soil systems

Article

Mixed Grazing Increases Abundance of Arbuscular Mycorrhizal Fungi in Upland Welsh Grasslands

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Abstract: Grasslands play a crucial role in exchanges between global ecosystems and the atmosphere and form an integral part of the agricultural industry. Arbuscular mycorrhizal fungi (AMF) are mutualistic symbionts of most grassland plant species and thereby influence the functional capacity of grassland systems. Agricultural grasslands are primarily used for livestock farming and are subjected to various management practices designed to increase production, but which also alter both plant and soil communities in the process. This research investigated the effects of a selection of management practices and environmental factors on the presence and abundance of AMF in upland Welsh grasslands. The aim was to identify how these management practices affected the abundance of AMF, assessed through microscopic observations of four AMF structures: spores, hyphae, vesicles and arbuscules. The results suggest grazing sheep and cattle together had the highest overall influence on AMF abundance compared to grazing sheep or cattle separately. High plant diversity correlated with high arbuscule and vesicle abundance, but conversely, the application of lime reduced vesicle abundance. These findings offer new insights into the effects of management practices on AMF. Mixing livestock, increasing plant diversity and reducing lime applications are shown here to improve the abundance of AMF and could, therefore, help to inform sustainable farm management decisions in the future.

Keywords: arbuscular mycorrhizal fungi; effects of management practices; mixed grazing

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1. Introduction

Grasslands are a globally important habitat with the potential to offer substantial resources and extensive ecosystem services, e.g., flood prevention, biologically diverse habitats and carbon storage [1–3]. Current research into the importance and value of these habitats focuses predominantly on their uses within the agricultural industry, a key area of which is the impact of livestock production, through direct grazing, silage or hay-making [1,2]. Multiple avenues of research are now seeking to understand how grasslands can be managed more appropriately by studying the complex interactions that occur both above and below ground [4–9].

Recent analysis suggests 70% of all agricultural grasslands are now used for livestock production, but these practices are also the main threat to many grassland habitats, with inappropriate management cited as the leading cause of grassland degradation and soil erosion [4,10–14]. However, if managed appropriately, livestock can be an essential tool in sustainable agricultural grasslands by increasing plant diversity and improving soil structure and function, to the extent that many conservation organisations now advocate the use of grazing animals in restoration projects [9,15–17].

Soils play a key role in healthy grasslands, containing a rich and varied diversity of life, including meso and macrofauna, as well as archaeal, bacterial and fungal communities [5,9]. The permanent vegetative canopy above buffers surface temperatures and evaporation, helping to regulate water filtration, decomposition rates and microbial activity in the soil below [5,9]. Soil health underpins many aspects of grassland health but is highly influenced by environmental and anthropogenic changes, both short-term and long-term. This research focuses on the soil microbial level, specifically arbuscular mycorrhizal fungi (AMF), and the influence of a range of management practices widely utilised within the livestock farming community [18,19].

1.1. The Role of Mycorrhizal Fungi within Healthy Grassland Soils

Mycorrhizal fungi are a major group of symbiotic soil fungi, predominantly from the phylum *Glomeromycetes*, but have also evolved independently in the phyla *Ascomycota* and *Basidiomycota* [20,21]. Approximately 80% of all terrestrial plant species form symbiotic relationships with mycorrhizal fungi, a phenomenon which is thought to have evolved around 410 Mya ago and is one of the reasons the plant kingdom has been so successful in colonising terrestrial environments [22–24]. The central benefit of these relationships is the exchange of nutrients, especially phosphorus to the plant and carbohydrates to the fungi, although continuing research is finding more varied and complex exchanges which are still not yet fully understood [25–27].

Arbuscular mycorrhizal fungi (AMF) are one of the most notable species in this group of symbiotic fungi and are unique in their appearance, due to the distinctive, highly branched 'tree-like' structure, the arbuscule, which forms inside plant root cells [24]. AMF are also the only group which form balloon-like storage structures, called vesicles [24]. The ability of the hyphal network to extend into the surrounding soil in order to absorb nutrients surpasses that of the host plant's roots [21,25,28,29]. Both the plant and fungal partner typically produce and obtain more carbon and nutrients together than they require individually, further supporting evidence that these associations are mutually beneficial [26,28,29]. The mycorrhizal network formed around the plant's root system has been strongly linked to improved soil aggregation through the release of glomalin, a glue-like deposit released by the hyphae [25]. Additionally, this hyphal network also creates a microscopic habitat for surrounding microbes which, in turn, release further micro-nutrients for the AMF to absorb, increasing the mutual benefits [30,31].

Although some species of AMF have been commercially produced as soil inoculants, most AMF cannot be synthetically cultivated [32]. This makes enhancing their abundance within agricultural soils highly dependent on appropriate management techniques as opposed to reliance on artificial enrichment [33,34]. This study focuses on how agricultural management practices change the abundance of four mycorrhizal structures (spores, hyphae, vesicles and arbuscules).

1.2. Impact of Livestock and Agricultural Management on Grasslands

Cattle and sheep are globally important animals with estimated numbers of 1.5 billion and 1.2 billion respectively [35]. They provide an important food source in places where the land and soil quality are not sufficient to support arable crops and, therefore, provide vital economic benefits [36]. However, their increasing numbers and influence on grassland ecosystems are now the focus of much attention, especially in light of global food security, climate change, environmental degradation and shifting dietary preferences.

Grassland plants have evolved to tolerate a degree of herbivory without long-term damage, and research has shown that the action of non-intensive grazing can promote biodiversity and stimulate plant growth through compensatory vegetative production [16,37]. Large grazing mammals can, therefore, play an important role in maintaining open grassland habitats, by reducing the encroachment of trees and scrub, varying feeding preferences to create a mosaic of plant species, contributing to the nutrient cycle with deposits of dung and urine, trampling the ground to open niche microhabitats and moving seeds both in and on their bodies [38].

However, overgrazing has been the most cited cause of grassland decline, and it is estimated that between 25 and 30% of global grasslands have been degraded by inappropriate levels of livestock grazing [4,39,40]. Multiple studies offer evidence that high-intensity grazing can negatively impact grasslands by excessive vegetation removal, adversely altering species composition and damaging the soils either directly or indirectly [41–44]. To combat these issues and maintain or even increase the productivity of agricultural grasslands, applications of fertilisers and other agrochemicals are used to artificially elevate grassland yields and livestock productivity [45,46]. However, the consequences of these agrochemicals are now emerging, as increasing application rates are required to compensate for diminishing soil health, so understanding how to readdress the natural balance of grassland soils is of vital importance to ensuring a sustainable future [46].

1.3. Agricultural Management

This research focuses on four agricultural practices within the livestock sector which are each controlled to a greater or lesser degree by management decisions of farmers. The aim of each practice is to increase agricultural output, but this study will investigate their effects on AMF.

Livestock type varies largely across the globe depending on the environmental, social and economic context and area of interest [47]. This study focuses on farmland in Wales, which covers 88% of the land area of this region [48]. Livestock production is central to Welsh farming, accounting for around 75% of total agricultural output [49]. It supplies 40% of the UK's sheep and cattle demand, despite only accounting for 10% of the agricultural land area for the whole of the UK. Currently, there is no published research specifically related to different livestock types and their effects on AMF, so this study aims to investigate this knowledge gap.

Grazing livestock on grasslands is one of the dominant agricultural features of Wales, as the low soil quality and the steep topography of the land is typically unsuitable for high arable output [48]. Almost 80% of Welsh agricultural land is used for either permanent of rough grazing, and Powys has one of the lowest arable land uses in Wales [48]. Only around 8% of farmers house their livestock year-round, with most choosing a mix of housing and grazing, highlighting the importance of healthy grasslands [49]. Currently, most research focuses on the effects of grazing intensity and AMF, commonly linking high-intensity grazing with reduced AMF abundance [43,44].

Lime is applied to soils in order to counteract the negative effects of soil acidification. The soils in Wales are predominantly acidic as a result of natural geological formation and, therefore, typically low in organic content [50,51]. Research has shown that different AMF taxa colonise soils of different pH; therefore, any alteration in the soil's pH by the application of lime could cause a disruption to or decline in the AMF community [52,53].

Plant diversity within livestock grasslands is commonly altered by farmers to maximise forage quantity and quality and compliment the application of agrochemicals, such as fertiliser [54,55]. Ryegrass and clover often dominate agricultural grasslands due to the quality and quantity of their forage, fast establishment and persistence within the field [55]. However, this reduces biodiversity and drought resilience. Research has shown that increased plant diversity can improve soil health and overall grassland resilience [55,56]. Plant diversity has also been linked with AMF, but more research is required as there are many compounding factors that influence this relationship, such as environment and soil composition.

Grasslands and their soils are of great importance to global food systems and environmental functions. Understanding the complexities with which these systems interact is vital for a sustainable and viable agricultural future. This research, therefore, aims to investigate the impact of widely used agricultural management practices upon the aforementioned four structures of AMF, a well-known but still relatively poorly understood element of healthy grassland soils. It is hypothesised that each of the management practices outlined in Section 1.3. will impact one or more of the AMF structures to a greater or lesser degree. It is predicted the practices which align more closely with sustainable practices, e.g., reduced grazing and inputs and increased plant diversity, will result in greater AMF abundance.

2. Materials and Methods

2.1. Site and Field Selection

This study focuses on farmland in Wales, with study sites chosen from a self-selected group of farmers consisting of 11 farms within the lower Wye Valley area of Powys. This area is dominated by livestock, consisting mostly of sheep and cattle and is, therefore, representative of the Welsh livestock industry [49]. The farms ranged in size between 12 hectares and 200 hectares and had varied management approaches with a mix of livestock including sheep and/or cattle.

The farmers were asked to identify two fields on their farm that had been under grass for at least five years and were currently used in a grazing rotation using a low or moderate grazing intensity. Information regarding management practices, such as type of livestock used in the grazing rotation (sheep or cows) and the application of lime, was also collected and recorded (Table 1). Each of the fields were sampled across three temporal periods to ensure representative data across the growing season: early spring (end of March 2022), late spring (late May 2022), and mid-summer (mid July 2022). During each temporal sample period, it was noted whether the fields were being actively grazed by livestock (Table 2). All the farmers were given an identification code and completed a consent form prior to research commencing.

Table 1. Field codes and their corresponding management practices.

Field Code	Livestock Type	Lime	Plant Diversity
A.1	Cattle	No lime	Medium
A.2	Sheep and Cattle	No lime	Medium
B.1	Sheep and Cattle	No lime	Medium
B.2	Sheep and Cattle	No lime	Medium
C.1	Cattle	Lime	High
C.2	Cattle	Lime	High
D.1	Sheep	Lime	Medium
D.2	Sheep	Lime	Low
E.1	Sheep	No lime	High
E.2	Sheep	No lime	High
F.1	Sheep and Cattle	No lime	High
F.2	Sheep and Cattle	No lime	High
G.1	Sheep and Cattle	Lime	Medium
G.2	Sheep and Cattle	Lime	Medium
H.1	Sheep and Cattle	Lime	Low
H.2	Sheep and Cattle	Lime	Low
J.1	Sheep and Cattle	Lime	Low
J.2	Sheep and Cattle	Lime	Low
K.1	Sheep	No lime	Medium
K.2	Sheep	No lime	Medium
M.1	Sheep	Lime	Low
M.2	Sheep	Lime	Low

Table 2. Field codes and their corresponding grazing practices per temporal period.

Field Code	Early Spring	Late Spring	Summer
A.1	No grazing	Active grazing	No grazing
A.2	Active grazing	No grazing	Active grazing
B.1	No grazing	No grazing	No grazing
B.2	No grazing	No grazing	No grazing

Table 2. *Cont.*

Field Code	Early Spring	Late Spring	Summer
C.1	No grazing	No grazing	No grazing
C.2	No grazing	No grazing	No grazing
D.1	No grazing	No grazing	No grazing
D.2	No grazing	No grazing	No grazing
E.1	No grazing	No grazing	No grazing
E.2	No grazing	No grazing	No grazing
F.1	No grazing	Active grazing	No grazing
F.2	Active grazing	Active grazing	No grazing
G.1	No grazing	Active grazing	No grazing
G.2	No grazing	No grazing	No grazing
H.1	No grazing	No grazing	Active grazing
H.2	No grazing	No grazing	Active grazing
J.1	No grazing	Active grazing	Active grazing
J.2	Active grazing	No grazing	No grazing
K.1	No grazing	No grazing	No grazing
K.2	No grazing	Active grazing	No grazing
M.1	No grazing	Active grazing	No grazing
M.2	No grazing	No grazing	No grazing

2.2. Plant Diversity

A 50 × 50 cm quadrat sample was taken at random from the selected fields, avoiding gateways, individual features such as trees, water troughs or footpaths and not within 20 m of hedgerows to ensure a representative sample (Table 1). The plants were identified using multiple keys to ensure accuracy [57–59].

The diversity was then ranked in one of three categories: low (indicating 6 or fewer species per quadrat), medium (indicating between 7 and 10 species per quadrat), and high (indicating over 10 species per quadrat).

2.3. Soil Samples

2.3.1. Collection and Storage

Three soil cores were taken from each of the selected fields per temporal period using a standardised auger, 5 cm in diameter and 15 cm deep. The cores were taken at random within the fields, but no cores were collected within 20 m of hedgerows, gateways or obvious access paths. At each sampling point, the three cores were mixed into a single field sample and any large vegetative matter was removed. In total, 66 samples were taken. The samples were kept cool and stored within 48 h in a freezer at a constant −18 °C.

2.3.2. Staining

Root staining preparations were adapted from the protocols of Wu et al. (2012) and Penn State, (2022) [60,61]. Blue Parker Quink™ ink was selected as the stain, due to its low toxicity and optimal staining performance comparable to other staining options which were deemed too noxious/carcinogenic [60,62].

Approximately half (600 g) of each field sample was removed from the freezer and defrosted thoroughly for a minimum of 24 h. These subsamples were then weighed and fractioned through 6 mm and 2 mm sieves to isolate the roots, which were separated and rinsed in a fine mesh strainer to remove the remaining soil. The isolated roots were then reweighed.

The roots were then cut into ~1 cm fragments and any large, thick or dead roots removed. The fragments were placed in a 10% potassium hydroxide mixture (KOH) and heated to 80 °C for 30 min. The fragments were rinsed three times in distilled water, placed in blue Parker Quink™ ink and clear white vinegar mix and left to stain for 15 min at room temperature, in alignment with the adapted protocols of Wu et al. (2012) and Penn State, (2022). The fragments were rinsed with distilled water and then submersed in distilled water with two drops of vinegar overnight at room temperature to de-stain further. A total of 660 root fragments were selected at random, mounted on plates with distilled water and observed using a compound microscope at ×250 and ×400 magnification.

2.3.3. Identification of Mycorrhizal Structures

The four AMF structures—spores, hyphae, arbuscules and vesicles—were identified using features standardised by Willis, Rodrigues and Harris, 2013 [25], Dixon et al., 2014 [63], and Walker et al., 2018 [64] (Figure 1).

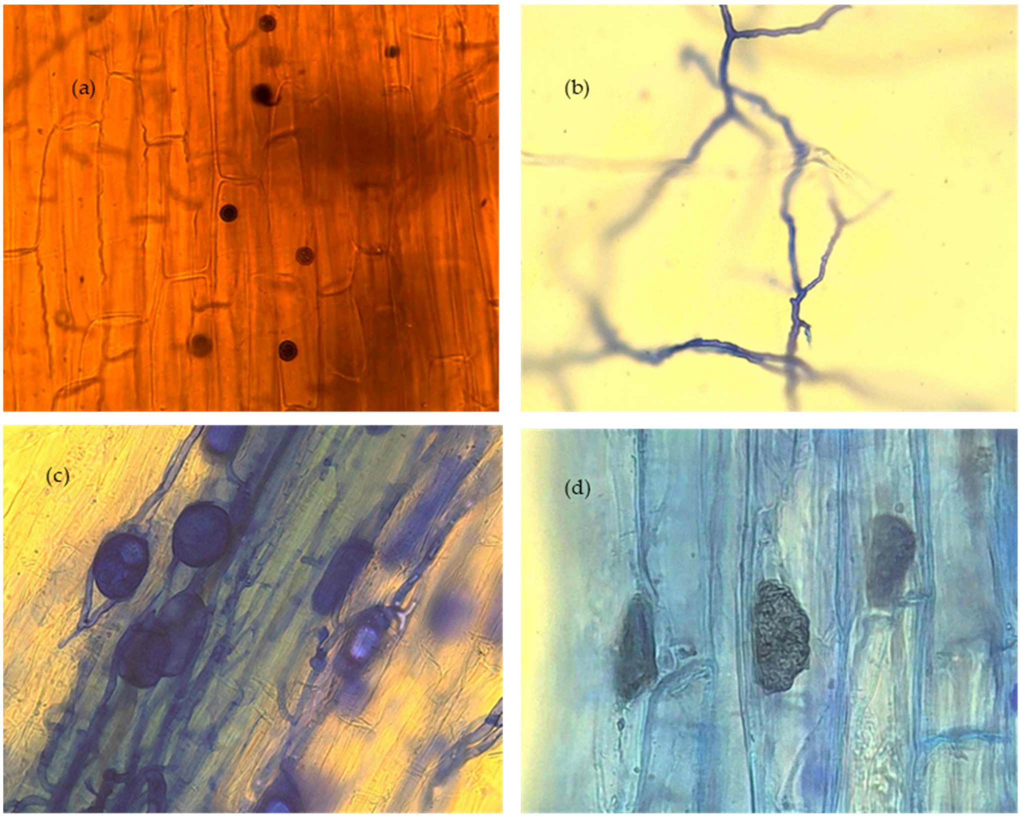


Figure 1. Microscopy images of AMF structures after staining and cleaning process for (**a**) spores, (**b**) hyphae, (**c**) arbuscules, (**d**) vesicles (Copyright Annie Buckle 2022).

For each root fragment selected, the spores, arbuscules and vesicles were assigned an abundance value, shown here in brackets, based on the amount observed through the microscope: none (0), between 1 and 4 (2), between 5 and 9 (5), and 10 or more (10). These scores were used due to the large differences between samples (and to reduce inaccuracy when counting large numbers on microscope slides). Hyphae were noted for their presence (1) or absence (0).

2.4. Data Analysis

All data were analysed in GenStat Version 20.1.2.24528 by VSN International Ltd., Rothamsted Research, St Albans, UK. The data were verified for normality and homoscedasticity prior to analysis and a transformation applied (log10 + 1) if necessary. Two-way sample *T*-tests were used to test for significance in pairwise comparisons, and Analysis of Variance (ANOVA) were used to determine significance for multiple pairwise comparisons. Graphs are presented with means of the abundance values, observed for each AMF structure, to provide an abundance assessment.

3. Results

Overall, all soil samples were found to contain root fragments which had at least one AMF structure, and 78% of the individual root fragments taken from the soil samples were found to contain more than one AMF structure. Significant results are denoted on the graphs by the use of an asterisk (*).

3.1. Livestock Type

There was a significant association between the type of livestock and the relative abundance of arbuscules and the presence of hyphae. When cattle and sheep were grazed together, there was a 43% higher abundance of arbuscules compared to when cattle or sheep were grazed separately (p = 0.036, Figure 2a). Similarly, when cattle and sheep were grazed together, there was a 30% higher rate of hyphae presence compared to when cattle or sheep were grazed separately (p = 0.001, Figure 2b). No significant differences were found in vesicle or spore abundance.

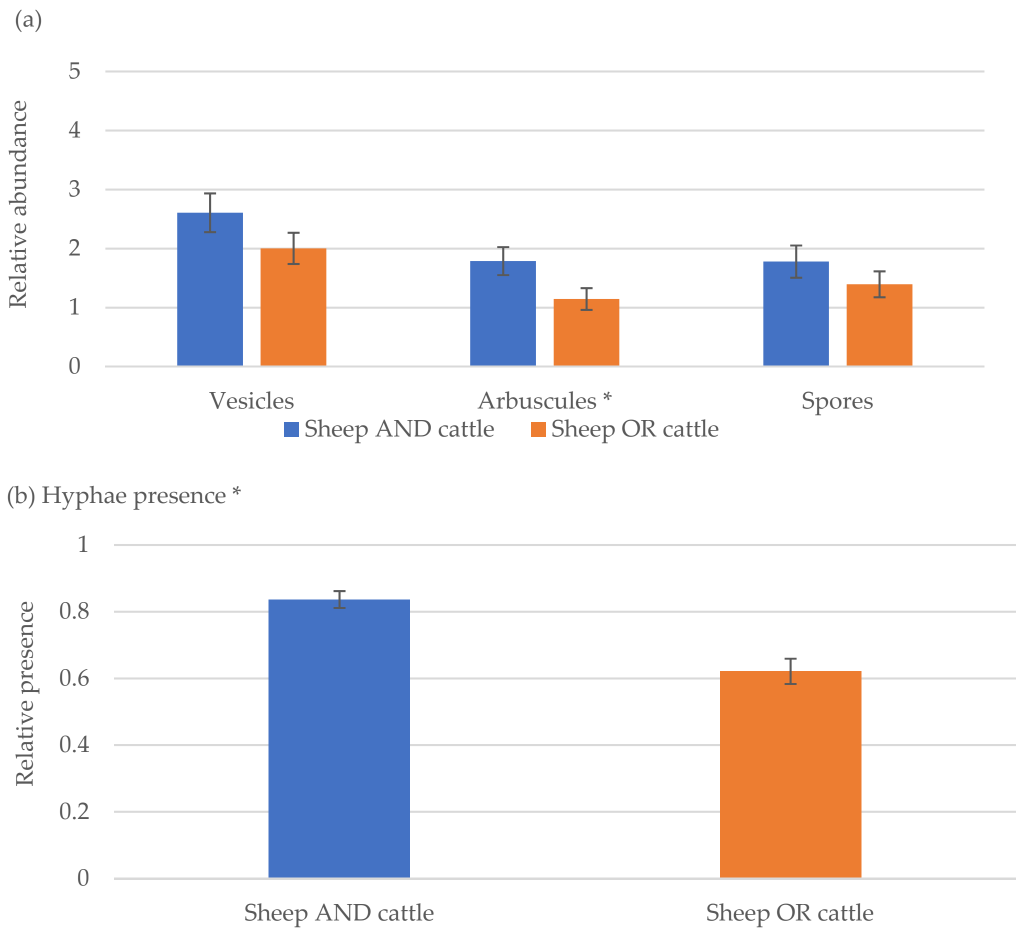
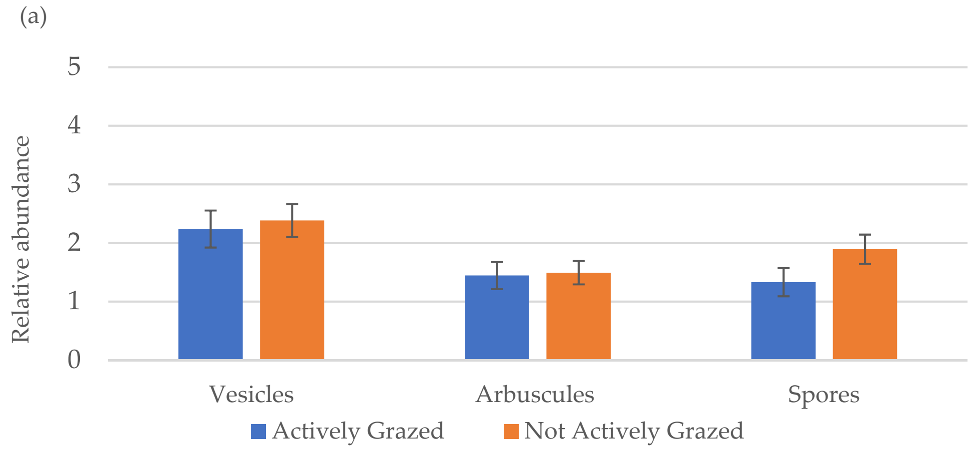


Figure 2. The abundance of (**a**) arbuscules and the presence of (**b**) hyphae were both significantly higher when both sheep and cattle grazed together compared to field systems where sheep or cattle grazed separately. No significant differences were found between vesicles or spores.

3.2. Active Grazing

The fields which were sampled whilst being actively grazed showed significantly lower hyphae presence than those which were not being actively grazed (p = 0.001, Figure 3b). No significant differences were found in the abundance of vesicles, arbuscules or spores (Figure 3a).



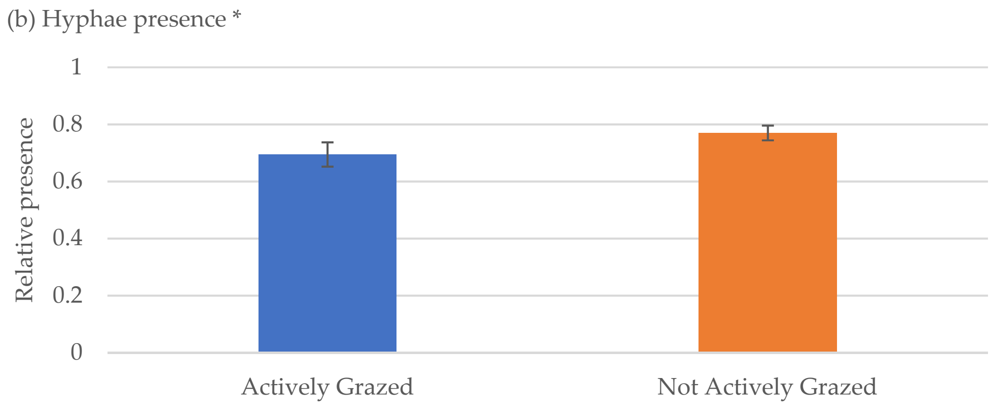


Figure 3. No significant differences were found for the abundance of (**a**) vesicles, arbuscules or spores, but (**b**) the presence of hyphae was significantly lower in fields which had active grazing compared to those which were not being actively grazed.

3.3. Application of Lime

There was a significantly lower abundance of vesicles when lime was applied to the sampled fields, compared to when lime was not applied (p = 0.014, Figure 4a). No significant differences were found between the abundance of arbuscules, spores or the presence of hyphae (Figure 4a,b).

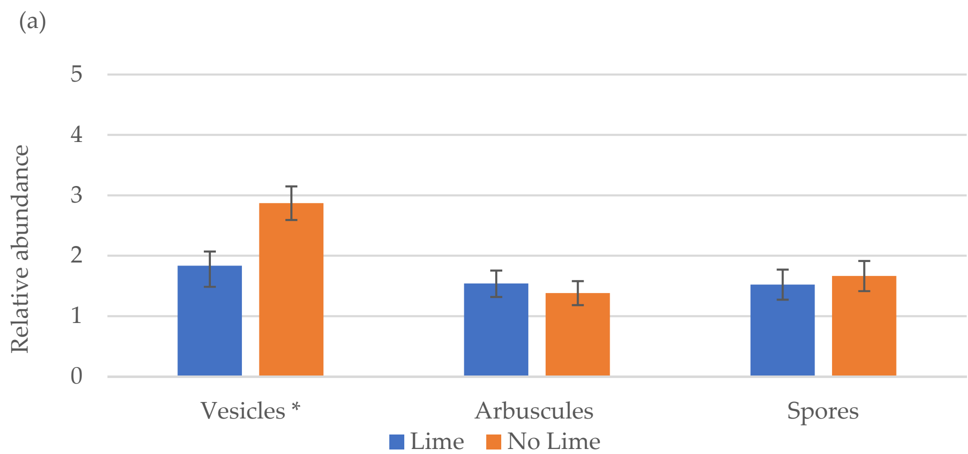


Figure 4. *Cont.*

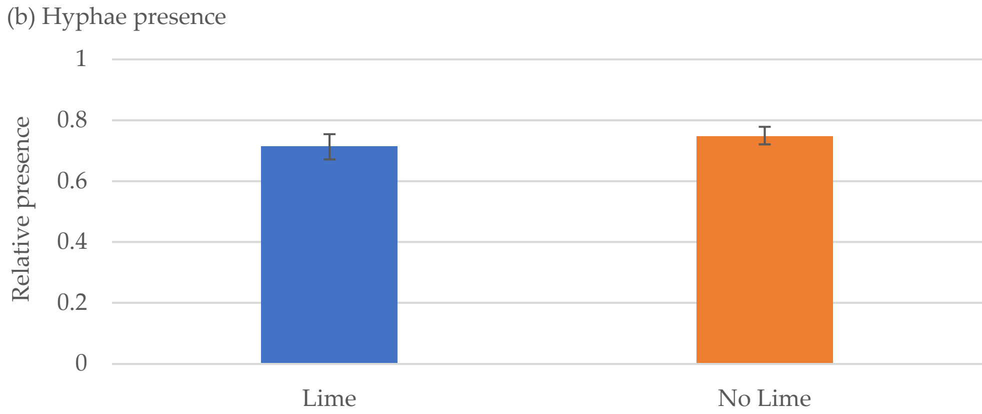


Figure 4. The abundance of (**a**) vesicles was significantly lower in fields which had received an application of lime within the management period of that field but no significant differences were found in the abundance of arbuscules, spores or (**b**) the presence of hyphae.

3.4. Plant Diversity

There was a significantly lower abundance of arbuscules with high plant diversity, compared to that of medium or low diversity ($p = 0.001$, Figure 5a). Conversely, there was a significantly lower abundance of vesicles with low plant diversity, compared to that of medium or high diversity ($p = 0.044$, Figure 5a). No significant differences were found in the abundance of spores or the presence of hyphae (Figure 5a,b).

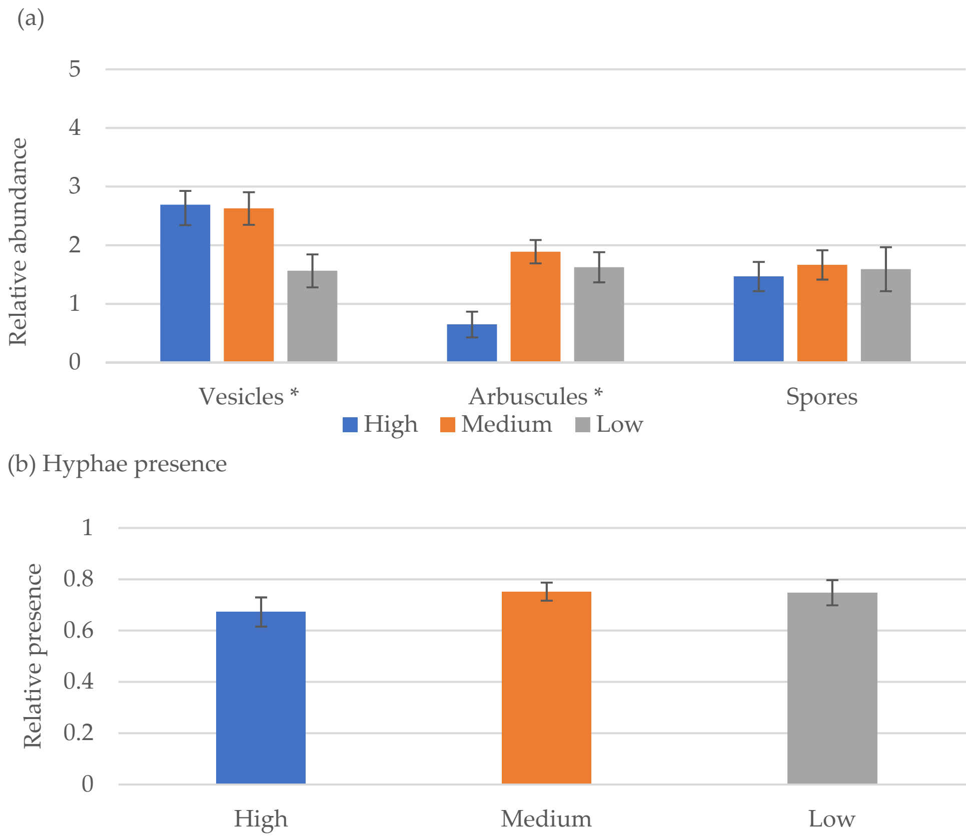


Figure 5. The abundance of (**a**) vesicles was significantly lower in fields with low plant diversity, and the abundance of arbuscules was significantly lower in fields with high plant diversity. No significance was found in the abundance of spores or (**b**) the presence of hyphae.

3.5. Farm Differences

The overall abundance of vesicles, arbuscules and spores, and the presence of hyphae were tested for significance across all eleven farms sampled (Figure 6). The abundance of vesicles showed a greater significant difference between farms D and B (p = 0.001), with farms E, G, H, J, K and M most similar to D, and farms A, C and F most similar to B. The abundance of arbuscules showed a greater significant difference between farm E and farms B, G and J jointly (p = 0.007), with all remaining farms otherwise unrelated to one another. The presence of hyphae had a greater significant difference between farms D and B (p = 0.001), with farms C, K, M and H most similar to one another. No significant differences were found in the abundance of spores (p = 0.761).

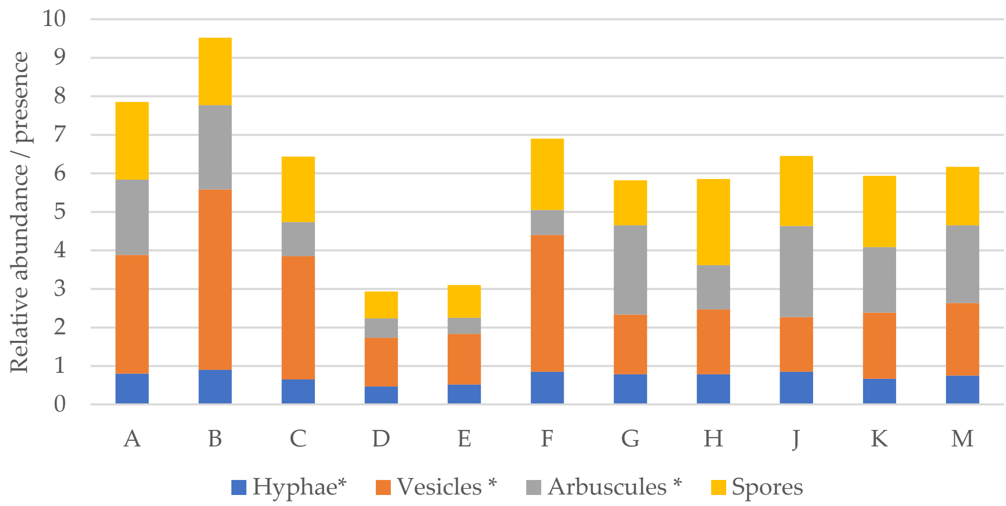


Figure 6. Farm B had a significantly higher abundance of arbuscules and vesicles and higher presence of hyphae compared to the other farms. Farm D had a significantly lower abundance of vesicles and lower presence of hyphae compared to the other farms. Farm E had the lowest abundance of arbuscules compared to the other farms.

It should be noted that farms C and F are organic, and the fields sampled on farms H and J were part of a crop rotation and, therefore, not permanent, despite being within the 5-year timespan stipulated in the selection process. However, these differences were not noticeable in comparison to the other farms (Figure 6).

Each farm showed considerable variation in the abundance and presence of the four AMF structures, and plant diversity. Here, the farms were ranked according to the highest abundance and presences of the four AMF structures (Figure 6) and are considered against each of the four agricultural interventions tested. It was observed that only the practice of grazing cattle and sheep together consistently appeared to correlate with the top-ranking farms (Table 3). No other treatments, which had previously yielded significant results, consistently appeared within these top performing farms.

Table 3. The farms which had the highest overall abundance or presence of the four AMF structures were ordered from highest to lowest (with farm B being the highest).

Overall Mycorrhizal Occurrence (by Farm)	Cattle and Sheep (Per Field)	Lime Applied (Per Farm)	Plant Diversity Ranked High (Per Field)	Actively Grazed (/Field/Temporal Period)
B	++	+	−	++++++
A	+	+	−	+++
F	++	+	++	+++
J	++	−	−	+++
G	++	−	−	+++++
H	++	−	−	++++
M	−	−	−	+++++

Table 3. *Cont.*

Overall Mycorrhizal Occurrence (by Farm)	Cattle and Sheep (Per Field)	Lime Applied (Per Farm)	Plant Diversity Ranked High (Per Field)	Actively Grazed (/Field/Temporal Period)
C	−	−	++	++++++
K	−	+	−	+++++
E	−	+	++	++++++
D	−	−	−	+++++

The two fields on each farm are marked with a (+) if they used both cows and sheep within their grazing rotation, or a (−) if they used either cows, or sheep. The farms which reported to use lime are marked with a (+) or a (−) if they did not use lime. The two fields on each farm are marked with a (+) if they ranked high on plant diversity or a (−) if they ranked either medium or low. The two fields are marked with a (+) if they had been actively grazed at each temporal period or (−) if they were not being actively grazed at each temporal period.

4. Discussion

This study investigated the effects of a selection of common agricultural management practices on the abundance of four structures of AMF. The results show that the most positive effect on AMF resulted from mixed grazing, while plant diversity had mixed results, and the application of lime, along with active grazing, negatively affected AMF. These variable results are discussed in more detail below.

4.1. The Effect of Mixing Livestock

The influence of mixing livestock types and the positive effect on the abundance of arbuscules and presence of hyphae is a novel outcome of this research as there is currently no other research into this particular phenomenon (Figure 1). Although research exists which investigates the influence of mixed grazing on other grassland functions, none refers specifically to the influence of mixed grazing on AMF. The results of this study, therefore, offer a new gateway for research to explore and examine the potential influence of mixed grazing on AMF.

Studies into the effects of mixed grazing or co-grazing on other aspects of grassland functions have found that combining cattle and sheep increases the soil organic content, reduces the bulk density and improves the species composition of the grassland [65–67]. Multiple studies conducted by Cuchillo-Hilario et al. have also shown that grazing sheep and cattle together alters the animals' grazing behaviours and, therefore, foraging selectivity and duration to the extent that plant diversity and botanical composition are also altered in a positive way [68–70]. A study by Zhang et al. (2022) also concluded that mixing livestock resulted in a higher turn-over of root growth and increased organic carbon soil content when compared to only one grazer type or no grazing at all [71]. Additionally, other influencing factors of mixing livestock may include the following: different grazing mechanisms such as ripping or biting, variable grazing duration and amounts consumed, body sizes and the associated weight impact on the soil, varying excretion composition and amount, and even potentially the sex of the animals as hormones within the excrements could affect the soil biome differently [67,72,73]. A recent meta-analysis of mixed grazing, conducted by Su et al. (2023) [65], highlighted the multi-dimensional benefits of mixing sheep and cattle, including effects on plant vegetation, both above and below ground, and the physical, chemical and biological effects on soil. However, with the exception of nematodes, the soil variables they investigated did not include any specific soil communities such as fungi or specifically AMF. As the role of hyphae and arbuscules are concerned predominantly with the transfer of nutrients between the fungi and the plant, the aforementioned beneficial effects exhibited within the soil by mixed grazing are, therefore, likely to be transferred to the AMF network also.

From the perspective of practical application, this initial analysis suggests mixing livestock types could be beneficial to AMF abundance in grassland soils. This study begins to fill this knowledge gap and highlights the need for further research to explore the value of mixed grazing alongside the existing and ongoing research which focuses on grazing intensity.

4.2. Active Grazing Impacts

The effect of grazing intensity on grasslands is one of the most widely studied areas in this field of research and the results from this study support previous findings which suggest grazing can negatively impact AMF (Figure 2) [43,45]. However, these results only indicated a reduction in the presence of hyphae, reflecting the findings of Faghihinia et al. (2020) [43], and support other suggestions [74] that the influence of grazing on AMF, whilst variable, may not necessarily be wholly detrimental.

In their study, Faghihinia et al. (2020) [43] found hyphal length was negatively correlated with increasing grazing intensity, but the number of root fragments colonised by hyphae and the amount of hyphal colonisation on those root fragments were not significantly affected. They also investigated arbuscule intensity, which did not express any significant correlation either. Earlier research by van der Heyde et al. (2017) [74] and Ren et al. (2017) [75] also showed hyphal length was negatively affected by grazing, but root colonisation itself was not. The results of this study appear to agree with those of Faghihinia et al. (2020) [43] and Heyde et al. (2017) [74].

Although it is unclear exactly why hyphal length is affected more severely than hyphal root colonisation, other studies have shown that carbon allocation to the plant roots decreases following the removal of above ground biomass by grazing, as available carbon is reserved for plant regrowth instead of being exchanged with the mycorrhizal associates [45,76]. A similar response also occurs with phosphorus, whereby it is absorbed by the plant for regrowth after vegetative removal, thereby altering the soil phosphorus availability [77]. Early research, supported by these later studies, also shows external hyphal growth is more sensitive to phosphorus availability than other nutrients, with phosphorus being a key trigger in hyphal growth [21,76]. This may go some way in explaining this phenomenon, but more research into the detailed mechanisms is needed. Additionally, the synergetic effects of grazing intensity and mixed grazing would be useful to explore as it is possible the impact of grazing intensity could be reduced or buffered by mixed stocking.

4.3. The Effects of Applying Lime

This research found that the application of lime negatively affected the abundance of both vesicles and root density, which appears to contradict the previous understanding of the benefits of liming (Figure 3).

Early research by Siqueira et al. (1984) [78] on the effect of soil acidity on mycorrhizal fungal colonisation suggests that reducing soil acidity improves colonisation, but it is now known that soil pH affects species of soil fungi differently, and this original assumption cannot be extrapolated across all AMF species [79]. There are currently no directly comparable data related specifically to the abundance of vesicles and the application of lime. However, research by Olsson et al. (2011) [80] identified phosphorus as a key element found within vesicles, along with calcium, sulphur and potassium. These particular nutrients become less available in soils with a pH of 6.5 or less, and therefore, the ability for AMF to provide a reserve of these nutrients for use by the host plants is highly advantageous in acidic soils. However, if lime is applied to readdress the pH balance, thus improving the availability of these nutrients, the need for vesicles is reduced as plants are able to receive them directly from the soil through their own roots or hyphal associations.

4.4. Plant Diversity

The relationship between plant diversity and AMF observed within this study appears to diverge from the published literature (Figure 4). Previous research has found that increased AMF correlates with increased plant diversity, but whilst both vesicle and arbuscule abundance occurred most frequently under medium plant diversity, arbuscule abundance significantly reduced under high plant diversity [81].

A study conducted by Horn et al. (2017) [82] found plant communities are not a strong predictor of AMF communities. Although their findings suggest plant community structure as a driver of AMF community structure, many compounding variables in the environment, such as soil requirements and temporal influences, meant the link was not necessarily universal across all plant and fungal interactions [82]. Research by Faghihinia et al. (2020) [42] also found plant diversity did not have a direct, linear effect on the presence or abundance of AMF, but was more variable and environmentally dependant. As plant diversity increases, the resource demands diversify, thus reducing inter-species competition and enabling the utilisation of different nutrients in different amounts. This trend has been observed in studies whereby increased plant species diversity improved soil resource use and correspondingly increased total plant biomass [83]. This theoretically leads to a reduced reliance on the AMF arbuscule exchanges sites within the root cells but an increase in storage vesicles, as excess nutrients are stored for later utilisation.

4.5. Implications and Limitations

The results of this study have highlighted further areas of potential research that could be applied to sustainable agricultural management practices related to both soil and grassland health within the livestock industry.

Considering the practical applications, utilising a mixed grazing approach could not only reduce reliance on artificial inputs and, therefore, reduce costs, but also offer farmers a more stable and resilient business model through diversification. Collaborations with other local farmers could also be encouraged through the sharing of livestock.

This study recommends more research is urgently required to better understand the effects of mixed grazing animals and AMF. The limitations of this study could be improved upon by increasing the sample size and the area of study to enable better extrapolation of the data, and by the addition of further parameters related to mixed grazing. This could include investigating the effectiveness of existing mixed grazing systems, such as 'co-grazing' or 'follow-on' grazing and the ability for mixed grazing to counteract the impact of grazing intensity.

5. Conclusions

This research aimed to investigate the impact of specific but widely used agricultural interventions on arbuscular mycorrhizal fungi in grazed grasslands.

The results of this research offer two novel insights: Mixed grazing promoted AMF, and the application of lime reduced AMF. The positive effect of mixed grazing is an important finding when considering the potentially negative effect of certain grazing intensities and, therefore, could have a significant impact on how livestock is managed in the future.

Grasslands play a key role within agriculture. As well as being an irreplaceable resource for the livestock industry, they provide invaluable habitats for wild species and multiple other ecosystem services. This research contributes to the knowledge required to inform best-practice grassland management, policy and governance and will help to inform the development and restoration of healthy, diverse and resilient grassland ecosystems.

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