in order to contrast with those affiliated to ASNW. This reverse approach to distinguishing ancient and recent woodland flora raises a question about colonisation limitation. AWI species colonisation in recent woodland is thought to be reduced by competition with ruderal species, thus creating distinct communities. However, seemingly few studies have considered the reverse process of recent woodland species colonisation in ancient woodland (Honnay *et al*., 2002b) and its contribution to community distinctiveness between ancient and recent woodland.

1.3.3 Utilisation of AWI species lists

It is important to emphasise the scope and limitations of the AWI list approach to ancient woodland identification. Firstly, it is only one of a number of methods used to identify woodland continuity (Rose, 1999). Primary field archaeology, secondary documentary evidence and historic cartographic resources are also employed to identify and map these woodlands (Rackham, 2003). Secondly, the AWI list approach is community-based – the presence of a single AWI species does not denote ancient woodland (Rose, 1999).

The AWI richness threshold required to reliably contribute to ancient woodland identification has been tested by very few studies. Honnay *et al*. (1998) found that a threshold of 25 AWI species, from a list of 66 generated by Fisher's exact test, indicated a much higher probability of a woodland being ancient rather than recent. However, the woodland sizes in the study varied substantially between 0.5ha up to 5216ha, and the authors emphasise that threshold numbers are scale-dependent. At the lowest end of range used, Kühn (2000) suggests a threshold of just two indicators. Glaves *et al*. (2009:15) researched how local authorities and organisations in the UK use AWI lists to assess woodland quality. Several quoted using thresholds of 8-12 for 'very good' quality ancient woodlands, 4-8 for 'good', and less than 4 was considered 'poor'. For woodlands under 2ha, five AWI species was considered as the 'good' quality threshold. However, these thresholds appear to have no evidenced research basis.

Glaves *et al*. (2009) raised a number of inconsistencies in the way AWI lists are used. Some organisations use the lists to identify ancient woodland, others to assess woodland biodiversity value. Respondents to the Glaves *et al*. (2009) survey note valid criticisms of the current use of AWI lists, particularly in terms of recognising underlying environmental variation. One respondent questioned the use of a single AWI list across different NVC communities, as NVC communities that are less associated with AWI species may be overlooked for conservation despite ancient status using the threshold approach. Another applied a weighting to the AWI scores to counter-balance the influence of variation in geology, although no further details were given on the methodology used to establish the weightings.

Although no one single species is known to be entirely restricted to ancient woodland (Schmidt *et al*. 2014; Glaves *et al.*, 2009; Wulf, 2003; Rose, 1999), it may be that individual species could be weighted as more effective indicators (Webb and Goodenough, 2018). Rackham (2003: 54-55) ranked the affinity of species with ancient woodland in the East of England, classifying each as 'strict, very strong, strong, moderate, weak, doubtful and none'. The best indicators are those with the strongest correlation with ancient woodland (Sciama *et al.*, 2009) and/ or those identified as indicators in more than one study (Wulf, 1997) (Table 1.5). In the UK, *Paris quadrifolia* has been noted for its near exclusivity to ancient woodland (Kirby and Morecroft, 2011; Spencer, 1990). Such particularly strong or recurrent indicators could be considered of higher ecological and indicator value than others. Additionally, the recurrence of species in studies over a long period of time, adds weight to its value as an indicator.

Scales covered by AWI lists vary from county up to supra-regional (Schmidt *et al*., 2014) Regional variation in the species associated with ancient woodland is well-recognised, with Kirby (2004) basing his regional lists on county data. However, there are gaps in coverage, for example Gloucestershire is not included in the most up-to-date list. Arguably, political county boundaries are not the most suitable basis for assessing preference of species for ancient woodlands: a combination of abiotic characteristics as used by the Forestry Commission's Ecological Site Classification (Pyatt *et al*., 2001) would be more suitable and worthy of future research. The supra-regional study carried out by Schmidt *et al*. (2014) aimed to establish a list of ancient woodland indicators that could be employed over north-western Germany. This scale of study could also be employed to understand the wider biogeography of AWI species, to assess their response to changing land use and climate. However, it is recognised that AWI species vary regionally according to climate and geology (Rose, 1999) and in support of this, Hermy *et al*. (1999) recommend the use of regional lists rather than any other scale.

As recognised by Glaves *et al.* (2009) AWI species are only one tool amongst many others used in the identification of ancient woodland. It is important to recognise that they are used as *indicators* only. The literature reveals that methods used to create and use AWI lists are highly variable, but as a widely recognised grouping of species their research is well-justified.

1.3.4 An ecological approach

Spencer (1990) presents an important critique of the ancient woodland indicator approach to woodland age identification. A strong case is presented for refocusing research onto the biology of ancient woodland plants and the ecological conditions of older woods rather than their management history. Spencer (1990:91) cautions against the 'circular logic' that ancient woodland indicators must mean ancient woodland, therefore the plants found in ancient woodland must be ancient woodland indicators. It is important that the AWI concept is critiqued using ecological evidence. The unquestioning use of the AWI approach, may expose unidentified ancient woodlands to the threats against which they should be protected (Department for Communities and Local Government, 2012; Corney *et al.*, 2008b). This risk is enhanced by reliance placed on the ancient woodland concept in conservation (Goldberg *et al.*, 2007). A focus on the species' biology and environmental requirements of AWI species could recognise woodlands of the highest ecological value rather than age *per se* (Spencer, 1990).

In agreement with Spencer (1990), Wright and Rotherham (2011) note that AWI species lists are more indicative of woodland conditions, than age. The lists contain early growing shade-evaders and later growing shade-tolerant species, but disregard light-tolerant species that may be found within meso-habitats, such as glades, streams and rock faces. They argue for AWI lists that better recognise the hetereogeneity of woodland habitats. Additionally, Wright and Rotherham (2011) observe that there is no consideration of AWI species' abundance in the current methodology for identifying ancient woodlands, which is a pertinent consideration.

It is possible that changes in canopy species and abiotic characteristics may have altered understorey communities since the original lists were developed. A wide-scale study of ancient woodland plants in northern and central Germany by Hermy *et al*. (1999) used pre-existing data sets from as early as 1952 up until 1994. Similarly, a recent AWI identification methodology developed by Schmidt *et al*. (2014) was based on data sets from between 1961 and 2012.

To evidence the need for updated lists, several important woodland species (including AWI species) experienced substantial shifts in abundance in Wytham Woods experimental plots between 1974-1999 (Kirby and Morecroft, 2011). Indicator species lists may require more frequent updating in response to concern over northwards range shift of species in response to climate change (Wesche *et al*., 2006; Skov and Svenning, 2004; Honnay *et al*., 2002a) not only due to alteration in the distribution of AWI species, but also of supporting species such as pollinators or mycorrhizae. Several important ecological changes have taken place in UK woodlands since the inception of AWI lists: pests and pathogens such as *Hymenoscyphus fraxineus (Chalara fraxinea)* and potentially *Agrilus planipennis* (Thomas, 2016); AWI competition with invasive species (e.g. *Hyacinthoides hispanica* and *L. galaeobdolon* subsp. *argentatum* (Rackham, 2008; Dines, 2005)); deer browsing damage (Kirby *et al*., 2016); coppice abandonment and restoration (Kirby *et al*., 2017; Kopecký *et al*., 2013: Vild *et al*., 2013); and biotic homogenisation (Naaf and Wulf, 2010; Keith *et al*., 2009).

1.4 Community ecology and life traits of ancient woodland vegetation

This section considers the community scale differences in the vegetation of ancient and recent woodland, in particular in terms of richness, abundance, and diversity.

1.4.1 Richness, abundance, and diversity

Floristic comparison between ASNW and recent woodlands is a well-established research area. Important aspects researched include: species richness (Kirby and Morecroft, 2011; Sciama *et al*., 2009; Graae, 2000; Hermy, 1985; Peterken and Game, 1984); woodland structure; community composition (Verheyen *et al*., 2003b; Dzwonko, 1993); dispersal (Dzwonko, 2001a); life traits (Sciama *et al*., 2009); affinity of species for ancient woodland (Schmidt *et al*., 2014; Graae, 2003; Hermy *et al*., 1999; Honnay *et al*., 1998; Peterken, 1974).

The vegetation of ASNW woodland is generally considered distinct from that of recent woodland, in terms of diversity (Kelemen *et al*., 2014; Sciama *et al*., 2009), species presence and abundance (Kelemen *et al*., 2014; Hofmeister *et al*., 2013; Orczewska, 2009), community composition (Berges *et al*., 2017; Hermy *et al*., 1999), and life traits (Verheyen *et al*., 2003a; Hermy *et al*., 1999).

However, until recently, very few studies had compared the vegetation of ASNW and ARW (Kirby, 1988). The potential of ARW for restoration has led to an increasing focus of ASNW-ARW vegetation comparison research in the UK (Berges *et al*., 2017; Atkinson *et al*., 2015; Brown *et al*., 2015), but publications are few compared to the longer-established research on ASNW versus recent woodland. Therefore, further research on the ecology of ARW is justified to address knowledge gaps.

A small number of studies have quantified the total plant species richness of ASNW in comparison to recently established woodlands. When plant species richness of canopy, shrub and herb layers is totalled and compared, results are not consistent in terms of any significant difference between ASNW and recent woodlands. An important Polish study in this field by Dzwonko (2001b) evidenced no significant difference in the mean richness of all species in ASNW and recent woodlands plots but found that the total number of species across all plots was significantly higher in ASNW. By contrast, in another Polish study, Sciama *et al*. (2009) found no significant difference in total species richness between ASNW and recent study sites.

The majority of studies exclude trees from the richness count due to their potential placement or management by humans. The significant findings of studies that investigate one or two woodland strata seem to corroborate the arguments of both Peterken (1993) and Rackham (2003) who argue that the composition of the canopy and understorey have little correlation and should be treated as separate communities. When Sciama *et al*. (2009) separately analysed shrub and herb layer species between ASNW and recent woodland, they found significant ($p<0.05$) differences in herb richness and shrub richness, with ancient having higher mean herb richness (18.7 versus 15.0), and recent higher shrub richness (9.4 versus 7.7). Supporting the findings of Sciama *et al*. (2009), Kelemen *et al*. (2014) found higher total herb richness in ASNW, compared to long-established recent forest. Likewise, excluding trees, Dumortier *et al*. (2002) identified a significantly higher species richness in older than younger woodland ($p<0.001$) but notes that degree of isolation may also be a contributory factor.

Basic richness counts are a simplistic measure of woodland biodiversity value. Instead, both Hermy *et al*. (1999) and Verheyen (2003a) promote the use of both qualitative (identity of species) and quantitative (number of species) when testing for difference in plant species composition between ASNW and recent woodland.

When a qualitative measure, such as 'woodland species' is applied to richness counts, study results consistently show that ASNW is richer than recent woodland. Brunet *et al*. (2011), Jacquemen *et al*. (2001), and Peterken and Game (1984) all reported higher richness of woodland species in ASNW compared to recent woodland. Dzwonko (2001b) compared both the general herb layer richness and woodland specialist richness, with results showing a significantly higher number of woodland species in ASNW than recent woodland, but no significant difference in general herb layer species richness. These results suggest that woodland specialists comprise a greater proportion of an otherwise similar species richness.

A further qualitative distinction is the richness of plants associated with ancient woodland specifically. Several studies show higher AWI richness in ASNW (Kelemen *et al*., 2014; Hofmeister *et al*., 2013; Orczewska, 2009), a pattern which holds true amongst countries. As another qualitative measure, Hofmeister *et al*. (2013) found a significantly higher number of red-list species in ASNW, which tallies with the concept of ancient woodland acting as refugia for rare species (Rose, 1999).

A small number of studies have considered measures of species diversity other than richness, such as richness + evenness (Vellend *et al*., 2007) and a comparison of percentage cover and richness (Orczewska, 2009). Vellend *et al*. (2007) found significantly higher beta diversity in ASNW compared to recent woodlands in 7/11 regions (6 in Europe and 1 in N.

America), and Orczewska (2009) found both significantly higher richness and abundance in ASNW compared to recent woodland in three different canopy types.

Where studies have involved a sequence of woodland ages instead of binary ancient/ recent classification, (ancient) woodland species richness (Brunet *et al*., 2011; Jacquemen *et al*., 2001) and abundance (Brunet *et al*., 2011; Bossuyt and Hermy, 2000) are shown to be significantly higher in older than younger woodlands. The studies do not necessarily show a perfect positive correlation between age and number of (ancient) woodland species, but the trend is clear, with significant differences between individual age categories. Brunet *et al.* (2011) tested both richness and abundance across age categories and by proximity to core older woodland. Richness and abundance measures produced conflicting results, demonstrating the need for both richness and abundance data collection to enhance understanding of ancient woodland species ecology: significantly higher abundance of woodland specialists was found across woodlands (13-82 years) in both isolated and proximate locations relative to old core (198-316 years) woodland, but richness was significantly higher only in recent woodlands in close proximity to old core woodland.

1.4.2 Life traits of ancient woodland plants

It has been established that plant species associated with closed canopy woodland have a distinct trait profile (Whigham, 2004; Bierzychudek, 1982) and that ASNW generally has a significantly higher richness of these species in comparison to recent woodlands (Kelemen *et al*., 2014; Hofmeister *et al*., 2013; Dzwonko, 2001b) AWI species have yet further distinctiveness of traits (Kimberley *et al*., 2013; Hermy *et al*., 1999). Hermy *et al*. (1999)

propose that such species be considered as a 'guild' due to their distinct ecological profile, summarised as: shade tolerant; avoiding very dry or wet soil; stress-tolerant; preferring weakly acid to neutral soils and intermediate soil nitrogen; regenerative form of geophytes and hemicryptophytes; summergreen; often myrmechorous; relatively large seeds; no persistent seed bank; specific germination requirements; clonal growth predominant; and limited fecundity.

The long lifespan of woodland herbs adapted to continuous and undisturbed conditions may partly account for their low ability to colonise new habitats. Honnay *et al.* (2002a) and Hermy *et al*. (1999) note that such species are less able to persist in high disturbance environments. Endels *et al*. (2007) established that plants with greater longevity have later age of first flowering, lower seed bank viability and shorter flowering periods. Additionally, age at first flowering was found to correlate positively with seed weight. A notable species exhibiting these traits is *P. quadrifolia,* a clonal, rhizomatous herb, that is noted to be one of the plants most closely confined to ASNW (Kirby and Morecroft, 2011; Hermy et al., 1999; Wulf, 1997; Spencer, 1990). *P. quadrifolia* can have a life span of hundreds of years (Kranczoch, 1997) and is slow to develop, with above ground growth only in the second year after germination and takes several decades to reach regenerative status (Jacquemen *et al.*, 2006; 2005). Genets of *A. nemorosa* have been found to be greater than 200 years old (Honnay *et al*., 2005). These species seem to illustrate links between longevity, reproductive limitations, and adherence to ancient woodland.

Low fecundity and reproductive effort of species associated with ancient woodland are considered significant factors in explaining their adherence to this habitat and slow colonisation of other woodland habitats (Kimberley *et al*., 2013; Endels *et al*., 2007; Kolb and Diekmann, 2005; Verheyen *et al*., 2003b; Graae and Sunde, 2000; Hermy *et al*., 1999).

In the seminal work by Bierzychudek (1982) woodland herbs are noted to produce heavier and fewer seeds per year compared to light-demanding species. In a comprehensive study of woodland plant species traits, Endels *et al*. (2007) distinguish spring-flowering species as having larger seeds with unassisted dispersal, which partly explains their reduced ability to colonise new habitats. In turn, this result is supported by findings that AWI species comprise a relatively high number of spring-flowering species (Brunet *et al*., 2011; Hermy *et al*., 1999) Woodland plants with heavier seeds and short-range dispersal mechanisms have been shown to have lower occupancy of woodland patches (Jacquemen *et al*., 2003a).

Short-range seed dispersal mechanisms contribute to the restricted range of AWI species (Hermy *et al*., 1999). Studies of dispersal mechanisms of specialist woodland species have identified a high proportion of barochorous (gravity dispersed) (Whigham, 2004; Bierzychudek, 1982) and myrmechorous species (ant dispersed) (Kelemen *et al*., 2014; Hermy *et al*., 1999) compared to other woodland plants. Both of these are short range dispersal mechanisms. By contrast Honnay *et al*. (1998) found no difference in dispersal mechanisms between ancient and other woodland species. Likewise, Kimberley *et al*. (2013) found that dispersal vectors were not effective in distinguishing AWI and non-AWI species.

The clonal reproduction of many ancient woodland herbs is considered an additional factor in accounting for slow dispersal and lack of new habitat colonisation (Hermy *et al*., 1999). Research by Verheyen *et al*. (2003a) established that annual and biennial woodland herbs display limited vegetative spread, and perennials with light seeds had intermediate vegetative spread, whereas perennials with heavy seeds demonstrated exhibited strong vegetative spread. Ancient woodland species are known to comprise more long-lived and seed-heavy species than recent woodland species (Bossuyt and Hermy, 2000; Hermy *et al.*, 1999). Again using *P. quadrifolia* as an example, Jacquemen *et al*. (2005) note that its rhizomes grow slowly at 2cm - >8cm per year. However, other studies have shown that the migration rate and genetic variability of *P. quadrifolia* points to greater dispersal by seed than once thought (Jogaite *et al*., 2005; Brunet and Von Oheimb, 1998). To account for this apparent conflict, Thomas and Packham (2007) hypothesise that occasional long-distance dispersal events may explain faster or further dispersal of predominantly clonal woodland herbs.

Colonisation rates of woodland species from ancient to recent woodland have been researched by Brunet and Von Oheimb (1998) in Sweden. The study concluded that c. 0.3-0.5 m year^{-1} is the rate at which herb layer vegetation, comparable to that of adjacent ASNW, disperses to and establishes in recent woodland (age 30-74 years). Myrmechorous species were found to have the lowest migration rates when calculated from distance to highest cover plot in recent woodland (corroborating Hermy *et al*. (1999)), but no difference was found when calculated from distance to furthest individual.

The relative contribution of dispersal and colonisation to the slow recruitment of AWI species in new woodlands has been questioned and tested. It is possible that even once dispersed, seedlings cannot easily establish a new population in a potentially sub-optimal habitat. The relative importance of seed dispersal limitation is thought to be higher than that of recruitment limitation in accounting for the restricted range of AWI species (Baeten *et al*. 2009; Hermy and Verheyen, 2007; Verheyen and Hermy, 2004). However, the processes of dispersal and recruitment are interdependent: Baeten *et al*. (2009) concluded that for AWI species to establish a significant population, a higher seed density would be required to compensate for poor recruitment at succeeding life stages, but woodland herbs tend to produce only low seed densities (Bierzychudek, 1982). Conditions in recent woodland have been shown to be suitable for the establishment of (ancient) woodland species (Hermy and Verheyen, 2007; Butaye *et al.*, 2001), which again indicates that dispersal rather than recruitment has the greatest influence.

Assessing AWI status by life traits provides an objective basis for the identification of AWI species, as opposed to techniques based on presence in ASNW (Herault and Honnay, 2005; Spencer, 1990). This method enables researchers to understand the relative influence of species life traits and environmental influences in determining species distribution (Kelemen *et al.*, 2014; Kimberley *et al.*, 2013).

1.4.3 Life traits community composition

Community comparisons of ASNW and recent woodland via Grime’s (1977) life strategies for herbaceous plants (Fig. 1.3) show consistent results. Stress-tolerant (S) strategists are more closely associated with ancient and older woodland (Sciama *et al.*, 2009; Bossuyt and Hermy, 2000; Hermy *et al.*, 1999). Competitive (C) strategists are generally associated with recent woodland (Sciama *et al.*, 2009; Hermy *et al.*, 1999). Very few AWI species are C-strategists (7.4%, compared to 39% S-strategists) (Hermy *et al.*, 1999), which has implications for their ability to colonise new habitats. De Keersmaeker *et al.* (2004) proved a negative correlation between cover of fast colonisers and woodland age, in addition to an increase in AWI species in both number and cover with age. AWI species are considered to be slow colonisers (Hermy *et al.*, 1999).

Primary strategies

C = competitive

S = stress tolerant

R = ruderal

Secondary strategies

C-R

S-R

C-S

C-S-R

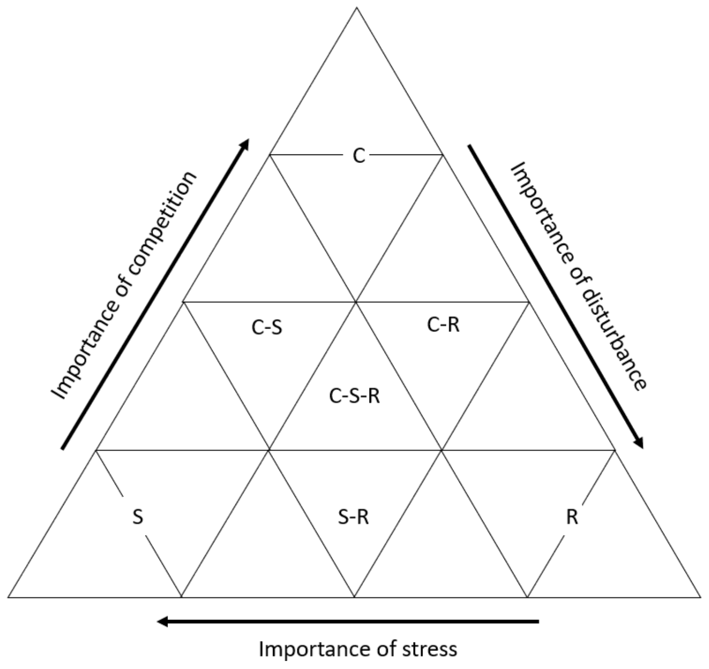

Figure 1.3 Grime’s life history strategies for herbaceous plants (after Krebs, 2008; Grime 2006; Grime, 1977)

Competitive exclusion is thought to inhibit the colonisation of ancient woodland species in recent woodlands. The proportion of competitive strategy plant species in recent woodlands is generally proved to be higher than in ancient woodlands (Sciama *et al*., 2009; Bossuyt and Hermy, 2000; Hermy *et al*., 1999). Herb layer species with vigorous growth, such as *Urtica dioica*, *Galium aparine* and *Poa trivialis* contributed to the reduced number and cover of AWI species in recent woodland (Orczewska, 2009). Hermy *et al*. (1993) found a significant negative correlation between *U. dioica* cover and AWI diversity, and Hermy *et al*. (1999) noted the competitive ability of *U. dioica* and *Pteridium aquilinum* in preventing the growth and development of woodland species. However, De Keersmaeker *et al*. (2004) concluded that there was no evidence for the exclusion of AWI species due to fast-colonisers, noting instead a significant negative correlation between the number of AWI species and total cover of light demanding species. Competition is not thought to act alone in restricting the establishment of AWI species in recent woodlands: competition, dispersal, and recruitment limitation have an aggregate effect as biological filters (Sciama *et al*., 2009).

Community composition by regenerative strategy can show distinction between ASNW and recent woodlands. Several studies have compared community composition according to Raunkiaer's (1937) life forms (Table 1.6), with a trend of geophyte association with ancient woodland in most studies (Kelemen *et al*., 2014; Verheyen *et al*. 2003b; Hermy *et al*., 1999; Wulf, 1997). Verheyen *et al*., (2003b) demonstrated an increase in the number and abundance of geophytes in a sequence of woodland age categories. A significant difference in community composition by life form was detected between ASNW and recent woodland by Hermy *et al*. (1999). Wulf (1997) identified three geophytes significantly associated with coppiced ancient woodland (*Anemone ranunculoides, Circaea lutetiana* and *P. quadrifolia*). In contrast to the above studies, Sciama *et al*. (2009) found no significant difference in herb layer regenerative strategy composition. Geophyte regenerative strategy may also account for slow colonisation of new habitats due to slower spread by vegetative structures such as rhizomes, tubers, bulbs and corms, rather than seed dispersal (Verheyen *et al.*, 2003a; Hermy *et al*., 1999).

Table 1.6 Raunkiaer's (1937) life forms.

Phanerophytes	Dormant buds on branches that project freely into the air eg. trees and shrubs
Megaphanerophytes	>30m
Mesophanerophytes	8-30m
Microphanerophytes	2-8m
Nanonpherophytes	<2m
Chamaephytes	Buds or shoot-apices perennate on the ground or just above it up to 25cm
Hemicryptophytes	Dormant buds just beneath the soil surface, aerial parts are herbaceous. Many native woodland and hedgerow species and rosette species.
Cryptophytes	Dormant parts subterranean
Geophytes	Bulbs, rhizomes, tubers and root buds subterranean.
Helophytes	Semi-aquatic dormant buds bottom of water or subterranean
Hydrophytes	Aquatic perennating rhizomes or winterbuds bottom of water.
Therophytes	Live through the unfavourable season as seeds – annual plants

1.5 Biotic, abiotic, and biogeographical factors

This section aims to explain the dynamics of ancient woodland vegetation within woodlands of different continuity histories, edaphic and climatic conditions, and spatial configurations, including isolation, patch size and edge effects.

1.5.1 Canopy characteristics

The phytosociological approach to woodland classification, such as the National Vegetation Classification, has raised the question of degree of canopy species influence on understorey vegetation. Several studies omit a survey of tree species due to the likelihood of their being planted, and/ or considering that the canopy layer has little impact on the understorey (Vallet *et al*., 2010; Honnay *et al*., 2002b; Graae, 2000). Rackham (2003) and Peterken (1993) criticise the phytosociological approach, emphasising a weak or inconsistent correlation between the canopy and understorey layers, not least that they respond to their environment at very different scales.

In support of Rackham's (2003) argument, research shows that canopy composition has no consistent influence on presence of AWI species amongs studies conducted. The presence of AWI species in both ASNW and recent woodlands is not easily predicted by the canopy species (Orczewska, 2009). Thomaes *et al*. (2012) reported no effect of tree species on AWI species in *Quercus* and *Populus* post-agricultural plantations. When diversity is considered, two studies conducted in the ancient deciduous forest Hainich National Park, Germany, evidenced greater herb layer diversity under greater canopy diversity (Vockenhuber *et al*., 2011; Mölder *et al.*, 2008). This variation in results may be accounted for by differing localities and methods.

Acidifying tree species in the canopy layer has been shown to indirectly influence herb layer species composition through soil pH. A field reintroduction experiment by Thomaes *et al*. (2014) showed that acid soil stands with acidifying trees species cover reduced the survival and abundance of *Primula elatior*, *O. acetosella, A. nemorosa*, *M. perennis*, and *P. aquilinum* compared to moderately acid stands. However, *H. non-scripta* was not affected.

Canopy percentage closure has a degree of direct influence on abundance of herb layer woodland species. A significant negative correlation was proved between total canopy closure (tree and shrub) and herb layer specialist species abundance in recent broadleaved woodlands in Sweden (Brunet *et al*., 2011). The same correlation was found for generalist species richness and total species richness. In contrast, Vockenhuber *et al*. (2011) concluded that the proportion of true woodland species increased with increasing canopy closure, but tallies with Brunet *et al*. (2011) in that general species richness and cover declined with increasing canopy closure. Kirby (1988) compared species richness under several canopy types in ARW and ASNW with a distinct trend towards higher herb layer species richness in open canopies than closed. However, the relative range of canopy closure varies among studies meaning they are not easily comparable.

1.5.2 Soil and environmental variables

Soil properties of ancient and recent woodland have been compared in order determine their contribution to limited AWI species colonisation in recent woodland. Several studies have concluded that soil properties, when considered in aggregate, provide a limited contribution to differences in ASNW versus recent woodland vegetation relative to other explanatory variables (Sciama *et al*., 2009; Herault and Hermy, 2005; Honnay *et al*., 1999b; Graae *et al*. 2004; Graae *et al*., 2003; Peterken and Game, 1984). However, the impact of individual soil properties on plant species have shown some significant results (Thomaes *et al*., 2014, 2013; Verstraeten *et al*., 2014; Orczewska, 2009; De Keersmaeker *et al*., 2004; Jacquemen *et al*., 2003a).

Weakly acid to neutral soils are preferred by AWI species, based on a synthesis of 22 European studies by Hermy *et al*. (1999). De Keersmaeker *et al*. (2004) found a significant positive correlation between pH (range KCl 3.9 – 5.0) and species richness, and significant positive correlations between pH and AWI species richness, and AWI species abundance. In terms of individual species, acid soils were linked to lower survival rates of *M. perennis*, *L. galeobdolon* and *P. elatior* (Thomaes *et al*., 2013) and lower regenerative performance in *G. odoratum* and *P. elatior* (Verstraeten *et al*., 2014). Woodland plant species, whether assessed individually or grouped by life- or functional traits, show a decided preference for soil pH at the higher end of the range present. However, studies show little or no difference in pH between ASNW and recent woodland soils, despite the preferences of ancient woodland species (Sciama *et al*., 2009; De Keersmaeker *et al*., 2004; Graae *et al*., 2003; Graae, 2000). In a scarce study considering ARW, Brunet *et al.* (2011) found significantly higher pH in post-arable woodland compared to ARW.

Soil phosphorous (P) has been found to significantly influence the colonisation of AWI species in recent woodland, but more likely due to indirect than direct effects. Honnay *et al*. (1999) proved a significant negative correlation between soil P and number of AWI species. High P levels have been shown to hinder establishments of AWI species in recent woodland due competitive exclusion by ruderal phosphateophiles such as *U. dioica* (Hermy *et al*., 1993). Studies have shown soil P to be higher in recent, particularly post-agricultural, woodlands than in ASNW (De Keersmaeker *et al*., 2004; Wilson *et al*., 1997). However, other studies show inconsistent or no significant differences in available P between ASNW and recent woodlands (Sciama *et al*., 2009; Orczewska, 2009; Graae *et al*., 2003). The differences in study outcomes could be explained by a number of factors, not least the quantity of prior P fertiliser input under different agricultural systems, but also other physical soil or geological characteristics. It is thought that there is a 30-40 year lag time between woodland establishment and normalisation of P levels (Honnay *et al*., 1999b).

Results of several studies suggest there is no consistent relationship between total nitrogen (N) and age or former land use (Sciama *et al*., 2009; Orczewska, 2009; De Keersmaeker *et al*., 2004). A small number of studies have tested for association between N and (ancient) woodland species /AWI diversity metrics. A study by Honnay *et al*. (1999) found no significant correlation between total N and number of ancient woodland species. Phosphate rather than nitrate has been found to more significantly affect the establishment of common and dominant woodland species, therefore causing competitive exclusion for ancient woodland species (Pigott, 1971).

Soil carbon (C) has been shown to be higher in ASNW compared to recent woodland, but too few studies exist to fully inform any trend. Orczewska (2009) evidenced a significantly higher percentage of organic carbon in ASNW compared to recent woodland under three canopy types. De Keersmaeker *et al*. (2004) reported a positive significant correlation between total C and a sequence of woodland ages. However, Graae *et al*. (2003) found no difference in organic matter content between ASNW and recent woodland. Seemingly no studies have explicitly analysed the effect of C on AWI species diversity. Overall, C in ancient woodland soils is not a widely studied area and is worthy of further investigation.

AWI and specialist woodland species are known to be shade-tolerant and adapted to shady environments (e.g. Whigham, 2004; Hermy *et al*., 1999; Bierzychudek, 1982). Sciama *et al*. (2009) found AWI species to have a higher specific leaf area than recent woodland species yet the same biomass, showing adaptation to shade. Richness and cover of woodland species have been found to be higher in plots with relative insolation < 8% (De Keersmaeker *et al*., 2004). In agreement with this finding, the more specialist ancient woodland species are noted to prefer < 5% insolation (Hermy *et al*., 1999). However, indirect influence of light via competitive exclusion by light-demanding species appears to partly account for low presence of AWI species in recent woodlands: De Keersmaeker *et al*. (2004) evidenced a significant negative correlation between number of AWI species and percentage cover of light demanding species.

1.5.3 Biogeographical factors

Many studies have considered the response of ancient woodland flora to biogeographical variables (Kimberley *et al*., 2016, 2014; Hofmeister *et al*., 2013; Brunet *et al*., 2011; Vockenhuber *et al*., 2011; Corney *et al*., 2004; Petit *et al*., 2004; Bailey *et al*., 2002; Graae, 2000; Peterken and Game, 1984). Biogeographical studies have researched how woodland species and their populations respond to processes such as fragmentation and connectivity, species-area-distance dynamics, edge effects and matrix impacts (Humphrey *et al*., 2013).

Woodland fragmentation is a process whereby ‘a large expanse of habitat is transformed into a number of smaller patches of smaller total area, isolated from each other by a matrix of habitats unlike the original’ (Wilcove *et al*., 1986:237). Four distinct effects of fragmentation on habitat configuration have been identified by Fahrig (2003): (a) reduction in habitat amount; (b) increase in number of habitat patches; (c) decrease in habitat patch size; and (d) increase in patch isolation. Jaeger (2000) theorises six stages in the process of fragmentation within a landscape (Fig 1.4) and uses the term ‘fragmentation’ to encompass the whole sequence. Fragmentation is a multi-faceted issue: it is not only a quantitative spatial problem, but also an issue of habitat quality reduction, which impacts on community composition (Bailey, 2007).

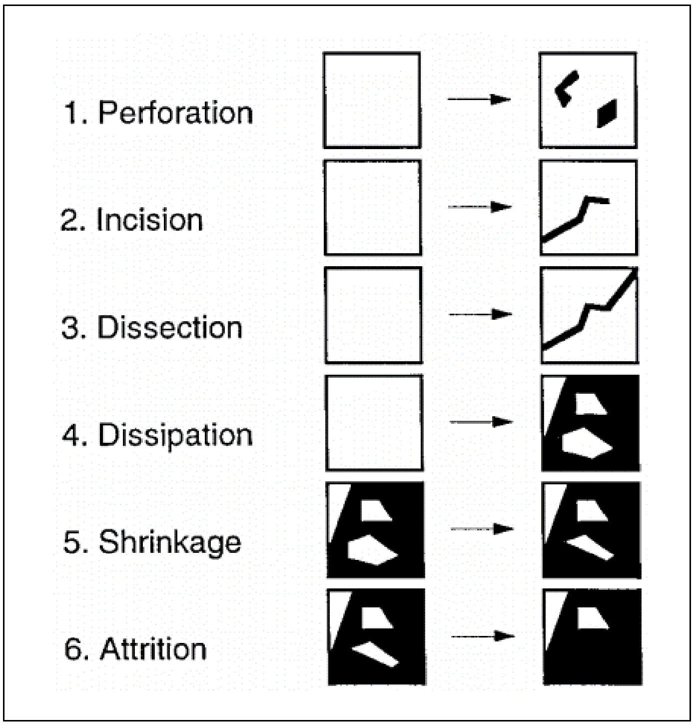

Figure 1.4 Jaeger (2000: 116). Phases of the fragmentation process, distinguished according to geometric characteristics (modified and extended after Forman 1995: 407).

The fragmented distribution of ancient woodland is considered a significant threat to the quantity, quality and distribution of its flora (Corney *et al.*, 2008b, 2004; Rackham, 2008; Harris, 1984). In the earlier part of the 20th century, approximately 38% of ancient woodland was felled for conifer plantation and 7% for conversion to agriculture (Atkinson and Townsend, 2011). Additionally, 48% of ancient woodlands are smaller than 5ha in size (Corney *et al.*, 2008b). Given that only patches over 2ha were mapped in the Ancient Woodland Inventory, this number could be higher (Thomas *et al.*, 1997; Spencer and Kirby, 1992) (Fig. 1.5).

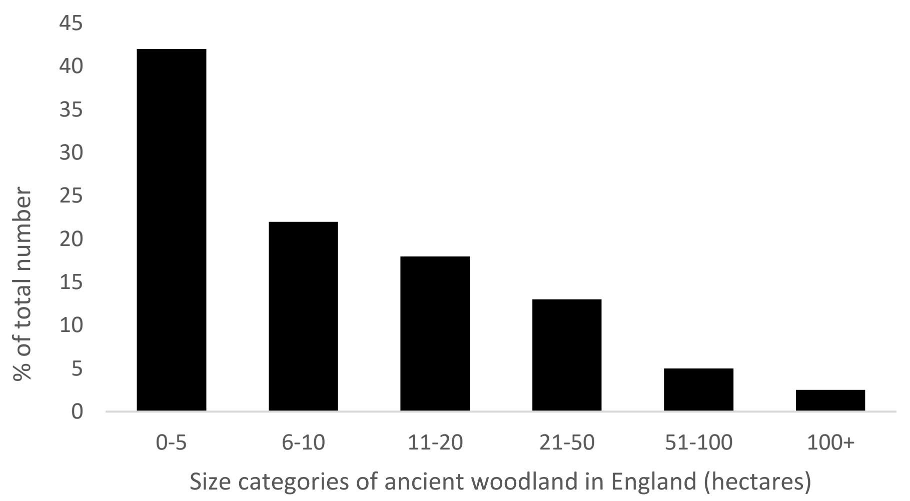

Figure 1.5 Size categories (hectares) of ancient woodland in England from the Ancient Woodland Inventory (After Thomas *et al.*, 1997:244)

Two over-arching themes are researched in this discipline - the impact of fragmentation on species dispersal, and on abiotic conditions in remnant woodlands. Both are studied in order to establish how loss and geographical reconfiguration of woodlands impact on community composition and population dynamics, often with a view to policy change or to inform restoration practices.

Reduced habitat continuity has been shown to cause an especially significant barrier to the dispersal and colonisation of ancient woodland species due to life traits such as few and heavy short-range dispersed seeds (Endels *et al.*, 2007; Hermy *et al.*, 1999; Bierzychudek, 1982) and clonal spread (Verheyen *et al.*, 2003a; Hermy *et al.*, 1999). These traits cause populations of these species to be sensitive to habitat fragmentation and loss, and even to the point of extinction (Flinn and Vellend, 2005; Peterken, 1977). Such traits are considered to be incompatible with survival in a dynamic landscape (Johst *et al.*, 2002), as alternative woodland habitats are not easily colonised. Frequency occurrence of woodland species in fragmented patches was linked to life form (which in turn relates to seed mass and dispersal mechanism) by Jacquemen *et al*. (2003a). This outcome corroborated findings of an earlier study that evidenced significant spatial aggregation of woodland species, which is indicative of severe dispersal limitation (Jacquemen *et al*., 2001).

The degree of isolation of recent woodlands from older or ancient woodlands has been shown to contribute to dispersal limitation of specialist or AWI species. Brunet *et al*. (2011) found significant negative correlations between distance from older core woodland and number and abundance of specialist woodland species ($p<0.001$ and $p<0.05$ respectively), and no correlation for generalist or open land species. These findings agree with an earlier study by Honnay *et al*. (2002a), where 85% of woodland species were identified as dispersal-limited and showed significantly decreasing colonisation ability with distance from source woodland. Isolation of recent woodland patches from ASNW had the strongest impact of a number of variables on the relative occurrence of spring flowering herbs with large seeds and unassisted dispersal (e.g. *P. quadrifolia*), and small, mainly vegetatively reproducing herbs (eg. *A. nemorosa*) (Endels *et al.,* 2007).

Considering connectivity instead of isolation, studies indicate that presence of specialist or AWI species correlated positively with degree of connectivity. Jacquemen *et al.* (2003a) demonstrated that patch occupancy of 35/59 woodland species was related to landscape connectivity. Likewise, AWI species richness in British lowlands was explained in a large part by length of hedgerows and lines of trees within the 1km square of a woodland plot, as well as the area of woodland within 500m of the plot (Petit *et al*., 2004). However, some hedgerows or tree lines may themselves be ASNW remnants (McCollin *et al*., 2000) and could potentially act as a source instead of, or as well as, a conduit for woodland species.

Conversely, Honnay *et al*. (1999a) discovered patch isolation to have negligible impact on woodland species richness, being of minor importance compared to internal habitat variables. Patch isolation has been demonstrated to be of varying importance for woodland species even when compared between landscapes in the same country: Graae (2000) assessed woodland species richness in response to isolation measures in two landscapes in Denmark, but only one site exhibited a significant decrease in woodland species richness with distance to large and ancient woodland. Similarly, Bailey *et al*. (2002) proved no link between the occurrence of four ancient woodland species and the distribution of ASNW woodlands at the regional scale, concluding that other factors must be more influential. Edge vegetation structure may be of greater importance than distance between woodlands in determining the permeability of edges for seeds (Cadenasso and Pickett, 2005).

Isolation combined with patch size may account for conflicting results regarding dispersal of specialist or AWI species across space: as emphasised by Bailey (2007) fragmentation is not simply a geographical distance problem. Two notable studies provide clear evidence that the aggregate effect of isolation and patch size influence species richness. Honnay *et al*. (2005) note that smaller habitats equal smaller populations, but this is potentially only problematic if small habitats are also isolated, reducing migration and increasing risk of local extinction. Petit *et al*. (2004) found the highest number of species in the largest and least isolated (and most shaded) woodland patches, with these factors being more important for species richness than total area of woodland in the vicinity, which ranked as the next highest factor.

Considering patch size alone, studies show conflicting results in terms of its impact on species richness. It would be expected that larger woodlands have higher species richness due to diversity of internal habitats (Honnay *et al*., 1999a) or that by being larger are more likely to encounter propagules (Dumortier *et al*., 2002). The landscape context may explain conflicting results among studies (Fahrig, 2003). Whilst some studies have found positive correlations between understorey species richness and presence with patch size (Gonzalez *et al*., 2010; Jacquemen *et al*., 2003a; Peterken and Game, 1984), the majority have evidenced no significant increase or little impact (Hofmeister *et al*., 2013; Dupre and Ehrlen, 2002; Graae, 2000; Honnay *et al*., 1999a). The 'single large or several small' debate (Diamond, 1975) was reviewed by Honnay *et al*. (1999a) with no evidence found that either a single large habitat or several smaller ones reduced species richness. Indeed, Graae (2000) found fewer species in large than in small woodlands in one study region, and *vice-versa* in another.

1.5.4 Edge effect

The concept of edge effect may partly account for the variation in results produced by studies of patch size *per se*. The impact of adjacent landuse on environmental gradients was recognised by Clements in 1897 (Farina, 1998). Edge effect is defined as the flux of matter, energy, and species directed from the landscape matrix into the habitat fragment (Ryszkowski, 1992). Small patch size combined with irregular shape resulting from landscape fragmentation is shown to cause a distinction in abiotic and consequently biotic variables in edge versus interior habitats. Smaller patches have a high perimeter to area ratio causing a proportionally higher area of edge habitat than in a larger woodland (Willi *et al*., 2005). The further the woodland patch deviates from the optimum circular shape, the more edge exists in proportion to interior (Laurance, 2008; Laurance and Yensen, 1991; Patton, 1975) (Fig. 1.6).

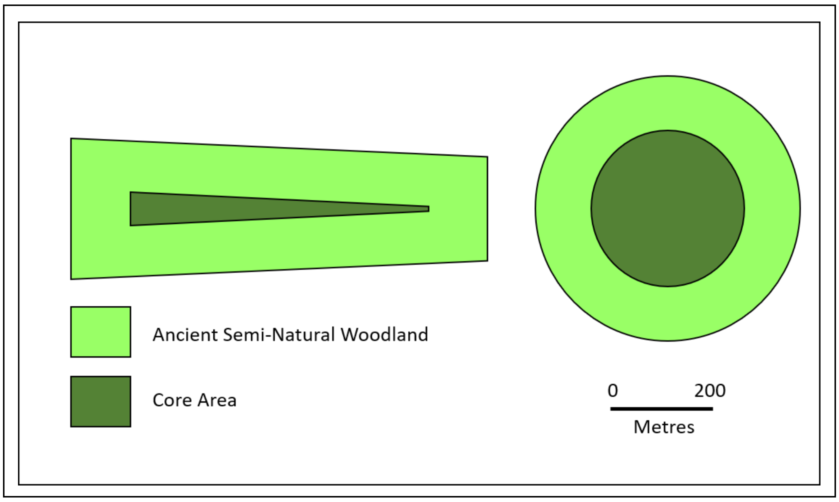

Figure 1.6 Woodland shape and edge effect. Two woodlands of the same area with markedly different proportion of 'interior' (after The Woodland Trust 2000:11).

A small number of studies have considered edge effects specifically in ancient woodland or explicitly concerning ancient woodland vegetation in northern Europe (Hofmeister *et al*., 2013; Willi *et al*., 2005; Honnay *et al*., 2002b) but generic woodland edge effects studies are well-established (e.g. Gonzalez *et al*., 2010; Vallet *et al*., 2010; Aune *et al*., 2005; Harper *et al*., 2005; Honnay *et al*., 2005; Ries and Sisk, 2004; Cadenasso and Pickett, 2001; Honnay *et al*., 1999a; Matlack, 1994). Woodland age (ASNW or recent) had no significant influence on the current edge width (Hofmeister *et al*., 2013), but there are seemingly no other studies with which to compare. Edge conditions are usually considered deleterious to populations of specialist species (Honnay *et al*., 2002b), which, when combined with small patch size, effectively become a form of habitat loss.

Murcia (1995) identifies three main edge impacts a) abiotic, b) direct biological caused by abiotic changes, and c) indirect biological, for example species interactions. Edge effects can include a change in environmental gradients perpendicular to the edge, such as wind speed, light penetration, humidity, temperature, and biotic impact such as ingress of weedy or non-specialist species. Additionally, edges may experience direct anthropogenic impacts such as pollution from agro-chemical spray-drift (Gove *et al*., 2007; Willi *et al*., 2005). Harper *et al.* (2005) theorise a sequence of edge impacts, being the primary response of the canopy structure to fragmentation, which then determines the abiotic gradients that cause secondary responses in terms of understorey processes, structure and composition.

Edge effect studies often aim to determine edge width in order to assess the proportion of the habitat influenced by external inputs. Murcia (1995) collated results from edge effect studies with nearly all variables exhibiting a width of between 10-60m in temperate forests. Similarly, Gehlhausen *et al*., 2002) found edge widths of between 10-80m for the majority of biotic and abiotic variables, and Honnay *et al*. (2002b) recorded 0-23m edge widths for the penetration of weedy species into ancient woodland. However, Hofmeister *et al*. (2013) found edge effects up to 200m for some variables, which they acknowledge as an unusual distance. Consequently, they emphasise that edge effects should be viewed as continuous rather than discrete variables, as there is no precise distance at which they cease.

Variation in edge width amongst studies may partly be accounted for by edge orientation, vegetation structure and the influence of multiple edges. Woodlands generally experience greater edge width on South and West than North facing edges in the northern hemisphere (Honnay *et al*. 2002b; Gehlhausen *et al*., 2000; Matlack, 1994). Hofmeister *et al*. (2013) recorded increasingly fewer ancient woodland species toward 150-200° orientation. This phenomenon is thought to be explained by the intensity of energy transfer between adjacent ecosystems (Harper *et al*., 2005; Ries and Sisk, 2004), which is influenced by solar radiation, light and wind energy inputs, often greater on South-facing edges and in the direction of the prevailing wind. Vegetation structure at the edge greatly influences edge width: sealed or high density vegetation structure at the edge reduced the magnitude and distance of edge width in comparison to edges with open vegetation (Harper *et al*., 2005).

An important but over-looked factor is the combined impact of effects caused by exposure of vegetation communities to multiple edges, for example in a woodland corner, and of these potentially being different matrix types. Ries and Sisk (2004) highlight that no studies have explicitly gathered primary data to investigate the effects of where multiple edge types converge, instead measuring linear distance to the closest edge only. In response, a recent study demonstrated the additive effect of dual-edge proximity on AWI richness in ASNW, which was lower in corner positions than near a single edge (Swallow and Goodenough, 2017). Specifically, presence of *A. nemorosa*, *P. quadrifolia*, *L. galaeobdolon* and *H. non-scripta* showed significant positive correlations with distance away from a single edge, and a stronger effect of proximity to dual-edges.

1.5.5 Relative influence of variables

To explain the phenomenon of distinctive ancient woodland vegetation, it is necessary to consider the relative contribution of variables that operate at different scales; from species-specific life traits, local environmental conditions, age and continuity, to the landscape scale geographical configuration of woodlands.

Age and geographical configuration of woodland seem have a high relative influence on vegetation characteristics (Kimberley *et al*., 2016). For studies focusing on ASNW, patch age ranks higher than other explanatory variables in explaining woodland species composition (Kimberley *et al*., 2016; De Keersmaeker *et al*., 2004). Patch age also has a high relative influence on the presence of geophytes and perennials with poor dispersal ability (Herault and Honnay, 2005). Petit *et al.* (2004), who did not test for the contribution of age, as being a previously established phenomenon, found patch size and isolation together accounted for 20% of the variation in AWI species richness in lowland British woodlands. Kimberley *et al*. (2016) concluded that management of the spatial configuration of woodland with suitable abiotic conditions would be the most effective method to influence woodland species diversity.

When considered relative to other explanatory factors, soil characteristics in ASNW and recent woodlands have a lower relative influence on understorey vegetation differences. Lack of long distance dispersal abilities, low seed availability, recruitment limitations and competition have all been shown to contribute more strongly to ASNW-recent woodland vegetation differences than soil characteristics (Sciama *et al*., 2009; Graae *et al*., 2004, 2003). Seemingly few studies have considered the relative influence of factors in ARW. However, in a national scale study across Britain, Corney *et al*., (2004) found pH to rank most highly in explaining vegetation composition.

1.6 Study scope and rationale

The ancient woodland concept is well-established internationally (Wirth *et al*., 2009b). The distinctiveness of ASNW plant communities is well-recognised in terms of richness, community composition, and presence of AWI species (Kelemen *et al*., 2014; Rackham, 2008; Kirby, 2004; Honnay *et al*., 1998; Rose, 1999; Wulf, 1997). The majority of existing research focuses on comparison of ASNW and recent woodland plant communities (e.g. Kelemen *et al*., 2014; Kirby and Morecroft, 2011; Sciama *et al*., 2009; Orczewska, 2009; Graae *et al*., 2003).

In the UK, ancient woodland holds especial protection in the National Planning Policy framework, which has been extended to accord equal status to ANSW and ARW (Forestry Commission and Natural England, 2014). Following the change in policy, a small but increasing number of studies have compared vegetation composition and dynamics of ASNW with ARW for restoration purposes (Berges *et al*., 2017; Atkinson *et al*., 2015; Brown *et al*., 2015).

Seemingly no previous study has drawn together the three woodland types (ASNW, ARW, and recent woodland) to understand the extent to which the vegetation of ASNW woodland may be supported in apparently sub-optimal habitats of semi-natural ARW and recent woodland. This thesis will address this gap in existing research.

The current thesis also addresses a research gap relating to the impact of dual edge effects. Ries and Sisk (2004:511) note a 'poor understanding' of multiple edge effects in the literature and that no studies using primary data are known. A search of the literature has revealed no studies considering the impact of multiple edge proximity on woodland plants. Given the current policy focus on ASNW conservation, it was appropriate to apply this theoretical gap to ancient woodland indicator species, which would in turn inform both fragmentation theory and application of AWI lists.

At the regional scale, the new Countryside Stewardship statement for the Cotswolds identifies ASNW and native woodland as priority habitats to maintain, and ARW for restoration and management (Natural England, Forestry Commission and DEFRA, 2015). The Cotswolds has twice as much ASNW cover than the average for England: 4.6% as opposed to 2.3% (Cotswold Conservation Board, 2018; Atkinson and Townsend, 2011). The Cotswolds is identified as an Ancient Woodland Priority Area for expansion and connectivity (Cotswold Conservation Board, 2018; Forestry Commission, 2006a). A search of the literature reveals seemingly no recent empirical studies of ancient woodland vegetation in the Cotswolds. Both Rose (1999) and Spencer (1990) mention the Cotswolds in published work, as a habitat for rare AWI species. Extensive survey data and grey literature exist, but there is a gap in the research for recent primary data collection and analysis of drivers of ancient woodland vegetation distinctiveness in this nationally important region.

1.6.1 Research aims and objectives

Research aim:

Using new primary data, to investigate the distinctiveness and community ecology of ancient woodland vegetation.

Research objectives:

1. To identify any distinctiveness in vegetation richness, diversity, and scarcity among ancient semi-natural, ancient replanted, and recent woodland.

2. To test the affiliation of herb layer plant species to ancient semi-natural and recent woodland, using a range of metrics.

3. To assess the degree to which the floristic community composition differs among ancient semi-natural, ancient replanted, and recent woodland.

4 To examine biotic influences on community composition in ancient semi-natural, ancient replanted, and recent woodland.

5 To examine the response of ancient woodland indicator communities to environmental and biogeographical variables, relative to woodland continuity status.

6 To assess the relative influence of single and dual-edge proximity on AWI species in a fragmented woodland.

1.6.2 Thesis structure

Chapter 1

This combined introduction and literature review established the following fundamental principles of ancient woodland vegetation research and identified research trends and gaps in knowledge. This basis was used to inform the research objectives of the thesis, which are presented in section 1.6.1.

- Woodland classification systems in Britain combine habitat continuity, naturalness and species composition. NVC is the current standard technique.
- A significant emphasis is placed upon the ancient woodland concept (ASNW and ARW) both in terms of conservation practice and legislation (HM Government, 2018; Forestry Commission and Natural England, 2014; Goldberg *et al.*, 2007).
- Ancient woodland covers just 2.3% of England's land area in a highly fragmented configuration (Atkinson and Townsend, 2011).

- The ecological value of ASNW is primarily due to its distinctive vegetation in comparison to more recently established woodland (Kelemen *et al*., 2014; Sciama *et al*., 2009).

- Prior studies have established differences between ASNW, ARW and/ or recent woodland in understorey richness, diversity, community composition, and/ or presence of AWI species, although outcomes among these metrics are inconsistent (e.g. Berges *et al*., 2017; Kelemen *et al*., 2014; Brunet *et al*., 2011; Sciama *et al*., 2009; Hermy *et al*., 1999).

- ARW vegetation (particularly that of semi-natural or mixed ARW, as opposed to coniferous or exotic) has been little studied relative to ASNW and recent woodland (Atkinson *et al*., 2015; Brown *et al*., 2013).

- The use of AWI species to identify ancient woodland and to measure conservation value is a widely accepted practice by governmental and non-governmental organisations. However, the methods of AWI list creation and application have been criticised for lack of objectivity and standardisation (Glaves *et al*., 2009). An increasingly favoured approach to is assess the ecological conditions of a woodland or to use weighted indicators.

- Several studies have concluded that woodland continuity and biogeographical factors, such as woodland connectivity, patch size and edge effects, are strong explanatory factors for the distinctive ecology of ancient woodland flora (Kimberley *et al*., 2016; Hofmeister *et al*., 2013; Petit *et al*., 2004).

Chapter 2

This study site and methods chapter details the generic methods used in all subsequent data chapters. Firstly, the woodland history and biogeography of the Cotswold Hills study area is outlined to set the context of the data collection. Secondly this chapter describes and justifies the site selection, sampling strategy, and methods of vegetation data collection. To reduce repetition, these generic methods are referred to within the methods section of individual data chapters.

Chapter 3

This data chapter addresses the following research objectives:

1. To identify any distinctiveness in vegetation richness, diversity, and scarcity among ancient semi-natural, ancient replanted, and recent woodland.
2. To test the affiliation of herb layer plant species to ancient semi-natural and recent woodland, using a range of metrics.

To underpin the later explanatory chapters, this first data chapter provides a baseline description of the relative distinctiveness of ASNW, ARW, and recent woodland vegetation. To establish whether there is any difference in the relative scarcity of herb layer species in ASNW, ARW and recent woodland, this chapter examines the frequency occurrence of species present in the three woodland continuity types within 10km squares in Britain (Hill *et al.*, 2004). The literature review identified inconsistent outcomes among the metrics used to distinguish ASNW vegetation from that of ARW and/ or recent woodland (Naaf and Wulf, 2010). To address this issue, Chapter 3 tests the consistency of alpha scale richness and diversity measures that are commonly used to differentiate the canopy, shrub, herb, AWI and terrestrial moss layer vegetation of ASNW, ARW, and recent woodland. Likewise, the literature highlighted inconsistencies in the creation of AWI lists (Kimberley *et al.*, 2013). Chapter 3 examines the consistency of AWI identification through the application of four indicator-identification metrics used in prior studies.

Chapter 4

This data chapter addresses the following research objectives:

3. To assess the degree to which the floristic community composition differs among ancient semi-natural, ancient replanted, and recent woodland.

4. To examine biotic influences on community composition in ancient semi-natural, ancient replanted, and recent woodland.

This chapter is distinguished from Chapter 3 in focusing on the beta and gamma scales of plant community differentiation. Unlike at the alpha scale, beta scale community comparisons recognise the identity of individual species and their phytosociological associations (Berges *et al.*, 2017; Atkinson *et al.*, 2015). Vegetation community comparisons are conducted for the canopy, shrub, herb (and AWI and non-AWI), and terrestrial moss layers among ASNW, ARW and recent woodland. Biotic explanations for any community distinctiveness are analysed. To investigate any strata interactions, AWI alpha richness, and the richness of the corresponding canopy, shrub, non-AWI herb, and moss layers are tested for correlation. Likewise, Jaccard community similarity at the beta scale is also tested for correlation between AWI communities and the other layers. The literature identified a lack of consensus on which life traits best explain ASNW, ARW, and/or recent woodland vegetation distinctiveness (Kimberley *et al.*, 2014; 2013). Chapter 4 compares the herb layer community composition of the three woodland types by life trait.

Chapter 5

This data chapter addresses the following research objective:

5. To examine the response of ancient woodland indicator communities to environmental and biogeographical variables, relative to woodland continuity status.

Chapter 5 uses a modelling approach to explain variation among ASNW, ARW and recent woodland for both alpha richness and beta community composition. Chapter 4 explains any biotic factors influencing community composition, whereas Chapter 5 focuses on abiotic environmental and biogeographical explanatory variables. The literature emphasised the importance of woodland continuity for AWI species presence, and identified a research trend towards ecological, rather than age-based, explanations for AWI presence (Barnes and Williamson, 2015; Wright and Rotherham, 2011; Spencer, 1990). Furthermore, biogeographical variables have been found to have significant influence on AWI communities in existing studies (Kimberley *et al.*, 2016; Petit *et al.*, 2004). Therefore Chapter 5 tests to what extent woodland continuity or habitat variables best account for the variation in AWI communities established in Chapter 4.

Chapter 6

The chapter addresses the following research objective:

6. To assess the relative influence of single and dual-edge proximity on AWI species in a fragmented woodland.

To research any additive effect of proximity to two edges on AWI presence, Chapter 6 focuses on a local scale, as opposed to the landscape scale of earlier chapters. The literature highlighted the influence of biogeographical variables resulting from fragmentation for AWI communities (Kimberley *et al*., 2016; Hofmeister *et al*., 2013; Petit *et al*., 2004), particularly the notable statistic of 48% of ancient woodlands being smaller than 5ha (Corney *et al*., 2008b). A search of the literature revealed few prior dual-edge effect studies for an ecological discipline (Fletcher, 2005; Ries and Sisk, 2004) and none in the context of ancient woodland vegetation. Chapter 6 tests for correlation between herb layer AWI species richness and individual AWI species presence with proximity to a single edge, and with combined proximity to two edges. Additionally, Chapter 6 describes the AWI community differences between a larger and smaller fragment at the local scale site. This chapter utilises primary data collected outside of the PhD but the analysis and write-up were completed entirely during, and as part of the thesis.

Chapter 7

This concluding chapter summarises the literature context of the thesis and provides a synopsis of each chapter. The original findings are presented in relation to individual research objectives, and the overarching conclusions of the thesis are summarised. This chapter presents a critique of the research conducted and indicates the next steps for publication and potential avenues for future research arising from the thesis.

2. Study site and generic methods

Mercurialis perennis dominated woodland. Castlett Wood, Guiting Strategic Nature Area 25/07/13, Grid ref. SP 07934 26469

Chapter scope:

This chapter outlines and justifies the selection of study sites and vegetation sampling methods within those sites. For Chapters 3, 4, and 5 the generic methods are considered here. Specific vegetation, soil, and biogeographical factors and statistical methods, are detailed in the relevant research chapters. For Chapter 6, an outline of methods is given here, with further details in the relevant chapter.

2.1 Woodland context of The Cotswold Hills

The Cotswold Hills region is located in southern England (Fig. 2.1), mainly within the county of Gloucestershire, centred on latitude 51.93N, longitude 1.96W. The region is categorised as hilly lowland with altitude of up to 300 metres above sea level. An area 78km in length and 2038km^2 is designated as an Area of Outstanding Natural Beauty (Cotswold Conservation Board, no date). The geological substrate is predominantly oolitic limestone, with smaller patches of mudstone, sandstone and lias clay (BGS, 2016). Soil cover is predominantly 0343d Sherborne Association (shallow, well-drained brashy calcareous clayey soils over limestone) (Cranfield University, 2016). Annual rainfall is 806.3mm and mean annual temperature is 5.7°C (min) to 14.0°C (max) (MET office, 1981-2010).

Figure 2.1. Cotswold Area of Outstanding Natural Beauty (Cotswold Conservation Board, undated)

The vegetative history of the area is complex. Circa 10,000 Cal. BP *Betula*, *Pinus* and *Corylus* recolonised the Cotswolds under post-glaciation tundra conditions, followed by *Quercus* and *Ulmus* and then other tree species as the climate warmed (Regini *et al*., 1987). Early woodland clearance of the Cotswold plateau began c. 5500 Cal. BP (Bathe *et al*., 1981), followed by a decline in *Ulmus* sp. and *Tilia sp.* and an increase in *Fraxinus excelsior* due to shifting cultivation ground disturbance (Pennington, 1969). Circa 2500 Cal. BP, the Cotswold plateau became more extensively cleared for sheep farming with some woodland remaining for timber (Rackham, 1990). Clearance continued until the c1000 Cal BP, with coppice management being initiated c500-800 Cal. BP. In the year 1700 through into 1900s, plantations were created on the plateau, with parks and woodland formed on large estates, such as Stowell and Cirencester (Regini *et al*., 1987). The replanting of ASNW for timber dates to the early 20th century, creating ARW (Forestry Commission, 2012).

The Countryside Stewardship agri-environment scheme identifies the Cotswolds as a priority region for ancient and native woodland (DEFRA, 2015). This is significant not only due to recognition of woodland habitats, but also due to the selection of the Cotswolds as a functioning landscape unit in which to administer habitat management strategies. As such, the present study is justified in researching ancient woodland in the Cotswold eco-region, rather than over a wider area, or within an administrative boundary, such as a county. Woodland covers 10.1% of the Cotswold AONB area, which reflects the England average of 10% woodland cover (Forestry Commission, 2017). The wooded area comprises 66% broadleaf and 34% coniferous woodland (Forestry Commission, 2001). The dominant broadleaf woodland types are *Fraxinus excelsior*, *Acer pseudoplatanus*, *Quercus robur*, under National Vegetation Classification (NVC) categories W8 and W10 and *Fagus sylvatica* under NVC categories 12 and 14 (Cotswold Conservation Board, 2018).

The Cotswold region offers significant scope to research the distinctiveness and community ecology of ancient woodland vegetation. The Cotswolds has a relatively high density, including some large landscape patches, of ancient woodland (Table 2.1; Fig. 2.2). Ancient woodland is twice as abundant in the AONB area than for England on average. Within the AONB area, 4.6% (9292ha) of land cover is ancient woodland (comprising 2.9% (5940ha) ASNW and 1.7% (3352ha) ARW) (Cotswold Conservation Board, 2018), compared to national coverage of 2.3% of ancient woodland (1.35% ASNW and 0.95% ARW, calculated from data in Atkinson and Townsend (2011)). As a result, a large proportion of the Cotswold region has been identified as one of four Ancient Woodland Priority Areas with potential for restoration and connectivity in the South-West UK (Forestry Commission, 2006b) (Fig. 2.1). Woodland patch size data for Gloucestershire shows a highly-fragmented landscape with dominance of small patch sizes, and only eight large landscape patches of woodland (Table 2.1) (Forestry Commission, 2001).

Table 2.1 Woodland patch size distribution in Gloucestershire (Forestry Commission, 2001)

Size class (hectares)	Number of woodlands	Percent of woodland area (%)	Mean area (ha)
<10	1083	15	4.1
10 - <20	136	6	14.0
20 - <50	94	10	30.6
50 - <100	36	9	74.0
100 - <500	31	19	178.5
500+	8	40	1475.1
Total	1388	100	21.1

Extensive primary field surveys and secondary documentary and cartographic sources research mean that the identification of ancient woodland in the region is as robust as possible. The earliest full woodland survey with conservation in view was conducted by Gloucestershire Trust for Nature Conservation in 1977 (Regini *et al*., 1987). Later, The Nature Conservancy Council (Spencer and Kirby, 1992; Kirby *et al*., 1984) compiled a national inventory of ancient woodlands at county level, including detailed flora surveys. Ancient woodlands over 2ha were recorded based on primary vegetation survey data, historical documents, tithe and estate maps, Domesday and Saxon charters, woodland names, owner information, woodland shape, location, earthworks and boundaries (Kirby *et al*., 1984). A study of ancient woodland archaeology in Gloucestershire was published by Grundy (1936), which assisted in dating woodlands.

More recently, The Forest Research Agency conducted a mapping exercise for ancient woodlands in South-West England under the Keepers of Time government policy for ancient woodland conservation (DEFRA and Forestry Commission, 2005). The exercise drew on published data sources such as the National Forest Inventory, National Biodiversity Network Habitat Inventories, the Ancient Woodland Inventory and Ordnance survey (Forestry Commission, no date). Significant woodland areas were identified as Strategic Nature Areas (SNA) for the South-West in order to address requirements of the Natural Environment and Rural Communities (NERC) Act (2006) (section 41). Smaller woodlands under 2ha are now also included in the Gloucestershire Nature Map.

The DEFRA MAGIC map provides definitive mapping of woodlands from the national to local scale. All ASNW, ARW and recent woodland sites surveyed in this thesis were identified by this standard and nationally accepted cartographic resource. Dedicated GIS layers for ASNW and ARW were superimposed upon Ordnance Survey base maps to identify the three woodland types. Using this standardised resource to identify ASNW and ARW, reduced the 'circular logic' of ancient woodland and AWI identification cautioned against by Spencer (1990). This means the research basis is as robust as possible and is comparable to other studies using MAGIC map at the national scale.

However, little empirical ancient woodland vegetation research has been published on the Cotswolds specifically. The region is mentioned by Rose (1999) as habitat for rarer ancient woodland plants, and Gloucestershire is noted for abundance of *Paris quadrifolia* in both ancient and plantation woodlands (Spencer, 1990).

2.2 Site selection, sampling and vegetation data collection

2.2.1 Selection of study locations

For all research chapters, study locations were located within the Cotswold Ancient Woodland Priority Area, and within the Cotswold Area of Outstanding Natural Beauty (Fig. 2.2).

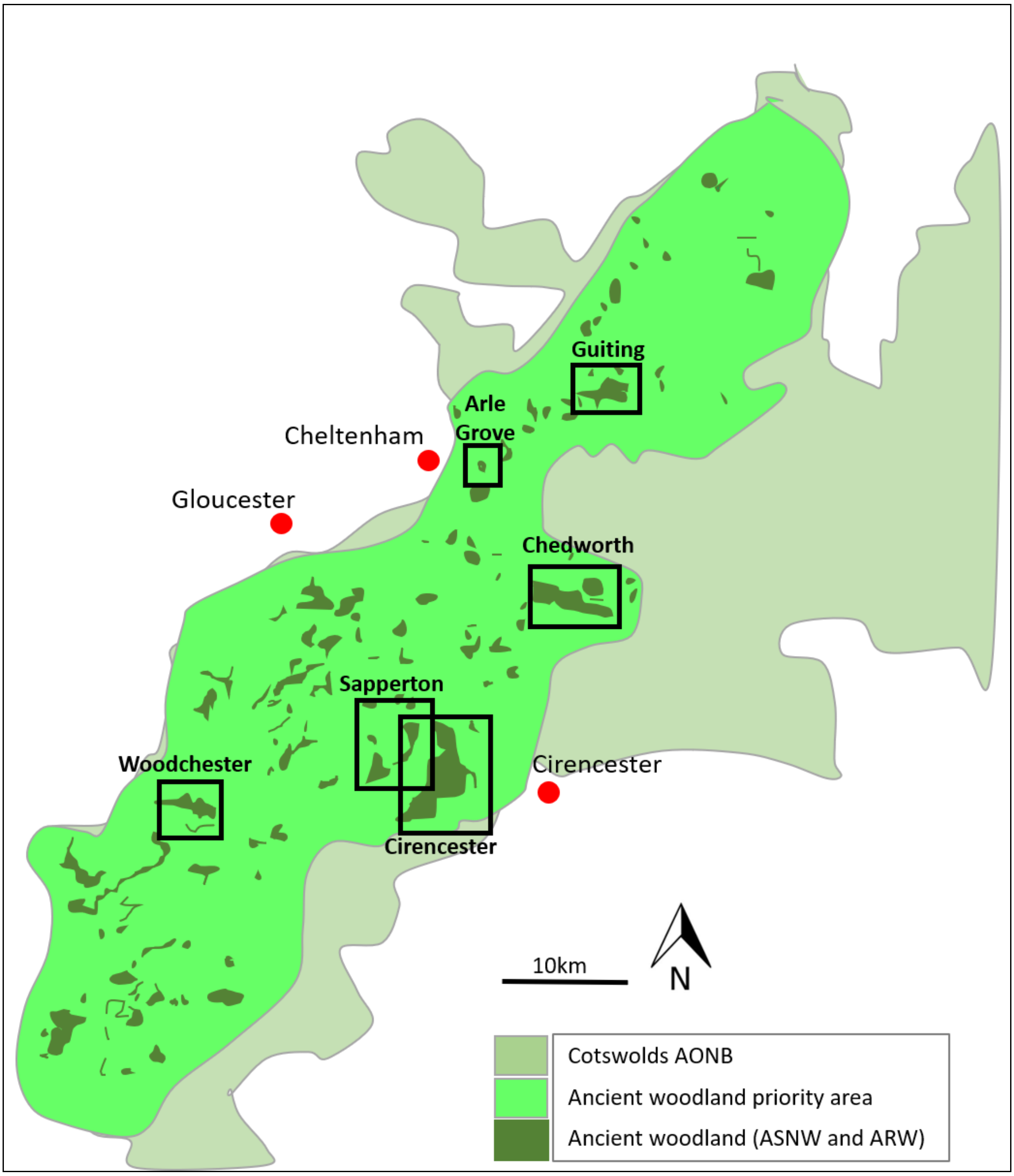

Figure 2.2. Cotswold Ancient Woodland Priority Area showing research locations: Strategic Nature Areas (SNA) of Chedworth, Cirencester, Guiting, Sapperton and Woodchester (Chapters 3, 4, and 5), and Arle Grove (Chapter 6) (After Forestry Commission and Cotswold Conservation Board (undated: 2).

To research the distinctiveness and diversity of ancient woodland flora, forty-five woodland sites were selected for study within five Strategic Nature Areas (SNAs) (Fig. 2.2), as identified by Gloucestershire Local Nature Partnership (2016). The study of sites within SNAs enables a practical application of the research outcomes. The rationale for selecting woodland site within SNAs, was that such landscapes have been identified as having potential for increased connectivity and ancient and native woodland management, such that the findings of this research will usefully contribute towards practical management. The selected SNAs spanned from the North to South Cotswolds (Fig. 2.2) being, Guiting, Chedworth, Cirencester, Sapperton, and Woodchester.

2.2.2 Sampling of woodland sites for Chapters 3, 4, and 5

A sample of forty-five woodland sites was split equally among the five SNAs, within categories of ASNW (n = 15), ARW (n = 15) and recent woodland (n = 15). To mitigate any effects of spatial autocorrelation (Legendre, 1993), three ASNW, three ARW and three Recent woodland sites were selected in each of the five SNAs (Fig. 2.3). The ASNW/ ARW / recent status of each site was ascertained by the definitive MAGIC map (DEFRA *et al*., 2016). The number of sites selected was informed by prior studies of similar scope (Hofmeister *et al*., 2013; Brunet *et al*., 2011; Graae *et al*., 2003; Honnay *et al.,* 1999b).

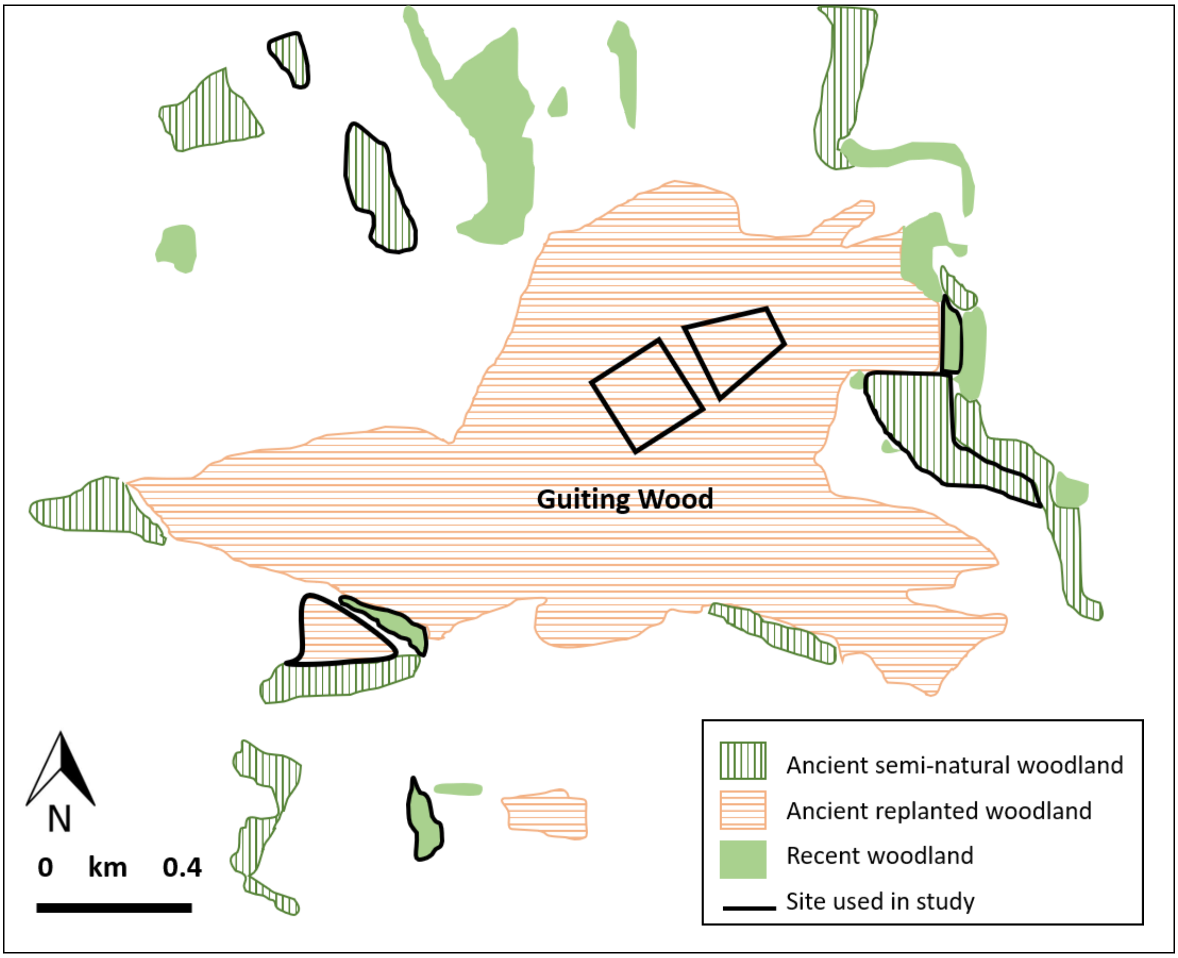

Figure 2.3. Example selection of study sites. Selection of 3x each ASNW, ARW and recent woodland sites within Guiting SNA. Sites surveyed indicated by black outlines (after DEFRA, 2016).

Variation in broad-scale environmental parameters was minimised through site selection. All sites were located on limestone geology, which was determined via layering the following Geographical Information System (GIS) layers: DEFRA (2016) MAGIC map ancient woodland dataset; Biodiversity Southwest SNA boundaries; geology maps; Ordnance survey base layer. Altitude was initially determined using Google Earth and later verified in the field using Geographical Positioning System (GPS). All sites were located between 170 and 270 m.a.s.l. Landowner permission was a further determining factor in site selection. A site was delineated by either being an isolated woodland patch surrounded by another land-use, or a compartment within a larger woodland separated from other compartments by a track.

To reduce bias in site selection, a random number generator was used to select grid co-ordinates from the GIS layers within each SNA. Numbers were generated until enough ASNW, ARW, and recent sites were selected within the permitted and suitable areas identified by GIS. Further selection was completed via ground-truthing to ensure accessibility of sites. Where a randomly selected site was inaccessible or unsuitable (for example unsafe, or recently felled) the next on the list was chosen until a suitable compartment was selected (Honnay *et al*., 1999a). The random sampling of study sites avoids any systematic bias.

All sites represent small habitat patches with each having a homogenous canopy cover of between 0.70ha and 20ha (Brunet *et al*., 2011). All sites selected were entirely or predominantly broadleaf, but due to scarcity of purely native deciduous canopy composition in ARW and some recent woodland, a degree of tolerance was permitted of up to 30% intermixed conifers (Kolk and Naaf, 2015; Kelemen *et al*., 2014), which was established during desk-based GIS assessment and ground truthing.

To ensure comparability among study sites, National Vegetation Classification was conducted for all sites (Hall *et al*., 2004; Rodwell, 1991). MAVIS software (Smart, 2000) and manual keying-out were used to identify the correct classifications. Where possible, these were verified against existing site surveys. For Chapters 3, 4, and 5, all sites were classified as W8, except one W12 (which had a close secondary classification of W8 using the MAVIS system). The matrix environment surrounding each site varied among woodland, arable, pasture, equine sport ground, parkland and semi-natural grassland. ASNW were largely managed as coppice and/or for conservation/ game purposes, whereas ARW, and most recent woodlands, were managed for timber (Brunet and Von Oheimb, 1998).

2.2.3 Plot-level sampling strategy and vegetation data collection for Chapters 3, 4 and 5

To achieve representative vegetation data, five plots were systematically located in each of the forty-five woodland sites. In total 225 plots were surveyed, equally divided among ASNW (n=75), ARW (n=75) and recent woodland (n=75). The plot-level sampling of canopy, shrub, herb and ground layers was based on the nested quadrat system of NVC centred on the smallest quadrat layer. Nested quadrats allow direct analysis of associations between vegetation strata and take account of the differing ecological scales at which the canopy and understorey layers function. Therefore, nested quadrats address Rackham's (2003) criticism of incomparable vegetation scales with phytosociological classification. For this reason, nested quadrats are commonly used in woodland studies (Kelemen, *et al*., 2014; Brunet *et al*., 2012; Graae *et al*., 2003).

To focus time and resources on the herb layer (which contains the majority of ancient woodland species), the NVC survey protocol was adapted. There was no detriment to the NVC classification, as Hall *et al*. (2004) explain that woodlands can be accurately classified using a range of quadrat sizes. Five plots, each containing nested quadrats, were surveyed within each woodland site (Hall *et al*., 2004). To avoid overlap of plots (and therefore samples that were not independent) canopy quadrat size was 30x30m (Hall *et al*., 2004) rather than the 50x50m initially recommended by Rodwell (1991. Shrub and herb layer quadrat sizes followed Rodwell (2006), 10x10m and 4x4m respectively. For efficiency, terrestrial moss ground layer vegetation was also surveyed within the herb layer quadrat (Hall *et al.*, 2004), rather than the 2x2m quadrat recommended by Rodwell (2006).

The area of canopy and herb layer surveyed per site exceeded that of similar published studies, enabling a robust and detailed analysis of vegetation composition among woodland sites (Hofmeister *et al.*, 2013; Brunet *et al.*, 2012, 2011; Coote *et al.*, 2012; Sciama *et al.*, 1999).

To ensure evenness in sampling across parcels of different size and shape, the five NVC plots were systematically located (Harmer and Morgan, 2009). Depending on the shape of each site, a plot was located in the most central position of each site and the remaining four positioned at equal distances from the central plot, or equally spaced from the nearest plot in a linear arrangement (Fig. 2.4). Distance between plots was proportional to the site size. The size of the site was assessed using Google Earth and spacing of plots planned *a priori*. The plot area surveyed was independent of site size, as per the majority of published studies (e.g. Kelemen, *et al.*, 2014; Brunet *et al.*, 2012; Petit *et al.*, 2004; Graae *et al.*, 2003).

Figure 2.4. Plot location examples in a square and linear parcel of differing area. Black outline = homogenous site area. White outline = 30x30m plot.

In the smallest woodlands, to minimise sampling non-representative vegetation at the woodland edge, a buffer of at least 2m was left around the woodland boundary before placement of the 30x30m canopy quadrat (Harmer *et al*., 2001), and therefore the shrub layer quadrat within was never less than 12m from the edge, and the herb layer quadrat never less than 15m. For larger woodlands, when systematic sampling indicated an internal microhabitat (e.g. stream, footpath or disturbance feature) for plot location, then an adjacent area was selected instead (Honnay *et al*., 1998), again with a 2m buffer around the feature before the 30x30m quadrat was placed (Hofmeister *et al*., 2013; Vockenhuber *et al*., 2011).

As NVC does not specify vegetation height to be surveyed in each stratum of a nested quadrat, these were classified in part by height, and in part by life traits or functional group (Hofmeister *et al*., 2013; Brunet *et al*., 2012, 2011; Van Calster *et al*., 2008):

- Canopy 30x30m: Woody species ≥7.0m high.
- Shrub layer 10x10m: Woody and semi-woody species 1.3-6.9m (woody shrubs, saplings, semi-woody climbers. *Corylus avellana*, *Rubus fructicosus* and *Hedera helix* at any height were included in the shrub layer).
- Field, herb and ground layer 4x4m: Vascular plant species (herbaceous, graminoids, pteridophytes, and semi-woody species) and terrestrial mosses ≤1.2m. Tree seedlings and liverworts excluded).

All vascular woody, semi-woody and non-woody plant species were surveyed, but tree seedlings were excluded (Kolk and Naaf, 2015; Mölder *et al*., 2008). In the present study canopy layer trees were surveyed both for NVC and strata-interaction analysis, but in other studies they are often omitted due to the likelihood of being planted and therefore not reflective of ecological conditions (Vallet *et al*., 2010; Gilliam, 2007; Honnay *et al*., 2002; Graae, 2000). In the present study, alien, planted or hybrid plants were surveyed, although some prior studies specifically exclude these (Jacquemen *et al*., 2001; Honnay *et al*., 1999a), and the majority do not specify. In terms of bryophytes, only terrestrial mosses were surveyed; epiphytic mosses and all liverworts were excluded. Bryophytes are recognised reliable indicators of woodland age and continuity (Mölder *et al.*, 2015; Coote *et al*., 2012), and moss is an important element of NVC classification (Rodwell, 1991).

As Gloucestershire has no published AWI list (Glaves *et al*., 2009), AWI species used for Chapters 3, 4, and 5 are those defined by Rose (1999) for South-West and South England, plus any additional from Kirby's (2004) list for Worcestershire, and the Avon, North Somerset and South Gloucestershire list submitted to Glaves *et al*. (2009) (Table 2.2).

Table 2.2 Ancient Woodland Indicator (AWI) species list for Chapters 3, 4, and 5.

These species are those found during surveys of sample sites. They are classified as AWIs in published lists for South and South-West England by Rose (1999), Kirby's (2004) list for Worcestershire, and from the list for Avon, North Somerset and South Gloucestershire (Glaves *et al*., 2009).

Acer campestre
Adoxa moschatellina
Allium ursinum
Anemone nemorosa
Carex pendula
Carex sylvatica
Colchicum autumnale
Conopodium majus
Convallaria majalis
Daphne laureola
Dryopteris carthusiana
Epipactis helleborine
Euphorbia amygdaloides
Galium odoratum
Hyacinthoides non-scripta
Ilex aquifolium
Lamiastrum galaeobdolon
Malus sylvestris
Mercurialis perennis
Orchis mascula
Oxalis acetosella
Paris quadrifolia
Platanthera chlorantha
Poa nemoralis
Polygonatum multiflorum
Polypodium vulgare
Polystichum setiferum
Primula vulgaris
Prunus avium
Rosa arvensis
Sanicula europaea
Tamus communis
Ulmus glabra
Viola reichenbachiana
Viola riviniana

For ease of identification, multiple season surveys were conducted (e.g. Kolk and Naaf, 2015; Kelemen *et al*., 2014; Brunet *et al*., 2011; Van Calster *et al*., 2008; Herault and Hermy, 2005; Graae, 2000; Wulf, 1997). Tree and shrub layer surveys were completed in full leaf July-August 2013. During April-May 2014, plots were surveyed for field, herb and ground layer vegetation, and identification verified again in April-May 2015, then a full summer re-survey July-August 2015. Spring and summer repeat surveys are justified by the phenology and life traits of woodland species. Woodland species comprise a high number of vernal species e.g. *A. nemorosa*, which complete flowering and leafing in the spring and wilt before summer (Brunet *et al*., 2011) whereas other woodland species are summergreen e.g. *Circaea lutetiana*. Plots were checked again during autumn and winter 2015 for any additional species e.g. *C. autumnale*, and bryophtyes.

To avoid bias during a time of rapid vegetation development for the spring surveys a systematic sampling rotation was followed: one each of ASNW, ARW and recent sites were surveyed in each SNA in turn and repeated until all 45 were complete. Highest species percentage cover values were taken from the full spring and summer surveys (Brunet *et al*., 2011; De Keersmaeker *et al*., 2004) or, for a very few species eg. *C. autumnale*, added from the autumn or winter plot checks. Vegetation data were recorded using the standard NVC recording form (Rodwell, 2006).

Tree (≥7.0m) and shrub (1.3-6.9m) species percentage cover was visually estimated with DOMIN classes as a vertical projection of the canopy onto the ground. This was for woody plants rooted within the 30x30m and 10x10m plots respectively (Palo *et al*., 2013; Brunet *et al*., 2011).

For plant species ≤1.2m, actual percentage cover was recorded within 16 1x1m subplots within the 4x4m plots. Only plants rooted within the quadrat were included (Graae *et al*., 2003). A 1x1m grid quadrat (each grid square = 1%) enabled a precise record of percentage cover, and the plot mean was calculated from all 16 subplots. However, in previous studies visual estimates of percentage cover are widely employed (Coote *et al*., 2012; Brunet *et al*., 2011; Vockenhuber *et al*., 2011; Mölder *et al*., 2008; Van Calster *et al*., 2008). Percentage cover data was necessary to assess the relative abundance of individual species among ASNW, ARW, and recent woodland, was therefore more appropriate than presence/ absence records alone (Graae *et al*., 2003; Jacquemen *et al*., 2001). Abundance scales (e.g. Hofmeister *et al*., 2013; Vallet *et al*., 2010; De Keersmaeker *et al*., 2004) were not used for vegetation ≤1.2m as the grid quadrat facilitated precise data collection. Additionally, DOMIN numbered categories were not suitable for statistical analysis, and choosing the median value would lead to many tied data.

2.2.4 Selection of study location for Chapter 6

To research the influence of edge effects on ancient woodland flora, an isolated and fragmented ASNW of approximately rectangular shape was selected. This was a single-site study based at Arle Grove, an AWI-rich reserve, situated centrally among the Strategic Nature Areas studied for Chapters 3, 4, and 5 (Fig, 2.2). Arle Grove is not within an SNA but met the environmental standardisation criteria as for the sites surveyed for prior chapters, being 256 m.a.s.l and with parent material of Jurassic limestone. Being a fragmented woodland, the size was 4.8ha plus a smaller remnant of 0.6ha (Fig. 2.5). As for sites in chapters 3, 4, and 5, Arle Grove classified as W8 in the National Vegetation Classification.

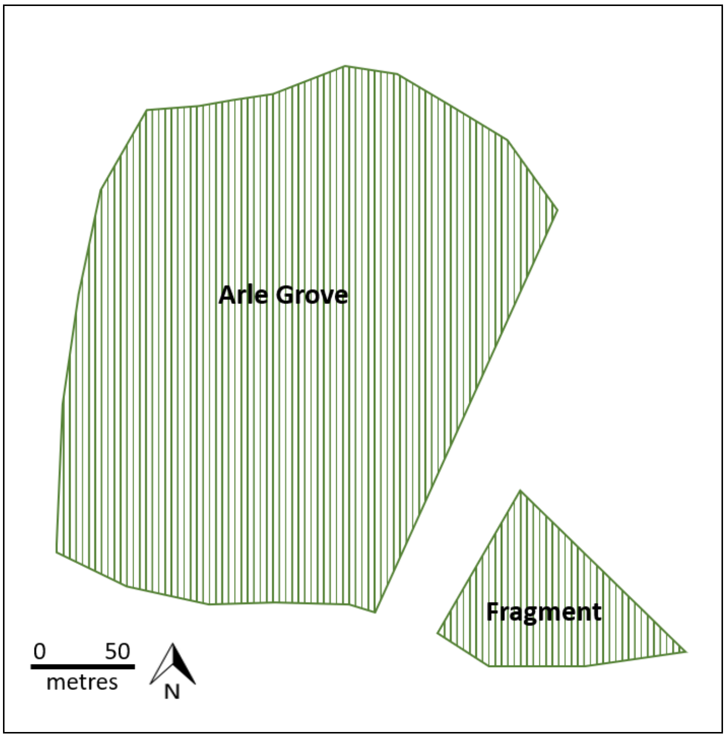

Figure 2.5 Arle Grove and fragment. Ancient semi-natural woodland.

2.2.5 Plot-level sampling strategy and vegetation data collection for Chapter 6

To assess any additive effect of single and dual woodland edges on herbaceous forb AWI richness (Brunet *et al*., 2011), plots were systematically located in transects at 0, 5, 10, 20, 30, 40 and 60m perpendicular to the East and West edges of Arle Grove and at 0, 5, 10, 20 and 30m for the remnant. Inter-transect distance was 10m, except at the edges where it was 5m. Plots were 2x2m. Fieldwork was carried out in May 2011. Both plot size and spacing was informed by earlier studies (Vallet *et al*., 2010; Honnay *et al*., 2002b; Gehlhausen *et al*., 2000; Murcia, 1995). For this chapter AWI species were defined by the South-West UK list (Rose, 1999). In each plot presence of herbaceous forb AWI species were recorded (Table 2.3).

Table 2.3 Ancient Woodland Indicator (AWI) species list for Chapter 6.

These species are those found during surveys of sample sites. They are herbaceous forbs classified as AWIs in published lists for South-West England by Rose (1999).

Anemone nemorosa
Hyacinthoides non-scripta
Allium ursinum
Lamiastrum galaeobdolon
Galium odoratum
Paris quadrifolia
Viola reichenbachiana
Conopodium majus
Primula vulgaris
Euphorbia amygdaloides
Orchis mascula
Veronica montana
Sanicula europaea

3. Distinctiveness of ancient woodland vegetation according to a range of diversity and indicator metrics.

Outputs arising from this chapter:

Swallow, K., Wood, M., and Goodenough, A. (2015) *Biodiversity value of ancient woodland flora: not quite as it seems*. British Ecological Society Annual Meeting. Edinburgh. 13-16 December

Swallow, K. (2015) *Woodland moss species richness in the Cotswolds: a pilot study*. Postgraduate conference. University of Worcester. 26 June.

Thuidium tamarisinum. Overley Wood. Cirencester Strategic Nature Area

27/04/2014. Grid ref: SO 97579 04644

3.1 Chapter scope

The literature review (Chapter 1) identified the scarcity, diversity, and distinctiveness of ancient woodland vegetation as strong justifications for its conservation. In response, the current chapter establishes the relative scarcity of species present in ASNW, ARW, and recent woodlands. A range of diversity metrics and methods of indicator species identification is employed in ancient woodland studies, but such methods have rarely been compared for consistency in outcomes. To address this, Chapter 3 evaluates the consistency of diversity metrics and indicator-identification techniques used in existing ancient woodland vegetation research. To inform development of the thesis in later chapters, the current chapter describes and compares baseline alpha richness and diversity among ASNW, ARW and recent woodland. Chapter 4 will compare gamma scale richness with the alpha richness established in the present chapter. AWI richness outcomes established in the present chapter will also form the basis of explanatory variable testing in Chapter 5.

3.2 Introduction

The distinctiveness and high biodiversity value of ancient woodland are long-established concepts and are widely used to justify its conservation (Honnay *et al*., 1999a; Wulf, 1997; Peterken, 1974). Ancient woodland in England is defined as land that has been continuously wooded since at least the year 1600 (Goldberg *et al*., 2007). This definition includes both Ancient Semi-Natural Woodland (ASNW) and Ancient Replanted Woodland (ARW), which are equally protected under UK planning law (Forestry Commission and Natural England,

2014) and woodland management policy (DEFRA, 2013). Recent woodland is any wooded area established since the 1600 threshold date.

However, there is little consistency in the choice of diversity metrics among studies that aim to compare the vegetation of ancient and recent woodland. Most studies apply one or two diversity metrics. The application of more than this has been considered poor practice, being indicative of unconsidered research design (Magurran, 2004). However, this view has been contested: the application of multiple indices has been commended for yielding greater insight into plant communities (Naaf and Wulf, 2010; Onaindia *et al*., 2004).

Richness, evenness and diversity metrics are frequently used to measure the alpha (α) diversity (sensu. Whittaker, 1972) of individual woodland sites (Heip *et al*., 1998). It is important to distinguish between the terms richness, evenness and diversity: diversity and richness in particular are employed differently and interchangeably among studies. Richness is defined as a count of species within a given area (Magurran, 2004). Evenness (equitability) measures show how equitably species abundance or cover is distributed among species (Morris *et al*., 2014). Diversity is often used as an overarching term but has a specific meaning: it is a compound measure of both richness and evenness (Morris *et al*., 2014; Tuomisto, 2012; Magurran, 2004; Smith and Wilson, 1996; Pielou, 1975). However, there is little consensus as to the interdependence of evenness and diversity, and the relative contributions of richness and evenness to diversity (Tuomisto, 2012; Jost, 2010; Magurran, 2004). As a result, a range of quantitative metrics are available that place different degrees of emphasis on richness and evenness.

Richness

Richness is defined as 'the number of species or attributes present at a specific site or sample area' (Morris *et al*., 2014:3515). Richness-based indices exist (such as Margalef's (1958) index and Menhinick's (1964) index) but are not commonly used in vegetation studies because they account for both the number of species and number of individuals of those species. The indices were originally developed for faunal studies where individuals are distinguishable, whereas individuals of clonal plant species are difficult to define (Magurran, 2004).

A number of ancient woodland studies have used richness as a method of distinguishing ancient from recent woodlands (e.g. Hermy and Verheyen, 2007; Borschsenius *et al*., 2004; De Keersmaeker *et al*., 2004). A simple count of species within a sample area is the most common richness measure employed in ancient woodland studies (e.g. Kelemen *et al*., 2014; Gotelli and Chao, 2013; Sciama *et al*., 2009; De Keersmaeker *et al*., 2004).

Measures of total species richness in all strata from canopy to ground layer show conflicting results as to whether ASNW flora is significantly richer than that of ARW or recent woodland (Sciama *et al*., 2009; Dzwonko, 2001b). Variation in outcomes may be due, in part, to the inclusion of trees in total plant species richness studies, which are potentially placed or managed by humans and therefore not reflective of the ecological capacity of the site (Vallet *et al*., 2010; Rackham, 2003; Honnay *et al*., 2002b). However, Kirby *et al*., (2016) advocate the inclusion of trees as an intrinsic element of a woodland's biodiversity. Additionally, studies vary in terms of moss species inclusion within total plant species richness: Dzwonko (2001b) and Sciama *et al*. (2009) surveyed only vascular plant species, whilst Matuszkiewicz *et al*. (2013) included mosses, but excluded liverworts. Few studies stipulate whether epiphytic, as well as terrestrial, mosses are surveyed, which has implications for the richness total as epiphytes are dependent on presence of woody species. Lichens tend to be studied separately (e.g. Ellis, 2015; Whittet and Ellis; 2013; Coppins and Coppins, 2002; Rose, 1993).

As a result of the difficulties identified in the total species richness approach, a more common approach is to restrict richness comparisons to the understorey vascular plant layers (Kelemen *et al*., 2014; Baeten *et al*., 2010; Dumortier *et al*., 2002). Sciama *et al*., (2009) compared outcomes between the total species approach and a separate shrub and herb layer count in ASNW and recent woodland. The total species count showed no significant difference in richness. However, the shrub layer was found to be significantly richer in recent woodland, whilst the herb layer was significantly richer in ASNW.

Evenness

Evenness measures show how evenly abundance or cover of a sample area is divided among the species that are present (Morris *et al*., 2014). A range of evenness indices are available (Magurran, 2004), each with attributes that might be viewed as strengths or weaknesses depending on the proposed application. Shannon's evenness (hereafter referred to as Shannon's E) (1949) is considered not to be sufficiently independent of richness, although is widely used in the literature (Magurran, 2004; Smith and Wilson, 1996). By contrast, Simpson's evenness (Simpson's E) (1949) is not sensitive to species richness (Magurran, 2004; Smith and Wilson, 1996). The emphasis placed on dominance or rarity by different indices may be useful in different environments and applications (Smith and Wilson, 1996). Smith and Wilson (1996) tested eight evenness indices, including their own, against four essential and ten desirable attributes. The Smith and Wilson (1996) evenness index fulfils a range of criteria that other indices lack, but requires a known number of individuals, which is difficult to apply to clonal plant species. However, Simpson's E, which does not require known numbers of individuals, ranks highly and is well-regarded, as it fulfils all the essential criteria and all but two of the desirable criteria (Smith and Wilson, 1996).

In the context of ancient woodland, evenness indices have been rarely applied, but despite this evenness appears to be a distinguishing factor between ASNW and recent woodland in this small number of studies (Baeten *et al*., 2010; Vellend, 2004; Verheyen *et al*., 2003a). Primary woodland was shown to have a significantly more even plant community than secondary woodland using Simpson's evenness index (Vellend, 2004). Post-agricultural recent woodlands were shown to have significantly decreased Simpson's E compared to ASNW between two timepoints (1980-2009) (Baeten *et al*., 2010). However, Verheyen *et al*. (2003a) found no significant difference across four age categories of ASNW and recent woodland using Shannon's E.

Diversity

Compound indices that integrate both richness and abundance/ cover of each species are widely used to distinguish the biodiversity value of habitats (Magurran, 2004). The different emphases placed on richness, rarity and abundance among indices means that none can be identified as the 'best' for all applications (Tuomisto, 2012). Onaindia *et al*. (2004) found Simpson's diversity index (hereafter Simpson's D) to be more effective than Shannon's diversity index (hereafter Shannon's H) in highlighting differences between old-growth woodland and recent woodlands with different levels of disturbance in Spain. Shannon's H has been shown to have many benefits: unlike alternatives, it is uninfluenced by uneven species richness among sites (Magurran, 2004), and if sufficient sites are sampled, it forms a normal distribution suitable for parametric statistical analysis (Taylor, 1978). However, Shannon's H is criticised for difficulty in distinguishing the extent to which a lower or higher value is the outcome of a change in richness versus evenness, which makes interpretation challenging (Magurran, 2004).

Despite the above issues, a small number of comparative ASNW-recent woodland vegetation studies have used compound diversity indices. Shannon's H was employed by Bossuyt *et al*. (1999) who found comparable diversity outcomes in adjacent ancient and recent woodland. Verheyen *et al*. (2003a) compared outcomes for Shannon's H and Shannon's E indices, finding no significant differences using either method across four age categories of ASNW and recent woodland.

Ancient Woodland Indicator species

Richness and/or cover of Ancient Woodland Indicator (AWI) species are accepted measures used to distinguish ASNW from recent woodland (Kirby, 2004; Rose, 1999). AWI species are a subset of herb (and some shrub) layer species particularly, but not exclusively, associated with ancient woodland on a regional scale (Glaves *et al*., 2009). They are likely to be slow-colonisers, and indicative of woodland interiors, habitat continuity, and potentially original woodland conditions (Hermy *et al*., 1999). The presence of a given number of AWI species contributes to the evidence used to designate a woodland as 'ancient' (Glaves *et al*., 2009), which lends it protection under UK law (Forestry Commission and Natural England, 2014; Communities and Local Government, 2012).

Studies consistently show that ASNW is significantly richer in those species more closely associated with older woodland, compared to recent woodland (Kelemen *et al*., 2014; Hofmeister *et al*., 2013; Brunet *et al*., 2011; Orczewska, 2009; Dzwonko, 2001b; Jacquemen *et al*., 2001; Peterken and Game, 1984). Total cover of AWI or woodland specialist species has also been shown to be significantly greater in ASNW compared to recent woodland

(Orczewska, 2009; Willi *et al.*, 2005; De Keersmaeker *et al.*, 2004). Orczewska (2009) analysed both richness and total cover of 'true woodland species' in ASNW and recent woodland, with the results of each metric corroborating the other.

The methods used to determine which species hold AWI status vary among studies. Initial UK lists were based on expert opinion and observation (Rose, 1999). However, European lists have used indices or proportion of occurrences in ancient versus recent woodland (Schmidt *et al.*, 2014; Thompson *et al.*, 2003a), statistical testing (Schmidt *et al.*, 2014; Baeten *et al.*, 2010; Thompson *et al.*, 2003a; Verheyen *et al.*, 2003b; Wulf, 1997; Honnay *et al.*, 1998) and specific indicator analysis techniques, such as Dufrene and Legendre's Indicator Analysis (Kelemen *et al.*, 2014).

Research gap

The designation, protection, and conservation management of ASNW and ARW is informed in part by vegetative assessment (Glaves *et al.*, 2009). However, lack of consistency in metrics used among studies is unhelpful for conservation decision-making, as it is difficult to establish overarching recommendations when it is unknown to what extent contrasting outcomes may be due to the metrics used and strata studied. Comparative studies of ASNW, ARW, and recent woodland some show significant differences in diversity while others show comparable outcomes (Sciama *et al.*, 2009; Verheyen *et al.*, 2003a; Brunet *et al.*, 1999). Richness in particular is used to differentiate ancient woodland from recent woodland, especially in conjunction with AWI lists. Diversity and evenness metrics have been applied in research in order to take account of abundance, as well as presence.

Despite the important conservation implications of metric choice few studies have empirically tested their outcomes in the context of ancient woodland vegetation (Onaindia *et al*., 2004; Verheyen et al., 2003a) and seemingly none in the UK. Moreover, very few studies have explicitly compared the diversity of ARW to ASNW or recent woodland, particularly of broadleaf or mixed ARW (Atkinson *et al*., 2015; Onaindia *et al*., 2013; Brunet *et al*., 2011; Kirby, 1988). Finally, although the metrics by which AWI species are identified have been criticised (e.g. Kimberley *et al*., 2013; Spencer, 1990) seemingly no studies have empirically tested the consistency of AWI identification among these metrics.

The research objectives for this thesis chapter are:

1. To identify any distinctiveness in vegetation richness, diversity, and scarcity among ancient semi-natural, ancient replanted, and recent woodland.

2. To test the affiliation of herb layer plant species to ancient semi-natural and recent woodland, using a range of metrics.

3.3 Methods

3.**3.1 Study location**

Cross-reference to sections 2.2.1 and 2.2.2.

3.3.2 Field methods and AWI definition

Cross reference to sections 2.2.3 and 2.2.4.

3.3.3 Statistical methods

Relative national prevalence of herb layer plant species in ASNW, ARW, and recent woodland was calculated from PLANTATT data (Hill *et al*., 2004). For each species recorded as part of this thesis, prevalence was taken to be the number of 10km squares in Britain in which the species is present (Hill *et al*., 2004). The mean prevalence was calculated across all vascular species (forbs, graminoids and pteridophytes) to give a value for the community of each woodland site (n=45). As data met assumptions for parametric testing, an independent t-test was used to test difference in mean species' prevalence between ASNW-ARW, ASNW-recent, and ARW-recent woodland.

To test for variation in diversity metric outcomes among ASNW, ARW, and recent woodlands, richness, evenness and diversity indices were calculated for plant communities in each woodland site (ASNW n=15, ARW n=15, recent n=15).

Richness was considered the baseline against which other diversity metrics would be compared, as it is the most commonly-used diversity measure. Richness was measured as a count of species within sample plots for: (a) all strata (canopy, shrub, herb, and moss) combined (Sciama *et al*., 2009): (b) canopy, shrub, herb and terrestrial moss strata separately; and (c) AWI species. AWI species richness (Orczewska, 2009; Willi *et al*., 2005; De Keersmaeker *et al*., 2004) was calculated for all (shrub+herb layer) AWI species and for herb layer AWI species only.

To compare the outcomes of evenness indices with richness and diversity metrics, Simpson's E (using the 1-D method) (Baeten *et al*., 2010; Smith and Wilson, 1996) and Shannon's E (Mölder *et al*., 2008; Verheyen *et al*., 2003a) were calculated for each woodland site. To compare diversity indices against other metrics, Simpson's D (also using the 1-D method) (Vellend, 2004) and Shannon's H (Mölder *et al*., 2008; Bossuyt *et al*., 1999) were also calculated for each woodland site. The choice of metrics was based on those used in prior studies and those that were appropriate for vegetation data including clonal species.

Due to the different scales of vegetation and ecological processes in woodland strata (Peterken, 1993), it was not logical to calculate a 'total' evenness or diversity indices across strata, as recognised in prior studies (e.g. Baeten *et al*., 2010; Vellend, 2004; Verheyen *et al*., 2003a; Bossuyt *et al*., 1999). Neither is there any precedent of calculating evenness or diversity indices for the subset of AWI species, so this was not performed in the present study.

For the evenness and diversity indices, transformations were conducted before analysis. DOMIN values for the tree and shrub layers were transformed to percentages using Currall's 2.6 rule (van der Maarel; 2007; Currall, 1987). For the herb and ground layer species all percentage cover data were transformed +1 because some values were <1%, which would have resulted in a lower rather than higher number when squared as part of index calculations. Simpson's E was calculated using percentage cover data normalised out of 1 (Baeten *et al*., 2010; Vellend *et al.,* 2005) within the Species Richness and Diversity programme (Seaby and Henderson, 2006). Shannon's E, Simpson's D and Shannon's H were calculated with the Diversity Excel add-in (University of Reading, 2010).

To investigate the distinctiveness of vegetation among ASNW, ARW and recent woodland, two-way multivariate comparative tests were applied for each metric to show interactions between strata and continuity (canopy, shrub, herb, and moss). A total richness value (the sum of all four strata) was also tested for difference among the three woodland types as part of the same test. Post-hoc analyses tested for pairwise differences between ASNW-ARW, ASNW-recent, and ARW-recent in each stratum separately, and for richness, AWI (all) and AWI (herb). Richness data was log transformed and subsequently met the assumptions for testing via two-way ANOVA with post-hoc Bonferroni correction to mitigate family-wise error. For the evenness and diversity metrics, the non-paremetric Scheirer-Ray-Hare tested for difference among the three woodland continuity types, with post-hoc Mann-Whitney U with Bonferroni correction.

To assess consistency among techniques used to identify indicator plants of ASNW, four established techniques were applied individually to herb layer species present in ≥20% of ASNW (n=15) plus recent woodland sites (n=15) (Schmidt *et al.*, 2014). The same indicator techniques were also used to identify potential negative indicators of ASNW (or positive indicators of recent woodland). ARW sites are not usually used when creating woodland indicator lists.

To examine consistency of results among different indicator identification metrics, four indicator metrics tested for higher species abundance/ frequency occurrence using different critieria. Firstly, the Kruskal-Wallis H non-parametric test tested for difference in the abundance of each species between ASNW and recent woodland sites (Matuszkiewicz *et al.*, 2013). Secondly, difference in frequency occurrence between ASNW and recent sites compared to the overall number of occurrences was assessed via Fisher's exact test (Wulf, 1997). Thirdly, the well-established Indicator Analysis Technique combines both abundance and frequency occurrence data to detect difference via a Monte Carlo test (Kelemen *et al.*, 2014; Dufrene and Legendre, 1997). The Indicator Analysis Technique was performed using the Indval function in the 'labsdsv' package for R (Roberts, 2016). Lastly, the percentage threshold frequency occurence technique was set at ≥75% of occurrences being in either ASNW or recent woodland sites (Schmidt *et al*., 2014) to register a species as an indicator, although this is not an inferential test. Due to multiple testing, the Bonferroni calculation was applied across the three interferential tests. Species with significant associations in at least one metric were tabulated, and those previously identified as AWI species (Kirby, 2004) were indicated with an asterisk. Those with ≥20% frequency occurrence in ASNW and recent woodland but were not significant, were listed.

3.4 Results

At the national scale, herb layer vascular species recorded in this thesis were found to be significantly less prevalent within ASNW than the species recorded in recent woodlands (p=0.030) (Fig. 3.3) (being present in significantly fewer 10km squares in Britain (Hill *et al*. (2004)). Mean species prevalence in ASNW was not significantly different to that of ARW (p=0.067). ARW versus recent woodland species showed no trend (p=0.570).

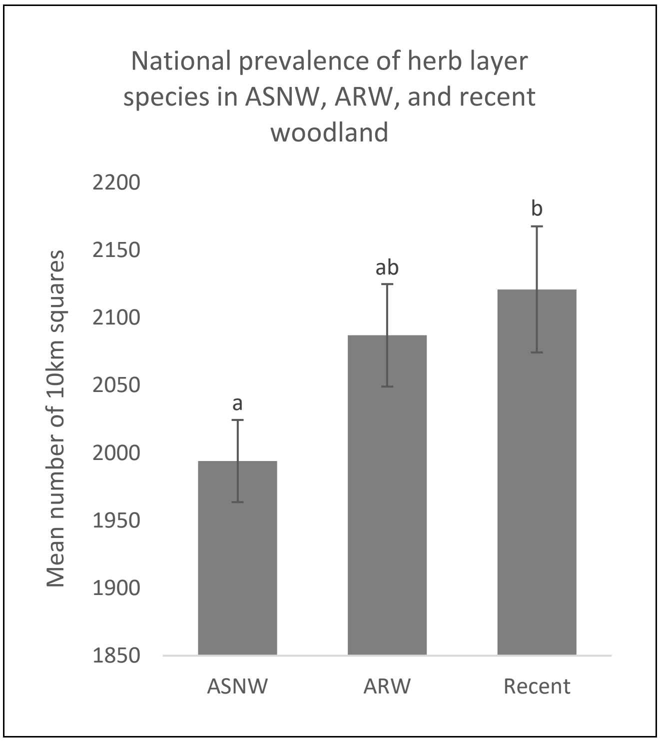

Figure 3.3. Mean national prevalence of the herb layer species recorded in this study in Ancient Semi-Natural Woodland (n=15), Ancient Replanted Woodland (n=15), and recent woodland (n=15). National data showing presence in 10km squares in Britain from PLANTATT (Hill *et al.*, 2004). Standard deviation. Independent t-test. ASNW/ ARW (p=0.067), ASNW/ recent (p=0.030), ARW/ recent (p=0.570). Different letters indicate significant difference.

The richness metric detected some significant differences among the woodland types (Fig. 3.4 and Table 3.1). ASNW exhibited significantly higher herb layer richness than ARW (p=0.028), and significantly higher richness of combined shrub and herb layer AWI species (p=0.039). Notably, the richness of herb layer AWI species was significantly higher in ASNW than in recent woodland. ASNW had a median of eight AWI herb layer species, ARW six and recent five. There were no significant differences in AWI species richness between ARW and recent woodland. In addition to Table 3.1, the total richness of canopy, shrub, herb, and moss layers combined, showed no significant difference among ASNW, ARW, and recent woodland (p=0.385).

Of the calculated indices, Shannon's E revealed a significant difference in evenness among the three woodland continuity types when all strata were considered. However, when multiple testing was conducted for each stratum separately, none was significant after correction for family-wise error. However, moss species evenness may be worthy of investigation in future research (p = 0.064). Simpson's E showed no significant differences. Neither of the diversity indices exposed any significant differences in any of the strata between any of the woodland types.

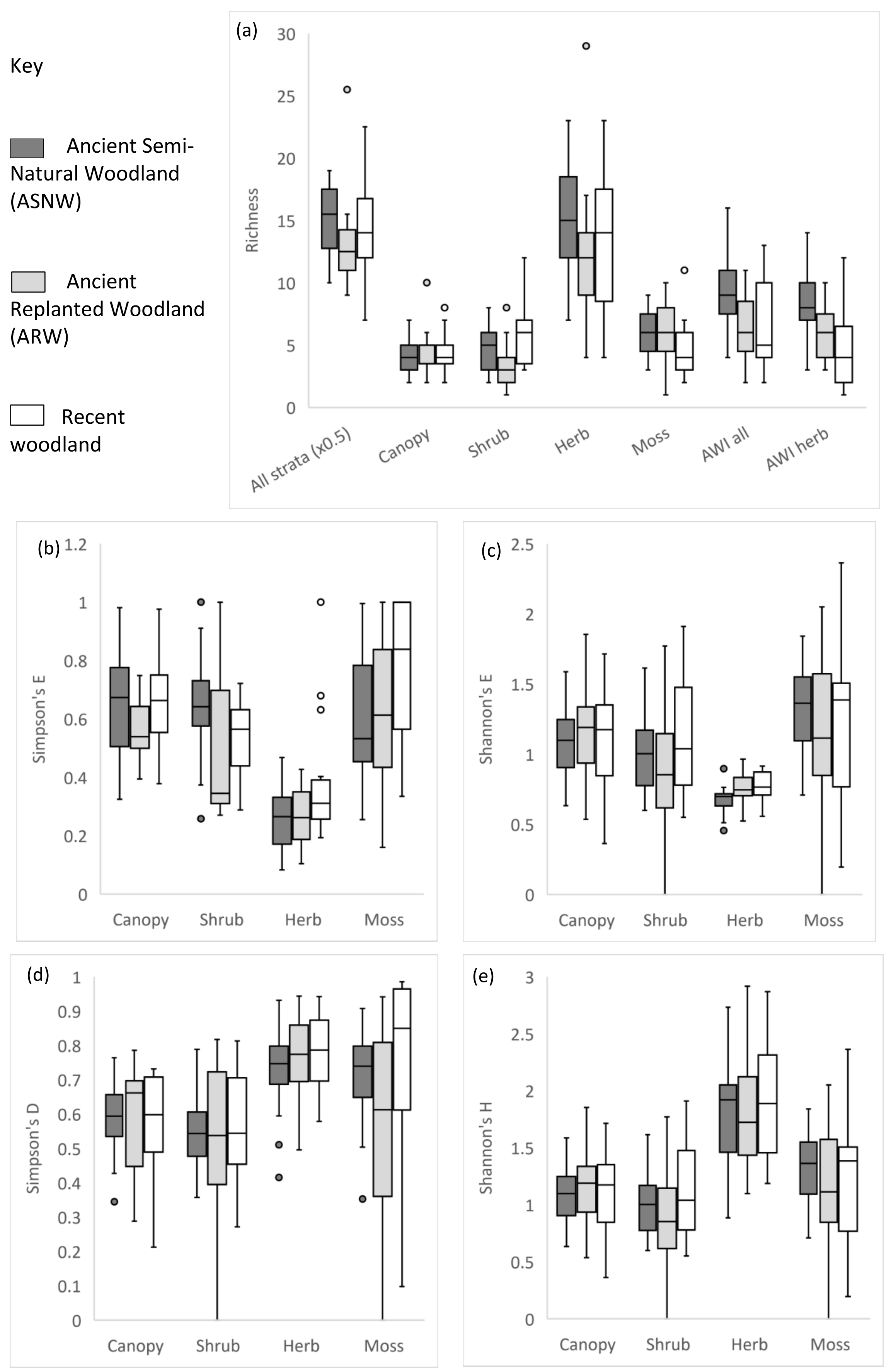

Figure 3.4. Alpha diversity metrics. Range, IQR, median. (a) Richness; (b) Simpson's E; (c) Shannon's E; (d) Simpsons D; (e) Shannon's H indices in ASNW (n=15), ARW (n=15) and Recent woodland (n=15). Outliers indicated as dot markers. See Table 3.1 for inferential test results.

Table 3.1. Metric choice influences interpretation of vegetation distinctiveness in woodlands with different continuity histories: Ancient Semi-Natural Woodland (ASNW) (n=15), Ancient Replanted Woodland (n=15) (ARW), and recent woodland (n=15). For richness only, overall difference in continuity among ASNW, ARW and recent woodland comprising canopy, shrub, herb and moss strata combined. For richness and all other metrics, difference in strata*continuity interaction among ASNW, ARW, and recent woodland. Pairwise tests between woodland continuity types separately for canopy, shrub, herb, moss. For richness only, pairwise tests for Ancient Woodland Indicators (AWI) (all = shrub and herb) and AWI (herb). Richness tested via two-way ANOVA with post-hoc Bonferroni correction. Evenness and diversity metrics tested via Scheirer-Ray-Hare with post-hoc pairwise Mann-Whitney U and Bonferroni correction **Bold text** p ≤ 0.05.

	All: Strata* Cont.	Pairwise: continuity	Canopy	Shrub	Herb	Moss	AWI (all)	AWI (herb)
Richness	0.421	ASNW-ARW	1.000	1.000	**0.028**	1.000	**0.039**	0.128
		ASNW-recent	1.000	1.000	0.970	1.000	0.062	**0.010**
		ARW-recent	1.000	1.000	0.313	1.000	1.000	1.000
Simpson's E	**0.040**	ASNW-ARW	0.608	0.272	1.000	1.000		
		ASNW-recent	1.000	0.372	0.206	0.064		
		ARW-recent	0.440	0.884	0.372	0.284		
Shannon's E	0.140	ASNW-ARW	1.000	1.000	0.176	1.000		
		ASNW-recent	1.000	1.000	0.084	0.520		
		ARW-recent	1.000	1.000	0.884	1.000		
Simpson's D	0.310	ASNW-ARW	1.000	1.000	1.000	0.764		
		ASNW-recent	1.000	1.000	0.884	1.000		
		ARW-recent	1.000	1.000	1.000	0.340		
Shannon's H	0.910	ASNW-ARW	1.000	0.764	1.000	1.000		
		ASNW-recent	1.000	1.000	1.000	1.000		
		ARW-recent	1.000	0.564	1.000	1.000		

Several species were identified as being significantly associated with ASNW or recent woodland according to one or more of the indicator metrics. Previously identified AWI species (Kirby, 2004) dominate the ASNW affiliation list, although several other AWI species showed no significant association with ASNW (Table 3.2). The Kruskal-Wallis test on species' abundance showed four species to be significantly associated with ASNW (*Brachypodium sylvaticum*, *Viola reichenbachiana, Carex sylvatica, Deschampsia cespitosa,*) (Table 3.2). Fisher's exact test on abundance showed only *V. reichenbachiana* to be significantly associated with ASNW. Dufrene and Legendre (1997) Indicator Species Analysis also showed three species to be associated with ASNW (*C. sylvatica, D. cespitosa and Hyacinthoides non-scripta*). Three species were also identified by ≥75% occurrence in ASNW method (*V. reichenbachiana*, *Primula vulgaris* and *Poa nemoralis*).

Some species are potentially acting as negative indicators of ASNW by exhibiting significant association with recent woodland. *Asplenium scolopenrium* was significant for all four tests. The Kruskal-Wallis test, Fisher's exact test, and the 75% threshold all identified *Urtica dioica*. The 75% threshold alone identified *Galium aparine* and *Geranium robertianum*.

Table 3.2. Species association/ indicator value between Ancient Semi-Natural Woodland (ASNW) (n=15) and recent woodland (n=15). Comparison of indicator metrics for species with p<0.05 or sufficient % in any category. (bracketed number indicates a negative correlation). Bonferroni correction applied across the three inferential tests. Species marked with * are previously identified Ancient Woodland Indicator (AWI) species (Kirby, 2004). Species with ≥20% frequency occurrence across both woodland types were tested. Additionally, those species with ≥20% frequency occurrence that were not significantly associated with either woodland type are listed for further information.

	Abundance. Kruskal-Wallis H (Matuszkiewicz *et al.*, 2013)	Frequency occurrence. Fisher's exact test (Wulf 1997)	Indicator Analysis. Dufrene and Legendre (1997)	75% frequency occurrence threshold. Schmidt *et al.* (2014)
Species with significantly higher abundance/ frequency occurrence in ASNW				
B. sylvaticum	**0.014**	0.075	1.000	71%
*C. sylvatica **	**0.015**	0.198	**0.024**	68%
D. cespitosa	**0.021**	0.081	**0.048**	73%
*H. non-scripta **	0.060	0.645	**0.030**	59%
*P. quadrifolia**	0.123	0.645	0.252	**75%**
*P. nemoralis**	0.210	0.240	0.972	**86%**
*P. vulgaris**	0.141	0.240	0.501	**86%**
*V. reichenbachiana**	**0.012**	**0.042**	(0.561)	**82%**
Species with significantly higher abundance/ frequency occurrence in recent woodland				
A. scolopendrium	**0.009**	**0.018**	**0.021**	**100%**
G. aparine	0.069	0.240	0.192	**86%**
G. robertianum	0.108	0.327	0.531	**78%**
U. dioica	**0.003**	**0.006**	0.543	**91%**

Species with ≥20% frequency occurrence with no significantly higher abundance/ frequency in either woodland type: *Allium ursinum*; Anemone nemorosa*; Arum maculatum; Circaea lutetiana; Euphorbia amygdaloides*; Fragaria vesca; Galium odoratum*; Geum urbanum; Glechoma hederacea; Lamiastrum galaeobdolon*; Mercurialis perennis*; Oxalis acetosella*; Sanicula europaea*; Vicia sativa; Viola riviniana*; Dryopteris filix-mas.*

3.5 Discussion

The application of a range of quantitative diversity metrics has given greater insight into the structure of plant communities in ASNW, ARW, and recent woodland, as well as identifying the most and least useful indices for differentiating the woodland types.

The mean national prevalence of herb layer species recorded in ASNW in the present research was significantly lower than for recent woodland (p=0.030) (Fig. 3.3). This indicates greater scarcity of the species present in ASNW, possibly as a result of limited habitat availability due to loss and fragmentation processes. Some species associated with ancient woodland hold conservation designations due to their threatened status, for example *P. vulgaris* and *H. non-scripta*, both of which were shown to be significantly more associated with ASNW than recent woodland using a range of indicator metrics (Table 3.2) (JNCC, 2010). This relative scarcity further justifies research of ASNW plant communities.

ARW is generally considered to be worthy of restoration due to its potential to recover the flora of ASNW (Thompson *et al*., 2003b; Pryor *et al*., 2002). However, the results of this study showed no significant differences between ARW and recent woodland communities across the metrics (Table 3.1). The richness metric identified two significant differences between the strata of ASNW-ARW, and only one between ASNW-recent woodland. Notably, there was no significant difference in number of AWI species between ARW-recent. These are important findings as so few studies have compared richness of ASNW and predominantly broadleaf ARW: many studies focus on ASNW-recent comparisons, and a very few on ASNW-ARW (Atkinson *et al*., 2015; Brown *et al*., 2015; Kirby, 1988).

AWI richness clearly differentiated ASNW from both ARW and recent woodland. ASNW was significantly richer in shrub+herb AWI species than ARW, and significantly richer in herb

AWI species than recent woodland. The significantly higher richness of shrub+herb AWI species in ASNW compared to ARW has possibly been influenced by management of the shrub layer in ARW. If AWI lists are used to identify ARW, a lower threshold allowance could be given to compensate for fewer woody AWI species.

The present study shows ASNW to have a median of eight shrub+herb layer AWI species (Fig. 3.4a). According to AWI thresholds used by some organisations to assess woodland quality (Glaves *et al*., 2009) 8-12 is considered ‘very good’; 4-8 ‘good’; < 4 ‘poor’, which means all ASNW woodland sites in this study would be rated as ‘good’ or ‘very good’. However, thresholds vary: in other organisations, 10 or even 12 AWI species were required to classify an ancient woodland (Glaves *et al*., 2009), which exceeds the number found in many ASNW in the present study (Fig. 3.4a).

Nonetheless, both ARW and recent woodlands in the present study are considered to have a ‘good’ (Glaves *et al*., 2009) number of AWI species when compared to the thresholds outlined above. For the purposes of biodiversity evaluation, their actual AWI richness is more important than their richness relative to ASNW. In a study of pine-dominated ARW, it was noted that half of sites (n=39) contained three or fewer AWI species (Brown *et al*., 2015). By contrast, in the present study the relatively high AWI richness in predominantly broadleaf ARW (median = 6) and recent woodland (median = 5) possibly reflects ease of colonisation due to the proximity and connectivity of ASNW, ARW, and recent woodland (Dumortier *et al*., 2002). within the densely wooded Strategic Nature Areas. As such, the purpose of the SNAs (conservation and restoration of priority ancient woodland habitat) is validated, as both ARW and recent woodland have potential for restoration or enhancement.

Of the two evenness metrics, Shannon's E was partially effective in distinguishing the woodland types in detecting a significant differences among the three woodland continuity categories when all strata were combined (Figure 3.4c; Table 3.1). Simpson's E detected no differences. Conversely, in a prior study, Simpson's E detected difference between ASNW and recent woodland (Vellend, 2004), but with higher evenness in ASNW (Fig. 3.4b). Another study using Shannon's E failed to detect difference across four woodland age categories (Verheyen et al., 2003a).

Of the two diversity indices, neither Simpson's D nor Shannon's H could be considered at all effective in differentiating ASNW, ARW, and recent woodland communities (Fig. 3.4d; 3.4e; Table 3.1). This outcome corroborates the findings of Bossuyt *et al*. (1999) who found no significant difference in Shannon's H between ASNW and recent woodland.

The lack of consistency in the methods used to create AWI lists may lead to different interpretations of the value of ASNW and indeed whether it is identified at all. When the results of all four metrics are viewed as a whole, many species identified as indicators by Rose (1999) and Kirby (2004) were shown to be significantly associated with ASNW in most cases (Table 3.2). However, when each metric is viewed individually, different species were identified and there was great variation in how many species were found to be significantly associated with ASNW or recent woodland. The Kruskal-Wallis test, based on abundance data, identified over twice as many species as being affiliated with ASNW, compared to Fisher's exact test, which is based on frequency occurrence. The Dufrene and Legendre (1997) analysis combines abundance and frequency occurrence but only identified three ASNW indicator species. *V. rechenbachiana* had significant associations with ASNW according to three metrics (more than any other species) but was found to be slightly associated with recent woodland (p=0.561) according to the Dufrene and Legendre

Indicator Analysis technique. The results suggest that one metric is unlikely to be sufficient when creating AWI lists.

Negative indicators (species significantly associated with recent woodland) could be further considered as a tool to distinguish ASNW and recent woodland. Prior studies have also found significant negative indicator results (Kelemen *et al.*, 2014; Kirby and Morecroft, 2011), and as reported by Glaves *et al.* (2009) some local authorities already employ negative indicators. The only species to demonstrate a consistently significant affiliation across all metrics was *A. scolopendrium* in recent woodland. Seemingly no prior list exists of species associated with ARW, which may impact on correct identification.

3.6 Conclusions and recommendations

Each diversity metric led to contrasting conclusions as to the distinctiveness of ASNW, ARW, and recent woodland. Richness most effectively distinguished between the woodland types, showing ASNW to have greater herb layer and shrub+herb AWI species richness than ARW, but not recent woodland. However, ASNW exhibited significantly higher richness in herb layer only AWI species than recent woodland. Simpson’s E and provided a potentially informative additional dimension, indicating an overall significant difference in diversity through strata*continuity interactions. Diversity metrics Simpson’s D and Shannon’s H were ineffective in identifying differences among the woodlands.

Allowance or recalibration of AWI thresholds could be considered when AWI richness is used to identify or measure the biodiversity value of ARW. The lack of distinction between ARW and recent woodland across all metrics has potential implications for restoration of

ARW within the SNA: future chapters will address the qualitative differences in community composition in terms of actual species present.

Established metrics used to identify AWI species classified different species, and different number of species, as indicators of ASNW, and of recent woodland. Inconsistency among the metrics has implications for the synthesis of studies based on AWI lists achieved by different means.

Outcomes of indicator species metrics indicate that a purely statistical approach may be inappropriate, and the integration of observation in the compilation of lists (as done for early lists) may be beneficial. More than one index, in addition to expert observation, is recommended, which would also permit scarce species to be included in AWI lists.

The metrics used to create AWI lists may lead to different interpretations of the ancient status of a woodland, which has policy and management implications. Greater consistency of metrics, where appropriate, could assist in formulating a more robust rationale for protection of ASNW and ARW, as well as for recent woodland sharing similar plant community characteristics.

4. Plant community differentiation and strata interactions among ancient semi-natural, ancient replanted, and recent woodlands

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Outputs arising from this chapter:

Swallow, K., Wood, M. and Goodenough, A. (2018) *Plant community differentiation among ancient semi-natural, ancient replanted, and recent woodland.* Royal Agricultural University research seminar. Cirencester, 25 January

Swallow, K. (2015) *Phytosociology of ancient woodland indicator species in the Cotswolds.* Postgraduate Research Student Conference. University of Gloucestershire. 22 June

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Primula vulgaris, Middleton Hill Plantation, Sapperton Strategic Nature Area

07/04/2015 Grid ref: SO 93777 02372

4.1 Chapter scope

The distinctiveness of ancient woodland vegetation is a well-established concept that provides a strong rationale for conservation. The previous chapter has shown ASNW to have significantly higher total species richness than ARW, and a significantly higher AWI species richness than recent woodland. Chapter 3 focused on alpha richness, whereas Chapter 4 shifts to consider gamma richness, and beta-scale community composition. The present chapter researches, for the first time, beta-scale plant community similarity for the canopy, shrub, herb (and subsets of AWI and non-AWI species), and terrestrial moss layers among ASNW, ARW, and recent woodland. Beta-scale AWI community analysis contributes substantially to Chapter 5, where explanatory variables are tested for their relative influence on AWI community composition. The present chapter assesses biotic explanations for AWI richness and similarity in the form of inter-strata interactions. Herb layer community composition by life trait is analysed among the three woodland types.

4.2 Introduction

When measured at the alpha diversity scale (sensu. Whittaker, 1972) it is well-established that woodlands with long continuity have a more distinctive and specialised flora than recent woodlands (Kelemen *et al*., 2014; Rackham, 2008; Honnay *et al*., 1998; Peterken and Game; 1984). As such, they are important refugia for the conservation of specialist and protected plant species (Corney *et al*., 2008b), and are a source for dispersal of these to other areas (Brunet *et al*., 2011; Petit *et al*., 2004; Jacquemen *et al.,* 2003a).

Alpha, beta and gamma diversity

Since the seminal work by Whittaker (1972), biodiversity is often measured at three major scales: alpha, beta, and gamma. Alpha diversity is a richness measure at the individual ecosystem scale (Magurran, 2004; Whittaker, 2001; Whittaker, 1972). Beta diversity measures spatial and/ or temporal difference in community composition between two or more ecosystems (Magurran, 2004; Whittaker, 2001; Whittaker, 1972). Beta diversity is increasingly recognised as comprising two components: (a) the mean difference in species composition; and (b) the within group variance in species present among communities at the beta scale (Baslega, 2010). Gamma diversity is the richness of species within a landscape or region, a wider scale measure than alpha diversity.

A common approach to floristic comparison among woodlands with different continuity histories is to compare alpha richness of species classified as woodland specialists, and/or AWI species (e.g. Stefanska-Krzeczek *et al*., 2016; Brown *et al*., 2015; Kelemen *et al*., 2014; Hofmeister *et al*., 2013; Brunet *et al*., 2011; Orczewska, 2009; Hermy *et al*., 1999). However, the alpha richness measure does not fully recognise the role of individual species in community composition: two ecosystems with very different species within their communities could have similar richness.

In response, beta community composition is attracting increasingly more research attention in the wider field of ecology (Anderson *et al*., 2011), and further progress in this area could be beneficial for ancient woodland restoration and connectivity in Britain. Globally, an increasing number of studies employ species-based ordination techniques to compare plant communities of woodlands with contrasting land-use histories at the beta landscape scale (Berges *et al*., 2017; Vukov *et al*., 2016; Atkinson *et al*., 2015; Coote *et al*., 2012; Naaf and Wulf, 2010; Keith *et al*., 2009; Vellend *et al*., 2007). However, only a small proportion of this research has been conducted on British woodlands: Vellend *et al*. (2007) incorporated one British data set from Peterken and Game (1984), while Atkinson *et al*. (2015) compared the effects of ARW restoration techniques on understorey communities.

Very few studies have compared alpha, beta and gamma measures for the same data set in the context of comparing ancient and recent woodland. A notable study by Baeten *et al*. (2010) found ASNW to have significantly higher richness of woodland herbs at the landscape-scale than post-agricultural woodland, but no significant difference in alpha species richness. Naaf and Wulf (2010) combined both alpha and beta measures to assess biotic homogenisation across 175 woodlands over 200 years old, finding no strong evidence of biotic impoverishment (alpha) but a trend towards increasing similarity among woodland communities (beta). Seemingly no studies have compared gamma richness of ASNW, ARW, and recent woodland at the landscape or regional scale.

Species-based community composition

Community comparison of ASNW, ARW, and recent woodland at the species level is especially relevant to ancient woodland restoration and connectivity (Vukov *et al*., 2016; Brown *et al*., 2015; Palo *et al*., 2013; Thompson *et al*., 2003b). Assessment of the existing community differentiation between these woodland types informs conservation management and resource prioritisation (Palo *et al*., 2013). Additionally, species-based community analysis is employed to assess biotic homogenisation (Naaf and Wulf, 2010; Vellend *et al*., 2007; Vellend, 2004), and to compare restoration or management influence on the understorey strata (Atkinson *et al*., 2015). Abiotic characteristics that may determine variation in community composition are increasingly studied and will be researched in a later chapter.

Studies of species-based community composition consistently report significant differences in the herb or understorey strata between woodlands of different ages, and/ or canopy structures. For example, significant differences in the understorey community composition were found between *Quercus*-dominated and *Fraxinus*-dominated ASNW, and between these and *Fraxinus* sp., *Larix* sp., *Picea stichensis* and *Picea abies* plantations (Coote *et al*., 2012). Likewise, significant differences were found in the herbaceous community pairwise among old ancient woodland, young ancient woodland and historical woodland cores (Kelemen *et al*., 2014). In a British study a significant difference was found in ground flora composition among native, thinned, clearfelled, and plantation woodlands (Atkinson *et al*., 2015). Although pairwise testing was not undertaken, Non-Metric Multidimensional Scaling (NMDS) plots showed considerable distance in woodland species communities on the ordination plot between native (ASNW) and the three other woodland management types.

Prevalence of AWI species has been proven to be associated with richness of other woodland species. Rose (1999) described the positive correlation between the number of AWI species and other woodland plants in southern England. A recent study by Stefanska-Krzaczek *et al*. (2016) tests the oft-cited belief that AWI prevalence is linked to higher species richness. Using a synecological grouping system of AWI species, they found that woodlands with an AWI group present had a highly significant greater number of herbaceous species than those without. Additionally, the higher the number of groups present, the higher the average richness of herbaceous species, shrubs and trees, ancient woodland species and closed woodland species.

Life traits community composition

To further understand community differentiation between ancient and recent woodland, herb layer community composition is often studied in relation to the proportion of species with different life strategies. Community comparisons of ASNW and recent woodlands via Grime's (1977) life strategies for herbaceous plants show consistent results in prior studies. The time required for colonisation is a differentiating factor in the community composition of ancient versus recent woodland. S-strategists appear to be associated with ancient woodland (Sciama *et al*., 2009; Hermy *et al*., 1999), and with increasing sequential categories of woodland age (Bossuyt and Hermy, 2000). Conversely, C- and R-strategists appear to be associated with recent woodland (Berges *et al*., 2017; Sciama *et al*., 2009; Hermy *et al*., 1999). To date, ARW has seemingly not been studied in this respect.

Dispersal mechanisms have been found to partly explain woodland species community composition. In particular, prior studies have shown an association between myrmechory (ant-dispersed - short-range dispersal vector) and AWI status (Kelemen *et al*., 2014; Hermy *et al*., 1999). Several studies have assessed the proportion of AWI or woodland species that are ant-dispersed (Kelemen *et al*., 2014; Kimberley *et al*., 2013; Hermy *et al*., 1999), but very few have conducted a community comparison. However, Kimberley *et al*. (2013) found that dispersal vectors were not a distinguishing factor between AWI species and other woodland plants.

There are few phenological studies of ancient versus recent woodland flora, but there is seemingly a link between vernal species (spring flowering and leafing, wilting before summer) and older woodland. In core woodland of 198-316 years continuous cover, Brunet *et al*. (2011) reported a significantly higher mean cover of vernal plants than in recent post-agricultural woodland. Vernal and wintergreen species are more frequent among AWI species than general woodland species (Brunet *et al*., 2011; Hermy *et al*., 1999). Vernal species tend to be geophytes (perennation from underground storage organ, such as a bulb), which in turn are associated with ASNW (Brunet *et al*., 2011). Again, comparisons with ARW are unavailable.

The regenerative life forms of woodland species can show distinction between ancient and other woodlands. Several studies have compared community composition according to Raunkiaer's (1937) life forms, with a clear trend of geophyte association with ancient woodland in most studies (Kelemen *et al*., 2014; Verheyen *et al*. 2003b; Hermy *et al*., 1999; Wulf, 1997). This regenerative strategy may also account for slow colonisation of new habitats due to slower spread by vegetative structures such as rhizomes, tubers, bulbs and corms, rather than seed dispersal (Verheyen *et al*, 2003a; Hermy *et al*. 1999).

Strata interactions

The phytosociology (plant community composition and relationships) of woodland flora is a debated concept, due to the stratification of vegetation and potential human intervention in some of those layers. Several studies omit a survey of tree species due to the likelihood of their being planted, and/ or considering that the canopy layer has little impact on the understorey (Vallet *et al*., 2010; Honnay et al., 2002; Graae, 2000). Peterken (1993) and Rackham (2003) critique the phytosociological approach, emphasising a weak or inconsistent correlation between the tree and understorey layers, not least that they respond to their environment at very different spatial and temporal scales.

Where analyses of strata interactions have been conducted, canopy richness and composition have been shown to have an inconsistent influence on understorey communities. When all herb layer species are considered, canopy richness has been shown to correlate positively and significantly with herb layer richness (Brunet *et al*., 2011; Vockenhuber *et al*., 2011; Mölder *et al*., 2008). However, when herb layer AWI or specialist species are considered, there is no consensus among studies. Orczewska (2009) concluded that both ancient and recent *Fraxinus-Alnus* carr had a significantly higher abundance, but not number, of AWI species, than ancient or recent *Quercus-Carpinus* and wet *Alnus* woodland. However, Thomaes *et al*. (2012) found no effect of tree species on strict woodland species nor AWI in ash and poplar post-agricultural plantations. This variation in results may be accounted for by differing localities and methods. Additionally, the capability of AWI species to survive under sub-optimal conditions has been noted by Honnay *et al*. (2005).

The influence of the understorey community on AWI or specialist woodland plant species has been considered by very few studies. When tree + shrub layer percentage cover was combined, no significant correlation with herb layer specialist nor generalist species richness was identified (Brunet *et al*., 2011). However, tree + shrub cover correlated significantly and negatively with herb layer specialist cover (Brunet *et al*., 2011).

Research gap

Beta and gamma diversity of ancient woodland receive comparatively little attention compared to alpha diversity, yet both are important in ancient woodland conservation: alpha diversity for correct designation of ancient woodland sites in order to protect them; and beta and gamma diversity for enhancing understanding of dispersal processes of specialist woodland species.

Seemingly no studies have conducted a comparison of community differentiation across all three of ASNW, ARW, and recent woodlands. Of studies that have researched some combination of these woodland types, very few have tested the within-woodland-type community variance relative to that of other woodland types (Coote *et al*., 2012). Likewise, there are seemingly no prior studies of ARW community differentiation by life traits.

A subset of AWI species or woodland specialists, rather than the whole plant community, is commonly surveyed in ancient woodland studies (e.g. Kelemen *et al*., 2014; Hofmeister *et al*., 2013; Brunet *et al*., 2011; Orczewska, 2009; Brunet *et al*., 1999; Dzwonko 2001b; Jacquemen *et al*., 2001). Fewer studies assess all woodland herbs or the flora of other strata in addition to woodland specialists (Atkinson *et al*., 2015; Coote *et al*., 2012; Orczewska, 2009; Sciama *et al*., 2009).

This chapter fulfils the following overarching research objectives:

1. To assess the degree to which the floristic community composition differs among ancient semi-natural, ancient replanted, and recent woodland.

2. To examine biotic influences on community composition in ancient semi-natural, ancient replanted, and recent woodland.

Within the above objectives, this chapter addressed the following research questions:

a. How distinct is ancient woodland flora at the alpha, beta and gamma scales?

b. How does the composition (including mean variance) of canopy, shrub, herb, and moss layer communities, vary among ancient semi-natural, ancient replanted, and recent woodland sites?

c. Is AWI richness and composition influenced by canopy, shrub, non-AWI or moss communities?

d. Is there is a difference in community composition by life traits among ancient semi-natural, ancient replanted, and recent woodland?

4.3 Methods

4.3.1 Study location

Cross reference to sections 2.2.1 and 2.2.2 for an extended description.

4.3.2 Field methods and AWI definition

Cross reference to sections 2.2.3 and 2.2.4.

NB: For this chapter all vegetation data was presence/absence, not percentage cover.

4.3.3. Statistical methods

All data analyses used presence-absence count data. Such data are commonly used to assess alpha richness, and constitute the most standard measure of beta community composition (Legendre *et al*., 2005; Koleff *et al*., 2003).

To assess alpha richness of ASNW (n=15), ARW (n=15), and recent woodland (n=15), richness was measured as a count of species per woodland site (Baeten *et al*. 2010) for: (a) all strata (canopy, shrub, herb, moss) combined (Sciama *et al*., 2009); and (b) canopy, shrub, herb, moss, and subset of AWI (herb layer) communities separately. Richness data was log transformed to meet assumptions for parametric testing. To test for any difference in alpha richness pairwise between ASNW-ARW, ASNW-recent, and ARW-recent woodland for all, and for separate strata a two-way ANOVA was used with post-hoc Bonferroni correction to mitigate family-wise error. Means reported are based on raw data for clarity.

To calculate gamma richness, presence of individual species was recorded per woodland site (Jamoneau *et al*., 2012; Naaf and Wulf, 2010; Vellend *et al*., 2007). The number of species present across all sites of each woodland type were tested for difference against total species richness of all woodland types via Fisher's exact test for: (1) all strata; and (2) for each stratum separately. These were pairwise tests between ASNW-ARW, ASNW-recent, and ARW-recent. Bonferroni was again applied to mitigate family-wise error.

Beta scale community difference was tested within ASNW, ARW, and recent woodland strata using Non-metric Multi Dimensional Scaling (NMDS) (Atkinson *et al*., 2015; Onaindia *et al*., 2013; Verheyen *et al*., 2003b). This test used matrices of pairwise Jaccard distances between all permutations of each of the 45 woodland sites. Strata were separately tested as canopy, shrub, herb, and terrestrial mosses. As AWI species are recognised to function as a guild (Hermy *et al*., 1999), the herb layer was subdivided and tested as AWI and non-AWI communities.

NMDS was the preferred test because it allows choice of distance measure, whereas alternatives of Principal Components Analysis or Canonical Correspondence Analysis use Euclidean distance. The Jaccard distance measure was selected (as per Oksanen, 2015; Naaf and Wulf, 2010; Jaccard, 1912), as the objective was to test community difference by presence of species, rather than abundance. Anderson *et al*. (2011) note that species presence is often preferred because species themselves are the usual units used in biodiversity conservation. These elements are appropriate in the present study, because the presence, rather than abundance, of particular species is an important factor in determining the ancient status of a woodland (Rackham, 2008; Kirby, 2004; Rose, 1999).

NMDS analysis was conducted using the 'metaMDS' function in the Vegan and MASS packages for R (Oksanen *et al*., 2017; Gardener, 2014; Hovanes, 2013), and plotted via the 'ordiplot' function in BiodiversityR (Oksanen *et al*., 2017; Kindt and Kindt, 2017; Gardener, 2014; Hovanes, 2013; Kindt and Coe, 2005). Stress was maintained <0.20 by using 3 dimensions (Gardener, 2014; Clarke and Warwick, 2001). Inferential testing for community difference was conducted pairwise between ASNW-ARW, ASNW-recent, and ARW-recent via PERMANOVA in the 'Adonis' (Analysis of Dissimilarity) function in Vegan for R (Oksanen *et al*., 2017; Atkinson *et al*., 2015; Hovanes, 2013; Thomaes *et al*., 2012; Baeten *et al*., 2010).

It is recognised that significant differences in community data tested via distance-based analysis (such as NMDS with PERMANOVA) may be influenced by within-group variance of the similarity scores, as well as different mean values (Warton *et al*., 2012; Anderson 2006). Beta diversity is considered to comprise both elements: the mean reflects the species composition, while the variance reflects the within-group variation in community composition at the beta scale. The variance is sometimes ignored, leading to misinterpretation of results (Warton *et al*., 2012). To establish whether significant results from distance-based analyses are the result of the mean or turnover, the variance must be tested separately.

In this chapter, the potentially confounding effects of variance were considered as informative rather than undesirable (Anderson *et al*., 2011). Variance was tested using the 'betadisper' function in the Vegan package for R with Jaccard distance measure (Oksanen *et al*., 2017; Anderson 2006). Pairwise testing for significant beta community difference among equal numbers of ASNW, ARW and recent woodland communities was conducted using the Tukey's HSD wrapper within the 'betadisper' function (Oksanen *et al*., 2017).

To detect any inter-strata influence on the AWI community within each woodland type, alpha richness and Jaccard similarity scores of the AWI community were correlated with the same factors for the canopy, shrub, non-AWI and moss communities for ASNW, ARW and recent woodland separately. It was important to include the non-AWI herb stratum community to understand the relative influence of each species grouping. Unlike the woody or moss strata, the non-AWI community functions at a similar spatial and temporal scale as the AWI grouping, and any influence provides a baseline against which to assess the influence of the other strata. The ARW canopy and non-AWI data were transformed (lg10), and raw mean values are reported. For richness, a paired *t* test was applied between each stratum and the corresponding AWI community for that site (n=45). SPSS was used to perform this analysis. To assess whether similarity of AWI communities was reflected in other strata, a two-tailed Mantel test with Pearson's correlation (randomisation method with 10,000 permutations) (McCune and Grace, 2002) was applied to Jaccard similarity matrix of the AWI community in each woodland type against canopy, shrub, non-AWI and moss matrices in XLSTAT (Addinsoft, 2017).

Community composition by life traits were compared pairwise among ASNW, ARW, and recent woodland for Grime's (1977) life strategies, Raunkiaer's life forms (1937), dispersal vectors, earliest flowering month, mean seed weight, and specific leaf area. Trait data was sourced from: LEDA database (Kleyer *et al*., 2008); Grime (1988), PLANTATT (Hill *et al*., 2005); Ecological Flora Database (Fitter and Peat, 1994). Where there were data gaps in these sources, a small number of species traits were found in Smith (2012), Tamis *et al*., (2004) and Chaideftou (2009). Grime's life strategies were condensed into seven major categories following Hermy *et al*. (1999). Categorical data were tested via a chi-squared contingency table, and continuous data were tested with independent t-tests where parametric assumptions were met (earliest flowering month, and specific leaf area), and by Mann-Whitney U where assumptions were not met (mean seed weight).

4.4 Results

The total number of species recorded in all plots across all sites was 131: 17 were canopy species; 19 shrub layer species; 71 herb layer species (of which 25 were AWI species); 25 terrestrial moss species.

Alpha richness (Table 4.1) of AWI species was significantly higher in ASNW than in recent woodland. The herb layer was significantly richer in ASNW than in ARW (Table 4.1)

Gamma richness indicated that ASNW was overall significantly less species rich across all woodland sites than both ARW and recent woodland (Table 4.1). For individual strata, there were no significant differences in any of the pairwise comparisons for each stratum, although herb layer gamma richness ASNW-recent (p=0.053) may be worthy of further investigation (Table 4.1).

Alpha	All strata	Canopy	Shrub	Herb	Moss	AWI (herb)
ASNW	29.87 ± 1.56 a	4.20 ± 0.38 a	4.80 ± 0.46 a	15.00 ± 0.33 a	5.87 ± 0.50 a	8.33 ± 0.85 a
ARW	26.67 ± 1.97 a	4.47 ± 0.49 a	3.47 ± 0.48 a	12.27 ± 1.45 b	5.47 ± 0.70 a	6.00 ± 0.54 ab
Recent	28.92 ± 2.04 a	4.47 ± 0.41 a	5.67 ± 0.68 a	14.07 ± 1.49 ab	4.73 ± 0.63 a	4.92 ± 0.83 b
Gamma	All strata 131 species	Canopy 17 species	Shrub 19 species	Herb 70 species	Moss 25 species	AWI (herb) 25 species
ASNW	82 a	9 a	14 a	43 a	17 a	21 a
ARW	99 b	14 a	14 a	51 a	21 a	20 a
Recent	106 b	12 a	19 a	57 a	18 a	19 a

Table. 4.1. Mean alpha richness of Ancient Semi-Natural Woodland (ASNW) (n=15), Ancient Replanted Woodland (ARW) (n=15) and recent woodland (n=15) ± s.e. tested with two way ANOVA with Bonferroni correction. Gamma richness of species of the total present across all sites tested with Fisher's Exact test. Shared lettering indicates no significant difference pairwise between ASNW, ARW and recent woodland (p<0.05).

Community analysis showed significant difference in the mean canopy composition of ASNW and ARW, while recent woodland shared elements of both (Fig. 4.1a). The status of ASNW versus ARW explained 16% of the difference in community composition. There was no significant difference of species variance for the canopy among the woodland types (Fig. 4.1a), therefore difference between ASNW and ARW can be attributed to the mean difference in species composition.

For the shrub layer ARW and recent woodland were significantly different in terms of mean species composition, and not significantly different in terms of variance (Fig. 4.1b). ARW/recent status explained 7% of the difference in community composition.

The herb layer showed significant community difference among all woodland types, although for ASNW-ARW and ASNW-recent, this significant difference is influenced by species variance and cannot be solely attributed to difference of mean (Fig. 4.2a). For ASNW-ARW 6% of the community variation was explained by woodland continuity status, 11% for ASNW-recent and 8% for ARW-recent.

When the subset of AWI communities was tested, recent woodland was significantly differently composed and more varied in its herb layer composition than either ASNW and ARW. There was no significantly different variance for AWI species suggesting that each woodland type contains AWI species drawn from largely the same group of species (although p=0.053 between ASNW and recent woodland suggesting this avenue may be worthy of further research) (Fig. 4.3a). The percentage of community variance explained by continuity status was 7% between ASNW-ARW, 9% ASNW-recent, and 6% ARW-recent.

The subset of non-AWI species showed significant differences in mean composition between ASNW-Recent, explaining 7% of the variation, but this may be partly attributable to a significant difference in variance (Fig. 4.3b). For the non-AWI community ARW-recent showed a significantly different mean, explaining 6% of the variation, and no significant result for variance (Fig. 4.3b). Non-AWI species between ASNW-ARW showed no significant difference of the mean, but did exhibit significantly different variance (Fig. 4.3b).

The terrestrial moss community exhibited significant differences in the mean composition of Recent woodland compared to both ASNW and ARW, none of which differed significantly for variance (Fig. 4.2b). For mosses, woodland continuity explained 10% of ASNW-recent 10%, and 7% of ARW community variation.

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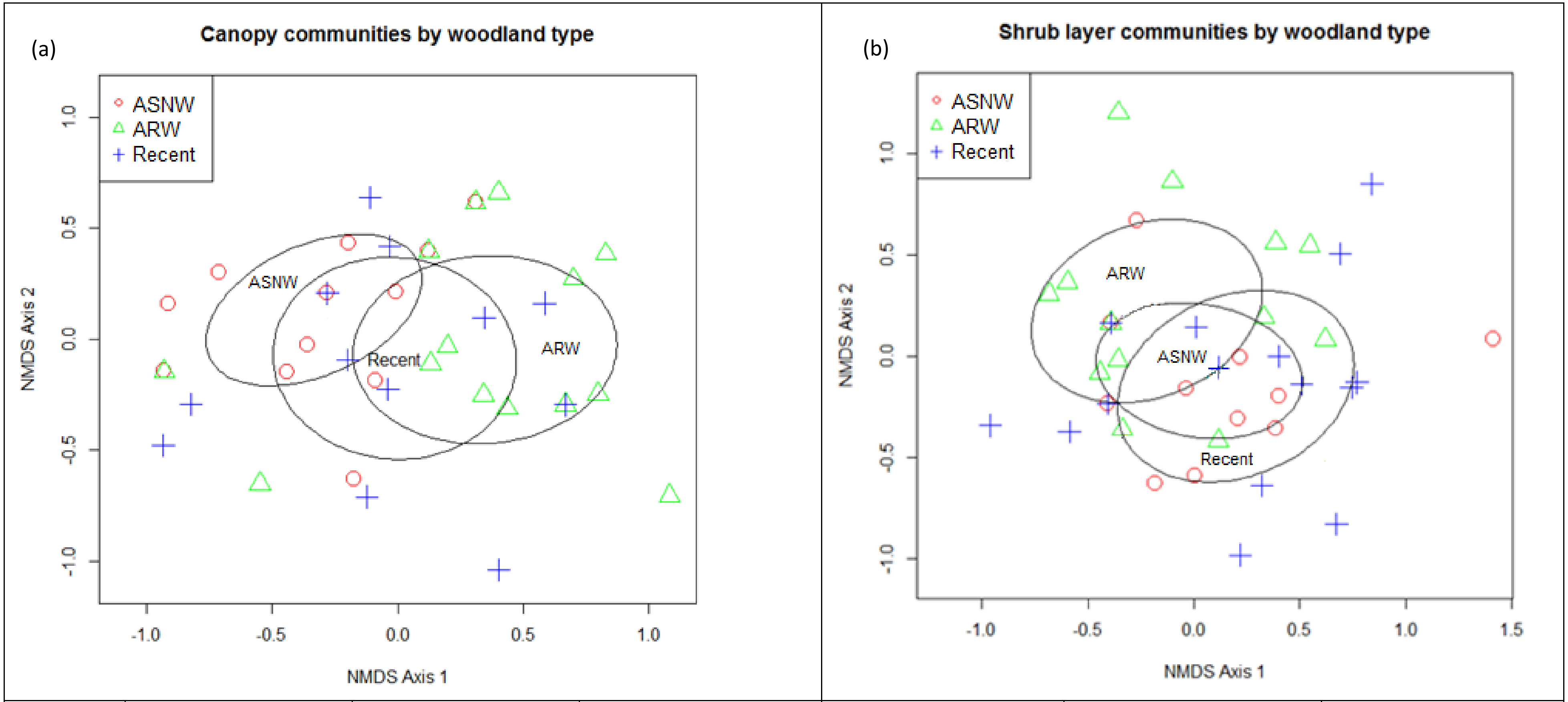
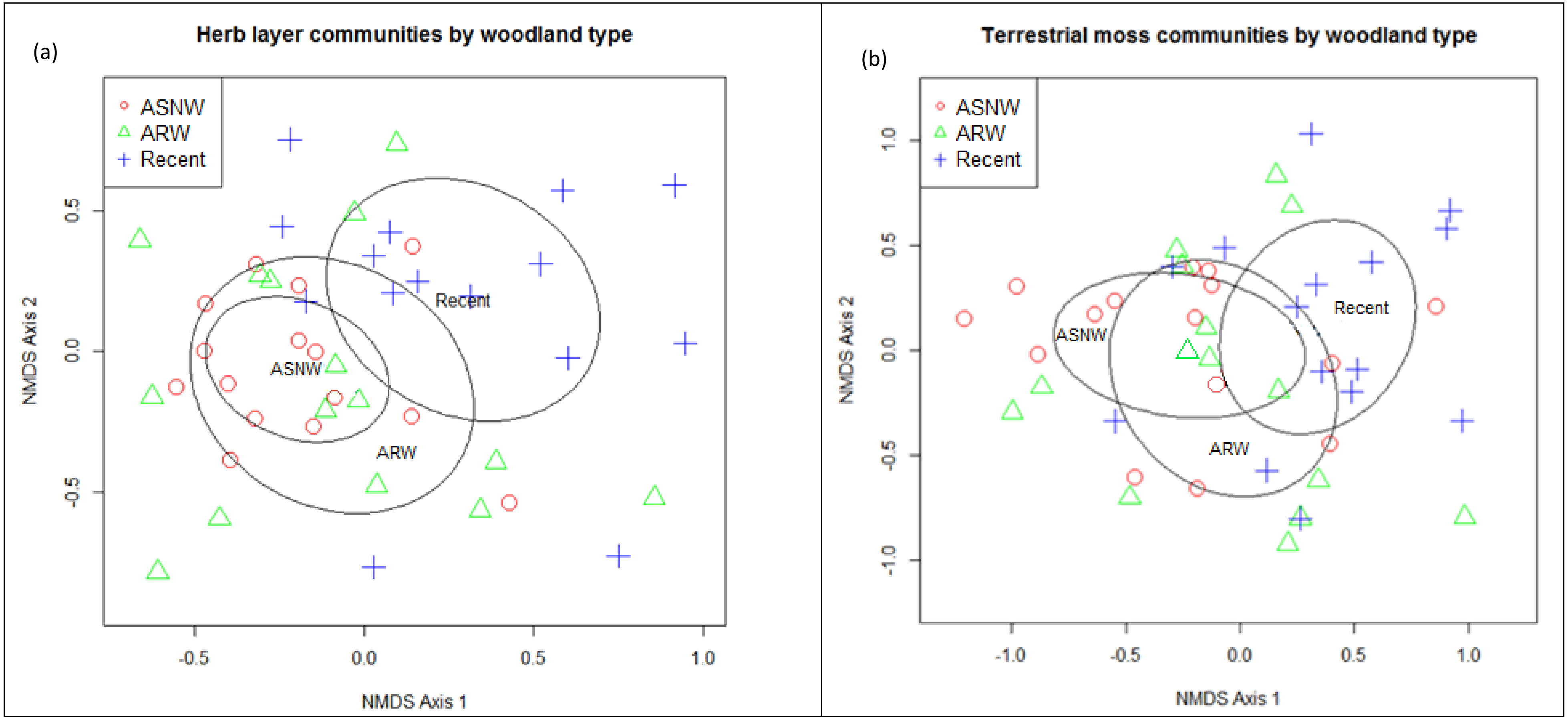


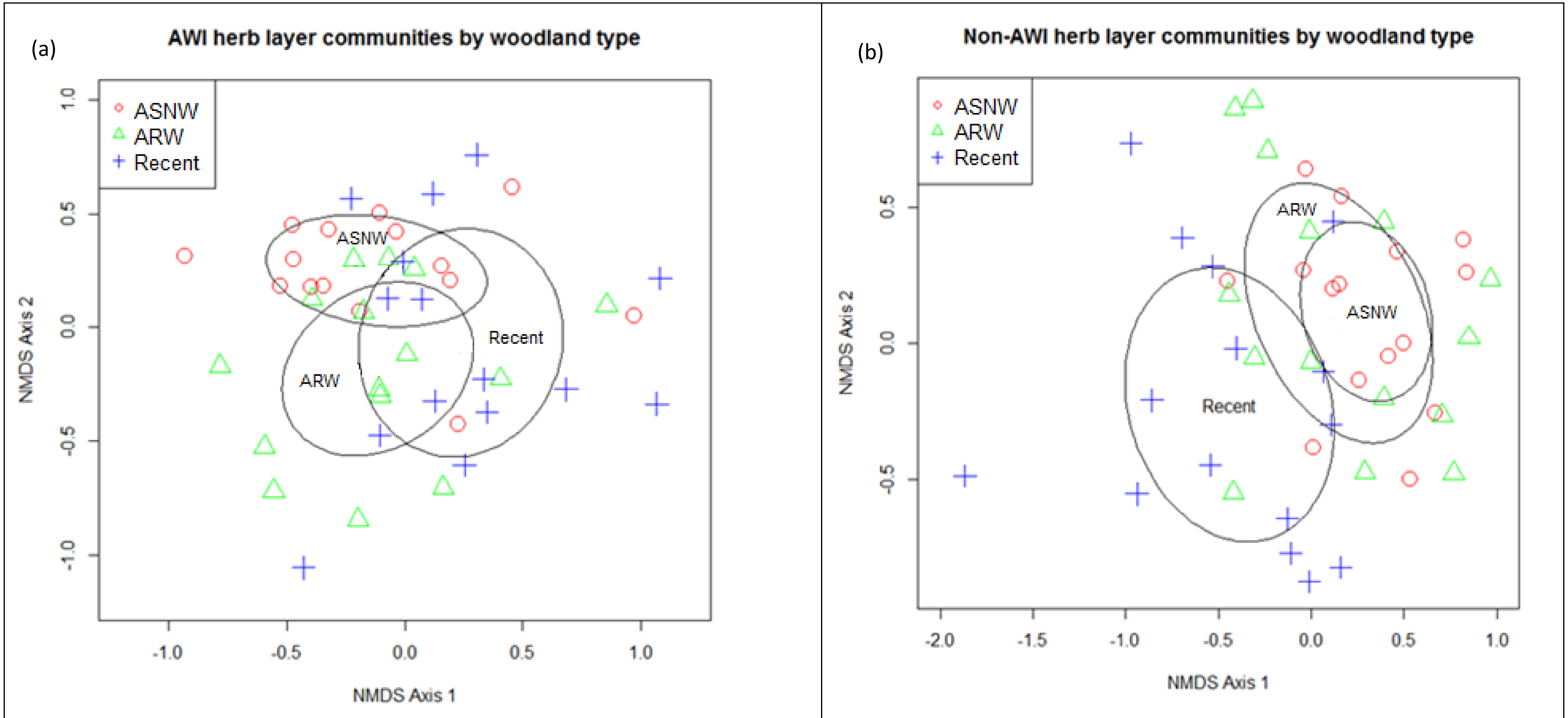
	ASNW-ARW	ASNW-Recent	ARW-Recent	ASNW-ARW	ASNW-Recent	ARW-Recent
Mean	**p = 0.001, R^2 = 0.159**	p = 0.128 R^2 = 0.060	p = 0.161 R^2 = 0.057	p = 0.104, R^2 = 0.058	p = 0.127, R^2 = 0.051	**p = 0.049, R^2 = 0.066**
Variance	p = 0.508	p = 0.771	p = 0.903	p = 0.069	p = 0.081	p = 0.996

Figure. 4.1 (a) canopy and (b) shrub layer community composition by woodland type by Jaccard distance. Ancient Semi-Natural Woodland (ASNW) (n=15); Ancient Replanted Woodland (ARW) (n=15); recent woodland (n=15). Canopy (17 species) (stress = 0.094). Shrub layer (19 species) (stress = 0.010). Mean community composition is the central point within standard deviation ellipse. Pairwise woodland types analysed with Non-metric Multi Dimensional Scaling. Difference in means tested using PERMANOVA with 'adonis' function in R. Variance tested with 'betadisper' function in R.



	ASNW-ARW	ASNW-Recent	ARW-Recent	ASNW-ARW	ASNW-Recent	ARW-Recent
Mean	**p = 0.014, R^2 = 0.064**	**p = 0.001, R^2= 0.106**	**p = 0.002, R^2 = 0.076**	P = 0.864, R2 = 0.018	**p = 0.002, R2 = 0.105**	**p = 0.032, R2 = 0.067**
Variance	**p = 0.015**	**p = 0.008**	p = 0.962	p = 0.352	p = 0.669	p = 0.837

Figure 4.2 (a) Herb and (b) moss layer community composition by woodland type by Jaccard distance. Ancient Semi-Natural Woodland (ASNW) (n=15); Ancient Replanted Woodland (ARW) (n=15); Recent woodland (n=15). Mean community composition is the central point within standard deviation ellipse. Herb layer (70 species) (stress = 0.167). Terrestrial moss (25 species) (stress = 0.126). Pairwise woodland types analysed with Non-metric Multi Dimensional Scaling. Difference in means tested using PERMANOVA with 'adonis' function in R. Variance tested with 'betadisper' function in R.



	ASNW-ARW	ASNW-Recent	ARW-Recent	ASNW-ARW	ASNW-Recent	ARW-Recent
Mean	**$p = 0.021$, $R^2 = 0.069$**	**$p = 0.005$, $R^2 = 0.089$**	**$p = 0.049$, $R^2 = 0.059$**	$p = 0.618$, $R^2 = 0.029$	**$p = 0.004$, $R^2 = 0.074$**	**$p = 0.004$, $R^2 = 0.062$**
Variance	$p = 0.332$	$p = 0.053$	$p = 0.603$	**$p = 0.028$**	**$p = 0.042$**	$p = 0.986$

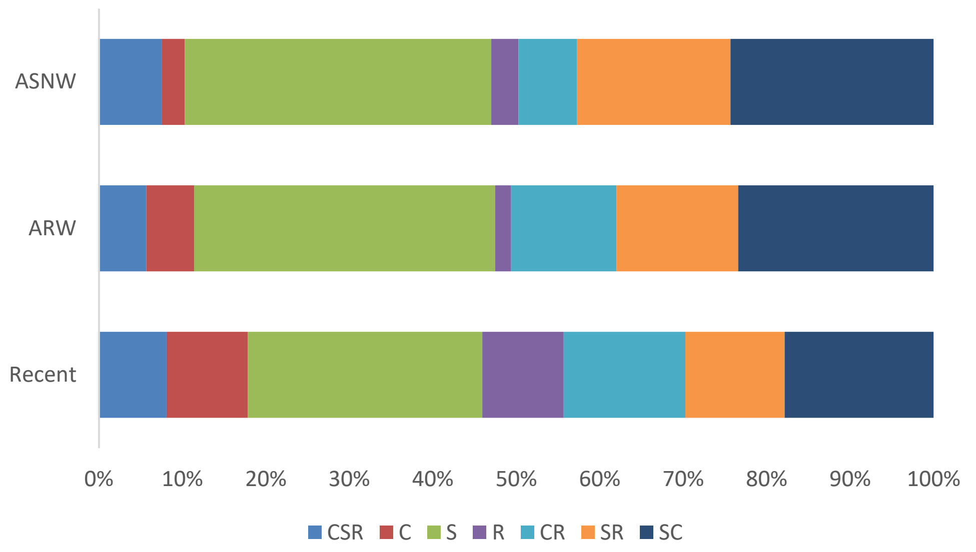
Figure 4.3 (a) Herb layer AWI and (b) non-AWI community composition by woodland type by Jaccard distance. Ancient Semi-Natural Woodland (ASNW) (n=15); Ancient Replanted Woodland (ARW); (n=15 Recent woodland (n=15). Mean community composition is the central point within standard deviation ellipse. AWI community (25 species) (stress = 0.164). Non-AWI community (40 species) (stress = 0.132). Pairwise woodland types analysed with Non-metric Multi Dimensional Scaling. Difference in means tested using PERMANOVA with 'adonis' function in R. Variance tested with 'betadisper' function in R.

Richness of ASNW canopy, shrub, and moss strata were significantly and negatively correlated with AWI richness (Table 4.2). For ARW, the canopy and shrub layer richness were significantly and negatively correlated with AWI richness. Recent woodland was the only type of woodland to show significant correlation between the non-AWI and AWI herb strata richness; this was a positive correlation (Table. 4.2). Jaccard similarity scores showed significant positive correlations between AWI and canopy, and shrub layer communities in ASNW (Table 4.2). For ARW AWI similarity correlated positively with non-AWI species (Table 4.2). Recent woodland exhibited no significant correlations.

Table 4.2. Strata interactions between AWI communities and canopy, shrub, herb layer non-AWI species and terrestrial moss communities in Ancient Semi-Natural Woodland (ASNW), Ancient Replanted Woodland (ARW), and recent woodland. Richness tested via paired t-test (d.f 208 for all tests). t statistics, p values, and direction of correlation. Similarity tested via two-tailed Mantel test (Pearson's) on matrices of Jaccard similarity (matrix 15x15 for all tests) (randomisation 10000 permutations), r(A/B) statistic, p values, and direction of correlation.

	Canopy			Shrub			Herb non-AWIs			Moss		
AWI	t	P	Dir.	t	P	Dir.	t	P	Dir.	t	P	Dir.
ASNW richness	**4.928**	**<0.0001**	**-**	**3.977**	**0.001**	**-**	2.076	0.057	-	**2.480**	**0.026**	**-**
ARW richness	**3.301**	**0.009**	**-**	**4.461**	**0.001**	**-**	0.249	0.807	-	0.597	0.560	+
Recent richness	0.498	0.626	-	0.817	0.428	+	**2.925**	**0.011**	**+**	0.295	0.772	-
	r	P	Dir.	r	P	Dir.	r	P	Dir.	r	P	Dir.
ASNW similarity	**0.205**	**0.033**	**+**	**0.225**	**0.022**	**+**	0.025	0.791	-	0.063	0.510	-
ARW similarity	0.051	0.617	-	0.140	0.158	+	**0.211**	**0.037**	**+**	0.108	0.279	+
Recent similarity	0.177	0.073	+	0.098	0.321	+	0.064	0.505	-	0.169	0.081	+

Herb layer community composition by life traits showed significant differences among woodland continuity types for Grime's (1977) life strategies and the dispersal vectors (Fig. 4.4; Fig. 4.5). None of the other life traits showed significant differences. For the life strategies, both ASNW and ARW differed significantly from recent woodland (Fig. 4.4). Stress-tolerant (S) strategists comprised a greater proportion of the community in ASNW and ARW compared to recent woodland. Competitive (C) and Ruderal (R) - strategists comprised a greater proportion of the community in recent. Likewise, the community comprised more SR- and SC-strategists, and fewer CR-strategists in ASNW and ARW compared to recent woodland. For dispersal vectors, ASNW was significantly different to both ARW and recent woodland (Fig. 4.5). ASNW and ARW comprised notably more myrmechorous plants than recent woodland, and fewer epizoochores. A greater proportion of anemochores was evident in ASNW, and fewer species were classed as 'unspecialised' than in recent woodland. ARW displayed some proportions intermediate between ASNW and recent woodland.



ASNW-ARW

p = 0.369

Chi = 6.50

ASNW-recent

p < 0.0001

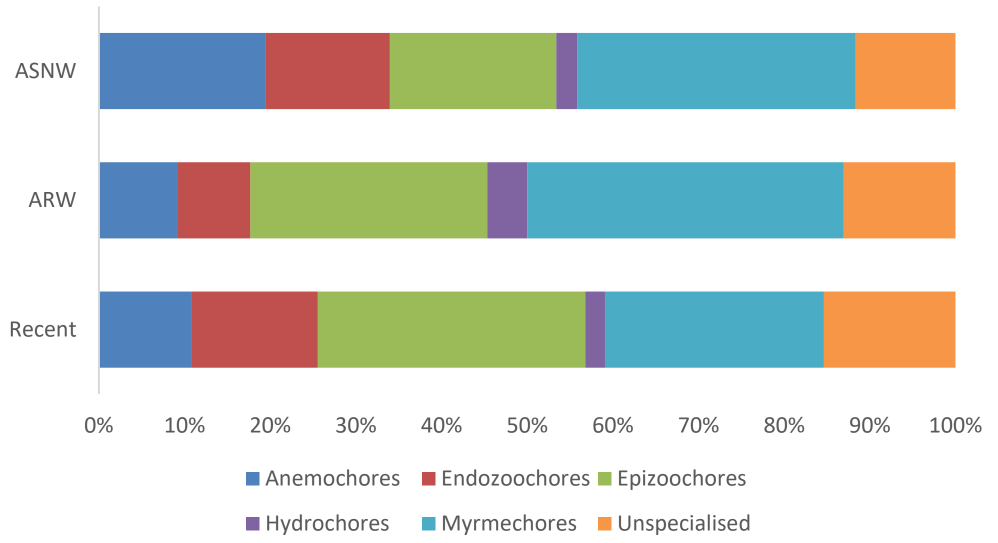
Chi= 24.8

ARW-Recent

p = 0.023

Chi = 14.7

Figure 4.4. Herb layer community composition by percentage frequency occurrence of the major plant life strategies at the gamma scale in Ancient Semi-Natural Woodland (ASNW), Ancient Replanted Woodland (ARW), and recent woodland. ASNW n=185 occurences in n=45 woodland, ARW n=158 occurences in n=45 woodlands, recent n=185 occurences in n=45 woodland) (Grime, 1977). C = Competitive, S = Stress-tolerant, R = Ruderal. Strategy types available for 58 of 70 species. Intermediate strategies were interpreted as C/CR=C; C/CSR=C; C/SC=C; CR/CSR=CR; R/CR=R; R/CSR=R; R/SR=R; S/CSR=S; S/SC=S; S/SR=S; SC/CSR=SC; SR/CSR=SR (Hermy *et al.*, 1999). Chi-squared χ^2 d.f 6.



ASNW-ARW

p = 0.045

Chi =11.3

ASNW-Recent

p = 0.029

Chi = 12.5

ARW-Recent

p = 0.176

Chi =7.66

Figure 4.5. Herb layer community composition by percentage frequency occurrence of the major dispersal strategies at the gamma scale. Ancient Semi-Natural Woodland (ASNW), Ancient Replanted Woodland (ARW), and recent woodland. (ASNW n=190 occurences in n=45 woodland, ARW n=130 occurences in n=45 woodlands, recent n=176 occurences in n=45 woodland). Dispersal mechanisms available for 56 of 70 species. Chi-squared χ^2 d.f 5.

4.5 Discussion

Results show that the biodiversity value of ASNW is not as straightforward as being simply richer than other woodland categories: although higher in alpha richness for some strata, ASNW exhibits no significant differences in gamma richness for the separate strata, and is significantly less rich when all strata are combined (Table. 4.1). This is in contrast to the small number of previous studies where ASNW was found to generally have higher richness at the landscape scale, or no significant difference with recent woodland (Baeten *et al*., 2010; Vellend, 2004). Plant community differentiation is evident among ASNW, ARW and/ or recent woodland. This is consistent with findings of previous studies where ancient/ plantation/ recent woodlands have been compared (Berges *et al*., 2017; Coote *et al*., 2012; Hermy *et al*., 1999). Additionally, the community composition of ASNW cannot simply be said to be ‘different’ to ARW or recent woodland: it is more complex due to the influence community variance on community differentiation.

The canopy stratum is considered the most influenced by management (Rackham, 2003; Peterken, 1993), and here ASNW-ARW showed significant community differentiation, whereas recent woodland was not significantly different to either ASNW or ARW (Fig. 4.1a). The deliberate planting of canopy species in ARW would account for the difference to ASNW. However, the mode of establishment of recent woodland, as plantation or via secondary succession, may explain its non-significantly different position relative to ASNW and ARW (Fig. 4.1a). Variance measures showed no significant difference in within-group consistency among the canopy strata of the three woodland types, indicating that the species present are largely the same species, but in a significantly different combination between ASNW-ARW.

The shrub stratum of ARW showed no significant differences at the alpha level. At the gamma scale, all of the shrub species found in ANSW and ARW were also found in recent woodland. As such, the lack of any significant differences in community variance (Fig. 4.1b) is not surprising; ASNW and recent can only exhibit the mean difference in community composition, with no scope for species variance. Kirby *et al.* (2016) note the strong effects of the shrub species composition and structure on other organisms, further justifying the importance of their inclusion in this study.

The herb stratum is less likely to be deliberately planted or managed than the canopy or shrub strata, and is therefore a truer reflection of environmental conditions and processes. The herb layer community is the most frequently studied stratum as an indicator of distinctiveness, difference, or change in habitat variables (e.g. Berges *et al*., 2017; Stefańska-Krzaczek *et al*., 2016; Kelemen *et al*,. 2014; Hofmeister *et al*., 2013; Brunet *et al*., 2011; Kirby and Morecroft, 2011; Bossuyt and Hermy, 2000; Hermy *et al*., 1999). The present results of significant community differentiation among ASNW, ARW and recent woodland (Fig. 4.2a) seem to support the use of the herb layer community as a sensitive indicator of habitat change.

The herb layer community was significantly different among all pairwise combinations of ASNW, ARW and recent woodland (Fig. 4.2a). However, the differentiation between ASNW-ARW and ASNW-recent is strongly influenced by species variance, as indicated by the significant results for variances in addition to the significantly different mean results (Fig. 4.2a). This indicates that the herb layer of ASNW has a significantly different group of species present compared to ARW or recent woodland, and larger standard deviation of recent woodland communities shows far greater variation in the species present. In a previous study, no significant differences in variance were found among the herb layer communities of ancient, plantation, clear-felled and restored woodland (Atkinson *et al.*, 2011).

Terrestrial moss communities of recent woodland differed significantly from both ASNW and ARW (Fig. 4.3b). There were no significant differences in variance, therefore any difference was due to mean community composition. Bryophyte communities are recognised as good indicators of ancient woodland, and of recent woodland (Mölder *et al.*, 2015). This is supported in the present study where there were no significant differences between ASNW and ARW, and the recent woodland moss community was significantly different to both categories of ancient woodland (Fig. 4.2b). There was no distinction among the woodland types in terms of moss alpha or gamma diversity (Table 4.1). Terrestrial moss communities are unlikely to be directly managed, and when studied in combination with herb layer AWI species, may provide a more robust identification of ancient woodland.

Where strata communities were significantly different between pairs of woodland types, a modest proportion of the community variation was explained by the woodland continuity categories themselves. The highest proportion was 16% of the difference in community composition explained by ASNW/ARW status for the canopy. As such, future studies may seek to establish further explanatory factors for woodland community differentiation.

AWI and non-AWI communities

The distinctive community of specialist woodland indicators in ancient woodland is a long-established concept (e.g. Kelemen *et al*., 2014; Schmidt *et al*., 2014; Hofmeister *et al*., 2013; Kirby, 2004; Rose, 1999; Peterken, 1974), and holds true in the present study. All pairwise combinations of ASNW, ARW, and recent woodland showed significant differentiation in community composition, but no significant variance in species consistency was detected. These outcomes align with the established concept that no single AWI species is confined to ancient woodland, and may exist in more recently established woodland (Rose, 1999). In addition, the presence of an AWI does not indicate ancient woodland, it is only with sufficient richness counts (Glaves *et al*., 2009) that they are considered indicators. While ASNW was significantly richer in AWI species than recent woodland at the alpha scale, the gamma scale showed no significant difference in the number present. Importantly, ASNW has been shown to have a distinctive community of AWI species, which justifies their protected status. While recent woodland does not support the same AWI community as ASNW at the alpha scale, it can support largely the same species over the landscape scale, some of which are scarce and protected (JNCC, 2017). This, in addition to no significant difference between ASNW-ARW in AWI alpha richness, indicates a high restoration potential for the conservation of specialist woodland plant communities (Brown *et al*., 2015; Palo *et al*., 2013; Pryor *et al*., 2002).

Non-AWI communities exhibited marked differences in both mean community differentiation and variance across the three woodland types. ASNW was significantly less varied in its non-AWI community than both ARW and recent woodland. However, between ASNW and ARW there was no significant difference in mean community, suggesting that ASNW non-AWI species were those also present within the significantly greater variety of species present in ARW. These findings show that the non-AWI community of ASNW deserves more research attention in terms of its unique consistency. The distinctiveness of ASNW non-AWI species is reflected in the gamma richness: 22 in ASNW; 31 in ARW; 38 in recent woodland. Prior research has shown that lack of AWI species in recent woodland is partly due to competitive exclusion (Sciama *et al*., 2009; Bossuyt and Hermy, 2000; Hermy *et al*., 1999). However, the present study raises the question of whether the narrow range of non-AWI species in ASNW is the result of a 'reverse' competitive exclusion process where efficient resource use by woodland specialist species hinders establishment of ruderals.

Strata interactions and community composition

Inter-strata correlations with AWI richness showed more significant relationships in ASNW compared to ARW and recent woodland, suggesting some degree of ecological interaction among the strata, which is less present in disturbed woodlands. A prior study found AWI presence to be associated with higher richness in all other strata (Stefanska-Krzaczek *et al*., 2016). In direct contrast, the present study found significant negative correlations between AWI richness and the canopy and shrub richness in ASNW and in ARW, and moss strata in ASNW. The only significant positive correlation was with the non-AWI community in recent woodland. The similarity indices showed significant positive correlations between AWI species and the canopy and shrub layer. The non-AWI community similarity of ARW also correlated with AWI similarity. Overall, these results show some evidence of phytosociological links among the strata, although coinciding with the views of Peterken (1993) and Rackham (2003) in that any linkages are inconsistent.

Life traits community composition

The significant difference in herb layer life strategies between ASNW and recent woodland, corresponds with findings of prior studies where S-strategist species are more commonly associated with ASNW, and C- and R-strategists are associated with recent woodland (Berges *et al*., 2017; Sciama *et al*., 2009; De Keersmaker *et al*., 2004; Hermy *et al*., 1999). The present study adds the extra dimension of ARW also exhibiting a significant difference to recent woodland in life strategy composition. The significant differences are likely to correspond with AWI richness: a large proportion of AWI species are S-strategists (39% stress-tolerant, compared to 7.4% competitive) (Hermy *et al*., 1999). De Keersmaeker *et al*. (2004) also proved a negative correlation between cover of fast colonisers and woodland age, in addition to an increase in ancient woodland specialists in both number and cover with age.

Community composition by dispersal vectors was significantly different between ASNW and ARW, and between ASNW and recent woodland. In particular, the myrmechorous component contributed to the difference. Myrmechory has been shown to be associated with dispersal of woodland herbs generally (Whigham, 2004), and particularly with AWI species. AWI communities were found to comprise 24% ant-dispersed species in one study and 50% in another (respectively Kelemen *et al*., 2014; Hermy *et al*., 1999). However, Kimberley *et al*., (2013) found that dispersal vectors were not a distinguishing factor between AWI species and other woodland plants. Myrmechory is a short-range dispersal mechanism, and the implications of this are reduced colonisation capacity, which may in turn link to the influence of individual species on community differentiation.

4.6 Conclusions and recommendations

This chapter finds that ASNW, ARW, and recent woodlands exhibit community differentiation in each of their strata, but in different pairwise combinations between the three woodland types. Both AWI and non-AWI communities are distinctive among the woodland types. Additionally, this chapter has demonstrated that any community differentiation among these woodland types may be in response to both the mean and variance aspects of beta community composition. The herb layer community composition of ASNW or ARW were significantly more varied in their composition than recent woodland. Herb layer community composition in terms of life strategies and dispersal mechanisms was significantly different between ASNW and recent woodland, and for life strategies also between ARW and recent woodland.

These findings assist in explaining why herb layer communities may differ among the woodland types, as a result of potentially limited dispersal, or reduced colonisation ability due to competitive exclusion. ASNW exhibited more significant correlations between AWI richness and similarity, and richness and similarity of other strata. The implications for conservation are twofold. Firstly, both ASNW and ARW categories of ancient woodland are shown to have distinct herb layer communities compared to recent woodland, which justifies their conservation and existing legal protection. Secondly, despite differences between ancient and recent woodland plant communities, there was no significant difference in richness of AWI species at the gamma landscape level.

The findings of this study will be of strong interest in the field of woodland connectivity and restoration, as it examines, for the first time, the community proximity of ASNW, ARW, and recent woodland. Further focus on the biodiversity value of the non-AWI species component of woodland communities is recommended. Lastly this study supports the use of moss species as indicators of ancient woodland, as the moss community clearly differentiated between ancient and recent woodland in this study.

5. Relative influence of habitat variables and continuity on ancient woodland indicator plant communities in ancient semi-natural, ancient replanted, and recent woodlands.

Outputs arising from this chapter:

Swallow, K., Wood, M. and Goodenough, A. (2018) Ancient woodland indicator communities in ancient replanted woodland: a shadow plant community? *European wood pastures*. Sheffield, 5-8 September

Swallow, K., Wood, M. and Goodenough, A. (2018) *Plant community differentiation among ancient semi-natural, ancient replanted, and recent woodland.* RAU research seminars. Cirencester, 25 January

Oxalis acetosella, Farmcote Wood, Guiting Strategic Nature Area.
03/08/14 Grid ref: SP: 06389 27190

5.1 Chapter scope

A review of the literature (Chapter 1) established that woodland plant communities rich in AWI species are considered to have high biodiversity value and are indicative of long habitat continuity. However, the literature also indicated that use of Ancient Woodland Indicator (AWI) species richness as a single-value indicator to inform conservation decisions is increasingly questioned due to lack of focus on the underlying ecological conditions. The present chapter seeks to explain the AWI alpha richness and beta community composition variation among ASNW, ARW and recent woodland established in Chapter 4. Furthermore, Chapter 4 identified only modest explanatory effects of woodland continuity status on plant community composition. As a result, the present chapter considers the relative influence of habitat continuity, biogeographical, soil, and canopy variables on AWI richness, community composition, and individual species' presence. The biogeographical elements of Chapter 5 inform the edge effect study conducted in Chapter 6.

5.2 Introduction

The herb layer vegetation of ASNW has been shown to be distinct from recent woodland in terms of specialist species richness (Kelemen *et al.*, 2014; Hofmeister *et al.*, 2013; Brunet *et al.*, 2011; Orczewska, 2009), and in terms of community composition (Vukov *et al.*, 2016; Brown *et al.*, 2015; Palo *et al.*, 2013). In Britain, ancient woodland comprises two categories: Ancient Semi-Natural Woodland (ASNW) and Ancient Replanted Woodland (ARW). They are accorded equal protection in the National Planning Policy Framework (Forestry Commission and Natural England, 2014), due, in part, to their distinctive ecology.

Ancient Woodland Indicator (AWI) species are woodland specialist vascular plant species particularly associated with, but not exclusive to, ancient woodland (Glaves *et al*., 2009). AWI species richness has long formed part of the assessment for woodland continuity (Rose, 1999; Rackham, 1980; Peterken, 1974), and has been increasingly used to gauge the biodiversity value of woodland (Glaves *et al*., 2009). Many AWI species have an intrinsic value beyond their indicator status, being relatively scarcer than woodland generalist species (Chapter 1), and in some cases endangered and protected under legislation (JNCC, 2017).

Increasingly, the binary ancient/ recent woodland explanation for floristic distinctiveness has come under scrutiny (Webb and Goodenough, 2018; Barnes and Williamson, 2015; Stone and Williamson, 2013; Wright and Rotherham, 2011; Spencer, 1990). Recent, interrupted and even felled woodlands have been found to have comparable AWI richness to known ancient woodlands (Webb and Goodenough, 2018; Stone and Williamson, 2013). There is a shift towards understanding the ecological conditions that determine the presence of AWI/ woodland specialist species (e.g. Kimberley *et al*., 2016; Kimberley *et al*., 2014). Re-focusing on the ecology of woodland specialist species' habitats enables a more objective assessment of the AWI approach, and may recognise the restoration or connectivity potential of recent woodlands (Palo *et al*., 2013).

Soil variables

Evidence presented in prior studies shows soil pH to be significantly associated with AWI presence (Orczewska, 2009; De Keersmaeker *et al*., 2004) and with woodland vegetation community differentiation (Corney *et al*., 2006). Significant positive correlations have been demonstrated between pH and both richness and abundance of AWI species (De

Keersmaeker *et al*., 2004; Kolb and Diekmann, 2004). Weakly acid to neutral soils, as opposed to strongly acidic, are preferred by AWI species, based on a synthesis of 22 European studies of AWI species by Hermy *et al*. (1999).

Plant available phosphorus has been shown to have a higher relative impact on woodland vegetation communities than other soil variables (De Keersmaeker *et al*., 2004). Significant negative correlations have been proven between soil phosphorus and AWI richness (Honnay *et al*., 1999). High phosphorus levels have been shown to indirectly hinder establishment of AWI species in recent woodland due to competitive exclusion by ruderal phosphateophiles such as *Urtica dioica (*De Keersmaeker *et al*., 2004; Hermy *et al*., 1993).

Total nitrogen and organic carbon seem to have inconsistent correlation with AWI communities, although studies are few. After soil P and pH, nitrate had the third most significant influence on woodland flora communities (De Keersmaeker *et al*., 2004). Yet a study by Honnay *et al*. (1999) found no significant correlation between total N and number of AWI species. Few studies have explicitly analysed any association between soil carbon and AWI richness or community composition.

Biogeographical variables

Fragmented habitat configuration is known to cause a significant barrier to the dispersal and colonisation of ancient woodland species due to life traits such as few and heavy short-range dispersed seeds (Endels *et al*., 2007; Hermy *et al*., 1999; Bierzychudek, 1982) and clonal spread (Verheyen *et al*., 2003a; Hermy *et al*., 1999). Increased connectivity has been shown to correlate with the presence of specialist or AWI species: AWI species richness in British lowlands was explained in a large part by length of hedgerows and lines of trees within the 1km square of a woodland plot, as well as the area of woodland within 500m of the plot (Petit *et al*., 2004). Some hedgerows or tree lines may themselves be ancient woodland remnants (Smart *et al*., 2001; McCollin *et al*., 2000) and could potentially act as a source instead of, or as well as, a conduit for woodland species.

Isolation of woodlands has been demonstrated to be of varying importance for woodland species relative to other factors. Distance from large core or ancient woodlands correlated negatively with richness of woodland plant species/ AWI species (Brunet *et al*., 2011; Petit *et al*., 2004; Honnay *et al*., 2002a). Isolation showed the strongest influence among several variables on AWI species richness (Petit *et al.*, 2004), and on occurrence of individual species, specifically spring flowering herbs with large seeds and unassisted dispersal (e.g. *Paris quadrifolia*), and small, mainly vegetatively reproducing herbs (e.g. *Anemone nemorosa*) (Endels *et al.,* 2007).

Conversely, habitat configuration has been found to have negligible impact on woodland species richness compared to internal habitat variables (Kolb and Diekmann, 2004; Honnay *et al*., 1999a). Bailey *et al*. (2002) proved no link between the occurrence of *A. nemorosa*, *Hyacinthoides non-scripta, Mercurialis perennis* nor *P. quadrifolia* and the distribution of ASNW at the regional scale, concluding that other factors must be more influential.

The influence of patch size on woodland species richness is inconsistent. It would be expected that larger woodlands have higher species richness due to diversity of internal habitats (Honnay *et al*., 1999a) or that by being larger are more likely to encounter propagules (Dumortier *et al*., 2002). Whilst some studies have found positive correlations between understorey species richness with patch size (Gonzalez *et al*., 2010; Jacquemen *et al*., 2003a; Peterken and Game, 1984), many have evidenced no significant correlation

(Hofmeister *et al*., 2013; Dupre and Ehrlen, 2002; Graae, 2000; Honnay *et al*., 1999a). The aggregate effect of isolation and patch size may account for conflicting results: as emphasised by Bailey (2007) fragmentation is not simply an issue of distance. Smaller habitats have been correlated with smaller populations, but this is potentially only problematic when they are also isolated, reducing dispersal and increasing risk of local extinction (Honnay *et al*., 2005). Petit *et al*. (2004) found the highest number of AWI species in the largest and least isolated woodland patches.

Canopy variables:

Specialist woodland species are known to be shade-tolerant and shade-adapted (e.g. Hermy *et al*., 1999; Whigham, 2004; Bierzychudek, 1982). Canopy closure has been found to have an inconsistent direct influence on presence of woodland flora (Vockenhuber *et al*., 2011): a positive correlation between true woodland species and canopy closure versus a negative correlation between tree+shrub layer closure and woodland specialist abundance (Brunet *et al*., 2011). However, the relative range of canopy closure varies among studies meaning they are not easily comparable. Richness and cover of specialist woodland species have been found to be higher in plots with relative insolation <5% (Hermy *et al*., 1999) and <8% (De Keersmaeker *et al*., 2004). However, indirect influence of light via competitive exclusion by light-demanding species appears to partly account for low presence of AWI species in recent woodlands: De Keersmaeker *et al*. (2004) evidenced a significant negative correlation between number of AWI species and percentage cover of light demanding species.

Research gap:

This thesis is the first study to explicitly compare woodland continuity versus ecological variables in explaining AWI richness and community composition across ASNW, ARW and recent woodland. An earlier study similarly applied a range of explanatory variables to the prediction of woodland species richness in plantations and native semi-natural (but not ancient) woodland, but the aim was not to compare these variables to continuity (Coote *et al*., 2012). Additionally, given the doubt cast over the use of AWI species to designate ancient woodland (Webb and Goodenough, 2018; Barnes and Williamson, 2015; Spencer, 1990), this chapter considers whether any habitat ecological variable(s) proves a significant indicator of high AWI richness that might be used as a viable alternative to continuity.

A very few studies have quantified the distinctiveness of ARW flora communities compared to ASNW or recent woodland (Atkinson *et al*., 2015; Palo *et al*., 2013; Corney *et al*., 2008b). Seemingly no prior study has examined all three categories of ASNW, ARW, and recent woodland in terms of the relative influence of continuity and ecological variables on AWI communities.

The underlying ecology of floristic differentiation is particularly important in the context of woodland restoration and conservation (Atkinson *et al*., 2015; Brown *et al*., 2015; Palo *et al*., 2013). Prior assessment of variables may be indicative of potential habitats to support the vegetation of ancient woodland (Coote *et al*., 2012), and therefore assist in site selection and resource allocation for restoration or conservation purposes.

This chapter fulfils the following overarching research objective:

5. To examine the response of ancient woodland indicator communities to environmental and biogeographical variables, relative to woodland continuity status.

Within the above objective, the following research questions are addressed:

a. Can habitat variables predict AWI richness as effectively as ancient semi-natural, ancient replanted, and recent woodland classification?

b. Can habitat variables predict AWI community composition as effectively as woodland continuity?

c. Can individual AWI species presence be predicted by habitat variables?

5.3 Methods

5.3.1 Study location

Cross reference to sections 2.2.1 and 2.2.2 for an extended description.

5.3.2 Field vegetation methods and AWI definition

Cross reference to sections 2.2.3 and 2.2.4.

NB: For this chapter all vegetation data was presence/absence, not percentage cover.

NB: This chapter employs AWI species data only.

5.3.3 Explanatory variables: field and laboratory methods

To explain any variation in AWI richness and community composition, a range of continuity, biogeographical and soil variables were measured for each woodland site (Table 5.1). To focus on the novel research question, and to avoid an excessive number of variables to cases, a list of variables was refined from a range of factors potentially influencing AWI communities. To assess, for the first time, whether any other variables could predict AWI communities equally or more effectively than a woodland's continuity status, it was logical to select variables that had previously been shown to influence AWI communities. In some cases, variables were not selected for other reasons. Management, for example, has been shown to influence AWI communities, but it was not selected because it was reflected in the continuity status of each woodland, with ASNW being predominantly coppice with standards and managed for conservation, whereas ARW and recent woodland were high forest managed for timber. It is recognised that many potentially interesting factors, such as deer browsing intensity, internal habitat heterogeneity, and surrounding non-woodland land-use are unexplored and therefore limitations of this chapter. However, once the seemingly most likely predictive variables have been tested in this novel analysis, future research could seek to extend the variables for inclusion.

Table 5.1. Explanatory variables tested for relative influence on Ancient Semi-Natural Woodland (ASNW), Ancient Replanted Woodland (ARW), and recent woodland AWI plant communities. Data acquisition: FM = field measurement; LM = laboratory measurement; GIS = geographical information system; CI = computed index (Herault and Honnay, 2005)	Data aquisition
Continuous variables:	
'Canopy' – percentage canopy closure	FM/ CI
'Wood' - woodland percentage cover within 500m of site edge	GIS
'ASNW' - ASNW percentage cover within 500m of site edge	GIS
'ARW' - ARW percentage cover within 500m of site edge	GIS
'N' - total nitrogen (%)	LM
'C' – total organic carbon (%)	LM
'P' – plant available phosphate (mg/l)	LM
'Hedge' - percentage hedgerow cover within 500m of site edge	GIS
'Area' – area of woodland site (m2)	GIS
'Shape' – shape of woodland site as departure from perfect circle of same area	GIS/ CI
'Size' – area of woodland site (m2)	GIS
Categorical/ ordinal variables:	
'Continuity' – classified as ASNW, ARW, and recent woodland (grouped 3, 2, 1 respectively)	GIS

Field and laboratory soil methods

In the same manner as for herb layer vegetation surveys, 45 woodland sites were sampled for soil variables (ANSW n=15, ARW n=15, recent n=15). For representative vegetation and corresponding soil samples, five 4x4m quadrats were systematically located within each woodland site. Within each quadrat, five soil samples were taken at 0-10cm depth below the litter layer at the centre and each corner then mixed, yielding one soil sample for each of the five quadrats per woodland site (Brunet *et al.*, 2011). These five samples were then homogenised in equal weights to give one bulk sample per woodland site (Hofmeister *et al*., 2013). Five samples per site enabled a fair representation in any soil variation across the site. The homogenisation of samples was conducted to avoid pseudoreplication – as all other explanatory variables yielded one data point per site, for example continuity, patch size, or shape.

To test for pH, total organic carbon (C), total nitrogen (N), and available phosphorous (P), samples were dried at 60°C (Graae, 2003). Samples were crushed in a mortar and sieved <2mm. For total organic C and total N a subsample was finely milled.

To test for pH, the ISO standard 10390 1:5 H_2O method was used: 5g of dried soil was added to 25ml deionised water and shaken for 12 hours (Herault and Honnay, 2005). The shaken sample was tested with a calibrated digital pH meter.

To test for total N and total organic C, samples were analysed using a Costech CNS automated elemental analyser whereby samples are combusted and percentage N and C are determined from gases released (Hofmeister *et al*. 2013; Vockenhuber *et al*., 2011). For each sample, 50mg of finely milled soil, with 5mg of tungsten to aid combustion, was weighed into a tin container. Quality was controlled by testing three replicates of a reference soil prior to sample analysis, against which the samples are calibrated. To maintain calibration, sulfanilic acid (5mg) was weighed into tin containers and analysed at intervals of 10 samples.

To test for available P, the Olsen sodium bicarbonate extraction method was used (Olsen, 1954). This was appropriate for the pH range established in earlier testing. Quality was controlled through analysis of a sequence of phosphorus standards, two reference soils and blanks. Available P was determined colourimetrically using a UV-visible spectrometer.

Biogeographical GIS methods

Patch size was measured in m^2 using the polygon function in MAGIC map. To calculate patch shape, the perimeter of each patch was measured using the distance function in MAGIC map, which was then divided by the circumference of a circle of the same area (Petit *et al*., 2004; Patton, 1975).

To establish connectivity of each woodland site, the percentage surrounding land use of ASNW, ARW, all woodland, and hedgerow were separately measured within 500m of the edge of the sampled site. To correct for site size, and therefore buffers of different sizes, it was important to measure land-use cover as a percentage of the 500m buffer. The polygon function in MAGIC map delineated the site area, and the buffer function was applied of a width of 500m around the site. The polygon function in MAGIC map was again used to measure the area of the 500m buffer surrounding the site, from which percentage cover of the landuse types was calculated. For hedgerows, length was first measured using a distance function in MAGIC map and then multiplied by a 2m width to give an area value that could be calculated as a percentage of the buffer area.

Biotic field and software methods

To measure canopy closure, the canopy immediately overhead was photographed in each of the five plots within each of the 45 woodland sites. To obtain a percentage canopy closure figure for each woodland site, photographs were processed using Canopydigi software (Goodenough and Goodenough, 2012), and the mean closure of the five plots was calculated. Canopydigi has been shown to produce comparable results to a densiometer (Fergot, no date).

5.3.4 Statistical methods

To assess the relative influence of habitat variables on AWI richness, a series of Generalized Linear Models (GLMs) was constructed (Coote *et al*., 2012). GLM was selected due to its ability to handle data with a range of statistical properties (Buckley, 2015; Bolker *et al*., 2009; Ripley and Venables, 2002). The dependent variable was count data (AWI richness in 45 woodland sites) and the model used poisson error term with log link function. Buckley (2015) notes a limitation of GLMs, in that count data may not fulfil the poisson distribution due to overdispersion or clustering. Prior to analysis, a Q-Q plot was created to check that residuals did indeed conform to the Poisson distribution. Most independent variables were continuous but woodland age was categorical (Table 5.1). Prior to analysis, percentage data for N, C, ASNW, ARW and hedgerow were arcsine square root transformed to reduce any effects of non-normal distribution. Although this did not achieve an entirely normal distribution for all variables, distributions were improved such that normality was approximate. Multicollinearity was assessed using Variance Inflation Factors (VIFs) and was below the threshold of <10 (Myers, 1990). All analysis relating to GLM was undertaken in SPSS version 24.

To test if any habitat variables were a stronger predictor of AWI richness than a woodland's continuity status as ASNW, ARW, or recent woodland, a series of univariate GLMs was created with each of the variables entered as a single predictor: continuity; area; shape; wood; ASNW; ARW; hedge; canopy; pH; P; C; N (Table 5.1).

Multivariate GLMs were then constructed based on ecological knowledge to model specific scenarios. To test whether any multivariate model could predict AWI richness as effectively as the univariate continuity model, variables were tested in multiple combinations. To understand which aspects of habitat ecology influenced AWI richness, GLMs were also constructed for subgroups of variables: connectivity (wood; ASNW; ARW; hedge); habitat configuration (area, shape); soil variables (pH; P, C, N). Canopy could not be logically grouped into these categories. For all multivariate models, interactions were only included where there was ecological justification.

Univariate and multivariate GLMs were compared for model fit and parsimony in terms of Akaike's Information Criterion (Burnham and Anderson, 2002). The lowest AIC was considered as $\triangle$AIC = 0. $\triangle$AIC continuity = relative to univariate continuity model. $\triangle$AIC non-continuity = relative to best non-continuity model. The contribution of each model relative to the best univariate and/ multivariate model was interpreted using AIC criteria established by Burnham and Anderson (2002:70), whereby the lowest AIC is the best model, and $\triangle$AIC < 2 shows substantial support for the model, 4-7 has considerably less support for the model, and > 10 means that the model essentially has no support.

To support model interpretation, scatterplots with trend line and R^2 values were created for each continuous variable with AWI richness. For continuity as a categorical variable, the mean AWI richness for each category was bar-graphed with the range. Additionally, an independent t-test determined the significance of any difference in mean AWI richness among the continuity categories.

To examine the relative influence of continuity and habitat variables on community composition (n=45), Non-Metric Multi Dimensional Scaling (NMDS) with Jaccard distance measure was conducted on the same variables as for richness. NMDS is recognised for its ability to handle a range of ecological data formats, with no requirement for a normal distribution (McCune & Grace 2002). Additionally, NMDS was selected because, unlike many ordination methods, it allows a choice of distance measure. The Jaccard distance measure (Atkinson *et al*., 2015; Naaf and Wulf, 2010; Jaccard, 1912), based on presence-absence data, was selected because the presence or otherwise of AWI species makes an important contribution to the designation of ancient woodland for protection under UK legislation (Kirby, 2004; Rose 1999).

NMDS output was plotted using the 'metaMDS' and 'ordiplot' functions in Vegan, MASS and BiodiversityR packages for R (Kindt and Kindt, 2017; Oksanen *et al*., 2017; Ripley et al., 2017; Gardener, 2014; Hovanes, 2013; Kindt and Coe, 2005). Stress was maintained <0.20 (0.164) by using 3 dimensions (Gardener, 2014; Clarke and Warwick, 2001). Significance was based on 999 permutations.

To test the relative influence of variables on AWI communities, the 'envfit' function in Vegan for R was applied in addition to the NMDS with Jaccard distance procedure (Oksanen, 2017). 'Envfit' fitted vectors to the NMDS plot to indicate the strength and direction of each explanatory variable. Variables with $p<0.05$ were displayed graphically. The R^2 values were used to assess the relative influence of variables on AWI communities. All variables comprised continuous data, except continuity which was entered as ordinal data and classified in descending order of continuity as 3 = ASNW, 2 = ARW, 1 = recent woodland.

To understand the response of individual AWI species to continuity and habitat variables, a series of binary logistic regression models was conducted for species present in ≥20% of the 45 woodland sites. Informed by the models generated for research question 1, Principal Components Analysis was used to condense area, shape (reciprocal), and surrounding ASNW, ARW, woodland and hedgerow data into one variable (PCA1 explained 41% of the variation), and the same was conducted for pH, P, N and C (PCA1 explained 70% of the variation). Continuity and canopy remained as single variables. Each variable was entered singly to test for correlation with presence of each AWI species. Continuity was entered as an ordinal variable. Wald chi-squared, p values and Nagelkerke pseudo R^2 were used to assess the influence of variables relative to continuity.

5.4 Results

Twenty-five AWI species were identified within forty-five woodland sites. The mean AWI richness of ASNW was 8.33 (n=15), for ARW 6.00 (n=15) and for recent woodland 4.92 (n=15) (Fig. 5.1a). AWI richness was strongly and significantly predicted by woodland continuity as a univariate variable ($p=0.001$ $\triangle$AIC=0).

Following Akaike’s criterion of $\triangle$AIC <2.0, the multivariate model comprising all biogeographical variables could essentially be treated as equal to the univariate continuity model in explaining AWI richness ($p=0.001$, $\triangle$AIC=0.160) (Burnham and Anderson, 2002:70).

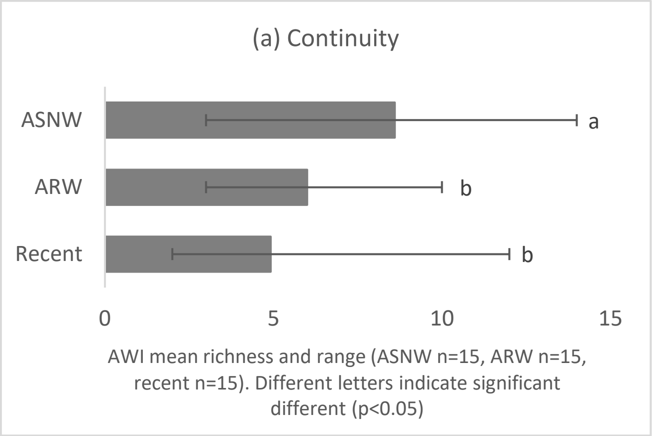
When biogeographical variables were subdivided into connectivity and habitat configuration variables, the connectivity variables did not provide strong support for the model, but were significant in their own right (p=0.020) (Table 5.2). No other univariate or multivariate model supported the strongest model or was a significant predictor of AWI richness.

Table 5.2. Generalized linear models for explanatory variables of AWI richness. (a) univariate single predictors. (b) multivariate models, including best overall model, and best models separately for connectivity, habitat configuration, and soil variables. Delta Akaike's Information Criterion scores are used to compare all models △AIC = 0. △AIC continuity = relative to univariate continuity model; △AIC non-continuity = relative to lowest non-continuity model. ASNW = Ancient Semi-Natural Woodland; ARW = Ancient Replanted Woodland.

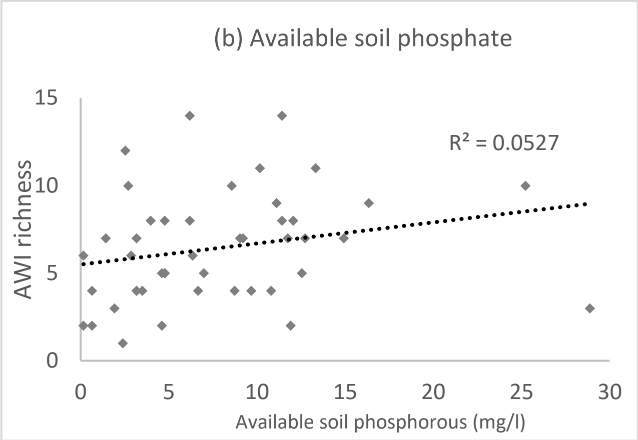
Model #	(a) Univariate		χ^2	d.f	Sig.	△AIC continuity	△AIC non-continuity
1	Continuity	ASNW/ARW/ recent site	13.849	2	0.001	0	n/a
2	P	Soil available phosphate (mg/l)	3.456	1	0.063	8.393	8.233
3	Hedge	% land use within 500m of site	2.897	1	0.084	8.862	8.702
4	Area	Area of site m^2	2.981	1	0.084	8.869	8.709
5	Canopy	% canopy closure	2.092	1	0.148	9.757	9.597
6	C	Soil total organic carbon %	0.720	1	0.396	11.121	10.961
7	ASNW	% land use within 500m of site	0.475	1	0.491	11.375	11.215
8	Shape	Deviation from circle of same area	0.332	1	0.565	11.518	11.358
9	N	Soil total nitrogen %	0.124	1	0.725	11.725	11.565
10	ARW	% land use within 500m of site	0.114	1	0.736	11.736	11.576
11	pH	Soil pH H_2O	0.015	1	0.904	11.835	11.675
12	Wood	% land use within 500m of site	0.001	1	0.981	11.849	11.689

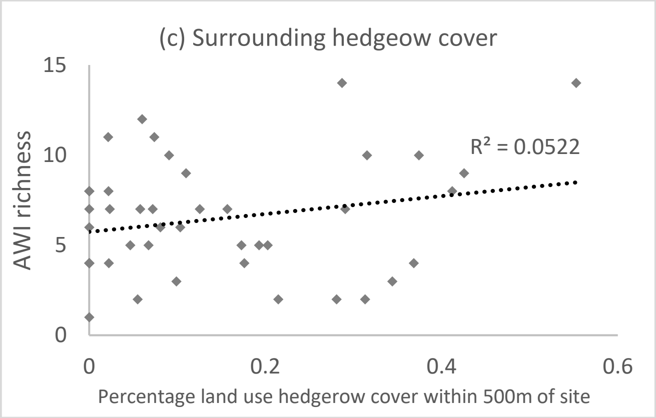
	(b) Multivariate models	χ^2	d.f	Sig.	△AIC continuity	△AIC non-continuity
13	Optimal multivariate model across all non-continuity variables/ biogeographical variables					
	Hedge	12.076	1	0.001		
	ARW	8.716	1	0.003		
	Area	7.177	1	0.007		
	ASNW	5.748	1	0.017		
	Wood	4.105	1	0.043		
	Shape	2.009	1	0.156		
	Area*Shape	4.872	1	0.027		
	ARW*Hedge	3.994	1	0.919		
	Wood*Hedge	0.010	1	0.046		
	Overall model	27.689	9	0.001	0.160	0
14	Connectivity variables					
	Hedge	6.234	1	0.013		
	ASNW	3.065	1	0.080		
	ARW	0.710	1	0.399		
	Wood	0.140	1	0.708		
	Wood*hedge	2.761	1	0.097		
	ARW*hedge	1.228	1	0.260		
	ASNW*hedge	0.394	1	0.530		
	Overall model	16.674	7	0.020	7.175	7.015
15	Configuration variables					
	Area	4.737	1	0.030		
	Shape	1.306	1	0.253		
	Area*shape	6.359	3	0.095	9.491	9.331
16	Soil variables					
	P	5.207	1	0.022		
	N	0.366	1	0.545		
	C	1.995	1	0.158		
	pH	0.345	1	0.577		
	Overall model	7.496	4	0.094	11.873	11.713

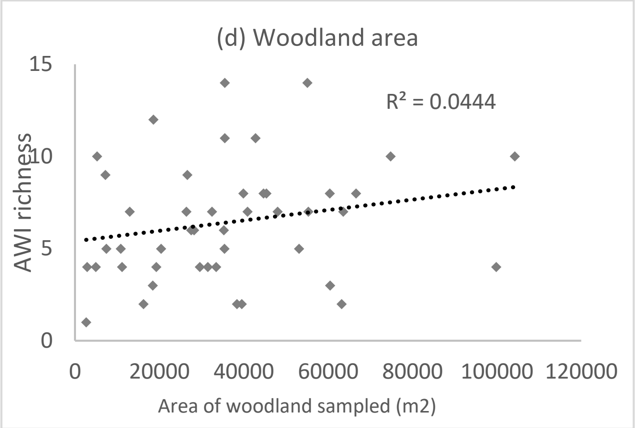
Graphical analysis showed AWI richness was significantly higher in ASNW than ARW or recent woodland (Fig. 5.1a). Graphical analysis of other explanatory variables showed no significant correlations between AWI richness any of the variables tested (Fig 5.1b-l).

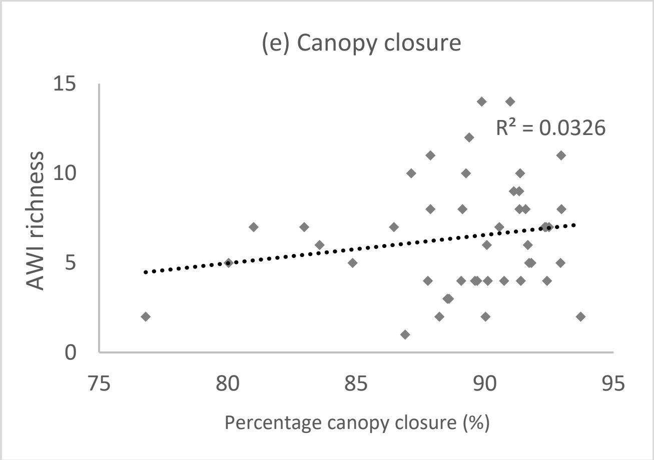

(a) Continuity

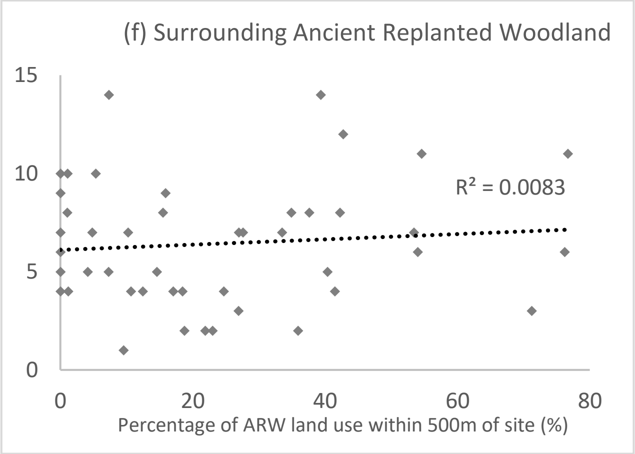
AWI mean richness and range (ASNW n=15, ARW n=15, recent n=15). Different letters indicate significant different (p<0.05)

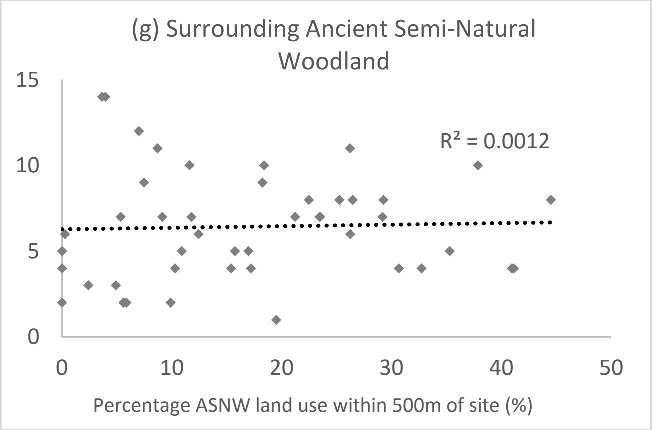

(b) Available soil phosphate

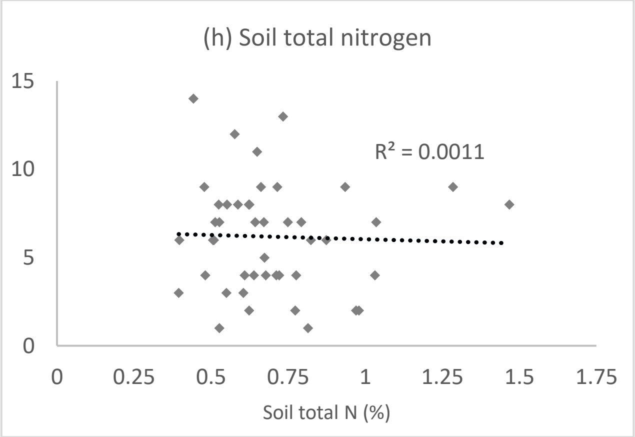

(c) Surrounding hedgeow cover


(d) Woodland area


(e) Canopy closure


(f) Surrounding Ancient Replanted Woodland


(g) Surrounding Ancient Semi-Natural Woodland


(h) Soil total nitrogen

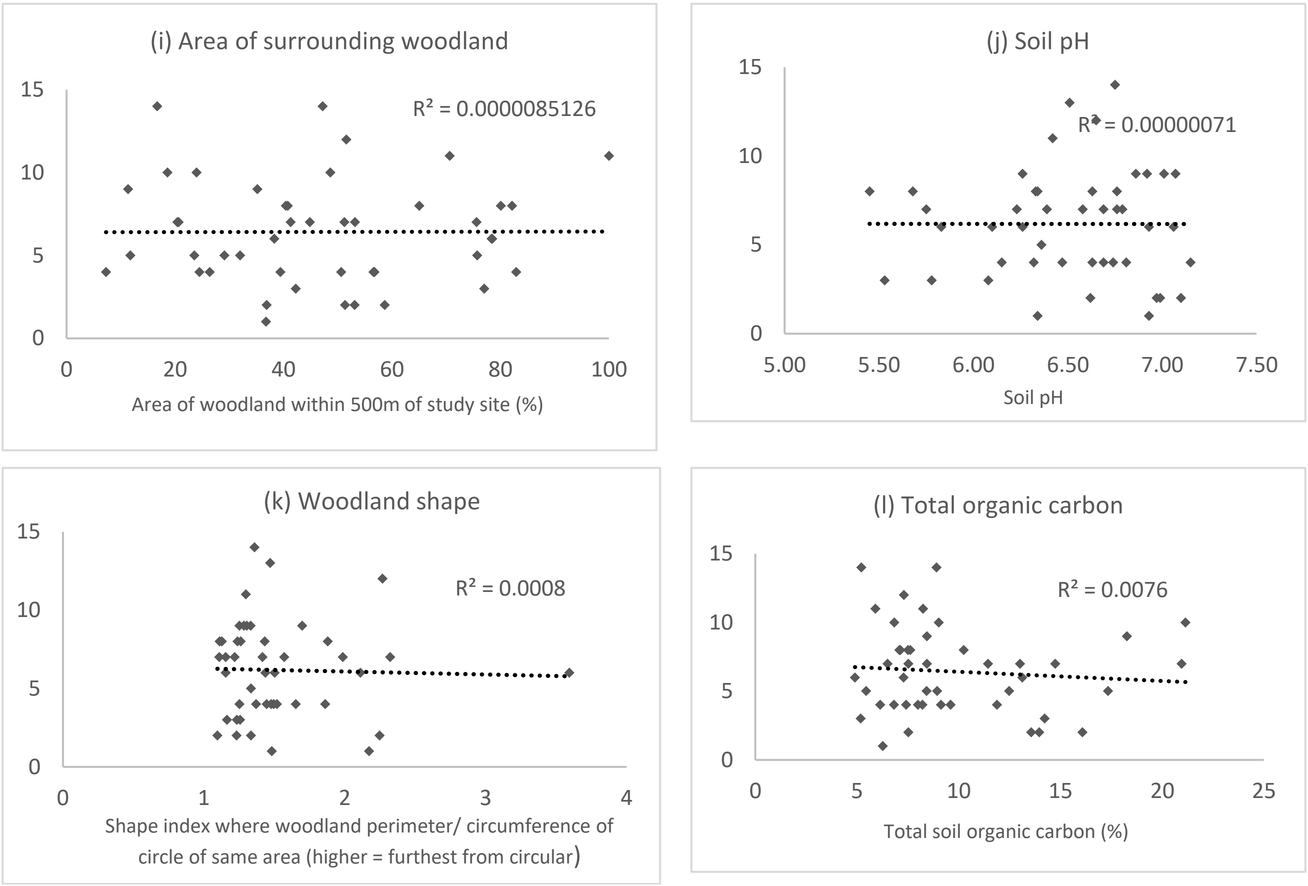
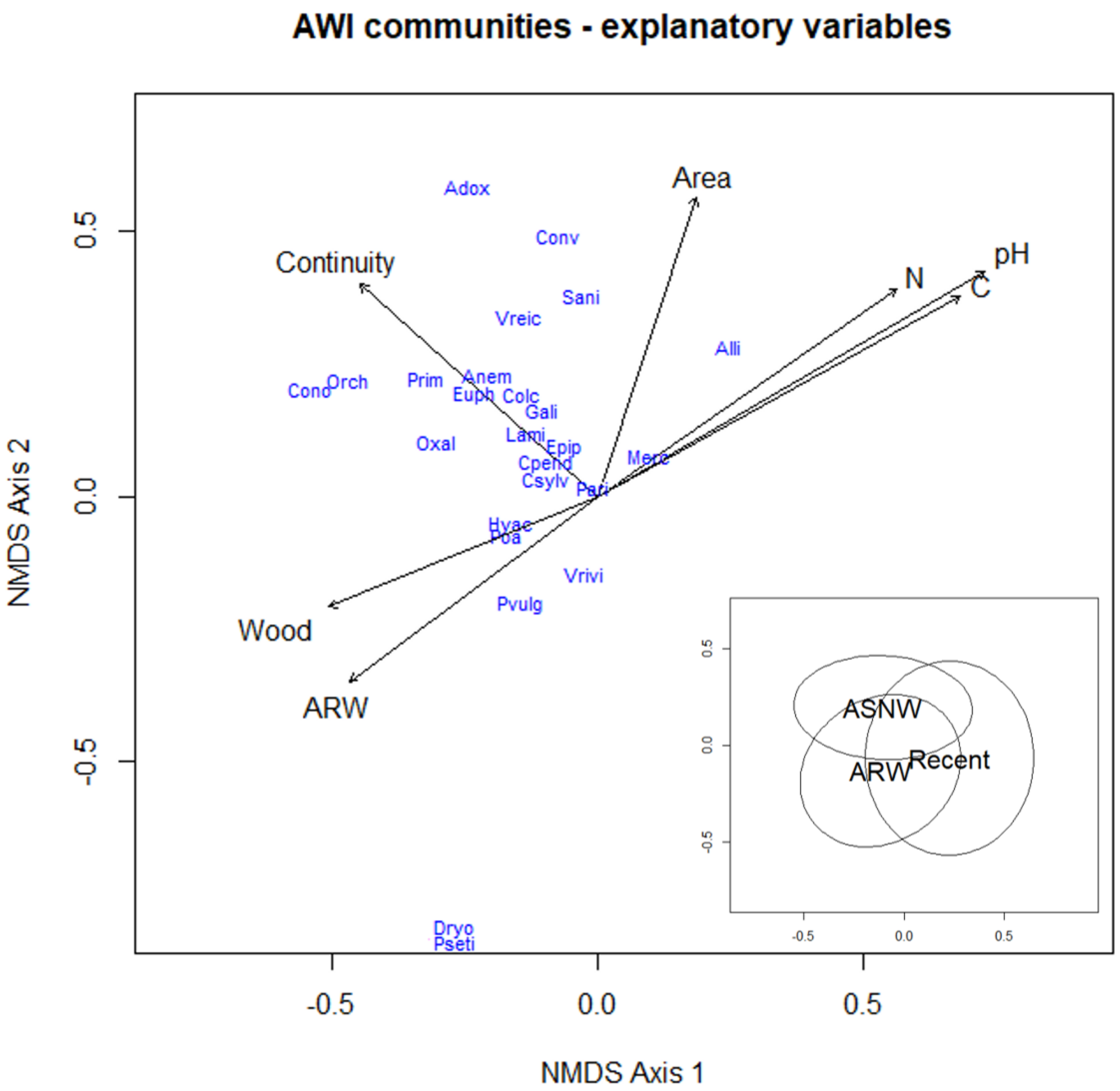


Figure 5.1. Explanatory habitat variables for AWI richness in 45 woodland sites. Ancient Semi-Natural Woodland (ASNW) (n=15); Ancient Replanted Woodland (ARW) (n=15); recent woodland (n=15). For categorical variable bar chart with range and Independent *t* test. For continuous variables scatterplots with trend line and R^2 value.

In contrast to AWI richness, community composition of AWI species was most strongly influenced by soil variables pH (p=0.001), N (p=0.003) and C (p=0.007) with R^2 values higher than continuity (Fig. 5.2). These three variables more strongly influenced community composition than continuity (p=0.024). Area, surrounding woodland and surrounding ARW also showed significant influence, but R^2 values were below that of continuity (Fig. 5.2). However, graphical analysis shows clustering of many AWI species around the continuity fitted variable, which corroborates the richness findings (Fig. 5.2). Indeed, the continuity variable occupies the same ordination space as the standard deviation of ASNW and ARW communities (Fig. 5.2). The soil variables appear in the same space as recent woodland communities, while connectivity variables occupy the same space as ARW communities. Sample size was too small to allow variable testing separately for ASNW, ARW and recent woodlands.



Species: *Adoxa moschatellina; Allium ursinum; Anemone nemorosa; Carex pendula; Carex sylvatica; Colchicum autumnale; Conopodium majus; Convallaria majalis; Dryopteris carthusiana; Epipactis helleborine; Euphorbia amygdaloides; Galium odoratum; Hyacinthoides non-scripta; Lamiastrum galaeobdolon; Mercurialis perennis; Orchis mascula; Oxalis acetosella; Paris quadrifolia; Poa nemoralis; Primula vulgaris; Polypodium vulgare; Polystichum setiferum; Sanicula europaea; Viola riviniana; Viola reichenbachiana.*

	R2 (%)	P		R2	P
pH (soil, H_2O)	**0.320**	**0.001**	**Wood (% land-use within 500m)**	**0.135**	**0.046**
C (soil total organic carbon C %)	**0.274**	**0.003**	P (plant available soil P mg/l)	0.056	0.335
N (total soil N %)	**0.213**	**0.007**	ASNW (% land-use within 500m)	0.053	0.339
Continuity (ASNW, ARW, recent)	**0.163**	**0.024**	Hedge (% land-use within 500m)	0.050	0.345
Area (of woodland site m2)	**0.160**	**0.019**	Canopy (% closure)	0.047	0.389
ARW (% land-use within 500m)	**0.156**	**0.038**	Shape (deviation from circle of same area)	0.041	0.409

Figure 5.2. AWI species community composition by Jaccard distance. Non-metric multidimensional scaling of AWI communities across all three woodland continuity types: Ancient Semi-Natural Woodland (ASNW) (n=15); Ancient Replanted Woodland (ARW) (n=15); recent woodland (n=15). 999 permutations. Dimensions = 3. Explanatory variables (p<0.05) fitted using ‘envfit’ function in R. 25 species. Stress = 0.164. Inset ellipses show s.d. of ASNW, ARW and recent woodland communities.

Individually, AWI species showed a range of responses to continuity, biogeographical, soil and canopy variables (Table 5.3). One third of the species (*A. nemorosa, P. vulgaris. P. quadrifolia, V. reichenbachiana, C. sylvatica*) correlated significantly and positively with greater continuity. In three cases biogeographical variables correlated significantly with species presence, positively for *H. non-scripta*, but negatively for *M. perennis* and *S. europaea*. Soil variables correlated significantly with *A. ursinum* and *M. perennis*. *G. odoratum* correlated significantly and positively with canopy closure. Three AWI species (*M. perennis, O. acetosella and S. europaea*) exhibited no correlation with continuity (p=1.000). Five species showed no significant correlations with any variable (*E. amygdaloides, L. galaeobdolon, O. acetosella, V. riviniana and P. nemoralis*).

Table 5.3. Influence of explanatory factors on AWI species presence (present in ≥20% of plots). Binary logistic regression. Continuity of site was measured as an ordinal variable (Ancient Semi-Natural Woodland (ASNW)= 3; Ancient Replanted Woodland (ARW) = 2; recent woodland = 1); connectivity; biogeographical comprises (PCA (40% var.) percentage of woodland, ASNW, ARW and hedgerow within 500m of the site and site area and reciprocal of shape; soil comprises (PCA (70% var.) P, N, C, pH; canopy closure of site (%). Wald chi squared, p value and Nagelkerke pseudo R2 value. Bold indicates significant correlation p<0.05.

Species (occurrence /45)	Continuity (d.f. 1)				Biogeographical (d.f. 1)				Soil (d.f. 1)				Canopy (d.f. 1)			
	Chi	P	R^2 (%)	Dir.	Chi	P	R^2 (%)	Dir.	Chi	P	R^2 (%)	Dir.	Chi	P	R^2 (%)	Dir.
A. ursinum (14)	1.370	0.242	4.4		1.514	0.218	4.8		**8.528**	**0.003**	**32.4**	**+**	9.210	0.051	15.9	
A. nemorosa (20)	**4.568**	**0.033**	**14.1**	**+**	0.347	0.556	1.00		0.109	0.741	0.3		0.935	0.334	2.9	
E. amygdaloides (19)	0.543	0.461	1.6		3.193	0.074	10.0		0.462	0.497	1.4		0.820	0.365	2.9	
G. odoratum (16)	0.577	0.447	1.8		2.312	0.128	7.3		0.959	0.327	2.9		**3.889**	**0.049**	**15.4**	**+**
H. non-scripta (35)	2.871	0.090	10.5		**5.414**	**0.020**	**21.3**	**+**	2.780	0.095	9.5		0.581	0.446	1.9	
L. galaeobdolon (13)	0.642	0.423	2.1		2.042	0.153	6.8		0.682	0.409	2.3		0.114	0.736	0.4	
M. perennis (39)	<.0.001	1.000	<0.001		**4.233**	**0.040**	**22.1**	**-**	**5.603**	**0.018**	**41.0**	**+**	1.960	0.162	7.5	
O. acetosella (9)	<.0.001	1.000	<0.001		0.568	0.451	2.0		3.342	0.068	14.8		0.007	0.934	<0.001	
P. quadrifolia (9)	**4.447**	**0.034**	**18.4**	**+**	1.048	0.306	3.8		0.203	0.653	0.7		0.014	0.907	<0.001	
P. vulgaris (9)	**4.477**	**0.034**	**18.4**	**+**	0.370	0.543	1.3		0.382	0.537	1.4		0.851	0.356	2.9	
S. europaea (9)	<.0.001	1.000	<0.001		**4.118**	**0.042**	**16.2**	**-**	3.098	0.078	11.0		2.999	0.083	14.6	
V. reichenbachiana (19)	**4.750**	**0.029**	**15.1**	**+**	0.148	0.700	0.4		0.722	0.395	2.2		0.392	0.531	1.2	
V. riviniana (13)	0.712	0.399	2.4		0.016	0.898	0.1		0.105	0.745	0.3		0.028	0.866	0.1	
C. sylvatica (27)	**4.637**	**0.031**	**14.7**	**+**	0.164	0.685	0.5		0.012	0.991	<0.001		0.539	0.463	1.6	
P. nemoralis (11)	2.720	0.099	9.5		1.007	0.316	3.4		2.122	0.145	8.0		0.141	0.707	0.5	

5.5 Discussion

Fundamental to understanding the ecological basis for high AWI richness, was that some ARW and recent woodlands exhibited AWI counts comparable to ASNW. In some cases, up to 12 AWI species (Fig. 5.1a). Usually the long continuity of ASNW is necessary to support a distinctive community of AWI species (e.g. Kelemen *et al*., 2014; Orczewska, 2009; Graae *et al*., 2003; Honnay *et al*., 1999; Wulf, 1997). Thresholds of 10 or even 12 AWI species have been employed by some organisations to classify woodland as ancient, and between 8-12 AWI species is considered an indicator of 'very good' woodland quality meriting conservation (Glaves *et al*., 2009). On this basis, there is strong rationale for understanding the ecological drivers of AWI richness to inform restoration of ARW, and for conservation of valuable recent woodland.

The continuity status of a woodland (ASNW, ARW, and recent) was a significant predictor of AWI richness (Table 5.2). The highest mean AWI richness was observed in woodlands of longest continuity (ASNW), which supports findings of prior studies (e.g. Kelemen *et al*., 2014; Sciama *et al*., 2009). Additionally, these findings suggest that AWI richness is, on average, a good indicator of ancient woodland status. However, as demonstrated by the high AWI counts of some recent woodlands there are exceptions, therefore AWI richness should be employed among other ecological and historic evidence bases for determination of ancient status.

A combination of biogeographical variables and their interactions proved a significant and equally strong predictor of AWI richness as continuity (Table 5.2). Both temporal continuity and biogeographical variables have been correlated with AWI richness in prior studies

(Kelemen *et al*., 2014; Brunet *et al*., 2011; Sciama *et al*., 2009; Petit *et al*., 2004). However, to date, no previous study has assessed the degree to which these could be treated as equal explanations for the distinctiveness of AWI rich communities. Woodlands with greater landscape connectivity and more regular shape significantly correlated with higher AWI richness (Table 5.2; Fig. 5.1c, d, f, g, i, k). The converse of these conditions has long been considered a threat to specialist woodland flora such as AWI species (Brunet *et al*., 2011; Rackham, 2008; Corney *et al*., 2008, 2004; Petit *et al*., 2004). The low dispersal ability of many AWI species (such as vegetative spread, or having few or heavy seeds dispersed by short-range vectors) has been found to be incompatible with a fragmented landscape (Jacquemen *et al*., 2003a; Hermy *et al*., 1999).

While biogeographical variables did not strongly contribute to the model as single predictors, when combined in multivariate models with appropriate interactions, they offered much stronger explanation of AWI richness (Table 5.2). Interactions between area*shape, and wood*hedge contributed particularly strongly. This is in line with prior studies where variables such as patch size and isolation were meaningful explanations of richness only when aggregate effects were tested (Bailey, 2007; Honnay *et al*., 2005; Petit *et al*., 2004).

Unlike a richness count, AWI community analysis recognises the identity of individual species and enables greater insight into the community ecology of AWI species. Soil variables (pH, C, and N) exhibited the strongest significant influences on AWI community composition (Fig. 5.2). Higher pH, C and N nutrient status diverged from the cluster of AWI species towards the ordination space of recent woodland AWI communities (Fig. 5.2). pH was the strongest significant determinant of community composition, explaining 32% of the community variation, which reflects the co-occurrence of species with the similar range of tolerance. N was found to be a significant explanatory variable in a similar community study (De Keersmaeker *et al*., 2004). In additional support of the findings of the present study, Hofmeister *et al*., (2013) found N enrichment to lead to competitive exclusion of woodland species by nitrophiles. Several studies have found soil C to be higher in ancient woodland soils (Brunet *et al*., 2011; Orczewska, 2009; De Keermaeker *et al*., 2004), but none have conducted comparable community analysis.

Continuity was again a significant predictor of AWI community composition, explaining 16% of community variation, and the majority of AWI species were clustered around the variable's trajectory and within the ordination space of ASNW communities (Fig. 5.2). As shown in Fig 5.1a, AWI species are more prevalent in ASNW, which accounts for the position of many AWI species in the ordination space. Numerous prior studies have found distinctive community composition in ancient woodlands (Atkinson *et al*., 2015; Kelemen *et al*., 2014; Coote *et al*., 2012; Chapter 4).

Similarly to the richness models, some biogeographical variables exhibited strong explanatory effects on AWI community composition. Area of surrounding woodland and surrounding ARW were associated with the AWI communities found in the ordination space overlapping ASNW and recent communities (Fig. 5.2).

The relative positioning of species in the ordination space gives insight into AWI community dynamics. The majority of AWI species such as *P. quadrifolia*, *Carex* sp., *L. galaeobdolon*, *M. perennis* and *H. non-scripta* are commonly co-occuring and occupy overlaps in three woodland continuity categories (Fig. 5.2). Two fern species, *D. carthusiana* and *P. setiferum*, are strongly disassociated from the majority of AWI species, occurring in the ordination space of ARW. The community composition of fern species, not only those that are AWI species, has previously been shown to indicate understorey succession in plantations (Onaindia *et al*., 2013). *A. ursinum* is positioned at a distance from the central cluster of AWIs in the same ordination space as the soil variables.

Analysis of variables influencing the presence of individual AWI species confirmed that the majority of individual AWI species are not significantly associated with long continuity (Table 5.3). For this reason, the current practice of treating them as a guild and with a certain richness threshold is appropriate when assessing woodland ancient status (Hermy *et al*., 1999; Rose, 1999). Habitat conditions other than continuity are significantly associated with the presence of several species (Table 5.3). These results enhance understanding of the ecological requirements of AWI species, which can be factored into interpretation of AWI studies, and have a practical management application.

Individual species' analysis enabled clearer understanding of their contribution to the AWI richness count under the different scenarios modelled in Table 5.2. Biogeographical factors were important for some species. *H. non-scripta* presence required high connectivity, while *M. perennis* and *S. europaea* were significantly and negatively correlated with connectivity. Although all three are recognised AWI species, the opposing preferences may be accounted for by different degrees of specialism/ generalisms: as noted by Brown *et al*., (2015), just over half of AWI species are in fact woodland generalists rather than specialists.

High soil factor values were significant ($p=0.003$) predictors of *A. ursinum* presence, which tallies with Ellenberg values of 7 for N (prefers richly fertile places) and 7 for reaction (weakly acid to weakly basic condition, never found on very acidic soils) (Hill *et al*., 1999). The ordination plot (Fig. 5.2) also associates *A. ursinum* with higher nutrient status. *M. perennis* was also marginally significantly associated with higher soil variables.

Only *G. odoratum* was marginally significantly associated with greater canopy closure. Canopy closure was shown to have no significant influence on either richness or community composition, but as there was low variation in this variable (Fig. 5.1e), this is not surprising.

5.6 Conclusions and recommendations

This chapter has provided novel contributions to the understanding of AWI community ecology. Individual ARW and recent woodlands have been shown to support equally rich AWI communities as ASNW. This holds important implications for the conservation of AWI rich recent woodlands, particularly as they are not as robustly protected as ASNW and ARW. With several AWI species of threatened status, an individual woodland scale approach may be worthwhile. However, mean AWI richness was highest in ASNW, which lends support to the continued use of AWI lists to identify them when supported by historic evidence. Additionally, the findings of this study support the use of AWI thresholds, as analysis of individual AWI species showed that only one third of those tested were significantly influenced by continuity. Instead, the presence of others was influenced significantly by biogeographical, soil or canopy factors.

An important outcome of this study is that biogeographical factors and woodland continuity are equally effective, significant, and positive predictors of AWI richness. To date, seemingly no study has tested alternative explanations of AWI richness relative to continuity. Woodland connectivity and restoration are currently important aspects of conservation management. This outcome strongly supports the implementation of greater landscape connectivity to support distinctive AWI communities whether in ASNW, ARW or recent woodlands. In terms of community composition, soil variables were a stronger predictor than woodland continuity.

Overall, the study has demonstrated significant responses of AWI species to a range of habitat ecological variables in addition to the single measure of continuity. Continuity remains a useful guide to locating the habitats of greatest biodiversity value, which can be supplemented with biogeographical and soil data to provide effective conservation of valuable AWI communities.

6. Impact of dual edge proximity on the distribution of ancient woodland indicator plant species in a fragmented habitat

This chapter is a replication of a paper forming part of this research that was published in Community Ecology. Referencing conventions have been changed to meet University requirements, and an appendix to the original paper has been incorporated into the results section. The original paper is available in Appendix II.

Outputs arising from this chapter:

Swallow, K. and Goodenough, A. (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), pp.31-36.

Swallow, K. and Goodenough, A. (2016) Double-edged effect? *Impact of dual edge proximity on the distribution of ancient woodland indicator plant species in a fragmented habitat*. RAU research seminars. Cirencester, 1 November

Woodland-arable interface. Arle Grove.
21/05/10 SO 99497 21572

6.5 Abstract

The influence of edge proximity on woodland plants is a well-established research area, yet the influence of dual edge exposure has rarely been investigated. This novel research aims to establish whether proximity to two edges has any additive influence on Ancient Woodland Indicator (AWI) species presence relative to proximity to a single edge. Several AWI species are threatened and thus specific conservation priorities, while Ancient Semi-Natural Woodland (ASNW) itself is often highly fragmented: almost half of remnant patches are less than 5ha, which increases the potential for dual edge effects. Here, systematic mapping of herbaceous AWI species was conducted in 310 vegetation plots in two formerly-connected ASNW fragments in South-West England. Linear regression modelling revealed that distance to nearest edge and distance to second nearest edge were both univariately positively correlated with AWI species richness. After distance from nearest edge was entered into a multivariate model first, distance from second edge was entered in a second optional step after meeting stepwise criteria. The resultant multivariate model was more significant, and explained more variance, than either variable in isolation, indicating an additive effect of dual edge exposure. Likewise, binary logistic regression modelling showed presence of individual AWI species (*Anemone nemorosa*, *Hyacinthoides non-scripta*, *Lamiastrum galaeobdolon* and *Paris quadrifolia)* was significantly related not only to distance from the nearest and second nearest edges in isolation, but significantly more strongly by the additive effect of distance from both edges in a single model. We discuss the implications of these findings from community ecology and conservation perspectives.

Abbreviations: AWI – Ancient Woodland Indicator, ASNW – Ancient Semi-Natural Woodland

Nomenclature: IPNI (2015) The International Plant Names Index http://www.ipni.org/

6.2 Introduction

The presence of scarce and range-restricted flora in Ancient Semi-Natural Woodland (ASNW) (Palo *et al*., 2013; Honnay *et al*., 1999b; Wulf, 1997; Peterken 1974) contributes to its status as an ecosystem of high conservation value (Goldberg *et al*., 2007; Rackham, 2003; Peterken, 1983). The ancient woodland concept is well-integrated into forest research and conservation practice, although definitions and date thresholds vary amongst countries (Wirth *et al*., 2009b; Hermy *et al*., 1999). In England, ASNW is defined as predominantly a native broadleaf canopy established through natural regeneration (Rackham, 2008) on land that has remained continuously woodland since at least the year 1600 (Goldberg *et al*., 2007).

Ancient woodland indicator (AWI) species are vascular plants that are particularly, but not exclusively, associated with ASNW (Glaves *et al*., 2009; Rose, 1999). Regional AWI lists were developed to assist in determining ancient woodland status and are additionally used to assess habitat quality (Glaves *et al*., 2009). AWI species are considered to have low colonisation potential due to poor seed production, low dispersal capability and short-term persistence in the seed bank (Honnay *et al*., 1998). As such, AWI species may not be able to colonise alternative woodland habitats if ASNWs are lost or conditions become sub-optimal (Hermy *et al*., 1999). The limited distribution of AWI species and their specific ecology has promoted extensive use in woodland research (Stefańska-Krzaczek *et al.,* 2016; Kimberley *et al*., 2014; Kirby and Morecroft, 2011; Rackham, 2003; Kirby and Goldberg, 2002; Hermy *et al*., 1999; Rose, 1999; Honnay *et al*., 1998; Wulf, 1997; Spencer, 1990; Peterken, 1974).

Landscape fragmentation is a significant threat to ASNW plant communities (Corney *et al*., 2008b; Rackham, 2008), not only due to reduction in dispersal potential of AWI species, but also due to increased edge effects. Edge width is defined as the outer part of a woodland compartment where environmental conditions differ significantly from the interior (Honnay *et al*., 2002b). Corney *et al*., (2008b) report that 48% of ancient woodlands are under 5 ha, which means they have a high edge: interior ratio and a large edge width, especially if they deviate from an optimum circular shape (Laurance, 2008). Edge environmental conditions are generally considered to be less favourable for persistence of specialist flora, including many AWI species, due to altered abiotic and biotic variables (Tinya and Odor, 2016; Hofmeister *et al*., 2013; Willi *et al*., 2005; Honnay *et al*., 2002b; Murcia, 1995; Matlack, 1993), as well as anthropogenic influences (Corney *et al*., 2008b).

Abiotic and biotic variables commonly exhibit an edge width of between 10-60m in temperate forests (Honnay *et al*., 2002b; Gehlhausen *et al*., 2000; Palik and Murphy, 1990; Matlack, 1994; Matlack, 1993). Additionally, studies seeking to avoid edge influence, have situated sample plots at >20m from the edge (Bossuyt and Hermy, 2000), >30m (Brunet *et al*., 2012), and >50m (Coote *et al*., 2012; Gehlhausen *et al*., 2000). Exceptionally, edge effects have been evidenced over 100m from the edge (Hofmeister *et al*., 2013; Pellissier *et al*., 2013) but such findings are not comparable to small ancient woodland fragments in the UK. Land use in the matrix (Gove *et al*., 2007), prevailing wind direction (Smithers, 2000) and aspect (Honnay *et al*., 2002b; Murcia, 1995) influence the extent to which edge effects permeate woodland.

This is the first study to investigate multiple edge effects in relation to AWI species and ancient woodland. The impact of multiple edges is an important but overlooked factor (Ries and Sisk, 2004). Few studies have explicitly gathered primary data to analyse this in relation to any species or ecosystem (Fletcher, 2005), instead measuring linear distance to the closest edge only. A small number of studies have been completed with explicit focus on AWI response to nearest edge proximity (Kimberley *et al*., 2014; Hofmeister *et al*., 2013; Pellissier *et al*., 2013; Willi *et al*., 2005). Despite the potential importance of edge effects on AWI species, both in their own right as specialist species, and in terms of their efficacy of indicators, a search of the literature revealed no studies relating to multiple edge effects on these species. This is surprising given that the highly-fragmented nature of ANSW means that the potential for exposure to multiple edge effects is considerable.

We test for relationship with the nearest edge, as well as any additional contribution of the second edge to take account of double exposure within fragment corners. We hypothesised that (1) AWI richness will increase with distance from any edge; (2) the second nearest edge would also correlate with AWI richness so that a multivariate model with both distances would be superior to a univariate model using either in isolation; (3) the patterns for AWI species richness would also hold true for specific AWI species analysed on a presence/ absence basis.

6.3 Methods

The study site was a fragmented species-rich ASNW in the South-West UK. The two discrete fragments comprising the site were situated near Cheltenham on the Cotswold Hills escarpment of Jurassic oolitic limestone, at 265m above sea level and centred on 51°53’35.5’’N, 2°00’34.60’’W (Fig. 6.1). The mean diurnal temperature was 8.6-14.7°C and annual precipitation was 843mm (MET office, 1981-2010). The fragments have comparable geology, edaphic variables and topography. The coppice-with-standards woodland classifies as National Vegetation Classification W8b (Rodwell, 1991), with a canopy dominated by *Fraxinus excelsior* and *Quercus robur*.

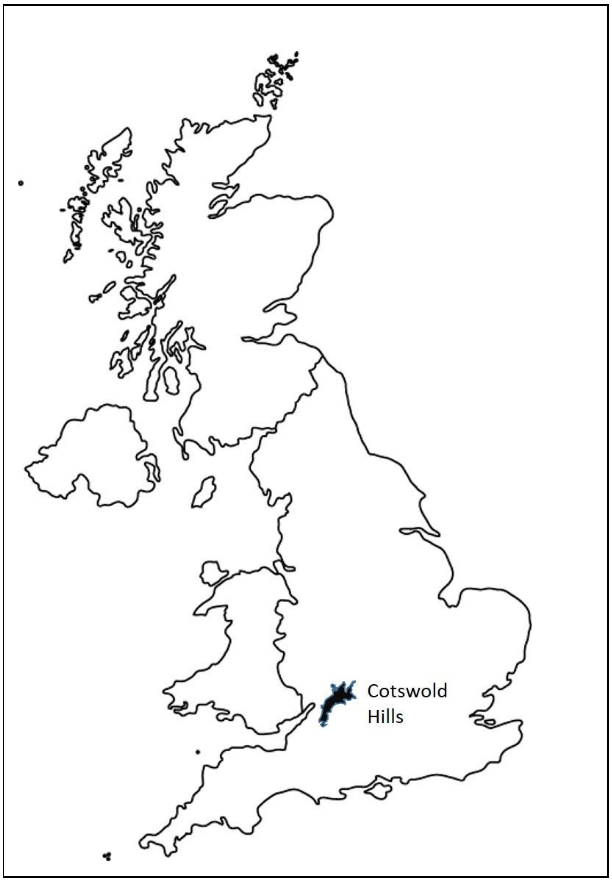


Figure 6.1. Study site location of the Cotswold Hills, UK.

Both fragments, henceforth referred to as Fragment 1a and 1b were located within an agricultural (arable and equine) matrix. Fragment 1a was 4.8ha and of approximately rectangular dimension (190x255m). Fragment 1b was a remnant of 0.6ha located 25m from the eastern edge of Fragment 1a. Historic map evidence showed that both fragments formed a single woodland until c1965. Both fragments are classified by DEFRA (2016) as ASNW.

In order to assess any influence of dual-edge effect in Fragment 1a, presence of AWI species was mapped and recorded via a total of 256 2x2m plots. Plots were located in the corners of Fragment 1a within 60m of both the nearest edge (Edge 1) and second nearest edge (Edge 2). The distance of 60m was deemed a conservative upper limit for detection of edge effects based on previous studies (Vallet *et al*., 2010; Honnay *et al*., 2002b; Gehlhausen *et al*., 2000; Murcia, 1995). Plots were located at 0, 5, 10, 20, 30, 40, 50 and 60m on transects perpendicular to the Western and Eastern edges, with 0m defined as the commencement of woody species' stems (Murcia, 1995). Changing the sampling distance from 10m to 5m at the edges of the fragment allowed small-scale change to be better detected (Honnay *et al*., 2002b). Recorded species were restricted to herbaceous and semi-woody plants (Brunet *et al*., 2011) identified as Ancient Woodland Indicators in the South-West UK (Rose, 1999). To complement analysis of the larger fragment and demonstrate any difference in species richness and presence between the two fragments, Fragment 1b was surveyed on the same system with plots at 0, 5, 10 and 20m from the Eastern and Western edges (n=54). All statistical analyses apply to Fragment 1a.

To predict the influence of Edge 1 and Edge 2 on AWI richness, separate univariate linear regression analyses were performed (n=256). To test any additive influence of both edges, a hierarchical multivariate model was created where Edge 1 was entered via forced entry and Edge 2 was available as a candidate variable in a second step using a stepwise approach (entry criterion α = 0.05, except *L. galaeobdolon* α = 0.1) (De Keersmaeker *et al*., 2004). Normality assumptions were met, and collinearity was within accepted limits: VIF < 10 (Myers, 1990) and tolerance >0.2 (Menard, 1995). The same principles were followed using binary logistic regression to test the influence of Edge 1 and Edge 2, separately and additively, on the presence AWI species (those found in >10% of plots) (n=256). The R^2 (linear regression) and Nagelkerke pseudo R^2 (logistic regression) statistics were calculated to measure the relative influence of single and additive edges on, respectively, AWI richness and species presence.

6.4 Results and analysis

Mapping of Fragment 1a showed clear spatial patterns in AWI richness in relation to edge proximity (Fig. 6.2). AWI richness was very low at the edge, and increased gradually up to 60m; this effect was most pronounced at the corners where a distinct edge effect was apparent up to 20-30m, rather than 5-10m on transects located mid-edge. Within the very small Fragment 1b, AWI richness is lower throughout than in Fragment 1a, with no clear edge or corner pattern (Fig. 6.2).

Regression analysis showed significant positive directional relationships between AWI richness and distance from the edge in Fragment 1a (Table 6.1). When tested independently, Edge 1 and Edge 2 were both shown to be significantly positively related to AWI richness, but Edge 1 was related more strongly than Edge 2. Used in a hierarchical framework, Edge 2 met the stepwise criteria for entry as a second variable into a multivariate model after Edge 1 had already been entered. This, together with the resultant multivariate model being more significant and explaining more variance than either Edge 1 or Edge 2 in isolation, strongly suggests dual-edge exposure is important for AWI richness.

Repeating the above analytical framework using hierarchical multiple logistic regression for the seven most prevalent species (those present in >10% of plots) showed that the presence of four species increased significantly with increasing distance from edge (Table 6.1). For each of these species (*A. nemorosa*, *H. non-scripta*, *L. galaeobdolon* and *P. quadrifolia)* Edge 1 and Edge 2 were both significant when analysed separately and again the R^2 statistic for Edge 2 was slightly lower than Edge 1. In all four cases, running a stepwise model with Edge 2 available as a candidate variable resulted in a multivariate model being created that had a substantially lower P value and substantially higher R^2 value than either edge tested alone. For where species Edge 2 was not entered using standard stepwise criteria ($\alpha = 0.05$ or 0.10), forcing this variable into the model did not improve it relative to using Edge 1 alone and all models were non-significant.

Thirteen AWI species were recorded within Fragment 1a sample plots and eight within Fragment 1b (Table 6.2). The four species significantly associated with distance showed clear reductions in prevalence in Fragment 1b, in comparison to 1a (Table 6.2). Presence of *A. nemorosa* and *H. non-scripta* in Fragment 1b was half of that in 1a, while *L. galaeobdolon* and *P. quadrifolia* were absent from Fragment 1b. Of prevalent species not significantly associated with distance, only *A. ursinum* occurred considerably more frequently in Fragment 1b than in Fragment 1a.

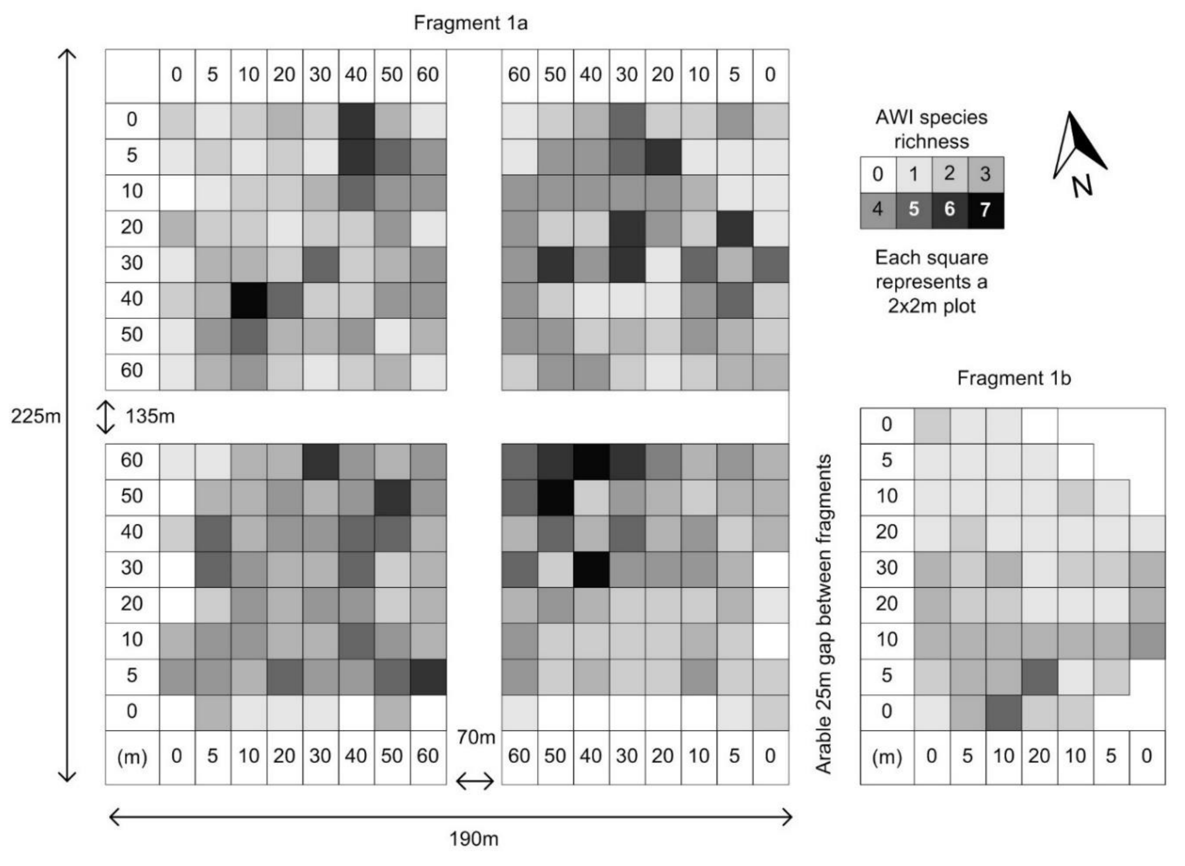


Figure 6.2. Richness of Ancient Woodland Indicator species in a total of 310 2x2m plots within two fragments of ancient semi-natural woodland.

Table 6.1. AWI richness (all species) and species presence (most frequently occurring species in >10% of plots) relationship with distance from Edge 1, Edge 2, and both edges together. In all cases, the additive model was a hierarchical one whereby Edge 1 was entered first and then Edge 2 was available as a candidate variable for inclusion following a stepwise approach; the model was not calculated if the addition of Edge 2 into the model did not significantly improve it.

		P	R^2	Dir.
AWI richness	Edge 1	<0.001	0.099	+
	Edge 2	<0.001	0.069	+
	Additive	<0.001	0.115	+

Species		Chi (df)	*p*	R^2	Dir.
A. ursinum	Edge 1	0.357 (1)	0.425	0.030	
	Edge 2	0.187 (1)	0.666	0.080	
	Additive	Model not calculated			
A. nemorosa	Edge 1	23.117 (1)	<0.001	0.126	+
	Edge 2	19.572 (1)	<0.001	0.107	+
	Additive	29.292 (2)	<0.001	0.158	+
G. odoratum	Edge 1	0.077 (1)	0.781	0.000	
	Edge 2	0.002 (1)	0.968	0.000	
	Additive	Model not calculated			
H. non-scripta	Edge 1	27.550 (1)	<0.001	0.141	+
	Edge 2	20.323 (1)	<0.001	0.105	+
	Additive	33.100 (2)	<0.001	0.168	+
L. galaeobdolon	Edge 1	11.866 (1)	0.001	0.064	+
	Edge 2	9.321 (1)	0.002	0.036	+
	Additive	14.417 (2)	0.001	0.077	+
P. quadrifolia	Edge 1	16.698 (1)	<0.001	0.095	+
	Edge 2	16.117 (1)	<0.001	0.092	+
	Additive	22.287 (2)	<0.001	0.126	+
V. reichenbachiana	Edge 1	0.699 (1)	0.403	0.005	
	Edge 2	2.044 (1)	0.153	0.014	
	Both	Model not calculated			

Dir. – direction of relationship for significant models. R^2 – Nagelkerke

Species present with conservation designations: *Hyacinthoides non-scripta* – Wildlife and Countryside Act, schedule 8; *Lamiastrum galaebdolon* – Vascular Plant Red List for Great Britain nationally scarce, vulnerable. Vascular Plant Red List for England, vulnerable; *Viola reichenbachiana* – Scottish Biodiversity List. *Primula vulgaris* (Wildlife Order Northern Ireland schedule 8); *Sanicula europaea* (Vascular Plant Red List for Great Britain, near threatened)

Table 6.2 Comparative frequency occurrence of AWI species in Fragments 1a and 1b. Total herbaceous AWI count of both fragments.

	Fragment 1a	Fragment 1b
Frequency occurrence		
A. nemorosa	73%	35%
H. non-scripta	66%	33%
A. ursinum	51%	96%
L. galaeobdolon	31%	Absent
G. odoratum	25%	Absent
P. quadrifolia	22%	Absent
V. reichenbachiana	15%	2%
Conopodium majus	4%	2%
P. vulgaris	2%	7%
Euphorbia amygdaloides	1%	Absent
Orchis mascula	1%	2%
Veronica montana	1%	2%
S. europaea	<1%	Absent
Total AWI count	13	8

6.5 Discussion

The above results show that not only are edge conditions less suitable for the majority of AWI species present, but the AWI community is vulnerable to a dual-edge effect whereby the combined influence of two edges is amplified and permeates further into a woodland near corners. The distance to the nearest two edges combined explained 11% of the variation in AWI richness and up to 17% of the variation in the presence/ absence of specific AWI species (Table 6.1). Dual-edge exposure explained a significant, and consistent, additional 1-3% of the variation in AWI richness and presence of some species than the single nearest edge alone (Table 6.1). The findings reinforce the need to protect ancient woodlands from fragmentation. Two species with conservation designations, *H. non-*

scripta and *L. galaeobdolon*, were especially adversely affected by edge proximity (Table 6.1). At 4.8ha, Fragment 1a is among the larger of the 48% of ancient woodlands that are smaller than 5ha (Corney *et al.*, 2008b), with a considerable area exposed to single and dual-edge effects. Fragment 1b is smaller still, and mapping suggests is influenced in its entirety by edge conditions (Fig. 6.2; Table 6.2).

Both woodlands reinforce the edge: interior ratio theory proposed by Laurance (2008). For this reason, some AWI species might not be appropriate indicators in small fragments where there is a high proportion of edge habitat, as they may be absent even from small ancient woodlands. Our findings show a lower richness count and predominantly lower prevalence of AWI species in Fragment 1b despite its adjacent position and history of connectivity with 1a. However, both fragments have what is considered to be an acceptable AWI score (Fragment 1a = 13; Fragment 1b = 8). Thresholds of 10-12 AWI species (including woody species, forbs and ferns) are used by organisations for allocating conservation priority, while ASNWs under 2ha with >5 AWI species were recommended for inclusion in a county ancient woodland inventory (Glaves *et al.*, 2009). If AWI species counts are used in small fragments, consideration should be given to only using the subset of species that are not seemingly affected by edge effects.

AWI species have been considered as a guild (Hermy *et al.*, 1999), but in this study the response of the community and individual species in relation to edge proximity indicates variation in niche requirements. Of the species significantly influenced by edge proximity, all increased in prevalence with distance from the edge (Table 6.1). The preference of *P. quadrifolia* for woodland interior may be accounted for by its adaptation for vegetative growth during low light periods (Bjerketvedt *et al.*, 2003). Similarly to this study, Honnay *et al.* (2002b) found *A. nemorosa* to have a positive edge-distance distribution in ancient woodland study sites in Belgium. Of those not exhibiting significant relationships with edge, only *V. reichenbachiana* decreased in prevalence with distance from either and both edges, possibly accounted for by its greater light requirement for a summer second leafing period (Rackham, 2003).

6.6 Conclusion and recommendations

This study has demonstrated dual-edge proximity has a substantial effect on AWI community composition, and has highlighted the species-specific nature of the response to different plants to the edge. It has also emphasised the effects of edge orientation and woodland size on floral response to edge conditions. Future research on the influence on multiple-edge biotic and abiotic variables in small ASNWs would be beneficial in further explaining spatial distribution of AWI species and for development of conservation management practices.

7. Conclusions and review of the research

Paris quadrifolia, Farmcote Wood, Guiting Strategic Nature Area.
03/08/14 Grid ref: SP: 06389 27190

Chapter scope:

The scope of the preceeding chapters is reiterated to provide a synthesis of the findings of the thesis. Original contributions are outlined in detail. The chapter also highlights avenues for future investigation.

7.1 Summary of the research context

This research considered the distinctiveness and community ecology of ancient woodland vegetation, and the implications for its conservation. The thesis was informed by the literature context summarised below.

The ancient woodland concept is recognised internationally (Wirth *et al*., 2009a) and holds an important place in conservation, policy and legislation. In England, ancient woodland is defined as an area of land that has been continuously wooded since at least the year 1600 (Kirby and Goldberg, 2002), and comprises both Ancient Semi-Natural Woodland and Ancient Replanted Woodland. These are both protected under planning law (Forestry Commission and Natural England, 2014), and their protection is reinforced in the recently published 25 year Environment Plan (HM Government, 2018). In England 2.3% of land area is ancient woodland (all woodland types comprise 10%) (Atkinson and Townsend, 2011).

ASNW is valued as the habitat nearest to natural climatic climax vegetation, which provides a baseline against which environmental changes can be measured (Corney *et al*., 2008b). The floristic biodiversity value of ASNW is widely considered to be higher than that of more recently established woodlands, providing further rationale for their protection (Honnay *et al*., 1999a; Wulf, 1997; Peterken, 1974). The vegetation of ASNW has long been considered distinctive to that of recent woodland (Kelemen *et al*., 2014; Rackham, 2008; Rose, 1999; Peterken, 1974). Very few studies have been completed on the vegetation of ARW (Atkinson *et al*., 2015).

Ancient woodlands are particularly valued as habitat for specialist AWI species, some of which are designated as protected species in their own right (JNCC, 2010; Kirby, 2004; Rose, 1999). They are particularly, but not exclusively, associated with ancient woodland (Glaves *et al.*, 2009). There is a degree of debate over the use of AWI species lists in the identification of ancient woodland, with some studies finding that a focus on ecological conditions or use of weighted indicators would be more logical (Webb and Goodenough, 2018; Rotherham, 2011; Spencer, 1990).

The distinctiveness of ASNW vegetation is due, in part, to the life traits of woodland-adapted plants, which tend to limit dispersal. A number of biotic factors such as poor dispersal, competitive exclusion, and poor recruitment are thought to have an aggregate effect in explaining the distinct communities of ASNW (Sciama *et al.*, 2009).

In the UK 48% of ancient woodlands are smaller than 5ha in size (Corney *et al.*, 2008b). Low habitat spatial continuity, combined with the life traits outlined above, is a contributing factor to the distinctiveness of ASNW flora (Brunet *et al.*, 2011). The small size of many ancient woodlands means they are prone to edge effects (Tinya and Odor, 2016; Hofmeister *et al.*, 2013), where altered environmental conditions mean a sub-optimum habitat for ancient woodland plant communities.

Habitat ecological conditions in ASNW have been found to be distinct from those in recent woodland, but findings are not consistent among studies. Ancient woodland plants are associated with higher pH (De Keersmaeker *et al.*, 2004; Hermy *et al.*, 1999) and with lower plant available phosphorous (De Keersmaeker *et al.*, 2004). Soil total nitrogen and carbon have not shown consistent results among studies (De Keersmaeker *et al.*, 2004; Graae *et al.*, 2003). Canopy closure and light factors also show varied results among studies (Brunet *et al.*, 2011; De Keersmaeker *et al.*, 2004).

7.2 Synopsis of the research

A combined introduction and literature review (Chapter 1) identified current issues in ancient woodland vegetation research and guided the aim and objectives of this study. Ancient woodland vegetation research currently demands a greater focus on the underlying ecological explanations for distinctive plant communities beyond the simple long-continuity reasoning (Barnes and Williamson, 2015; Wright and Rotherham, 2011). The impact of fragmentation on plant communities is a topical issue (e.g. Kimberley *et al*., 2016, 2014; Brunet *et al*., 2011), following extensive ASNW loss and conversion to ARW prior to late 20th century conservation measures (Glaves *et al*., 2009). AWI species have been shown to be poorly adapted to fragmentation and disturbance, being generally poor dispersers and colonisers due to vegetative reproduction, few and heavy seeds, with short range dispersal mechanisms (Berges *et al*., 2017; Hermy *et al*., 1999). Consequently, restoration of ARW and increased woodland connectivity are high profile research areas in the field (Atkinson *et al*., 2015; Brown *et al*., 2015; Sutherland *et al*., 2006).

Chapter 2 outlined the overarching site selection and vegetation data collection methods used to address the thesis and chapter research aims and objectives. The study was conducted in the under-researched region of the Cotswold Hills. This is an Ancient Woodland Priority Area, with twice the land cover of ancient woodland than England on average (4.6% compared to 2.3%, calculated from data in Atkinson and Townsend (2011)). Current standard woodland survey techniques were evaluated and adapted to the research project. These techniques included the industry standard National Vegetation Classification (Hall *et al*., 2004; Rodwell, 1991), use of the DEFRA and Natural England MAGIC map ancient woodland inventory mapping layers, and application of currently accepted AWI lists (Kirby, 2004; Rose, 1999). Use of nationally recognised techniques gives the research wider applicability and facilitates comparisons with other studies. More specific methods were detailed in individual data chapters.

Chapter 3 tested for the relative scarcity of herb layer speies among ASNW, ARW and recent woodland. Community distinctiveness in ASNW, ARW and recent woodland was tested according to a range of diversity metrics. There is debate in the literature relating to which, and how many, measures of diversity should be used to determine difference between ecological communities (Naaf and Wulf, 2010; Magurran, 2004; Onaindia *et al.*, 2004). Richness, diversity and evenness indices were applied to canopy, shrub, herb (and AWI species separately) and terrestrial moss layer plant communities to establish any quantitative differences among the three woodland continuity types. The literature review established a lack of consistency in how AWI species are identified (Kimberley *et al.*, 2013). To test any impact of this variation, four indicator analysis metrics were applied to herb layer plant species in ASNW and recent woodland. It was important to establish any basic quantitative differences among ASNW, ARW, and recent woodland study sites early in the research process to provide a baseline for subsequent chapters.

Chapter 4 extended the preliminary analysis of Chapter 3 by assessing species richness at the beta and gamma scales in addition to the alpha scale. This provided a landscape level interpretation of ancient woodland distinctiveness, which has applicability to connectivity projects (Berges *et al.*, 2015; Anderson *et al.*, 2011). In addition to simple richness measures, it was important to apply community analyses that recognise individual species' identities. Chapter 4 compared the relative community composition of ASNW, ARW, and recent woodlands, for all woodland layers. Moving away from descriptive towards explanatory analysis, biotic influences on community composition were investigated. AWI richness and AWI community similarity were tested for correlation with richness and community similarity of the canopy, shrub, non-AWI herb layer, and moss communities. Informed by the literature (Berges *et al*., 2017; Kimberley *et al*., 2014, 2013), community composition was compared by life traits to determine any differences between the woodland continuity categories.

Having identified differences in richness and community composition in the previous chapters, Chapter 5 focused specifically on the response of AWI species to biogeographical and environmental variables. An increasing body of literature calls for greater focus on ecological processes underpinning AWI community ecology, rather than reliance on the binary ancient/ recent woodland classification to account for any distinctiveness (Barnes and Williamson, 2015; Stone and Williamson, 2013; Wright and Rotherham, 2011). In response to this shift, Chapter 5 investigated whether any other variable or group of variables could predict AWI richness, community composition or individual species presence as effectively as woodland continuity status of ASNW, ARW or recent woodland.

Informed by the importance of biogeographical variables for AWI communities evident in the literature (Hofmeister *et al*., 2013) and the outcomes of the previous chapter, Chapter 6 investigated AWI community response to edge effects and fragmentation. This was conducted on a small isolated, fragmented woodland, which was located near to, but did not form one of the sites analysed for Chapters 3, 4 or 5. The objectives here were to: (a) test the relative and additive influence of proximity to a single or dual-edges on herbaceous AWI richness and (b) compare AWI richness in a larger woodland fragment of 4.8ha and an adjacent remnant of 0.6ha. Sampling units for this study were individual plots, whereas for earlier chapters, each woodland site formed a unit of study. The research conducted for Chapter 6 also enabled analysis of edge effect influence on individual AWI species, which further enhanced the understanding of individual AWI ecology developed in earlier chapters.

The present Chapter 7 outlined the broad context of the study and gave a synopsis of each chapter. Links between the chapter sequence and increasing specificity of the research was outlined. The original contributions of the research project were discussed relative to the research objectives. A critique of the research identified how the process of research could be refined and improved. Chapter 7 also detailed the practical implications of the research findings and recommendations for future conservation practice. Lastly, the thesis concluded by identifying questions for future research arising from the present study, and suggesting additional ways the new primary data set could be used for future research projects.

7.3 Conclusions and original contributions

7.3.1 Thesis aim

Thesis aim:

- Using new primary data, to investigate the distinctiveness and community ecology of ancient woodland vegetation.

The overall aim of the research has been met through the collection of new primary data, subsequently applied to research questions in four data chapters.

For Chapters 3, 4 and 5, vegetation data were collected in a total of 225 plots within 45 woodland sites spanning the Cotswold Ancient Woodland Priority Area. These sites were also situated within Strategic Nature Areas – landscape areas that have been identified as having potential for greater habitat connectivity. Within each site, five nested NVC plots per site (canopy 30x30m, shrub 10x10m, herb 4x4m, moss 4x4m) were used to record plant species presence and abundance. Likewise, soil samples were collected within each plot and were homogenised to create one sample for each of the 45 sites. Samples were tested for pH (H_20), plant available phosphorus, total nitrogen and total organic carbon. For each of the 45 woodland sites, biogeographical characteristics of area, shape, and surrounding woodland and hedgerow connectivity were measured using GIS tools.

For Chapter 6 high granularity herbaceous AWI presence data was collected in 256 2x2m plots in an isolated fragmented woodland.

The new datasets collected for this study are in themselves an original contribution to knowledge of woodland ecology in the under-researched region of the Cotswolds. To the author’s knowledge no recent published study has collected extensive and high granularity descriptive and explanatory data for this region. The data have application to future conservation management of woodlands, particularly ancient woodlands, not only in this region, but also nationally, and potentially within other temperate regions at the international scale.

The overarching research aim and original contributions to knowledge were met through the research objectives discussed in sections 7.2.2, 7.2,3, 7.2.4 and 7.2.5 below.

7.3.2 Research objectives 1 and 2

Chapter 3 fulfilled these research objectives:

RO1. To identify any distinctiveness in vegetation richness, diversity, and scarcity among ancient semi-natural, ancient replanted, and recent woodland.

RO2. To test the affiliation of herb layer plant species to ancient semi-natural and recent woodland, using a range of metrics.

Chapter 3 summary of findings:

Of the four diversity metrics applied to woodland strata communities in Chapter 3, richness identified the most significant differences. ASNW exhibited higher richness than ARW for herb layer species, and herb+shrub layer AWI species. Importantly, ASNW exhibited significantly higher richness of herb layer AWI species than recent woodland. This result corroborates numerous earlier studies that identified the distinctiveness of ASNW in this respect (Kelemen *et al*., 2014; Hofmeister *et al*., 2013; Orczewska, 2009).

ASNW herb layer species occupied on average significantly fewer 10km squares in Britain than herb layer species found in recent woodland ARW herb layer species, showed an intermediate prevalence and were not significantly different to either woodland category. To conduct this analysis, secondary data was used from PLANTATT (Hill *et al*., 2004).

The range of indicator metrics applied to herb layer species present in ASNW and recent woodland, all yielded different outcomes. The Kruskal-Wallis test on species abundance

(Schmidt *et al.*, 2014) and the 75% frequency occurrence threshold identified more indicators than Fisher's Exact test and Indicator Analysis (Dufrene and Legendre, 1997).

Original contributions - Chapter 3:

Original contribution	Prior to this study	Application of findings
Of richness, diversity and evenness indices, richness was the most effective in identifying differences among ASNW, ARW, and recent woodland.	Few prior studies have empirically tested diversity metrics in relation to ancient woodland flora (Onaindia *et al.*, 2004; Verheyen *et al.*, 2003a), and seemingly none in the UK.	The intensity of plant species abundance data collection required for diversity and evenness indices detected very few differences among the woodland continuity categories. Richness, therefore, is recommended as the most efficient metric when distinguishing ancient woodlands.
The mean national prevalence of herb layer species in ASNW was significantly lower than for recent woodland.	To the author's knowledge, no prior study has conducted this analysis.	Essentially, the herb layer species found in ASNW within this region are relatively scarce at the national scale. This adds weight to the justification of ASNW protection. There is also scope for ARW restoration to encourage communities of these species.
Metrics currently used to identify AWI species for research and policy yield contrasting outcomes.	Although some studies have critiqued the variation in quantitative and expert opinion approaches to indicator identification (Kimberley *et al.*, 2013; Spencer, 1990), very few have explicitly compared outcomes of a range of metrics (Schmidt *et al.*, 2014).	Based on the current findings, caution is recommended when comparing AWI studies or using lists to inform policy. It is important to fully appreciate the methods that were used to create the AWI lists on which research is based before comparing or making conservation decisions. Although, the use of expert opinion to identify AWI species has been questioned, this has a valuable role particularly in the identification of scarce AWI species with insufficient prevalence for empirical testing.

7.3.3 Research objectives 3 and 4

Chapter 4 fulfilled these research objectives:

RO3. To assess the degree to which the floristic community composition differs among ancient semi-natural, ancient replanted, and recent woodland.

RO4. To examine biotic influences on community composition in ancient semi-natural, ancient replanted, and recent woodland.

Chapter 4 summary of findings

AWI richness at the gamma scale (where the landscape is the study unit) revealed contrasting results to alpha scale (woodland scale study unit) richness measures. Importantly, there was no significant difference in the gamma richness of herb layer AWI species found in ASNW, ARW, or recent woodland sites. Recent woodland was significantly richer than ASNW and ARW when species in all strata were taken into account.

Community composition comparisons were conducted at the beta scale (the degree of change or difference between woodland sites). For canopy, shrub, herb, non-AWI and terrestrial moss communities there was always at least one pairwise significant difference among the three continuity categories. For AWI species, all three woodland categories exhibited significantly different community composition. An additional measure of community variance was carried out simultaneously with compositional analysis. This measure found a significantly more varied herb layer (and subset of non-AWI species) in recent woodland compared to both categories of ancient woodland. Therefore, in some respects the flora of ancient woodlands is more consistent among sites than in recent woodlands

In terms of biotic explanations for community differences, results show more inter-strata interactions in ASNW (which have the longest continuity), and progressively fewer interactions in ARW and then recent woodlands (with shorter continuity). The richness of AWI species was found to be significantly and negatively correlated with the richness of canopy, shrub and moss layers in ASNW. In ARW this decreased to two significant results, and in recent woodland only once significant interaction. Likewise, where AWI communities had high similarity in ASNW, then they correlated significantly and positively with the similarity of the canopy and shrub layers in ASNW. For ARW, there was only one significant interaction and none for recent woodland.

When herb layer plant species were grouped by life trait, significant differences were found in Grime's life strategies between ASNW and recent woodland. Community composition by dispersal strategy was significantly different between and ASNW and recent woodland. No significant differences were found for life forms, dispersal vectors, earliest flowering month, mean seed weight, nor specific leaf area. However, the significant result for dispersal strategies was not surprising based on several prior studies with similar results. The life strategy results showed more stress-tolerant species in ancient woodland, again corroborating the existing theory.

Original contribution	Prior to this study	Application of findings
AWI richness is significantly higher in ASNW than recent woodland at the alpha (site) scale but not at the gamma (landscape) scale.	To the author's knowledge no prior study has compared alpha and gamma richness among ancient and recent woodlands.	These results show that ARW and recent woodland sites have the potential to support a valuable AWI community among them at the landscape (gamma) scale. These are positive outcomes that justify restoration of ARW or creation of valuable new woodlands that are capable of supporting scarce AWI species.
The within group variation of ASNW herb layer community composition is significantly more consistent than for ARW or recent woodland.	Coote *et al*., (2012) compared within group community composition in plantation study sites, and semi-natural sites. However seemingly no study has tested within-woodland category community variation across all three of ASNW, ARW and recent woodland categories.	The finding provides new supporting evidence relating to the distinctiveness of ancient woodland plant communities. ASNW plant communities are more consistent in their composition than sites with short continuity, such as ARW or recent woodland. This suggests a degree of community development that is not easily replicated in other woodland types and adds weight to the justification for conservation.
ASNW, ARW, and recent woodland exhibit differences in canopy, shrub, herb, and moss layer community among the three continuity categories.	A small number of studies have established differences in community composition between (ancient) semi-natural woodlands and plantations (Berges *et al*., 2017; Atkinson *et al*., 2015; Coote *et al*., 2012). However, the analysis of all three woodland categories has not previously been conducted, nor any division between AWI and non-AWI communities.	This detailed analysis of community composition could be applied to monitoring of restoration and connectivity projects to assess vegetation progress towards communities found in ASNW. Additionally, community composition analysis may further inform species dispersal and colonisation dynamics.

AWI communities in ASNW exhibit more correlations with communities in other layers, compared to ARW and recent woodland.	A small number of studies have tested richness interactions among strata (Thomaes *et al*., 2012; Orczewska, 2009) of ancient and recent woodlands, but seemingly none have included ARW.	An area worthy of further research, as there are potential implications for the use of phytosociological classifications such as NVC. This initial analysis suggests the layers of ASNW may function more as one community, whereas for ARW, and recent woodland this is less likely to be the case.
Community composition by life strategies and dispersal mechanisms differed among ASNW, ARW, and recent woodland, which in part may account for ancient woodland distinctiveness.	Life traits have been well-studied in relation to ancient woodlands and their flora (eg. Kimberley *et al*., 2016, 2014, 2013; Kelemen *et al*., 2014; Brunet *et al*., 2011). However, no prior study has encompassed all three woodland categories of ASNW, ARW, and recent woodland.	This finding tested and supported existing theory. The ARW element is novel and shows an intermediate position between ASNW and recent woodland.

7.3.4 Research objective 5

Chapter 5 fulfilled this research objective:

RO5. To examine the response of ancient woodland indicator communities to environmental and biogeographical variables, relative to woodland continuity status.

Chapter 5 summary of findings

Woodland continuity was found to be the strongest univariate predictor of AWI richness. However, a multivariate model of biogeographical factors and their appropriate interactions (including surrounding woodland, surrounding ASNW, surrounding ARW, surrounding hedgerow, site area and site shape) were found to be equally good predictors of AWI richness. A multivariate model of soil factors was found to be the least effective predictor.

When explanatory variables were fitted to AWI community composition across all three categories of ASNW, ARW, and recent woodland, soil pH was the strongest predictor, along with total nitrogen and total organic carbon. Continuity was the fourth strongest predictor and, linking to the richness models described above, was associated with a cluster of commonly co-occurring AWI species. Several biogeographical factors were also significant predictors, but less strong than continuity.

The presence of only one third (5/15) of the individual AWI species tested were significantly and positively predicted by woodland continuity (*Anemone nemorosa, Primula vulgaris. Paris quadrifolia, Viola reichenbachiana, Carex sylvatica*). Biogeographical variables accounted for three species, then soil variables for two and finally canopy for one. Four species had no significant predictor, and *Mercurialis perennis* had two. These findings give a detailed insight into the ecology of AWI species and reinforce that they should be treated as a group or guild when used to identify ancient woodland, as there was great variation in the degree of association with continuity. This chapter also found that some recent woodlands are capable of supporting equal AWI community richness as some ASNW, which is supported by the 10/15 AWI species that were not significantly associated with continuity.

Original contributions - Chapter 5

Original contribution	Prior to this study	Application of findings
Combined biogeographical factors are equally effective as predictors of AWI richness as woodland continuity.	Earlier studies have tested the relative influence of ecological habitat variables on AWI richness (e.g. Coote *et al*., 2012; Brunet *et al.,* 2011; Petit *et al*., 2004) but seemingly none have explicitly tested for alternative predictors to woodland continuity.	Woodland connectivity and site configuration factors have been confirmed as significantly important for AWI species richness. This outcome can be used to inform woodland connectivity projects. Literature has cast some doubt on the accuracy of ancient woodland identification (Webb and Goodenough, 2018; Barnes and Williamson, 2015; Stone and Williamson, 2013; Wright and Rotherham, 2011), so biogeographical assessment may provide support, or an alternative, to using continuity alone.
Individually, only one third of AWI species tested were significantly associated with woodland continuity, either being associated instead with biogeographical, soil, canopy, or no specific variables.	Various prior studies have identified lack of weighted value for AWI species (e.g. Webb and Goodenough, 2018; Kelemen *et al*., 2014; Wulf, 1997). However, seemingly none has identified the relative contribution of explanatory variables to AWI presence across ASNW, ARW, and recent woodland.	Overall AWI richness was significantly and positively predicted by woodland continuity but this did not hold true when AWI species were analysed individually. Therefore, this lends support to the continued use of AWI richness, rather than presence of individual species, as an indicator of ancient woodland. Additionally, the results suggest that many populations of AWI species, including scarce or protected species, may be conserved not only in ancient woodland, but also in woodlands with a shorter continuity history. Conservation and protection of AWI-rich recent woodlands is therefore recommended.

7.3.5 Research objective 6

Chapter 6 fulfilled this research objective:

RO6. To assess the relative influence of single and dual-edge proximity on AWI species in a fragmented woodland.

Chapter 6 summary of findings

Mapping of AWI richness across a small, isolated woodland showed low AWI richness at the edges compared to the woodland core, with low AWI richness extending further into the woodland in corners. The descriptive map outcomes were empirically supported by modelling, which showed positive and significant correlations between herbaceous AWI richness and distance from the nearest edge and second nearest edge when tested separately. Importantly, an additive effect of proximity to both edges was shown to affect AWI richness more strongly than either edge individually. For small woodlands in a matrix of contrasting land-use, edge effects are therefore stronger than previously thought.

Individual AWI species responded differently to distance from a single or dual-edge position. *A. nemorosa, Hyacinthoides non-scripta, Lamiastrum galeobdolon,* and *P. quadrifolia* all showed significant and increased presence with distance from both the nearest and second nearest edges, and with a stronger response to both edges combined. *Allium ursinum*, *Galium odoratum* and *V. reichenbachiana* exhibited no significant response. Additionally, the 4.8ha fragment supported greater AWI richness that the 0.6ha remnant (13 compared to 8 species respectively). Mapping showed low AWI richness throughout the remnant, which was effectively all edge habitat comparable to the corners of the larger fragment.

Original contributions - Chapter 6

Original contribution	Prior to this study	Application of findings
AWI richness was significantly reduced by proximity to dual-edge exposure, compared to a single edge.	This is the first study to consider dual-edge effects for AWI species specifically, and one of very few dual-edge studies that have been conducted in any ecological context (Fletcher, 2005; Ries and Sisk, 2004).	48% of ancient woodlands in the UK are <5ha in size (Corney *et al*., 2008b), therefore this study has significant implications for understanding the detrimental effect of fragmentation and can inform future woodland protection and conservation measures.
Individual AWI species responded differently to edge proximity.	Prior studies have researched individual woodland species' responses to edge effects (eg. Vallet *et al.,* 2010; Willi *et al*., 2005), but none have assessed the influence of dual-edges.	AWI species that were less influenced by edge effects (single and dual) are recommended as better indicators for small ancient woodlands. This is especially relevant as ancient woodlands of <2ha are now identified and mapped as part of the ancient woodland inventory. AWI species that are greatly affected by edge, may be entirely absent from very small woodlands. This finding lends support to the threshold of 5 AWI species used by some organisations to determine the ancient status of a small woodland (Glaves *et al*., 2009).

7.4 Overarching findings

Based on the findings and original contributions of individual chapters above, the following overarching conclusions have been drawn from the research:

- ASNW, ARW, and recent woodlands frequently exhibit distinct plant communities in each of their strata in terms of richness and community composition, but diversity and evenness metrics were not effective in differentiating between the woodland categories.

- Individual ARW, and often recent, woodlands are capable of supporting AWI richness equal to some ASNWs, and this is accounted for by differing habitat preference of individual AWI species, some of which are more affiliated with biogeographical or environmental conditions than the continuity of a woodland.

- The mean alpha AWI richness of all three categories of woodland is considered a reflection of 'very good' or 'good' woodland biodiversity value according to thresholds currently used by some organisations, which justifies conservation within the Strategic Nature Areas studied.

- Gamma richness showed no significant differences between ASNW-ARW, ASNW-recent, and ARW-recent for any of the individual strata. This outcomes suggests good restoration potential of ARW and recent at the landscape scale.

- The metrics by which AWI species are identified yield widely varying results, and a combination of metrics as well as expert opinion is recommended for determining appropriate regional lists (the latter particularly for rare species with too low occurrence for statistical testing).

- The herb layer species of ASNW have a significantly lower mean prevalence at the national scale, than those found in recent woodlands, adding weight to the justification for conservation of ancient woodlands.

- Biotic explanations for the distinctiveness of AWI communities among ASNW, ARW, and recent woodlands are inter-strata interactions, and some life traits (life strategy and dispersal mechanisms).

- AWI richness and community composition is significantly influenced by biogeographical and environmental factors, which, given some existing criticism of the way ancient woodlands are identified, could provide a substitute or additional measure in identifying woodland with distinct and valuable plant communities.

- ARW commonly exhibits an intermediate status between ASNW and recent woodland for measures of distinctiveness.

- For very small woodlands, AWI species less prone to edge effects could be weighted as more appropriate indicators.

- Dual-edge effects have been shown to significantly influence AWI distribution, which for small woodlands potentially means a higher edge:interior ratio than previously thought.

7.5 Recommendations for policy and practice

The research findings led to several recommendations for conservation policy and practice:

Policy recommendations:

For use of Ancient Woodland Indicators:

- AWI list generation using a standardised statistical method, supplemented with expert opinion to identify rare or regionally distinct AWIs is recommended. Current AWI lists vary considerably in terms of the balance of statistical methods and expert opinion used to create them. Even where statistical testing is applied to address the concerns surrounding the use of expert opinion (Kimberley *et al.*, 2013; Rolstad *et al.*, 2002), the tests applied are not comparable among studies. Chapter 3 demonstrated notable variability in the species identified as AWIs according to a range of statistical techniques.

- AWI lists for ecological regions rather than political county boundaries are recommended. Currently Gloucestershire has no list, and the lists of many other counties are based on now historic data. This provides opportunity to re-examine the geographical parameters of AWI list creation, basing them within biogeoclimatic regions, for example the Forestry Commission's Ecological Site Classification (Pyatt *et al.*, 2001). Linked to this, recalibration of the AWI count thresholds to suit National Vegetation Classification communities is also recommended within the broader regional list. This practice would reduce under-representation, and therefore lack of protection, of communities that, whilst ancient, do not naturally feature as many AWI species as other NVC categories.

- The use of negative or reverse indicators is recommended in conjunction with standard AWI lists. Chapter 3 demonstrated that several species are as strongly affiliated with recent woodland, as others are with ancient woodland.

- For small woodlands or those with high edge: interior ratio, AWI thresholds could be lowered to take account of AWI species that are susceptible to edge effects and many not be found in a woodland that is predominantly edge habitat, as shown in Chapter 6. Some regions already use a threshold of five AWI species (as opposed to 8-12) as evidence for long continuity (Glaves *et al.*, 2009); a similar practice could be applied through all regions.

For management of the landscape and matrix:

- To conserve population of woodland specialist species, particularly those with conservation designations such as *H. non-scripta*, protection of recent woodland with high botanical quality is recommended. Chapter 3 showed than some recent woodlands are capable of supporting equal AWI richness as some ancient woodlands.

- Development within 500m of an ancient woodland are already considered within the National Planning Policy framework. It is recommended to extend this to protection of all woodland and hedges within 500m of an ancient woodland. Chapter 5 demonstrated that proportion of woody habitats within 500m of a woodland were as strong a predictor of AWI richness as the continuity of the central habitat.

Practice recommendations:

- Herb layer and AWI richness is recommended over abundance for distinguishing ancient woodland. Chapter 3 showed that richness was more effective than abundance-based diversity measures in differentiating ancient woodlands and is more efficient in data collection, requiring only presence data.

- Small ancient woodlands, or those with high edge: interior ratios could be managed with a soft edge buffer within the surrounding land use to conserve populations of woodland specialist species. Chapter 6 showed increased edge effects on such species in woodland corners, meaning reduced core woodland area. An external buffer could mitigate how far these effects permeate a small woodland. Where possible, avoid hard edges or strongly contrasting matrix land-use.

- When assessing habitat quality, the area of core woodland should take into account the greater edge effect in woodland corners. Chapter 6 showed that the area of core woodland may be less than previously thought due to increased influence of dual edges.

- When determining the ancient status of woodland, surveyors should continue to triangulate evidence from historical sources and archaeological evidence in addition to AWI lists (Glaves *et al*., 2009). The research has shown that AWI lists do indeed distinguish ancient woodland, but some individual recent woodlands can support a high richness of AWI species. As noted by Spencer (1990) the ‘indicator’ aspect of AWI species should be emphasised: they are indicators to be used in context with further information, they are not a stand-alone measure.

- Restoration and connectivity of ARW is recommended the wider landscape scale of the Cotswolds and the smaller landscape of the Strategic Nature Areas. Chapter 4 showed that gamma richness of ASNW, ARW, and recent woodlands was not significantly different for individual woodland layers, including a subset of AWI species. This means that at the gamma landscape scale, woodlands that are generally considered individually sub-optimum for AWI populations, can in fact support equal richness of AWI species when they are combined. Restoration of ARW, and a general increase in connectivity among all broadleaf woodland types, could enable the already present species to disperse into restored woodlands, increasing population viability.

7.6 Critique of the research

In terms of the research conducted, a lower granularity and greater breadth of data could have been collected by reducing the number of nested plots per woodland site (x5) to a single nested plot for canopy and shrub layers, with up to five 4x4m plots for the herb and ground layers within it. However, this would not have been compatible with the NVC procedure, which was also required for each woodland site in order to standardise the study sites. Should prior NVC classification be available for any future studies, an adapted vegetation data collection would be advised in order to increase number of woodland surveyed. Alternatively, the high granularity data that has been collected could be further tested for within-site variation.

Although study sites were as standardised as far as possible, even more stringent standardisation, or even use of experimental sites, could be recommended to fully test the ecological processes underpinning vegetation distinctiveness in ancient woodland. However, the purpose of the study was to examine woodland vegetation as it exists in the working landscape within the Ancient Woodland Priority Area of the Cotswolds. This usage has more application for practical intervention, whereas a more experimental approach would be more effectively used to generate theory.

Finally, determining the specific age of each study site, rather than categorising as ASNW, ARW, or recent woodland could have been beneficial. There are several criticisms of the year 1600 threshold for ancient woodland, as detailed in the literature review. However, it was not feasible to research the history of each woodland over several hundred years. Additionally, The National Planning and Policy framework recognises these categories of woodland, with both of the ancient categories being equally protected by law. Nationally, conservation strategies and management advice are directed particularly at ancient woodlands according to their categorisation rather than specific age. Therefore, the research was justified in taking the categorical approach to woodland continuity.

7.7 Future research avenues

The short to medium term plan is for publication or conference presentation of the chapters presented in this thesis. Additionally, several further research avenues have been identified with a view to future original contributions:

- The distinctiveness of the non-AWI herb layer community among ASNW, ARW, and recent woodland, in terms of national prevalence and community composition.
- Within-site scale analysis of variation in species presence and abundance – are ASNWs more consistent in their community composition within-site as between-sites, relative to ARWs and recent woodlands?
- Dual-edge effects on the vegetation of isolated ASNW in an agricultural matrix, and environmental and life trait explanatory variables. This would be a new study scaling up Chapter 6 to a larger sample size to further test the existing findings.
- What determines AWI rich flora in recent woodlands? This would require additional data collection for a larger sample size of recent woodlands suitable for testing with several explanatory variables.

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Appendix 2 – Chapter 6 original published paper

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Double-edged effect? Impact of dual edge proximity on the distribution of ancient woodland indicator plant species in a fragmented habitat

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Abstract: The influence of edge proximity on woodland plants is a well-established research area, yet the influence of dual edge exposure has rarely been investigated. This novel research aims to establish whether proximity to two edges has any additive influence on Ancient Woodland Indicator (AWI) species presence relative to proximity to a single edge. Several AWI species are threatened and thus specific conservation priorities, while Ancient Semi-Natural Woodland (ASNW) itself is often highly fragmented: almost half of remnant patches are less than 5 ha, which increases the potential for dual edge effects. Here, systematic mapping of herbaceous AWI species was conducted in 310 vegetation plots in two formerly-connected ASNW fragments in South-West England. Linear regression modelling revealed that distance to nearest edge and distance to second nearest edge were both univariately positively correlated with AWI species richness. After distance from nearest edge was entered into a multivariate model first, distance from second edge was entered in a second optional step after meeting stepwise criteria. The resultant multivariate model was more significant, and explained more variance, than either variable in isolation, indicating an additive effect of dual edge exposure. Likewise, binary logistic regression modelling showed presence of individual AWI species (*Anemone nemorosa, Hyacinthoides non-scripta, Lamiastrum galaeobdolon* and *Paris quadrifolia*) was significantly related not only to distance from the nearest and second nearest edges in isolation, but significantly more strongly by the additive effect of distance from both edges in a single model. We discuss the implications of these findings from community ecology and conservation perspectives.

Abbreviations: ASNW – Ancient Semi-Natural Woodland, AWI – Ancient Woodland Indicator.

Nomenclature: IPNI (2015) The International Plant Names Index http://www.ipni.org/

Introduction

The presence of scarce and range-restricted flora in Ancient Semi-Natural Woodland (ASNW) (Peterken 1974, Wulf 1997, Honnay et al. 1999, Palo et al. 2013) contributes to its status as an ecosystem of high conservation value (Peterken 1983, Rackham 2003, Goldberg et al. 2007). The ancient woodland concept is well-integrated into forest research and conservation practice, although definitions and date thresholds vary amongst countries (Hermy et al. 1999, Wirth et al. 2009). In England, ASNW is defined as predominantly a native broadleaf canopy established through natural regeneration (Rackham, 2008) on land that has remained continuously woodland since at least the year 1600 (Goldberg et al. 2007).

Ancient woodland indicator (AWI) species are vascular plants that are particularly, but not exclusively, associated with ASNW (Rose 1999, Glaves et al. 2009). Regional AWI lists were developed to assist in determining ancient woodland status and are additionally used to assess habitat quality (Glaves et al. 2009). AWI species are considered to have low colonisation potential due to poor seed production, low dispersal capability and short-term persistence in the seed bank (Honnay et al. 1998). As such, AWI species may not be able to colonise alternative woodland habitats if ASNWs are lost or conditions become sub-optimal (Hermy et al. 1999). The limited distribution of AWI species and their specific ecology has promoted extensive use in woodland research (Peterken 1974, Spencer 1990, Wulf 1997, Honnay et al. 1998, Hermy et al. 1999, Rose 1999, Kirby and Goldberg 2002, Rackham 2003, Kirby and Morecroft 2011, Kimberley et al. 2014, Stefańska-Krzaczek et al. 2016).

Landscape fragmentation is a significant threat to ASNW plant communities (Rackham 2008, Corney et al. 2008), not only due to reduction in dispersal potential of AWI species, but also due to increased edge effects. Edge width is defined as the outer part of a woodland compartment where environmental conditions differ significantly from the interior (Honnay et al. 2002). Corney et al. (2008) report that 48% of ancient woodlands are under 5 ha, which means they have a high edge:interior ratio and a large edge width, especially if they deviate from an optimum circular shape (Laurance 2008). Edge environmental conditions are generally considered to be less favourable for persistence of specialist flora, including many AWI species, due to altered abiotic and biotic variables (Matlack 1993, Murcia 1995, Honnay et al. 2002, Willi et al.

2005, Hofmeister et al. 2013, Tinya and Ódor 2016), as well as anthropogenic influences (Corney et al. 2008).

Abiotic and biotic variables commonly exhibit an edge width of between 10-60 m in temperate forests (Palik and Murphy 1990, Matlack 1993, Matlack 1994, Gehlhausen et al. 2000, Honnay et al. 2002). Additionally, studies seeking to avoid edge influence, have situated sample plots at > 20 m from the edge (Bossuyt and Hermy 2000), > 30 m (Brunet et al. 2012), and > 50 m (Gelhausen 2000, Coote et al. 2012). Exceptionally, edge effects have been evidenced over 100 m from the edge (Hofmeister et al. 2013, Pellissier et al. 2013) but such findings are not comparable to small ancient woodland fragments in the UK. Land use in the matrix (Gove et al. 2007), prevailing wind direction (Smithers 2000) and aspect (Murcia 1995, Honnay et al. 2002) influence the extent to which edge effects permeate woodland.

This is the first study to investigate multiple edge effects in relation to AWI species and ancient woodland. The impact of multiple edges is an important but overlooked factor (Ries and Sisk 2004). Few studies have explicitly gathered primary data to analyse this in relation to any species or ecosystem (Fletcher et al. 2005), instead measuring linear distance to the closest edge only. A small number of studies have been completed with explicit focus on AWI response to nearest edge proximity (Willi et al. 2005, Hofmeister et al. 2013, Pellissier et al. 2013, Kimberley et al. 2014). Despite the potential importance of edge effects on AWI species, both in their own right as specialist species, and in terms of their efficacy of indicators, a search of the literature revealed no studies relating to multiple edge effects on these species. This is surprising given that the highly-fragmented nature of ANSW means that the potential for exposure to multiple edge effects is considerable.

We test for relationship with the nearest edge, as well as any additional contribution of the second edge to take account of double exposure within fragment corners. We hypothesised that (1) AWI richness will increase with distance from any edge; (2) the second nearest edge would also correlate with AWI richness so that a multivariate model with both distances would be superior to a univariate model using either in isolation; (3) the patterns for AWI species richness would also hold true for specific AWI species analysed on a presence/ absence basis.

Methods

The study site was a fragmented species-rich ASNW in the South-West UK. The two discrete fragments comprising the site were situated near Cheltenham on the Cotswold Hills escarpment of Jurassic oolitic limestone, at 265 m above sea level and centred on 51°53’35.5”N, 2°00’34.60”W (Fig. 1). The mean diurnal temperature was 8.6-14.7°C and annual precipitation was 843 mm (MET office, 1981-2010). The fragments have comparable geology, edaphic variables and topography. The coppice-with-standards woodland classifies as National Vegetation Classification W8b (Rodwell 1991), with a canopy dominated by *Fraxinus excelsior* and *Quercus robur*.

Figure 1. Study site location of the Cotswold Hills, UK.

Both fragments, henceforth referred to as Fragment 1a and 1b were located within an agricultural (arable and equine) matrix. Fragment 1a was 4.8 ha and of approximately rectangular dimension (190 m × 255 m). Fragment 1b was a remnant of 0.6 ha located 25 m from the eastern edge of Fragment 1a. Historic map evidence showed that both fragments formed a single woodland until c. 1965. Both fragments are classified by DEFRA (2016) as ASNW.

In order to assess any influence of dual-edge effect in Fragment 1a, presence of AWI species was mapped and recorded via a total of 256 2 m × 2 m plots. Plots were located in the corners of Fragment 1a within 60 m of both the nearest edge (Edge 1) and second nearest edge (Edge 2). The distance of 60 m was deemed a conservative upper limit for detection of edge effects based on previous studies (Murcia 1995, Gelhausen et al. 2000, Honnay et al. 2002, Vallet et al. 2010). Plots were located at 0, 5, 10, 20, 30, 40, 50 and 60 m on transects perpendicular to the Western and Eastern edges, with 0 m defined as the commencement of woody species' stems (Murcia 1995). Changing the sampling distance from 10 m to 5 m at the edges of the fragment allowed small-scale change to be better detected (Honnay et al. 2002). Recorded species were restricted to herbaceous and semi-woody plants (Brunet et al. 2011) identified as Ancient Woodland Indicators in the South-West UK (Rose 1999). To complement analysis of the larger fragment and demonstrate any difference in species richness and presence between the two fragments, Fragment 1b was surveyed on the same system with plots at 0, 5, 10 and 20 m from the Eastern and Western edges (n = 54). All statistical analyses apply to Fragment 1a.

To predict the influence of Edge 1 and Edge 2 on AWI richness, separate univariate linear regression analyses were performed (n = 256). To test any additive influence of both edges, a hierarchical multivariate model was created where Edge 1 was entered via forced entry and Edge 2 was available as a candidate variable in a second step using a stepwise approach (entry criterion $\alpha = 0.05$, except *L. galaeobdolon* $\alpha = 0.1$) (De Keersmaeker et al. 2004). Normality assumptions were met, and collinearity was within accepted limits: VIF < 10 (Myers 1990) and tolerance >0.2 (Menard 1995). The same principles were followed using binary logistic regression to test the influence of Edge 1 and Edge 2, separately and additively, on the presence AWI species (those found in > 10% of plots) (n = 256). The R^2 (linear regression) and Nagelkerke pseudo R^2 (logistic regression) statistics were calculated to measure the relative influence of single and additive edges on, respectively, AWI richness and species presence.

Results and analysis

Mapping of Fragment 1a, showed clear spatial patterns in AWI richness in relation to edge proximity (Fig. 2). AWI richness was very low at the edge, and increased gradually up to 60m; this effect was most pronounced at the corners where a distinct edge effect was apparent up to 20-30 m, rather than 5-10 m on transects located mid-edge. Within the very small Fragment 1b, AWI richness is lower throughout than in Fragment 1a, with no clear edge or corner pattern (Fig. 2).

Regression analysis showed significant positive directional relationships between AWI richness and distance from the edge in Fragment 1a (Table 1). When tested independently, Edge 1 and Edge 2 were both shown to be significantly positively related to AWI richness, but Edge 1 was related more strongly than Edge 2. Used in a hierarchical framework, Edge 2 met the stepwise criteria for entry as a second variable into a multivariate model after Edge 1 had already been entered. This, together with the resultant multivariate model being more significant and explaining more variance than either Edge 1 or Edge 2 in isolation, strongly suggests dual-edge exposure is important for AWI richness.

Repeating the above analytical framework using hierarchical multiple logistic regression for the seven most prevalent species (those present in > 10% of plots) showed that the presence of four species increased significantly with increasing distance from edge (Table 1). For each of these species (*A. nemorosa*, *H. non-scripta*, *L. galaeobdolon* and *P. quadrifolia)* Edge 1 and Edge 2 were both significant when analysed separately and again the R^2 statistic for Edge 2 was slightly lower than Edge 1. In all four cases, running a stepwise model with Edge 2 available as a candidate variable resulted in a multivariate model being created that had a substantially lower P value and substantially higher R^2 value than either edge tested alone. For where species Edge 2 was not entered us-

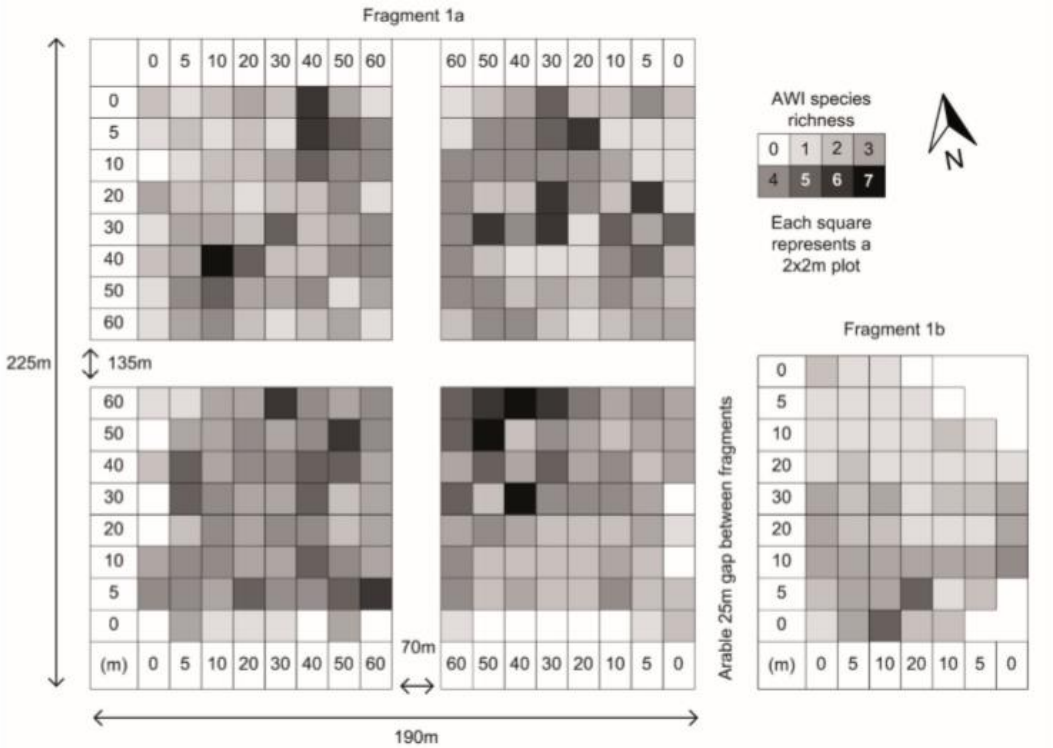

Figure 2. Richness of Ancient Woodland Indicator species in a total of 310 2 m × 2 m plots within two fragments of ancient semi-natural woodland.

ing standard stepwise criteria ($\alpha = 0.05$ or 0.10), forcing this variable into the model did not improve it relative to using Edge 1 alone and all models were non-significant.

Thirteen AWI species were recorded within Fragment 1a sample plots and eight within Fragment 1b (Appendix 1). The four species significantly associated with distance showed clear reductions in prevalence in Fragment 1b, in comparison to 1a (Appendix 1). Presence of *A. nemorosa* and *H. non-scripta* in Fragment 1b was half of that in 1a, while *L. galaeobdolon* and *P. quadrifolia* were absent from Fragment 1b. Of prevalent species not significantly associated with distance, only *A. ursinum* occurred considerably more frequently in Fragment 1b than in Fragment 1a.

Discussion

The above results show that not only are edge conditions less suitable for the majority of AWI species present, but the AWI community is vulnerable to a dual-edge effect whereby the combined influence of two edges is amplified and permeates further into a woodland near corners. The distance to the nearest two edges combined explained 11% of the variation in AWI richness and up to 17% of the variation in the presence/ absence of specific AWI species (Table 1). Dual-edge exposure explained a significant, and consistent, additional 1-3% of the variation in AWI richness and presence of some species than the single nearest edge alone (Table 1). The findings reinforce the need to protect ancient woodlands from fragmentation. Two species with conservation designations, *H. non-scripta* and *L. galaeobdolon*, were especially adversely affected by edge proximity (Table 1). At 4.8 ha, Fragment 1a is among the larger of the 48% of ancient woodlands that are smaller than 5ha (Corney et al. 2008), with a considerable area exposed to single and dual-edge effects. Fragment 1b is smaller still, and mapping suggests is influenced in its entirety by edge conditions.

Both woodlands reinforce the edge:interior ratio theory proposed by Laurance (2008). For this reason, some AWI species might not be appropriate indicators in small fragments where there is a high proportion of edge habitat, as they may be absent even from small ancient woodlands. Our findings show a lower richness count and predominantly lower prevalence of AWI species in Fragment 1b despite its adjacent position and history of connectivity with 1a. However, both fragments have what is considered to be an acceptable AWI score (Fragment 1a = 13; Fragment 1b = 8). Thresholds of 10-12 AWI species (including woody species, forbs and ferns) are used by organisations for allocating conservation priority, while ASNWs under 2 ha with > 5 AWI species were recommended for inclusion in a county ancient woodland inventory (Glaves et al. 2009). If AWI species counts are used in small fragments, consideration should be given to only using the subset of species that are not seemingly affected by edge effects.

Table 1. AWI richness (all species) and species presence (most frequently occurring species in > 10% of plots) relationship with distance from Edge 1, Edge 2, and both edges together. In all cases, the additive model was a hierarchical one whereby Edge 1 was entered first and then Edge 2 was available as a candidate variable for inclusion following a stepwise approach; the model was not calculated if the addition of Edge 2 into the model did not significantly improve it.

		p	R^2	Dir.
AWI richness	Edge 1	< 0.001	0.099	+
	Edge 2	< 0.001	0.069	+
	Additive	< 0.001	0.115	+

Species		Chi (df)	*p*	R^2	Dir.
A. ursinum	Edge 1	0.357 (1)	0.425	0.030	
	Edge 2	0.187 (1)	0.666	0.080	
	Additive	Model not calculated			
A. nemorosa	Edge 1	23.117 (1)	< 0.001	0.126	+
	Edge 2	19.572 (1)	< 0.001	0.107	+
	Additive	29.292 (2)	< 0.001	0.158	+
G. odoratum	Edge 1	0.077 (1)	0.781	0.000	
	Edge 2	0.002 (1)	0.968	0.000	
	Additive	Model not calculated			
H. non-scripta	Edge 1	27.550 (1)	< 0.001	0.141	+
	Edge 2	20.323 (1)	< 0.001	0.105	+
	Additive	33.100 (2)	< 0.001	0.168	+
L. galaeobdolon	Edge 1	11.866 (1)	0.001	0.064	+
	Edge 2	9.321 (1)	0.002	0.036	+
	Additive	14.417 (2)	0.001	0.077	+
P. quadrifolia	Edge 1	16.698 (1)	< 0.001	0.095	+
	Edge 2	16.117 (1)	< 0.001	0.092	+
	Additive	22.287 (2)	< 0.001	0.126	+
V. reichenbachiana	Edge 1	0.699 (1)	0.403	0.005	
	Edge 2	2.044 (1)	0.153	0.014	
	Both	Model not calculated			

Dir. - direction of relationship for significant models. R^2 - Nagelkerke

Species present with conservation designations: *Hyacinthoides non-scripta* - Wildlife and Countryside Act, schedule 8; *Lamiastrum galaebdolon* - Vascular Plant Red List for Great Britain nationally scarce, vulnerable. Vascular Plant Red List for England, vulnerable; *Viola reichenbachiana* - Scottish Biodiversity List. *Primula vulgaris* (Wildlife Order Northern Ireland schedule 8); *Sanicula europaea* (Vascular Plant Red List for Great Britain, near threatened)

AWI species have been considered as a guild (Hermy et al. 1999), but in this study the response of the community and individual species in relation to edge proximity indicates variation in niche requirements. Of the species significantly influenced by edge proximity, all increased in prevalence with distance from the edge (Table 1). The preference of *P.*

quadrifolia for woodland interior may be accounted for by its adaptation for vegetative growth during low light periods (Bjerketvedt et al. 2003). Similarly to this study, Honnay et al. (2002) found *A. nemorosa* to have a positive edge-distance distribution in ancient woodland study sites in Belgium. Of those not exhibiting significant relationships with edge, only *V. reichenbachiana* decreased in prevalence with distance from either and both edges, possibly accounted for by its greater light requirement for a summer second leafing period (Rackham 2003).

This study has demonstrated dual-edge proximity has a substantial effect on AWI community composition, and has highlighted the species-specific nature of the response to different plants to the edge. It has also emphasised the effects of edge orientation and woodland size on floral response to edge conditions. Future research on the influence on multiple-edge biotic and abiotic variables in small ASNWs would be beneficial in further explaining spatial distribution of AWI species and for development of conservation management practices.

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