The Sustainable Use of Species-Rich Grasslands for Forage and Biogas Production



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Thesis submitted for the degree of
Doctor of Philosophy
at the
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Abstract

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The rapid loss of species-rich grasslands in the UK may have profound environmental and socio-economic ramifications. Although they were once the foundation of livestock production systems, the agricultural use of these landscapes has rapidly declined since the 'Green Revolution' of the 1950s due to increased economic incentives to replace species-rich grasslands with agriculturally improved leys and arable fields. In this thesis, I examined the agricultural value of species-rich grasslands in lowland England and the potential ecological impact of losing these landscapes. My research had four main objectives: (1) to determine whether the nutritional composition of forage from species-rich grasslands was comparable to commercial livestock feeds, (2) to assess the bioenergy output of biomass from species-rich grasslands compared to current bioenergy feedstocks, (3) to determine the effect of land use change and agricultural intensification on microbial diversity, and (4) to determine whether grassland biodiversity was stable or changed (temporally and spatially) over time. I show that the forage quality of species-rich grasslands is comparable to that of forage from improved leys and contains extra nutrients (minerals and phytochemicals) which contribute to livestock health. I demonstrate that biomass from species-rich grasslands can compete with other bioenergy crops, especially Miscanthus (the primary bioenergy feedstock in the region) and that species richness and species composition contributes strongly to the biogas yield of grasslands. Through soil metagenomic analysis I found that while bacterial diversity does not decline with agricultural intensification, fungal diversity decreases and plant, human and livestock microbial pathogens shown to be pathogenic in other ecosystems dramatically increase on agriculturally improved fields compared to species-rich grasslands. Finally, by combining palaeoecological and modern vegetation data I demonstrate that grassland biodiversity was temporally and spatially linked to long-term human management and would revert to oscillating between open grassland and woodland if this management ceases. Grassland biodiversity is thus a finite natural resource with a strong economic and environmental value that can contribute to the development of more sustainable agricultural and bioenergy production systems in the UK.

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CHAPTER 1

INTRODUCTION

1. The 'Grassland Problem'

Reviving the agricultural use of species-rich grasslands in the UK could help maintain the biodiversity they harbor while boosting local livelihoods. Species-rich grasslands are anthropogenic ecosystems that are used for livestock grazing ('pastures') or hay-making ('meadows') (Barnes and Nelson 2003). These can be a hundred or more years old. Although they once formed the backbone of pastoral production, today these grasslands are in a crisis: increasingly species-rich grasslands are ploughed up and sown with arable crops or abandoned (Kemp and Michalk 2011; Young et al. 2005). The UK National Ecosystem Assessment estimates that arable fields have replaced 90% of lowland species-rich grasslands since 1945 (Bullock et al. 2011). Determining the agricultural and economic value of species-rich grasslands in modern agriculture is thus critical given the pace at which these landscapes are disappearing.

This thesis will focus on the viability of producing forage and bioenergy from species-rich grasslands and the potential ramifications of losing these landscapes in regards to soil microbial diversity and plant genetic resources. This research is important given that the majority of studies on species-rich grassland focus on their conservation, as opposed to agricultural, value (Oglethorpe and Sanderson 1998; Pykälä et al. 2005). It is also timely due to recent proposals that we increase the use of grasslands to preserve ecosystem services, boost food security, and achieve national biodiversity goals (Lemaire et al. 2014; O'Mara 2012; Schader et al. 2015). These results can help inform how farmers, graziers, and landowners manage and use species-rich grasslands. At the same time, they can inform conservation activities at these sites.

This chapter will provide a brief overview of the causes of grassland biodiversity decline in the UK. It will then describe renewed interest in grassland biodiversity due to recent initiatives to maintain ecosystem services, to increase food security through reliance on lower-input systems, and to develop a national 'bioeconomy.' Subsequently, it will provide a succinct overview of previous research conducted on the effect of biodiversity on forage quality, the use of grassland biomass for bioenergy, the effect of agricultural intensification on microbial diversity, and the role of long-term ecology in grassland conservation. Finally, it will present the objectives, structure and scope of this thesis.

2. Decline of Grassland Biodiversity: From Hyper-diversity to Homogeneity

Until the 19th century, grasslands were characterized by a wide variety of plants and what we consider 'species-rich grasslands' today are a product of both environmental and anthropogenic influences. Palaeoecological evidence (pollen, spores and plant remains from soil cores or archaeological sites) from the UK and Europe suggests that what we consider 'traditional' hay meadows or unimproved grasslands developed roughly 2,000 years ago (Derreumaux 2005; Hodgson et al. 1999; Tereso et al. 2013) and were characterized by a variety of grasses, legumes, and herbs characteristic of modern hay meadows (e.g. *Lolium perenne* L., *Sanguisorba officinalis* L., etc.) (Robinson 2011). Natural shifts in vegetation, grazing and anthropogenic management all likely influenced the diversity of early species-rich grasslands and this development likely varied from place to place given a mix of these factors. By the 16-17th century, printed books indicate grasslands were dominated by a variety of grasses and herbals from this period refer to a variety of medicinal plants found in pastures and meadows (Turner [1658] 1995). By the 18th and 19th centuries, agronomic texts extolled the value of particular grasses, legumes, and forbs (Preston 1887). While some plants were rated higher than others, planting one or a few species as opposed to a mixture was not promoted. A guide to pasture and meadow plants produced by the Sutton Seed Company in 1888 promoted the sowing of 28 grasses, 12 legumes and 8

medicinal plants (Sutton 1888). Samples of agricultural grasses framed by the same company indicates a wide variety of grasses were still cultivated at this time. Throughout this period, grassland diversity was taken for granted and, in some cases, encouraged.

However, the perception of the agricultural and economic value of semi-natural grasslands, and growing multi-species swards, changed sharply during the 'Green Revolution' following World War II. Four key developments contributed to the decline of grassland biodiversity we see today. First, from the early 20th century onwards plant breeders focused on creating high-yielding forage crops, primarily ryegrass (*Lolium perenne* L.) and clover (*Trifolium pratense* L.) (Hodgson et al. 2005; Humphreys 2003). Grasslands with multiple-species were considered 'inferior' and lower yielding (Organization for European Economic Co-operation 1954). Second, agronomists encouraged farmers to grow short-term leys (e.g. of clover, etc.) in rotation with other arable crops (e.g. wheat, *Triticum aestivum* L.) to help maintain soil fertility and decrease microbial pathogens in the soil (Boyd 1949; Davies 1952). This decreased the area of land covered by semi-natural grasslands. Third, the use of chemical fertilizers and pesticides increased dramatically after WWII. For example, 37 pesticides were available in 1955 and by 1997 there were 344 different products (Batary et al. 2015; Robinson et al. 2002; Tallowin et al. 2005). This caused a decline in plant species requiring low soil fertility to persist (e.g. some orchid species). Finally, livestock breeders focused on creating larger breeds which reached the right size, weight and body composition for slaughter/dairy production more quickly than their predecessors (Peeters 2009). These breeds largely replaced older 'traditional' livestock breeds that were often smaller, had lower nutritional requirements, and were well suited to the local terrain (Bowles et al. 2014; Dawson et al. 2011). However, this came at a price: these new breeds had higher nutritional requirements (e.g. protein, sugar) and to meet these needs farmers turned to nutrient-dense feeds (e.g. wheat, soy) and reduced the use of grass-based feed. Grasslands which have undergone

these modifications since the 1950s are known as 'improved grasslands.' They now make up the majority of the grasslands used in livestock production (Hopkins 2008).

Today, remaining species-rich grasslands are often protected as Sites of Special Scientific Interest (SSSIs). They are protected (and many cases managed) for the rare plants they contain (Spash and Simpson 1993; Porter and Leach 2013). In many cases, these are colorful charismatic species (e.g. snake's head fritillaries, *Fritillaria meleagris* L.), plants considered endangered in the UK according to the UK Biodiversity Action Plan (e.g. green hound's-tongue, *Cynoglossum germanicum* Jacq.), and plants found in only a handful of locations in the UK (e.g. Creeping marshwort, *Apium repens* L.; Pasqueflower, *Anemone pulsatilla* Mill.).

3. Renewed Interest in Grassland Biodiversity: Ecosystem Services, Food Security and the 'Bioeconomy'

Interest in grassland biodiversity has renewed in recent years. In the UK, and more broadly in the European Union (EU), governments are investing in biodiversity, primarily due to the 'ecosystem services' they provide. Ecosystem services are the services provided by ecosystems to humans (Costanza et al. 1997; Mace 2012; Millennium Ecosystem Assessment 2003, 2005). They fall into four main categories: provisioning (e.g. natural products, food), regulating (e.g. control of climate), supporting (e.g. pollination), and cultural (e.g. historic landscapes). Many have challenged the concept of ecosystem services since it was first proposed in the 1980s and whether we need to place a value on biodiversity at all (Comberti et al. 2015; Silvertown 2015). However, in the case of grasslands ecosystem services are increasingly used to justify maintaining, restoring and recreating species-rich pastures and meadows (Gibson 2009; Lamaire et al. 2011; Peeters 2009; Wrage et al. 2011). These services range from flood control (Posthumus et al. 2010), to carbon sequestration (De Deyn et al. 2011), to provision of forage for pollinators (Martins et al. 2015), to maintaining cultural heritage

(Lindborg et al. 2008). From 2007-2013, the UK government spent £375 million pounds a year on agroenvironmental schemes (Batary et al. 2015). Six of these schemes focused on the maintenance, restoration and recreation of species-rich grasslands (Natural England 2012). In the EU, farmers also receive payments through the Common Agricultural Policy (CAP) for maintaining species-rich grasslands (Batary et al. 2015).

National (and global) efforts at achieving food security have also driven the recent push to conserve grassland biodiversity (O'Mara 2012). Although 'food security' is often associated with the developing world (and especially, alleviating poverty and starvation), in Europe food security primarily relates to producing crops and livestock in a sustainable manner. That entails using natural resources more efficiently and minimizing the damage of agriculture (including livestock production) on the natural environment. Increasing the use of native grasslands is viewed as a means of reducing nitrogen (N) and phosphorus (P) inputs on grasslands and arable fields (Bol et al. 2011). This would help (1) reduce the runoff of fertilizers into waterways and native ecosystems (thereby reducing eutrophication) and (2) increase the adoption of low-input livestock production systems, which may become the norm in the future given predictions that inputs like N and P will become increasingly scarce and more expensive (Cordell et al. 2009). Godfray et al. (2010), Foley et al. (2011), and Schader et al. (2015) propose that returning to grassland-based livestock systems could help reduce the amount of crops fed to livestock which could instead be used for human consumption. Other studies have looked at the big picture and argue increasing grasslands in livestock production systems could reduce greenhouse gas (GHG) emissions by up to 20% (Dawson et al. 2011; Lemaire et al. 2015).

More recently, UK investment in building a national 'bioeconomy' has also renewed interest in grasslands, specifically for their potential role as a source of sustainable renewable energy and a source of new genes for crop improvement (Birch 2009; Burns 2016; DBIS 2015; Jenkins 2008;

McCormick 2014; Smolker 2008). The idea of the 'bioeconomy' developed in the early 21st century primarily in America quickly spread through Europe. It refers to an economy which uses and manipulates biological resources to meet the needs of society and to solve global problems such as food security and sustainable energy production (Cook 2006; EC 2006; Karp et al. 2015; McCormick and Kautto 2013). The bioeconomy emphasizes rural landscapes and rural development and using the natural resources found there to create jobs and the raw materials needed for innovation. In the UK, Defra's 2013 On Farm Anaerobic Digestion Fund allocated £3 million to assist farmers in setting up anaerobic digestion plants (Defra 2013). This fund (and accompanying policy documents) promoted the use of lignocellulosic bioenergy feedstocks such as *Miscanthus* and Short Rotation Coppice. Biomass from grasslands was also referred to as a potential future source of energy which required further research and several UK universities are now leading BBSRC projects on this (I. Donnison, *pers. comm*.). Other initiatives have provided financial support to research stations such as the National Institute of Agricultural Botany (NIAB), the Royal Botanic Gardens (Kew), and individual universities to initiate research on the use of plant genetic resources in forage, cereal, and bioenergy crop breeding. Many of these wild relatives are currently found on species-rich grasslands (Fielder et al. 2015; Maxted et al. 2007).

Despite increased interest in both grasslands and biodiversity in the UK, at the moment it is unclear whether these initiatives will be enough to stem the loss of species-rich grasslands to intensive arable cultivation. Although there are few studies on farmer reception of grassland biodiversity conservation policies in the UK, research among farmers in Europe suggests biodiversity policies are generally unpopular with farmers because they go against traditional ways of land use. For example, Toro-Mujica et al. (2016) found that even though Common Agricultural Policies (CAP) policies in Spain supported the maintenance of permanent pastures, farmers abandoned their traditional pastoral practices when these payments were de-coupled from payments given for sheep farming. Farmers

replaced these grasslands with improved pastures, orchards, and cereal fields and the area covered by native pastures decreased by up to 76% in some regions. In contrast, Jitea and Arion (2015) found that without CAP payments from agroenvironmental schemes, 30% of farms within the High Nature Value Areas (regions dominated by Alpine meadows) in Romania would be abandoned. Although Kirchner et al. (2016) have argued that recent changes to CAP policies (which require farmers to maintain pre-existing permanent pastures as of 2014) could lead to grassland expansion, they argue that this land will be 'set aside' and not actively incorporated into current agricultural systems. This would negate the potential benefit actively using species-rich grasslands might provide to reducing the environmental impact of agriculture (e.g. by providing a source of low-input fodder).

More research on the economic, agricultural, and social value of grassland biodiversity is needed to make grassland biodiversity conservation economically viable for farmers. Quantitatively, little is known about the effect of biodiversity on grassland nutritional composition, biomass composition, soil microbial community composition, and availability of plant genetic resources. This is particularly important in order to compare how well (or how poorly) species-rich grassland compare to other sources of livestock feed and bioenergy crops and the potential economic effects of losing these landscapes. Qualitatively, little is known about the value of grassland biodiversity to farmers and graziers, the response of these stakeholders to current grassland conservation policies in England, and how these policies might affect their willingness to remain in these agroenvironmental schemes. Understanding the challenges they face in managing grasslands under these policies is critical to making sure that conservation, agriculture, and economic growth are balanced. Research projects on the value of species-rich grasslands also need to make clear recommendations to the stakeholders who manage these landscapes: farmers, graziers, land owners and conservation groups. Together, this quantitative and qualitative data can ensure these 'new futures' for grasslands are (1) tangible and (2)

fit into pre-existing agricultural systems. The failure to incorporate both into agroenvironmental schemes may have limited the success of previous government initiatives and conservation policies.

4. Overview of Previous Research

4.1 Biodiversity and Forage Quality

Research on biodiversity and forage quality is rare. Although people have written about the nutritional value of particular plants for centuries, few studies have looked at the effect of biodiversity (species-mixtures) on forage quality. The majority of research has focused on how biodiversity can improve yield (Bullock et al. 2007; Duru et al. 2015; Tilman et al. 1996) and resilience to environmental change (Caldeira et al. 2005; Isbell et al. 2015; Sanderson et al. 2005; Vogel et al. 2012). A few studies specifically looking at the forage quality of forage from species-rich grasslands suggest that the nutritional value is low, in part, due to the presence of grasses with high lignin content (which is presumed to reduce palatability) and woody forbs (Balasko and Nelson 2003). However, Reiné et al.'s (2014) research on Alpine grasslands suggests that biodiversity positively impacts forage quality by raising dry matter and protein content. More research has been done on the plant secondary compounds found in forage from species-rich grasslands. For example, Buchin et al. (2009) have demonstrated that metabolites from the Asteraceae and Lamiaceae families have a positive impact on dairy and meat quality by raising fatty-acid levels and by affecting sensory qualities (e.g. aroma). However, few studies have compared the nutritional quality of forage from species-rich grasslands to commercial feeds, examined the value of grassland biodiversity to farmers, or identified the social and practical issues which might limit using species-rich grasslands under conservation for fodder production.

4.2 Bioenergy Production from Grassland Biomass

Studies assessing the suitability of grassland biomass for bioenergy production began in the early 21st century. Earlier bioenergy research focused on ethanol production from primary energy crops like maize and wheat. The recent realization that these sources of bioenergy are unsustainable and can have long-term negative ecological impacts has increased research into the use of excess biomass from lignocellulosic materials (e.g. crop waste, coppice, grass), from the search for new microbes which can break down lignin to the optimization of anaerobic digestion technology (Frigon and Giuiot 2010; Halford 2015; Prochnow et al. 2009). However, currently opinions on the bioenergy potential of grassland biomass are divided. For example, Russelle et al. (2007) and Steubing et al. (2010) suggest that the amount of ethanol and/or biogas from grasslands cannot compete with that of primary feedstocks like maize. In contrast, research by Tilman et al. (2006) on low input-high diversity grasslands in the United States and Fischer et al. (2009) on conservation grasslands in Germany suggest that grasslands can produce levels of bioenergy comparable to Miscanthus and Switchgrass. To date, few studies have compared biomass from species-rich grasslands and other feedstocks based on lignocellulosic composition and bioenergy output. In addition, few studies have estimated (for a given area or region) the amount of potential bioenergy that could be produced from species-rich grasslands and how this compares with land currently used to grow bioenergy crops.

4.3 Grassland Microbial Diversity and Agricultural Intensification

Few studies have examined how the conversion of species-rich grasslands to arable cultivation will affect microbial diversity, including whether specific species will be lost and whether diversity can be restored. Previous research has primarily focused on characterizing grassland soil microbial diversity. For example, Oehl et al. (2010) and Verbruggen et al. (2013) demonstrated that species-rich grasslands

are rich in arbuscular mycorrhizal fungi. Within arable systems, Aeron et al. (2011), Hartmann et al. (2014), Fox et al. (2007) and Kiers et al. (2003) have shown that crop choice, rotation, and increased nitrogen can negatively affect soil microbial diversity. However, Saia et al. (2015) has shown that increased soil nitrogen does not always lead to a decline in beneficial microbiota (Plant-Growth Promoting Rhizobia and arbuscular mycorrhizas) while Shafiani and Malik (2003) demonstrated that pesticide application on agricultural fields does not affect bacterial diversity. Further research on how agricultural intensification affects microbial diversity, and whether this can be reversed through arable reversion, would help inform how soil microbial diversity from species-rich grasslands can be managed and used.

4.4 Palaeoecology and Grassland Conservation

Traditionally, palaeoecology (the study of fossilized pollen, spore and plant remains) provided a descriptive study of how vegetation changed in a given area over time, and in the case of archaeobotany (the study of plant remains from archaeological sites), how particular communities used plants and natural resources at a specific point in time. More recently however, palaeoecologists have argued that long term ecological data can inform conservation. Willis et al. (2007, 2010) have shown that palaeoecology can highlight the biotic and abiotic factors driving changes in biodiversity over time while Jeffers et al. (2015) have argued that this data can also be used to monitor changes in ecosystem services. In the UK, Chambers et al. (2007) and Groves et al. (2012) have used palaeoecology to inform blanket bog mire and heathland restoration, respectively. To date, few studies have used palaeoecological data to inform species-rich grassland conservation. For example, research by Robinson (2011) in England and Berglund et al. (2008) and Lindbladh (1999) in Sweden suggest the strong role of humans in the creation of species-rich grasslands, yet few studies have examined whether grassland biodiversity is stable or changes over time. Additional research on temporal and

spatial changes in grassland biodiversity could contribute to the management of these landscapes and the natural resources they contain.

5. Objectives, Thesis Structure, and Scope

Based upon previous research and the overall aim to determine the agricultural potential of species-rich grasslands in the 21st century, this thesis has four main objectives:

1. To determine whether the nutritional composition of forage from species-rich grasslands is comparable to commercial livestock feeds

2. To assess the bioenergy output of biomass from species-rich grasslands compared to current bioenergy feedstocks

3. To determine the effect of land use change and agricultural intensification on microbial diversity

4. To determine whether grassland biodiversity is stable or changes (temporally and spatially) over time and identify the extent to which the plant genetic resources found there are dependent upon human management

The present research takes a multidisciplinary approach to the problem of how to balance grassland biodiversity with agricultural production. Chapter 2 combines ecology, forage analysis and ethnobotany to look at the effect of biodiversity on the nutritional composition of grassland biomass. Chapter 3 estimates the bioenergy output of biomass from species-rich grasslands based on lignocellulosic composition and land cover area (ha) and compares this output to that of current bioenergy feedstocks such as Miscanthus and crop waste. Chapter 4 uses soil metagenomics to assess the impact of agricultural intensification (converting grasslands to improved leys and arable crops) and arable reversion on soil microbial diversity. Chapter 5 combines palaeoecological data and modern vegetation survey data to look at the stability of grassland biodiversity over time and the extent to

which it is reliant on anthropogenic disturbance and to identify hotspots of long-term biodiversity for conservation.

The scope of this study is primarily on the agricultural value of grasslands in lowland England. The ecological, ethnobotanical, soil metagenomic and long term ecological research was conducted on sites in Oxfordshire, England. However, in all chapters this thesis draws upon examples in the UK and EU. In addition, the analysis confines itself two potential uses of species-rich grasslands, for forage and bioenergy production, although other uses are possible (e.g. solar farms). Finally, it confines the analysis of the potential ramifications of the loss of species-rich grasslands to microbial and vegetation diversity, and will not discuss other potential ramifications (e.g. loss of ecosystem services such as flood control).

References

Aeron, A. et al., 2011. Emerging role of plant Growth Promoting Rhizobacteria in agrobiology. In D. K. Maheshwari, ed. *Bacteria in Agrobiology: Crop Ecosystems*. Berlin: Springer-Verlag, pp. 1–36.

Balasko, J.A. & Nelson, C.J., 2003. Grasses for northern areas. In *Forages. An Introduction to Grassland Agriculture*. Iowa State Press, pp. 125–148.

Barnes, R.F. et al., 2003. Forages and grasslands in a changing world. In R. F. Barnes, ed. *Forages. An Introduction to Grassland Agriculture*. Iowa State Press, pp. 3–23.

Batary, P. et al., 2015. The role of agri-environment schemes in conservation and environmental management. *Conservation Biology*, 29(4), pp.1006–1016.

Birch, K., 2009. The knowledge–space dynamic in the UK bioeconomy. *Area*, 41(3), pp.273–284.

Bol, R., Dunn, R. & Pilgrim, E., Managing C and N in grassland systems: the adaptive cycle theory perspective. In G. Lemaire, J. Hodgson, & A. Chabbi, eds. *Grassland Productivity and Ecosystem Services*. Wallingford: CABI, pp. 73–82.

Boyd, D.A., 1949. Experiments with leys and permanent grass. *Grass and Forage Science*, 4(1), pp.1–10.

Bowles, D., Carson, A. & Isaac, P., 2014. Genetic distinctiveness of the Herdwick sheep breed and two other locally adapted hill breeds of the UK. *Plos One*, 9(1), p.e87823.

Buchin, S. et al., 1999. Influence of the composition of Alpine highland pasture on the chemical, rheological and sensory properties of cheese. *Journal of Dairy Research*, 66(04), pp. 579–588.

Bullock, J.M., Pywell, R.F. & Walker, K.J., 2007. Long-term enhancement of agricultural production by restoration of biodiversity. *Journal of Applied Ecology*, 44(1), pp.6–12.

Bullock, J. et al., 2011. Semi-natural grasslands. In UK National Ecosystem Assessment, ed. *UK National Ecosystem Assessment : technical report*. Cambridge: United Nations Environment Programme World Conservation Monitoring Centre, pp. 161–197.

Burns, C., Higson, A. & Hodgson, E., 2016. Five recommendations to kick-start bioeconomy innovation in the UK. *Biofuels, Bioproducts and Biorefining*, 10(1), pp.12–16.
Caldeira, M.C. et al., 2005. Species richness, temporal variability and resistance of biomass production in a Mediterranean grassland. *Oikos*, 110(1), pp.115–123.
Chambers, F.M. et al., 2007. Palaeoecology of degraded blanket mire in South Wales: Data to inform conservation management. *Biological Conservation*, 137(2), pp.197–209.
Comberti, C. et al., 2015. Ecosystem services or services to ecosystems? Valuing cultivation and reciprocal relationships between humans and ecosystems. *Global Environmental Change*, 34, pp.247–262.
Cordell, D., Drangert, J.-O. & White, S., 2009. The story of phosphorus: Global food security and food for thought. *Global Environmental Change-Human and Policy Dimensions*, 19(2), pp.292–305.
Costanza, R. et al., 1997. The value of the world's ecosystem services and natural capital. *Nature*, 387(6630), pp.253–260.
Davies, W., 1952. *Composition and value of ley farming: address to the Staffordshire Agricultural Valuers' Association, January 5th, 1952*, Grassland Research Station.
Department of Business, Innovation & Skills (DBIS), 2015. *Building a high value bioeconomy: Opportunities from Waste*, London: HM Government, Department of Business, Innovation and Skills.
Department of the Environment, Food and Rural Affairs (Defra), 2013. Loan fund to turn farm waste to energy.Webpage accessible at: https://www.gov.uk/government/news/loan-fund-to-turn-farm-waste-to-energy. Accessed: 05 May 2016.
Derreumaux, M., 2005. How to detect fodder and litter? A case study from the Roman site "Le Marais de Dourges," France. *Vegetation History and Archaeobotany*, 14(4), pp.373–385.
European Commission (EC), 2005. *New perspectives on the knowledge-based bio-economy: conference report*, Brussels: European Commission.
Fischer, G. et al., 2010. Biofuel production potentials in Europe: Sustainable use of cultivated land and pastures, Part II: Land use scenarios. *Biomass and Bioenergy*, 34(2), pp.173–187.
Foley, J.A. et al., 2011. Solutions for a cultivated planet. *Nature*, 478(7369), pp.337–342.
Fox, J.E. et al., 2007. Pesticides reduce symbiotic efficiency of nitrogen-fixing rhizobia and host plants. *Proceedings of the National Academy of Sciences*, 104(24), pp.10282–10287.
Frigon, J.-C. & Guiot, S.R., 2010. Biomethane production from starch and lignocellulosic crops: a comparative review. *Biofuels, Bioproducts and Biorefining*, 4(4), pp.447–458.
Gibson, D., 2009. *Grasses and Grassland Ecology*, Oxford: Oxford University Press.
Godfray, H.C.J. et al., 2010. Food Security: The Challenge of Feeding 9 Billion People. *Science*, 327(5967), pp.812–818.
Groves, J.A. et al., 2012. Long-term development of a cultural landscape: the origins and dynamics of lowland heathland in southern England. *Vegetation History and Archaeobotany*, 21(6), pp.453–470.
Halford, N.G., 2015. *An Introduction to Bioenergy*, Imperial College Press.
Hartmann, M., B. Frey, J. Mayer, P. Mäder, and F. Widmer. 2014. Distinct soil microbial diversity under long-term organic and conventional farming. *The ISME Journal* 2014: 1-18.
Hodgson, J.G. et al., 2005. The impacts of agricultural change (1963–2003) on the grassland flora of Central England: processes and prospects. *Basic and Applied Ecology*, 6(2), pp.107–118.
Hopkins, A., 2008. *Country Pasture/Forage Resource Profiles: United Kingdom*, Rome, Italy: FAO.
Humphreys, M.O., 2003. Utilization of plant genetic resources in breeding for sustainability. *Plant Genetic Resources*, 1(01), pp.11–18.
Jeffers, E.S., Nogué, S. & Willis, K.J., 2015. The role of palaeoecological records in assessing ecosystem services. *Quaternary Science Reviews*, 112, pp.17–32.

Jenkins, T., 2008. Toward a biobased economy: examples from the UK. *Biofuels, Bioproducts and Biorefining*, 2(2), pp.133–143.
Jitea, M.I. & Arion, F.H., 2015. The Role of Agri-Environment Schemes in Farm Economic Sustainability from High Natural Value Transylvanian Areas. *Environmental Engineering and Management Journal*, 14(4), pp.943–953.
Karp, A. et al., 2015. Growing innovations for the bioeconomy. *Nature Plants*, 1(12), p.15193.
Kemp, D. & Michalk, D., 2011. Livestock production styles and managing grassland ecosystems. In G. Lamaire, J. Hodgson, & A. Chabbi, eds. *Grassland Productivity and Ecosystem Services*. Wallingford: CABI, pp. 53–64.
Kiers, E.T., West, S.A. & Denison, R.F., 2002. Mediating mutualisms: Farm management practices and evolutionary changes in symbiont co-operation. *Journal of Applied Ecology*, 39(5), pp.745–754.
Kirchner, M., Schönhart, M. & Schmid, E., 2016. Spatial impacts of the CAP post-2013 and climate change scenarios on agricultural intensification and environment in Austria. *Ecological Economics*, 123, pp.35–56.
Kleijn, D. et al., 2001. Agri-environment schemes do not effectively protect biodiversity in Dutch agricultural landscapes. *Nature*, 413(6857), pp.723–725.
Lemaire, G. et al., 2014. Integrated crop-livestock systems: Strategies to achieve synergy between agricultural production and environmental quality. *Agriculture Ecosystems & Environment*, 190, pp.4–8.
Lemaire, G. et al., 2015. Grassland–cropping rotations: An avenue for agricultural diversification to reconcile high production with environmental quality. *Environmental Management*, 56(5), pp.1065–1077.
Lindborg, R. et al., 2008. A landscape perspective on conservation of semi-natural grasslands. *Agriculture, Ecosystems & Environment*, 125(1–4), pp.213–222.
Mace, G.M., Norris, K. & Fitter, A.H., 2012. Biodiversity and ecosystem services: a multilayered relationship. *Trends in Ecology & Evolution*, 27(1), pp.19–26.
Martins, K.T., Gonzalez, A. & Lechowicz, M.J., 2015. Pollination services are mediated by bee functional diversity and landscape context. *Agriculture Ecosystems & Environment*, 200, pp.12–20.
Millennium Ecosystem Assessment, 2003. *Ecosystems and Human Well-being: A Framework for Assessment*, Island Press.
Millennium Ecosystem Assessment, 2005. *Ecosystems And Human Well-Being : Synthesis : A Report For The Millennium Ecosystem Assessment*, Washington, D.C.: Island Press.
Oehl, F. et al., 2010. Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. *Soil Biology & Biochemistry* , 42, pp.724–738
Oglethorpe, D.R. & Sanderson, R., 1998. Farm characteristics and the vegetative diversity of grasslands in the North of England: a policy perspective. *Biodiversity & Conservation*, 7(10), pp.1333–1347.
O'Mara, F.P., 2012. The role of grasslands in food security and climate change. *Annals of Botany*, 110(6), pp.1263–1270.
Organization for European Economic Co-operation, 1954. *Pasture and fodder production in north-west Europe*, Paris: Organization for European Economic Co-operation.
Porter, K. & Leach, S.J., 2013. Botanical records and their role in shaping nature conservation priorities and actions – an English perspective. *New Journal of Botany*, 3(3), p.148.
Posthumus, H. et al., 2010. A framework for the assessment of ecosystem goods and services; a case study on lowland floodplains in England. *Ecological Economics*, 69(7), pp.1510–1523.
Preston, S., 1887. *Pasture grasses and forage plants, and their seeds, weeds, and parasites*, London.
Prochnow, A. et al., 2009. Bioenergy from permanent grassland – A review: 1. Biogas. *Bioresource*

Technology, 100(21), pp.4931–4944.
Pykälä, J. et al., 2005. Plant species richness and persistence of rare plants in abandoned semi-natural grasslands in northern Europe. *Basic and Applied Ecology*, 6(1), pp.25–33.
Reiné, R. et al., 2014. Pyrenean meadows in Natura 2000 network: grass production and plant biodiversity conservation. *Spanish Journal of Agricultural Research*, 12(1), pp.61–77.
Robinson, M. 2011. "The paleoecology of alluvial hay meadows in the Upper Thames valley." *Fritillary* 5: 47-57.
Russelle, M.P. et al., 2007. Comment on "Carbon-negative biofuels from low-input high-diversity grassland biomass". *Science (New York, N.Y.)*, 316(5831), p.1567; author reply 1567.
Saia, S. et al., 2015. Soil inoculation with symbiotic microorganisms promotes plant growth and nutrient transporter genes expression in durum wheat. *Frontiers in Plant Science*, 6.
Sanderson, M.A. et al., 2005. Forage mixture productivity and botanical composition in pastures grazed by dairy cattle. *Agronomy Journal*, 97(5), p.1465.
Schader, C. et al., 2015. Impacts of feeding less food-competing feedstuffs to livestock on global food system sustainability. *Journal of the Royal Society Interface*, 12(113), p.20150891.
Silvertown, J., 2015. Have ecosystem services been oversold? *Trends in Ecology & Evolution*, 30(11), pp.641–648.
Smolker, R., 2008. The new bioeconomy and the future of agriculture. *Development*, 51(4), pp.519–526.
Spash, C.L. & Simpson, I.A., 1993. Protecting Sites of Special Scientific Interest: Intrinsic and utilitarian values. *Journal of Environmental Management*, 39(3), pp.213–227.
Steubing, B. et al., 2010. Bioenergy in Switzerland: Assessing the domestic sustainable biomass potential. *Renewable and Sustainable Energy Reviews*, 14(8), pp.2256–2265.
Sutton, M.J. 1888. *Permanent and temporary pastures*, London: Hamilton.
Tallowin, J.R.B. et al., 2005. Spatial and structural uniformity of lowland agricultural grassland in England: a context for low biodiversity. *Grass and Forage Science*, 60(3), pp.225–236.
Tereso, J.P. et al., 2013. Crops and fodder: evidence for storage and processing activities in a functional area at the Roman settlement of Monte Mozinho (northern Portugal). *Vegetation History and Archaeobotany*, 22(6), pp.479–492.
Tilman, D., Wedin, D. & Knops, J., 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature*, 379(6567), pp.718–720.
Tilman, D., Hill, J. & Lehman, C., 2006. Carbon-negative biofuels from low-input high-diversity grassland biomass. *Science*, 314(5805), pp.1598–1600.
Toro-Mujica, P.M. et al., 2015. Changes in the pastoral sheep systems of semi-arid Mediterranean areas: association with common agricultural policy reform and implications for sustainability. *Spanish Journal of Agricultural Research*, 13(2), p.e0102.
Young, J. et al., 2005. Towards sustainable land use: identifying and managing the conflicts between human activities and biodiversity conservation in Europe. *Biodiversity & Conservation*, 14(7), pp.1641–1661.
Watkins, F. et al., 2012. Antimicrobial assays of three native British plants used in Anglo-Saxon medicine for wound healing formulations in 10th century England. *Journal of Ethnopharmacology*, 144(2), pp.408–415.
Willis, K.J., Gillson, L. & Knapp, S., 2007. Biodiversity hotspots through time: an introduction. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 362(1478), pp.169–174.
Willis, K.J. et al., 2010. Biodiversity baselines, thresholds and resilience: testing predictions and assumptions using palaeoecological data. *Trends in Ecology & Evolution*, 25(10), pp.583–591.
Wrage, N. et al., 2011. Phytodiversity of temperate permanent grasslands: ecosystem services for

agriculture and livestock management for diversity conservation. *Biodiversity and Conservation*, 20(14), pp.3317–3339.

Vogel, A., Scherer-Lorenzen, M. & Weigelt, A., 2012. Grassland resistance and resilience after drought depends on management intensity and species richness. *PLOS ONE*, 7(5), p.e36992.

CHAPTER 2

BIODIVERSITY ENHANCES FORAGE QUALITY AND SUSTAINABILITY OF PASTORAL FARMING IN LOWLAND ENGLAND

Abstract

Grassland biodiversity contributes to the provision of ecosystem services but it may also promote livestock health and the creation of more sustainable livestock production systems. Current commercial livestock production relies on intensively managed low-diversity leys and/or commercial feeds such as cereals, soy, and rapeseed meal. In contrast, species-rich grasslands contain a variety of grasses, forbs and legumes. However, few studies have characterized the nutritional profile of species-rich grasslands, how these forages compare nutritionally to commercial feeds, and the feasibility of producing forage from grasslands under current conservation and agro-environmental schemes. This study conducted ecological and ethnobotanical research at a series of species-rich grasslands in Oxfordshire, England and demonstrates that forage from species-rich grasslands is comparable in macronutrient content to commercial feeds and contains plants with established antibiotic and anthelmintic properties which may affect livestock health. It also suggests that these fields could be increasingly used for agriculture (as opposed to solely for conservation) but this will rely on increasing the flexibility of conservation guidelines around their management. This contradicts previous studies which claim that the forage quality of species-rich grasslands is too low or which promote conserving grassland biodiversity by restricting agricultural use of these fields. Increasing the agricultural use of species-rich grasslands could lower the environmental impact of livestock production, reduce reliance on commercial antibiotics and anthelmintics, and ultimately, help conserve grassland biodiversity through active use. Although based on research in the UK, these results provide quantitative and qualitative support for the potential benefits of increasing the use of native grasslands in global livestock production systems.

1. Introduction

Grassland biodiversity contributes to the provision of ecosystem services but it may also promote livestock health and the creation of more sustainable livestock production systems. In Europe, farmers historically raised livestock on native, semi-natural grasslands (e.g. pastures, common lands, and meadows). Today however, intensive livestock production systems rely on agriculturally improved leys and commercial crops such as cereals, rapeseed meal and soy to produce more meat and milk in less time (Dawson et al. 2011; Knaus 2013; Naylor et al. 2005). These shifts in livestock production began in the 1950s and have intensified with the rise of global meat consumption (Hodgson 2005a, 2005b; Ridding 2015; Peeters 2009; Sala et al. 2000). These changes in livestock rearing practices

have had significant widespread environmental and socio-economic ramifications, including increased competition between land used to feed humans and livestock (Foley et al. 2011; Godfray et al. 2010; O'Mara 2012; Noor 2014; Taube et al. 2014), declined botanical and zoological diversity due to increased homogenization of the landscape and use of inputs (O'Mara 2012; Robinson and Sutherland 2002; Tscharntke et al. 2013), and increased global livestock antibiotic and anthelmintic resistance due to the overuse of these pharmaceuticals to maintain livestock health and increase productivity (Brooker and Acamovic 2005; Gallagher 2015; Makkar et al. 2009).

Increasing the use of semi-natural, species-rich grasslands for grazing and hay-making could help decrease the potential negative effects of livestock production on the environment while promoting the conservation of grassland diversity. However, at the moment very little is known about their nutritional value and the feasibility of producing forage from grasslands under current agro-environmental schemes. A few studies indicate that biodiversity contributes to the nutritional quality of forage by increasing dry matter content and protein content (Hammond et al. 2014; Hofmann and Isselstein 2005). In contrast, other studies suggest that the forage from species-rich grasslands is deficient in macro and micro nutrients and is thus inadequate for commercial livestock production (Tallowin and Jefferson 2008). However, the results of these studies are often based upon grass taken from experimental plots in agricultural research stations where the number of species used to represent species-rich grasslands rarely reflects the diversity found in real grassland ecosystems. In addition, few studies have compared the nutritional value of forage from species-rich grasslands to commercial feeds currently used to feed livestock. Finally, there is little qualitative research on the agricultural value of this biodiversity to farmers and the viability of using species-rich grasslands for forage production.

In order to establish the effect of biodiversity on forage quality and to determine whether species-rich grasslands can provide a sustainable alternative to commercial livestock feeds, I conducted ecological

and ethnobotanical research at a series of farms in Oxfordshire, England. This region was selected due to the number of species-rich grasslands in the county under active farm management as well as high levels of local interest in the agricultural use and conservation management of these landscapes. I conducted vegetation surveys, forage analysis, stakeholder interviews and participant observation to determine the effect of species-richness and particular plants on forage quality. I then compared the macro and micronutrient composition of species rich-grass to that of commercially available feedstocks. Finally, I used stakeholder interviews to identify the value of grassland biodiversity to farmers and what barriers limit the incorporation of these grasslands into current livestock production systems. The goal of this research is to inform agricultural and conservation policies regarding the active use of species-rich grasslands for forage production.

2. Background

2.1 Geography and Local Land Use

The county of Oxfordshire is located in south-east England. The county has a maritime temperate climate with an annual rainfall of between 570-750 mm (depending on elevation) (Killick et al. 1998). The county is divided into four 'National Character Areas' which represent distinct regions marked by particular geographical features (e.g. elevation, soils, precipitation): the Cotswolds, the Upper Thames Clay Vales, the Midvale Ridge, and the Chilterns.[1] The Cotswolds and Chilterns are characterized by, higher rainfall and elevation, sedimentary and chalk soils (respectively), and grade 1 and 2 agricultural

[1] The National Character Areas are a system of land division in England to designate "areas that share similar landscape characteristics, and which follow natural lines in the landscape rather than administrative boundaries making them a good decision-making framework for the natural environment" (Natural England 2013).

land.[2] In the central part of the county the Upper Thames Clay Vales and the Midvale Ridge are characterized by lower elevation and rainfall, clay and alluvial soils, and grade 3 and 4 agricultural land. On grade 1 and 2 land, farmers grow wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), and rapeseed (*Brassica napus* L.) for both human and livestock consumption. The county is covered by an area of 54% grassland. [3]

2.2 Grassland types

2.2.1 Improved leys

The majority of grasslands in the county are improved leys where fertilizers (inorganic and organic) and commercially available, legume-based seed mixtures are deliberately sown (e.g. ryegrass (*Lolium perenne* L.), red clover (*Trifolium pratense* L.), and white clover (*Trifolium repens* L.). These fields are ploughed and re-sown annually or biannually depending on the yield of the first season and if a rotation system is used. Farmers apply inorganic Nitrogen (N), Phosphorus (P) and Potassium (K) to these fields, and to a lesser extent, manure or slurry. Improved grasslands correspond to MG7 of the National Vegetation Classification (NVC) system.[4]

[2] In the UK, land is graded on a scale of 1-5 with 1 being the 'best.'

[3] The total area of grassland (not including arable fields) was calculated by the arable data provided for each NCA by Natural England. The total area of grassland varies by NCA, with the Upper Thames Clay Vales having the highest value (61%) and the Chilterns having the lowest (41%). From this data, the amount of grassland actually used for grazing is unknown.

[4] This system is widely used in Britain and divides the landscape up into 286 vegetation communities based on species composition and phyto-social associations. Although NVC numbers will not be referred to in the text, they are referred to here for those familiar with British grassland ecology (Cooper 1998; Rodwell 1992, 1995).

2.2.2 Species-rich grasslands

Species-rich grasslands can be divided into two categories: 'unimproved grasslands' which have not been agriculturally improved or cultivated as arable land for several hundred years and 'conservation grasslands' where fields formerly used for arable cultivation are re-sown with species-rich seed mixtures (either from commercial seed companies or harvested from nearby unimproved meadows) in an effort to restore/recreate unimproved grasslands. The three major semi-natural grassland types in the county are neutral lowland grassland (NVC5, MG1-5, 8, 11, 13), floodplain meadows (NVC M22-27), and calcareous grassland (NVC CG1-10) and are protected under the UK Biodiversity Action Plan (BAP) (Maddock 2011; Defra 2011). These grasslands contain a diverse array of grasses, forbs, legumes, and nationally rare plants protected under the UK Biodiversity Action Plan (e.g. creeping marshwort (*Apium repens* L.), wild celery (*Apium graveolens* L.), and birthwort (*Aristolochia clematitis* L.)) (F. Watkins and C. Lambrick, pers. comm). In this paper, 'species-rich grasslands' is used to refer to both unimproved and conservation grasslands due to the high levels of vegetation diversity found on these sites. **Table 1** provides an overview of the key differences in management and use between the three site categories.

Table 1 Main characteristics distinguishing unimproved, conservation, and improved grasslands. Grassland types are separated based on whether the vegetation is 'natural' (unsown) or man-made ('sown'), the source of seeds (if applicable), grassland use, whether inputs are applied, whether grasslands are permanent or rotated, and whether they are grazed (G), mown (M), mown and grazed (MG) or cut without grazing.

Description	Unimproved grassland	Conservation grassland	Improved grassland
Vegetation	Unsown	Sown	Sown
Seed type	n/a	Commercial, local grasslands	Commercial
Use	Agricultural	Conservation	Agricultural
Application of N, P, K	No	No	Yes
Permanent/Rotation	Permanent	Permanent	Rotation
Grazed, Mown, or Cut	G, M, MG	G, MG	Cut

Although the management of species-rich grasslands varies among farms, some generalizations can be made. Overall, farmers do not add anything to these fields, other than manure from livestock which is naturally deposited during grazing. Neutral grasslands and floodplain meadows tend to be high in soil fertility due to manure from livestock, the high frequency of legumes in the grassland swards, and the deposition of nutrient-rich alluvial soils during winter and spring floods. Cows and/or sheep graze pastures and farmers cut meadows for hay between mid-June and early August and livestock subsequently graze these fields. National biodiversity regulations created by Natural England regulate the timing of many of these activities and are reinforced by annual checks. Grasslands designated as Sites of Special Scientific Interest (SSSIs) or managed under Entry or Higher Level Stewardship (ELS and HLS) schemes must follow these guidelines. Entry and Higher Level Landscape Stewardship schemes pay farmer to maintain and restore species-rich grassland. **Table 2** presents a few of these schemes.

Table 2HLS schemes relating to species-rich grassland management. The higher payment for creating new-species rich grasslands is one of the prime drivers of species-rich grassland expansion in the region. Data source: Natural England 2012.

Scheme	Payment (£ per ha)
Maintenance/restoration of species-rich grassland	200
Creation of species-rich grassland	280
Maintaining/restoring traditional water meadows	350
Haymaking supplement	75
Grazing cattle	up to 35
Grazing native breeds at risk	up to 70

Natural England's *Lowland Grassland Management Handbook* (Second edition) (2012) outlines national policies on the timing of hay meadow cutting, the use of fire to control vegetation, and the application of inputs on grasslands. Guidelines are presented as flexible in the handbook, although the conservation of botanical and avian diversity is given priority. For example, in regards to hay cutting,

the handbook states that guidelines aim to 'maintain a flexible approach' but state that the date of cut should 'ensure cutting does not take place before breeding birds have hatched or populations of 'desirable' plants, particuarly those which depend on seed production for regeneration have set seed (e.g. yellow rattle) ' (Natural England 2012: Chapter 6, p.12). Grasslands classified as SSSIs are under stricter guidelines and all management practices must be approved by Natural England (Natural England 2012: Chpater 6, p. 22). Failure to manage grasslands under these guidelines can lead to fines (which can reach up to £20,000 for farmers with SSSIs) (Natural England 2012).

3. Methods

3.1 Selection of Study Sites and Participants

Research was conducted at 30 grassland sites across Oxfordshire. I selected study sites from published lists of Sites of Special Scientific Interest (SSSIs), commonlands (land designated as communal grazing areas based on historical precedent) (Aitchison *et al.* 2000), and National Nature Reserves (NNRs) in Oxfordshire. Three criteria were used for selection: (1) geographic location; (2) grassland management type; and (3) willingness of land managers (farmers and/or graziers) to participate in the study. **Appendix Table 1** provides the following information for each of the study sites: site name, location, management type, and type of livestock (if applicable) and **Figure 1** shows their locations.

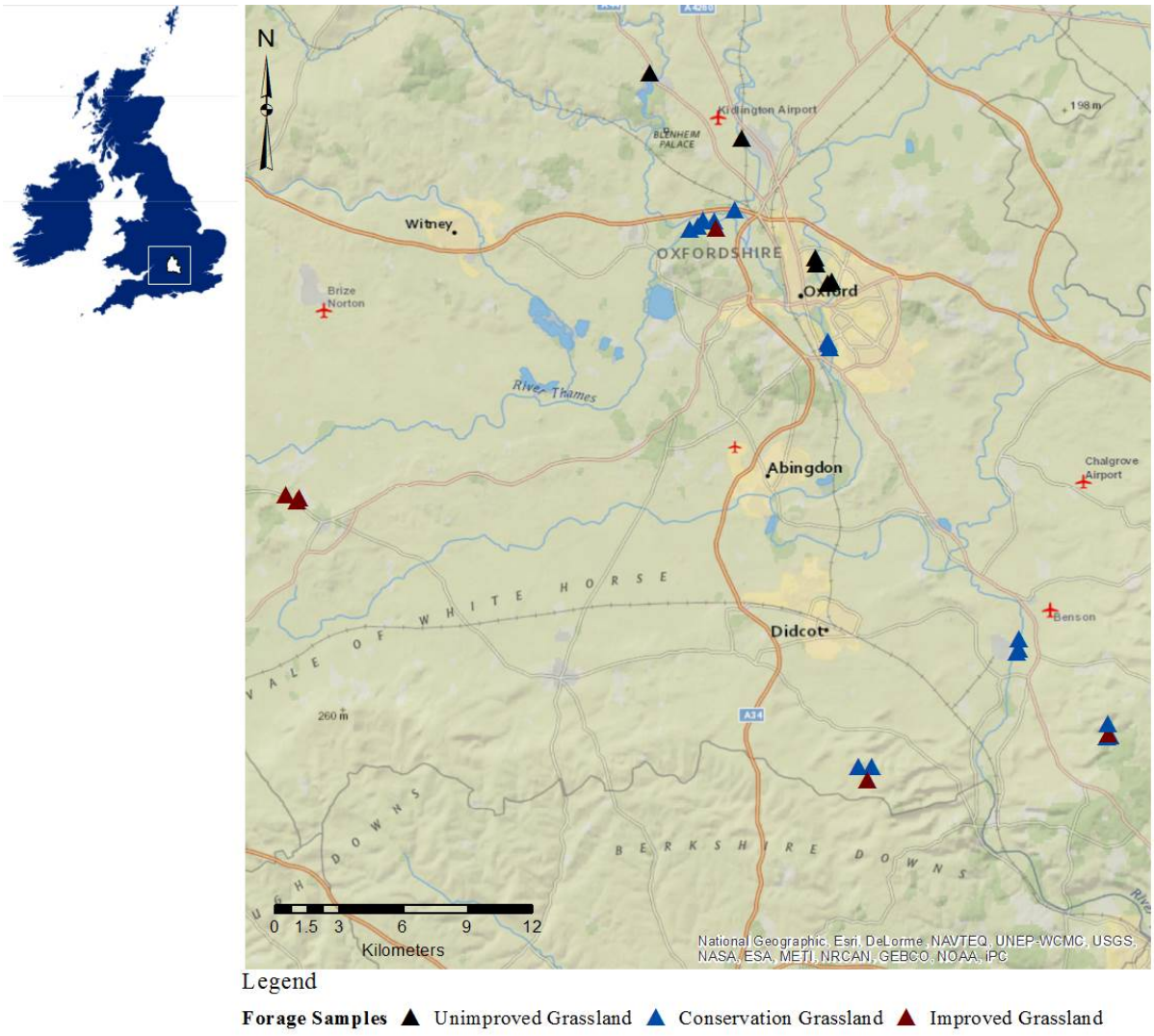


Figure 1 Location of grassland sites involved in the study. Forage sampling was conducted at a subset of these locations.

3.2 Vegetation Surveys

From June-July 2015, I conducted vegetation surveys at each site to determine the species-composition and richness for each field. Care was taken to avoid the edges of sites (which can reflect 'disturbed' vegetation as opposed to the vegetation character of the main field from which the soil samples were taken) by excluding a 1 m margin around each field. To determine species composition, five $1m^2$

quadrats were randomly placed within each field and the species present in each quadrat were recorded. Abundance was determined as the number of quadrats each species occurred in. Quadrats were not selected beforehand because the specific field sampled was based on the farmer's decision on the day of the site visit. The presence of grazing livestock, fertilizer application, specific conservation regulations, and farmer interest in the forage quality of specific fields influenced farmer choice. Plants were identified using Fitter et al. (1984) and Rose (1989).

3.3 Forage Sample Collection and Analysis

At a limited number of sites (13 in total), I collected forage samples of ca. 150 grams. Initially, the research design was balanced and sought to collect samples from five unimproved, five conservation and five improved grasslands due to lack of funding to sample all study sites. However, during fieldwork it became clear that three sites initially identified as conservation grasslands were unimproved and three sites identified as improved had recently undergone arable reversion. In addition, on visiting two of the conservation grassland study sites, volunteers had cut the hay before the site was visited, excluding these sites from the analysis.

I collected forage samples following the guidelines provided by the Agri-Food and Biosciences Institute (AFBI) (Belfast, Northern Ireland) (http://www.afbini.gov.uk/). I sampled grasslands at the same time (10 am) during each site visit to ensure comparability between samples (e.g. because levels of sugar can rise throughout the morning). Handfuls of grass were cut ca. 10 cm from the ground with shears at ca. 20 different locations within each field to capture the overall forage quality of each field. To ensure an accurate representation of the forage quality of the field, all species collected in the process of sampling were included in the sample (e.g. if nettle or meadowsweet was cut alongside grass it was not removed) because the quality of pasturage and hay is based on this complex botanical

composition. During collection, grass was placed in a canvas bag to prevent sweating (and the start of any 'ensiling' process, which can change the nutrient content of the sample).

At the end of each day of fieldwork, I sent the forage samples to the Agri-Food and Biosciences Institute agricultural research center in Hillsborough for wet chemistry and mineral analysis. To ensure the nutrient content of the hay was captured, samples were mailed on the same day of collection. Samples were then oven dried at 60^{o}C and milled. Wet chemistry was used to establish Dry Matter (DM) content, sugar content (WSC), fiber (NDF, ADF), protein (CP) and lignin (ADL) content of each sample. Mineral analysis was used to determine Phosphorus (P), Magnesium (Mg), Calcium (Ca), Sodium (Na), Potassium (K), Chlorine (Cl), Manganese (Mn), Copper (Cu), Zinc (Zn), Selenium (Se), Cobalt (Co), and Iodine (I) levels. Mineral analysis was also used to determine the levels of antagonists (Iron (Fe), Aluminum (Al), Molybdenum (Mo), Sulphur (S), and Lead (Pb)) in the samples. Antagonists can have a negative effect on livestock health in large quantities by blocking the absorption of minerals (Gupta and Gupta 1998; López-Alonso 2012).

Table 3 Key macro and micro minerals important to livestock health. Medium and large-scale farmers producing meat and milk products for commercial industries primarily focus on the levels of P, Ca, Mg and Na in livestock feed in order to optimize livestock growth and development. Smaller-scale farmers do not usually pay attention to the mineral content of livestock feeds or perform forage analysis on the feeds they use. Data source: Barnes et al. 2003.

Category	Element	Abbreviation	Importance for livestock health
Macronutrients	Calcium	Ca	Growth
	Phosphorus	P	Growth and milk production
	Magnesium	Mg	Hypomagnesium
Micronutrients	Manganese	Mn	Growth, reproduction and birth
	Chlorine	Cl	Vigor, eye sight, respiratory
	Copper	Cu	Immune function
	Zinc	Zn	Growth, anti-perakaratosis
	Nickel	Ni	Growth
	Sodium	Na	Diet, coat quality
	Iodine	I	Thyroid functioning
	Cobalt	Co	Muscle tone and weight gain
	Selenium	Se	Prevents white muscle disease

3.4 Collection of Data on Commercial Livestock Feedstocks

I collected data on 30 conventional feedstocks from *Feedapedia*. INRA (Institut National de la Recherche Agronomique, French National Institute for Agricultural Research), CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement, French Agricultural Research Center for International Development, AFZ (Association Française de Zootechnie, French Association for Animal Production) and the FAO (Food and Agriculture Organization of the United Nations) run this database and provide frequently-updated data on over a hundred plants used to feed livestock around the world (http://www.feedipedia.org/). I collected data on five categories of feedstocks: cereals (wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.) and maize (*Zea mays* L.); n=12); monoculture hay (Timothy (*Phleum pratense* L.), orchard grass (*Dactylis glomerata* L.); Triticale; and haylage; n = 4); legumes (pea (*Pisum sativum* L.), Lucerne

(*Medicago sativa* L.), red clover (*Trifolium pratense* L.), bird's foot trefoil (*Lotus corniculatus* L; n = 4); and soybean (*Glycine max* (L.) Merr.); n = 9); oilseed products (rapeseed (*Brassica napus* L.) and flaxseed (*Linum usitatissimum* L.) meal; n = 4), and sugar beet byproducts (*Beta vulgaris* L. tops and roots; n = 4). I selected these feeds because these are the main commercial crops used to feed livestock in the UK (and Europe more broadly).

3.5 Ethnographic Work and Stakeholder Interviews

To understand the value of species-rich grasslands to local stakeholders, I interviewed farmers, graziers, and landowners who owned and/or managed species-rich grasslands. Before fieldwork began, all participants were given a short survey to complete in their own time (**Appendix II**). The survey covered history of land use, number and type of livestock, and grassland management practices. To gather additional information on the management and use of each site, I also interviewed study participants during field visits. During these visits, farmer(s) and/or grazier (s) showed me the different grasslands under their management and I accompanied them while they were out doing their daily work. Although much of this information was gleaned from the written surveys, in many cases farmers and graziers provided additional information during site visits. To ensure the protection of participants' identity, intellectual material, and privacy, the project was evaluated and cleared by the University of Oxford Central University Research Ethics Committee [CUREC Ref No: SSD/CUREC1A/15-023].

3.6 Statistical Analysis

To determine whether there were significant differences in species richness and forage quality among unimproved, conservation and improved grasslands, I used One-way Analysis of Variance (ANOVA).

To establish the effect of plant species composition on forage quality, I used indirect gradient analysis using Redundancy Analysis (RDA) followed by a Monte Carlo permutation test with 499 permutations on log-transformed data. In the figures samples were coded by management type (see **Appendix I**). I created Generalized Additive Models (GAMs) from this data to determine the effect of botanical diversity (using the Shannon Wiener Index) on macronutrient composition (Yee and Mitchell 1991). These models were also used to determine the relationship between the abundance of particular species and particular macronutrients. To compare the overall nutritional composition of species-rich grass to other feedstocks, I performed Correspondence Analysis on log-transformed data and used ANOVA to determine whether there were significant differences in specific macro and micronutrients among the different feed types. In all analyses, I performed ANOVA in R (version 3.2.2, "Fire Safety") and RDA and CA in Canoco (version 4.5, Lepš and Šmilauer 2003).

4. Results

4.1 Overview of Stakeholders and Sites

I interviewed a total of 33 stakeholders regarding 30 different grassland sites throughout the county. The majority of respondents were male (58%) and over the age of 50 (61%). They were divided into arable farmers (46%), graziers (37%), dairy farmers (14%), and landowners who managed grasslands but were not farmers (3%). 40% of farms had unimproved and improved grasslands, 33% had unimproved grasslands, and 27% managed conservation and improved grasslands. 47% of the grasslands were SSSIs and 43% of all sites in the study were managed under HLS schemes. Among the study participants, 43% raised cattle, 37% raised sheep, 17% raised cattle and sheep, and 3% did not own livestock. Local farmers raised ‘traditional’ cattle breeds, including Ayrshire, Dexter, and

Hereford. One farm raised Salers cattle, a French breed well-suited to wetland grazing.[5] The sheep breeds raised in the region were Coopworth, Welsh Mountain, West County Mules, and Bellulah Speckled Face.

In terms of general variability in sward composition among grasslands, I identified a total of 54 taxa across all sites. *Dactylis glomerata* L., *Lolium perenne*, *Poa annua* L., *Poa pratensis* L., *Rumex acetosa* L., *Trifolium pratense* L., and *Trifolium repens* L. were ubiquitous. Unimproved grasslands were characterized by a wide range of grasses, forbs and legumes, 59% of which were not found on the other site types (**Figure 2**). Conservation grasslands contained 37% of the species found on unimproved grasslands. Improved grasslands were dominated by ryegrass and clover and contained several species not found on unimproved or conservation grasslands (e.g. lucerne (*Medicago sativa* L.)). Species richness varied significantly among unimproved, conservation and improved grasslands ($F_{2, 27} = 13.27$, $P < 0.001$). Unimproved grasslands had the highest average species richness (17 ± 1.574 species). The species richness of conservation grasslands was slightly lower (12.5 ± 2.611 species) but did not vary significantly from that of unimproved grasslands ($t = -1.724$, $P = 0.115$). In contrast, the species richness of improved grasslands was on average three times lower than that of unimproved grasslands (4.5 ± 3.340 species).

[5] Commercial dairy and beef cattle breeds (e.g. Frisian, Holstein, Belgian Blue) also grazed two of the SSSIs in the study area (which were common lands) but were owned by commercial livestock breeders who were not involved in the study.

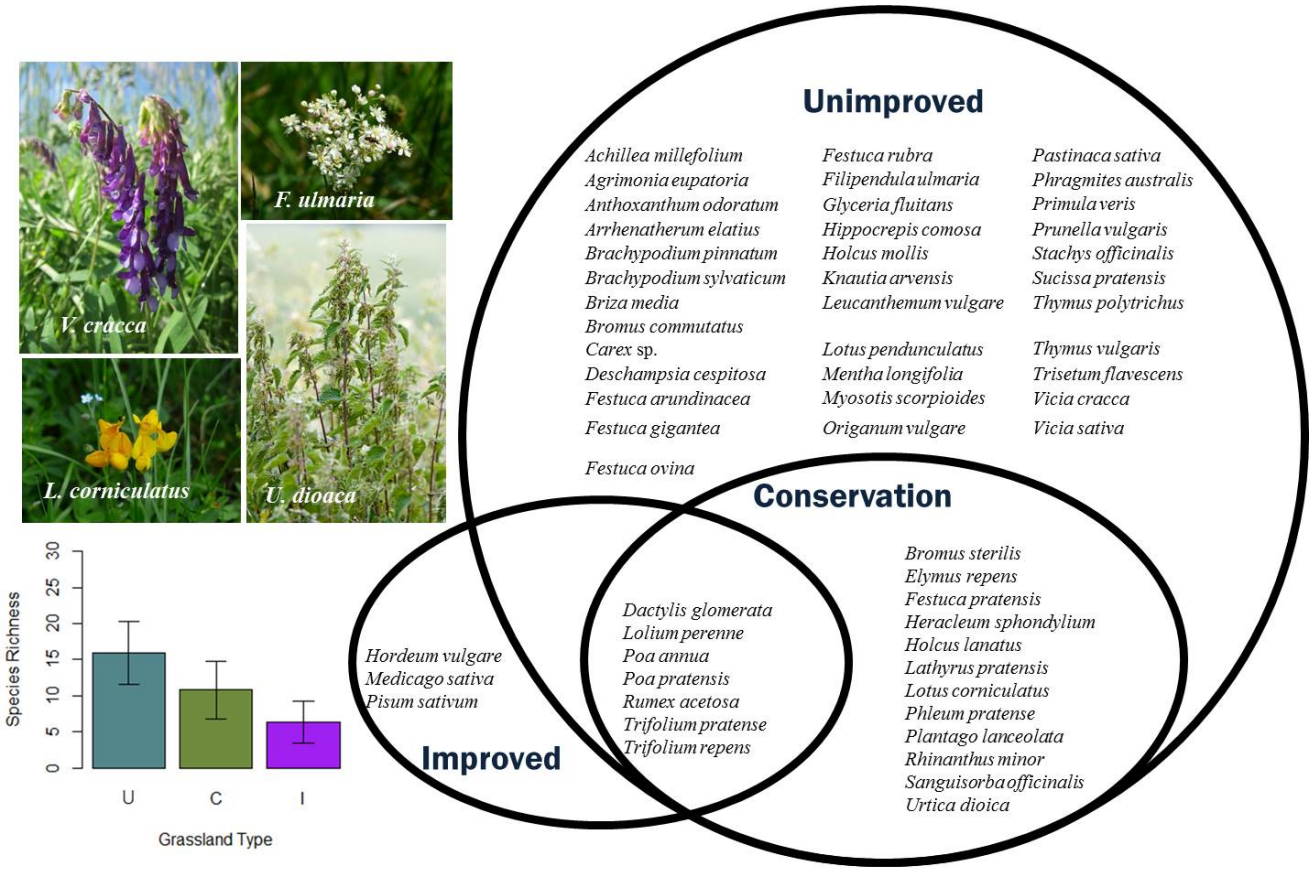


Figure 2 Venn diagram showing the botanical composition of unimproved, conservation and improved grassland sites in Oxfordshire. Unimproved grasslands contained 94% of the taxa found on all sites. Conservation grasslands had many of the plant species found on unimproved grasslands while only a handful of highly productive grasses and legumes were found on improved grasslands. Authorities for each Latin name are left out for clarity. In the bar chart, U = unimproved grasslands, C = conservation grasslands, and I = improved grasslands. Bars show mean ± one standard error.

4.2 Variation in Forage Quality among Oxfordshire Grasslands

Table 4 compares the nutrient composition of forage among grassland sites (results for each sample are in **Appendix I Table 2**). There was no difference in DM content among grassland types ($F_{2, 10}$ = 0.10, P = 0.91) but ADF ($F_{2, 10}$ =5.512, P = 0.024), NDF ($F_{2, 10}$ = 5.79, P = 0.021), and protein ($F_{2, 10}$ = 15.01, P < 0.001) content varied significantly among grasslands (**Appendix I Figure 1**). The ADF content of forage between unimproved grasslands and conservation grasslands was highly similar (t = 0.153, P = 0.881) while that of the improved grasslands was significantly lower (t = -3.136, P = 0.011).

The NDF content of unimproved and conservation grasslands was also similar (t = 0.525, P = 0.611) while improved grasslands had a significantly lower NDF content compared to unimproved grasslands (t = -3.074, P = 0.012). The protein content of forage from improved grasslands was twice as high as that from unimproved grasslands. Although lower than improved grasslands, the protein content of unimproved and conservation grasslands was highly similar (t = 0.818, P = 0.433). Sugar content varied marginally among grassland types ($F_{2, 10}$ = 3.698, P = 0.063) and forage from conservation grasslands had the highest sugar-content out of all the samples. There was no difference in lignin content among the samples ($F_{2, 10}$ = 2.408, P = 0.14).

Table 4 Comparison of the nutrient composition of forage from unimproved, conservation and improved grasslands. Forage from unimproved and conservation grasslands were highly similar and were high in sugar and Phosphorus. Forage from improved grasslands was high in protein and iodine.

	Unimproved Grassland	Conservation Grassland	Improved Grassland
DM (% of wet weight)	33.64 ± 1.8	33.45 ± 2.99	19.45 ± 3.82
Protein (% DM)	11.553 ± 0.839	10.418 ± 1.392	20.385 ± 1.780
Sugar (% DM)	9.126 ± 0.795	10.388 ± 1.318	5.47 ± 1.686
ADF (% DM)	34.46 ± 1.006	34.205 ± 1.669	27.765 ± 2.135
NDF (%DM)	57.357 ± 2.39	59.437 ± 3.964	41.77 ± 5.070
Lignin (% DM)	10.357 ± 2.274	12.525 ± 3.771	1.35 ± 4.824
Phosphorus (P) g/kg DM	0.374 ± 0.024	0.370 ± 0.039	0.235 ± 0.059
Iodine (I) mg/kg DM	0.576 ± 0.059	0.614 ± 0.098	0.99 ± 0.126

The mineral composition between the samples was similar apart from levels of P ($F_{2, 10}$ = 4.14, P = 0.049) and I ($F_{2, 10}$ = 33.08, P < 0.001). There was no significant difference in P content of forage from unimproved and conservation grasslands (t = -0.11, P = 0.915). However, the P content of forage from improved grasslands was significantly lower than that of unimproved grasslands (t = -2.788, P = 0.019). Levels of I in forage from unimproved grasslands and conservation grasslands were similar (t = 0.399, P = 0.698). However, the levels of I in forage from improved grasslands was twice as high as that from unimproved grasslands (t = 2.902, P < 0.001). All levels of antagonists (Fe, Al, Mo, S, Pb)

were low in the samples and there was no statistical difference in levels of antagonists among grassland types.

4.3 Effect of Botanical Diversity and Species Composition on Forage Quality

Redundancy Analysis (RDA) explains 35.7% of variation in the species data among samples and 100% of the correlation between environmental-species data (F =1.8, P = 0.05) (**Figure 3**). Generalized Additive Models (GAMs) derived from this analysis indicate that botanical diversity (using the Shannon-Wiener Index) was negatively correlated with forage protein content (F =15.72, P < 0.001). Forage sugar content initially increased with increased botanical diversity but declined at the highest levels of diversity (F =7.06, P = 0.012). Lignin content increased significantly as botanical diversity increased (F =10.37, P < 0.001). Specific plants were associated with high levels of protein, sugar and lignin and specific minerals. Increased levels of protein were associated with *D. glomerata* (F = 6.12, P = 0.018) and *M. sativa* (F = 262.49, P < 0.001). Increased levels of sugar were associated with increased abundance of *L. perenne* (F = 4.12, P = 0.049). Six plants, *D. glomerata* (F = 4.63, P = 0.037), *H. lanatus* (F = 4.64, P = 0.037), *P. australis* (F = 33.78, P < 0.001), *Carex* sp. (F = 33.78, P < 0.001), *F. arundinacea* (F = 6.60, P = 0.01) and *G. fluitans* (F = 33.78, P < 0.001), were associated with increased levels of lignin. Increased levels of Mg were associated with increases in abundance of *A. elatius* (F = 7.68, P = 0.009) and *D. cespitosa* (F = 11.42, P = 0.002) (F = 11.42, P = 0.002). Increased levels of Ca were associated with increased abundance of *L. pratensis* (F = 7.01, P = 0.013), *D. cespitosa* (F = 446.48, P < 0.001), *H. comosa* (F = 6.54, P = 0.015), *F. arundinacea* (F = 5.56, P < 0.02) and *L. pedunculatus* (F = 446.48, P < 0.001). Increased levels of K were associated with increases in the abundance of *F. arundinacea* (F = 9.37, P = 0.004), *H. comosa* (F = 8.3, P = 0.007), *L. corniculatus* (F = 20.3, P < 0.001), and *L. pratensis* (F = 5.99, P = 0.019).

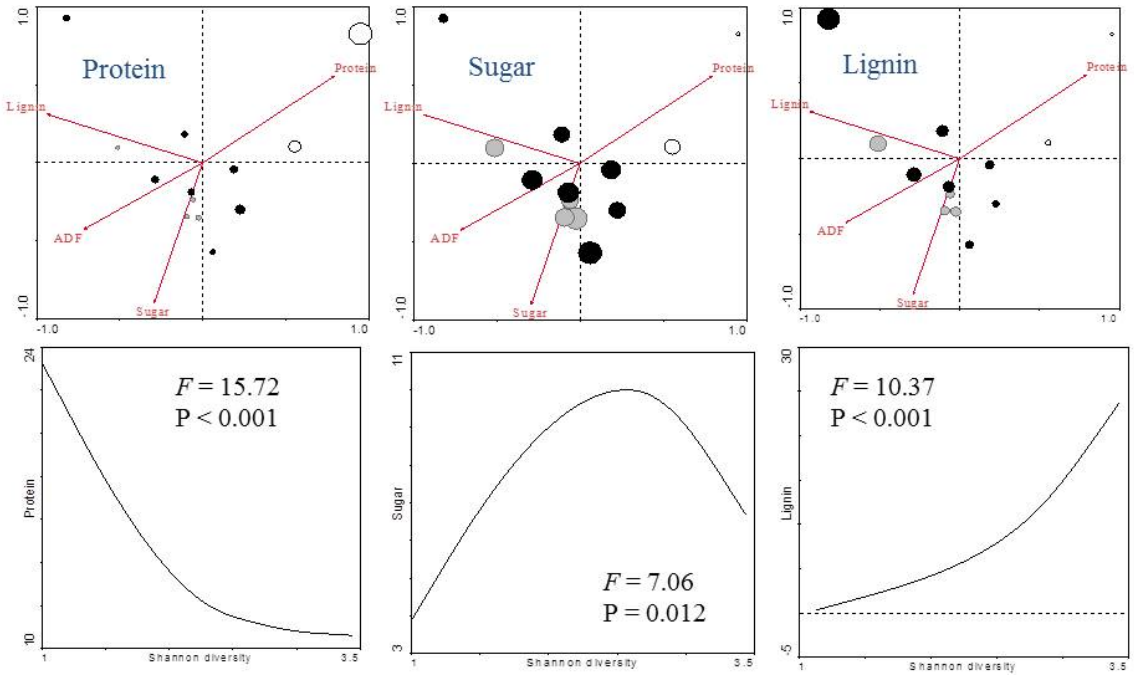


Figure 3 RDA showing variation of forage nutrient composition among samples. A. Improved leys were high in protein and protein content decreased with increasing diversity. B. There was little difference in terms of sugar content among samples. Sugar content increased initially with increasing diversity and then sharply declines. C. Lignin content did not vary significantly among the samples but increased with diversity. In the biplots, the abundance of protein, lignin and sugar in each sample is represented by symbol size. In the GAMs, Shannon diversity is used as a proxy for diversity. RDA explains 35.7% of variation in the species data among samples and 100% of the correlation between environmental-species data (F = 1.8, P = 0.05).

4.4 Comparison of Forage from Species-Rich Grasslands to Commercial Feedstocks

Overall, forage from species-rich grasslands, cereals and hay from monoculture-based grass leys were highly similar in nutritional composition (**Table 5, Figure 4**). DM content ($F_{5, 38}$ = 3.927, P = 0.005), protein content ($F_{5, 38}$ = 14.61, P < 0.001), and sugar content ($F_{5, 38}$ = 3.48, P = 0.01) varied significantly among livestock feeds. The average DM of species-rich grass was two to three times greater than other feedstocks but did not differ significantly from the DM content of cereals, hay and oilseed meal. The average protein content of forage from species-rich grasslands was comparable to that of cereals and hay but about 50% less than that of oilseed meal and legume based leys. The sugar content of forage from species-rich grasslands was similar to all other feeds apart from sugar beets, which on average contained three-times more sugar than species-rich grass. There was only a

marginally significant difference in the average ADF content ($F_{5, 38} = 2.16$, P = 0.08) and lignin content ($F_{5, 38} = 2.17$, P = 0.08) among feeds. Species-rich grass contained the highest average ADF content which was comparable to that of cereals and hay but up to 157% more than that of oilseed meal, legumes, and sugar beets. The lignin content of species-rich grass was comparable to all other feeds and was only marginally different from cereals. Surprisingly, sugar beets contained the highest lignin content, twice the average amount found in forage from species-rich grasslands.

Table 5 Comparison of macronutrient and mineral content of forage from specie-rich grasslands to other commercial feeds. The main differences between species-rich grass and other feeds were in regards to DM, Protein, Sugar, P, Mg, K, and Zn. All macro and micronutrients are represented as % DM.

	Species-Rich Grass	Cereals	Hay	Legumes	Oilseed Meal	Sugar Beet
DM	33.52 ± 6.41	76.9 ± 10.26	84.33 ± 14.35	65.58 ± 11.1	70.68 ±14.35	35.6 ± 14.42
Protein	11.14 ± 1.94	8.77 ± 2.68	10.63 ± 3.75	25.32 ± 2.89	33.25 ± 3.75	12.48 ± 3.75
Sugar	9.59 ± 4.35	6.02 ± 7.17	11.58 ±8.42	4.57 ± 6.48	7.3 ± 8.42	38.38 ± 8.42
ADF	34.56 ± 3.62	25.47 ± 5.01	28.28 ± 7	21.93 ± 5.39	17.88 ± 7	17.18 ± 7
Lignin	11.15 ± 3.25	4.5 ± 3.53	4.25 ± 6.29	4.04 ± 4.84	6.83 ± 7.03	21.4 ± 6.29
P	0.375 ± .03	0.24 ± 0.05	0.273 ± 0.07	0.35 ± 0.05	0.10 ± 0.09	0.19 ± 0.09
Ca	0.68 ± 0.01	0.24 ± 0.18	0.27 ± 0.25	0.73 ± 0.19	0.58 ± 0.33	0.87 ± 0.33
Mg	0.16 ± 0.01	0.13 ± 0.02	0.13 ± 0.03	0.22 ± 0.02	0.56 ± 0.04	0.16 ± 0.05
K	0.24 ± 0.02	0.12 ± 0.04	0.21 ± 0.05	0.21 ± 0.04	0.12 ± 0.07	n/a
Zn	0.30 ± 0.04	0.24 ± 0.07	0.24 ± 0.09	0.35 ± 0.07	0.58 ± 0.01	n/a

The amount of P ($F_{5, 38} = 15.1$, P < 0.001), Mg ($F_{5, 38} = 24.48$, P < 0.001), K ($F_{4, 29} = 2.63$, P = 0.05), and Zn ($F_{4, 29} = 2.85$, P = 0.04) varied significantly among the different feeds. On average, species-rich grass contained more P than cereals and sugar beet but roughly three times less than the amount found in oilseed meal. The Mg content of species-rich grass was similar to cereals, hay and sugar beet but three times less than oilseed meal. The average K content of forage from species-rich grasslands was comparable to all other feeds and contained double the amount found in cereals. The amount of Zn in forage from species-rich grasslands was comparable to other feeds but roughly 44% less than the amount of Zn in oilseed meal. The Ca content differed only marginally significantly among feeds ($F_{5, 38} = 2.11$, P = 0.08). The average Ca content in species-rich grass was comparable to all other feeds

and contained triple the amount found in cereals. All other minerals were similar among the different feed types.

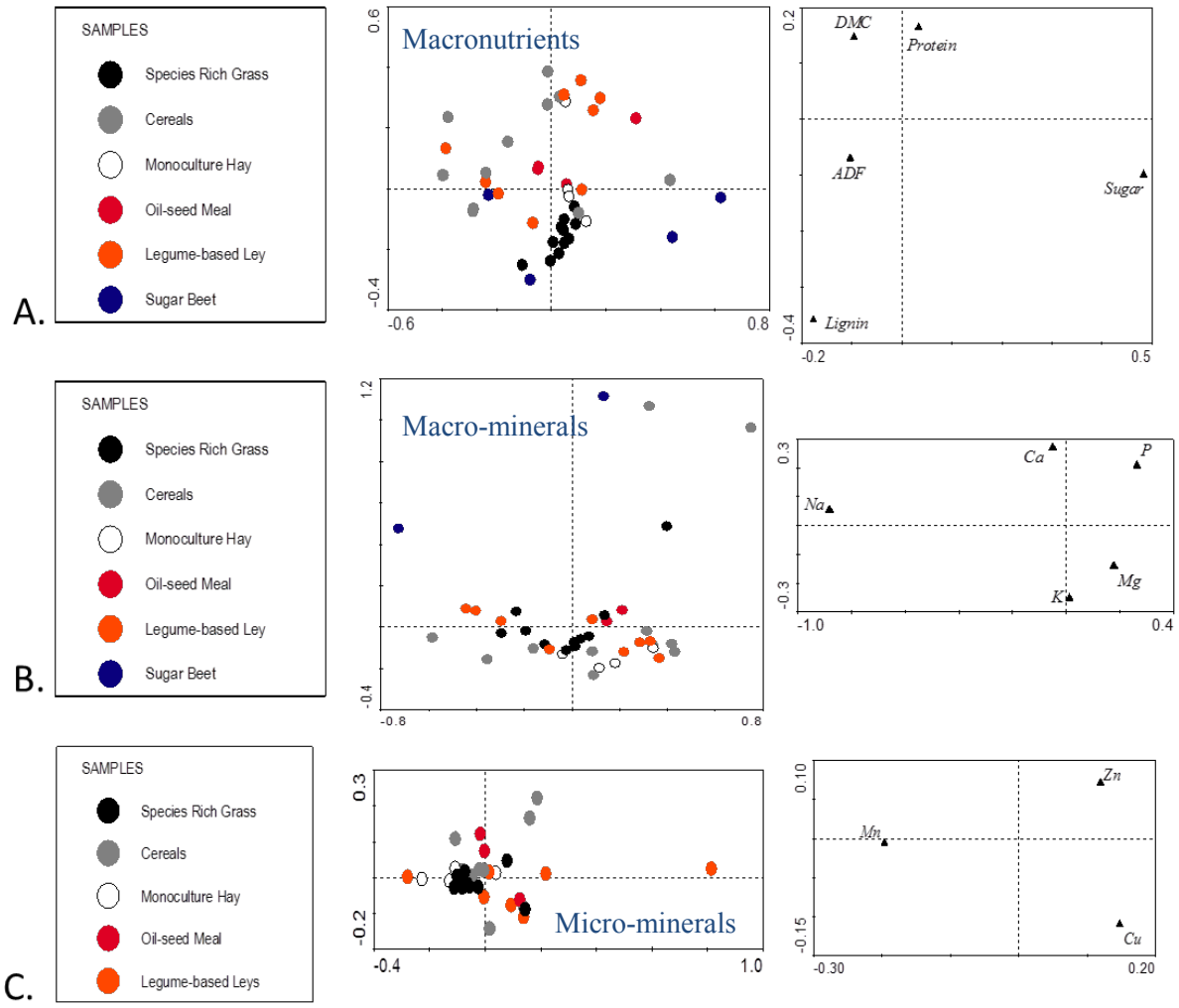


Figure 4 Correspondence Analysis showing the relationship in nutritional composition between the different types of livestock feeds. In terms of macronutrients, species-rich grass is closest in macro nutrient composition to monoculture hays, legume-based leys, and sugar-beet (4A). There was much less distinction in macro and micro-mineral composition between samples. All correspondence analysis diagrams represent 100% of variation in the samples.

4.5 Value of Grassland Biodiversity to Farmers and Graziers

Farmers valued grassland biodiversity primarily for its impact on forage quality. 32% of farmers believed that specific plants had a general positive effect on livestock when grazed. These included

willow (*Salix* spp.), holly (*Ilex* spp.), 'wild mint' (identified as *Thymus praecox, Origanum vulgare, Mentha arvensis*, and *Mentha sylvestris* at different sites) and 'vetches' (identified as *L. corniculatus*, *V. cracca*, and *L. pratensis* at different sites). Specifically, these plants were said to control livestock intestinal parasites. Several plants with medicinal properties not identified by farmers and graziers were also seen in the fields during fieldwork (e.g. betony (*Stachys officinalis*)). 29% of farmers also valued forage from species-rich grasslands as an inexpensive, low input source of livestock feed because these grasslands were permanent and did not need to be reseeded. In several cases, farmers were also allowed to graze local SSSIs (which they did not own) for free. Related to cost effectiveness, 21% of farmers valued their species-rich grasslands due to increased environmental resilience to drought and flooding. Compared to their improved leys, respondents reported that species-rich swards always provided a yield under adverse weather when their other fields sown with crops for livestock (e.g. improved leys, wheat) failed. 12 % of respondents valued grassland biodiversity for conservation reasons (e.g. to maintain rare plants such as orchids). A small number of farmers (17%) stated that grassland biodiversity contributed to meat and dairy quality and the marketability of these products (e.g. by connecting grassland biodiversity to 'local flavor' and/or sustainability).

4.6 Limitations to Incorporating Species-Rich Grasslands into Current Livestock Productions Systems

The main factors inhibiting the (continued or increased) agricultural use of species-rich grasslands were restrictions on time of grazing (23%), restrictions on time of hay-cut (36%), and reduced grassland yield and forage quality due to recent increases in invasive plants (40%). Farmers believed that current restrictions on spring grazing led to increases in coarse grasses like Tor grass (*B. pinnatum*), bramble (*Rubus* spp.), yew (*Taxus* sp.), and juniper (*Juniperus communis*). Farmers also believed late hay cuts led to increases in meadowsweet (*F. ulmaria*) and yellow rattle (*Rhinanthus* spp.) (**Figure 5**). 20% of farmers also viewed open-access to grasslands (e.g. in the case of SSSIs) by

members of the general public as a barrier to the agricultural use of species-rich grasslands, primarily due to trampling of meadows by the general public and dog worrying. 85% of farmers viewed current agroenvironmental and conservation schemes as having a negative impact on their ability to use species-rich grasslands for agriculture and 17% said they would leave their current HLS agreements once they ran out in 2020.

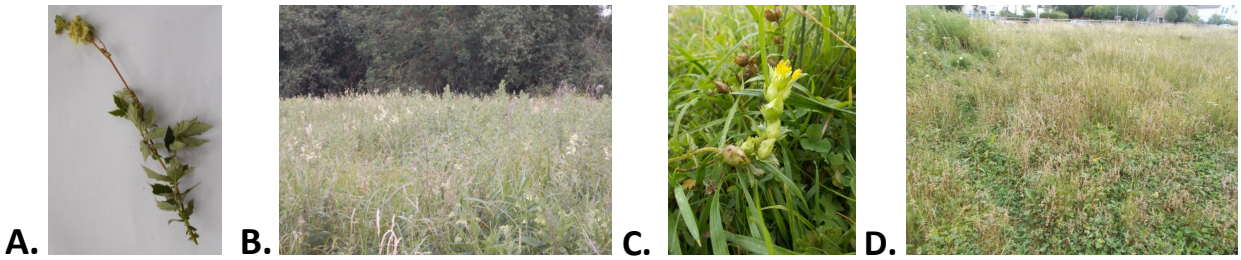


Figure5 Barriers to the agricultural use of species-rich grasslands. Respondents viewed increases in 'invasive' plants such as Meadowsweet (A and B) and Yellow rattle (C and D) due to 'lax' management as a significant barrier to using species-rich grasslands in current agropastoral systems because they reduced forage yield and/or quality.

5. Discussion

This study found that vegetation diversity, and potentially specific species, affected forage macro and micro nutrient levels. Comparison of the nutrient composition of species-rich grass to commercial feeds demonstrated the comparability between the two. Vegetation surveys and ethnographic research indicated local farmers and graziers valued species-rich grasslands as a source of low-input nutritional forage which may have a positive effect on livestock health, meat and milk quality. Qualitative data among Oxfordshire farmers suggests that increasing the agricultural use of species-rich grasslands is feasible and could help reduce reliance on commercial feeds, but only if agroenvironmental and conservation policies around grassland management become more flexible.

5.1 The Nutritional Value of Forage from Species-Rich Grasslands

Grassland biodiversity directly affected the macro and micro nutrient composition of forage samples from Oxfordshire, particularly in relation to DM, protein, sugar, and P content. Forage DM and fiber content (ADF, NDF) was higher on unimproved and conservation grasslands compared to improved grasslands due to the high abundance and variety of grasses and forbs (e.g. *F. ovina*). These plants are higher in cellulose, hemicellulose and lignin than legumes. This agrees with Hofmann and Isselstein (2005) who found that increased species-richness on experimental grassland plots increased DM and fiber content. Protein content was highest in the forage samples from the improved grasslands because of the abundance of legumes (e.g. *T. pratense*) which are rich in protein. This disagrees with a study by Reiné et al. (2014) which found that the protein levels in hay from unimproved meadows in the Central Spanish Pyrenees was positively correlated with species richness and contained more protein than forage from improved grasslands. Surprisingly, this study found that conservation grasslands had the highest sugar content. Although the reasons for this are unresolved, it could be due to the high abundance of clover and wildflowers used to restore these sites. The increased levels of P in forage from species-rich grasslands was also unusual and may be due to the presence of arbuscular mycorrhiza (which increase plant uptake of soil P) and are known to be particularly abundant on species-rich grasslands (Parniske 2008; Garg and Chandel 2010). Overall, these results disagree with previous studies which suggest forage from species-rich grasslands in lowland England contains less DM content, P and Mg compared to grass from improved leys (Tallowin and Jeffers 1999). The conflicting results on the extent to which biodiversity affects forage quality may be due to the wide variability in species composition among species-rich grasslands and potentially differences in when samples were taken (e.g. because the nutritional composition of grass changes throughout the summer).

Although experimental, I also identified several plant species which may positively contribute to forage quality. For example, one Generalized Additive Model showed that increased abundance of *D. glomerata* was associated with increased forage protein content, a factor well known from previous forage analyses (Aufrere et al. 2003; Davies 1982). Other models suggested that particular plants were associated with increased levels of Mg, Ca, P and K. Although these results are preliminary, previous studies suggest that the plants found on species-rich grasslands do contain elevated levels of these nutrients (**Table 7**).

Table 7 Protein, phosphorus, calcium and magnesium content of common plants found on species-rich grasslands. Two legumes commonly sown on improved grasslands are included for comparison and marked with a *. The highest values of each component are highlighted in bold. Quantities are given as mg/g. All data is taken from the Phytochemical Database hosted by the USDA-ARS National Agricultural Library (http://www.ars-grin.gov/duke/).

Species	Protein (mg/g)		Phosphorus (mg/g)		Calcium (mg/g)		Magnesium (mg/g)
	Seed	*Leaves*	*Seed*	*Leaves*	*Seed*	*Leaves*	*Leaves*
A. millefolium	369	144		2.95		8.67	1.92
****M. sativa***	**385**	199		3.1		17.2	2.3
P. lanceolata	190		0.6		**23.4**	1.34	1.06
R. acetosa		260				4.3	4.6
****T. pratense***	380	280		4.5		22.9	8.1
U. dioica	201	**304**		**6.8**		**41.9**	**8.6**

When compared to commercial feeds, I found that species-rich grass was most comparable to monoculture grass leys, cereals, and cereal by-products in macro and micro nutrient composition. Although the protein content of species-rich grass was lower than well-known protein rich feedstocks like soy and rapeseed meal, it had higher levels of protein than both cereals and hay composed solely of a single species (e.g. *D. glomerata*). I also found no difference in the fiber and lignin content between species-rich grass and commercial feedstocks, contradicting previous claims that species-rich grass is less digestible and palatable to livestock due to high lignin content (Bruinenberg et al. 2002; Waghorn and McNabb 2003). I also found that species-rich grass contained elevated levels of specific

minerals associated with livestock growth and health (Ca, Mn and K) compared to other feeds (Barnes et al. 2003; Yatoo et al. 2013).

Although the comparability of fodder from species-rich grasslands to commercials feeds is important, whether this fodder can meet the nutritional needs of livestock is the most important concern to farmers. **Table 8** indicates that fodder from species-rich grassland can meet most of the dietary needs of livestock. Farmers could adequately raise beef cattle, sheep and horses on fodder from species-rich grasslands. Dairy cows require higher levels of sugar and Mg. However, fodder from species-rich grasslands could serve as a supplementary source of protein, P, and Ca, all of which contribute to growth and milk production. Species rich hay also meets the nutritional needs of horses, many of which require low-sugar feeds to prevent diseases such as laminitis (Pollitt 2004).

Table 8 Nutrient requirements of beef cattle, dairy cows, and sheep, and nutrient content of forage from species-rich grasslands. The nutritional content of species-rich grass is based on the mean value for unimproved and conservation grasslands from Oxfordshire. Source of livestock dietary requirements: Brown et al. 2008; Fisher 2013; Kavanagh 2015; National Research Council of the Academies 2007; Parish 2016; Ralston 2015.[6]

Nutrient (% DM)	Beef cattle	Dairy cows	Sheep	Horses	Species-rich grass
Protein	6-9%	7-11%	3-7%	8-13%	**11.14 %**
Sugar	< 20%	25-30%	3-8%	< 10%	**9.58 %**
ADF	< 35%	< 35%	< 40%	< 60%	**34.55 %**
Ca	0.15-0.25%	0.4-0.8%	0.20-0.82%	0.25-0.5%	**0.68 %**
P	0.13-0.24%	0.3-0.4%	0.16-0.38%	0.2-0.4%	**0.37 %**
Na	0.06-0.08 %	0.1-0.2%	0.09-0.18%	0.16-0.18%	**0.12 %**
Mg	0.10-0.2%	0.25-0.3%	0.12-0.18%	0.1-0.3%	**0.17 %**

[6] The dietary requirements of cattle and sheep vary according to animal size, age, and whether or not they are lactating. As such, these requirements are general estimates.

5.2 Grassland Biodiversity and Livestock Health

In Oxfordshire, farmers primarily valued forage from species-rich grasslands due to its perceived effect on livestock health. Out of all the plant species identified on the study sites, 12 (22% of all species) have established medicinal properties. **Table 9** lists these plants, the chemicals they contain, and their effect on livestock health. Previous research indicates that these plants contain specific compounds (e.g. alkaloids, phenolics, and terpenes) associated with antibiotic (agropyrene, avicularin, carnosol, coumarin, cysteine, flavonoids), anthelmintic (carvacrol, harmine, thymol, geraniol, mannitol, tannins), anti-inflammatory (adenosine, matricin, rutin), and antioxidant (lutein, anthocyanin) activities (Barry and McNabb 1999; Durmic and Blache 2012; Cornara et al. 2015; Githiori et al. 2006; Kemp et al. 2001; Provenza and Villalba 2010; Villalba and Provenza 2009). This list is provisional and based solely on the medicinal plants found on species-rich grasslands during a very short window of time (June-July). Additional medicinal plants may be found on these grassland sites at other times of the year.[7] Interviews with Oxfordshire farmers also identified other plants, specifically wild legumes referred to as 'vetches,' which may have antibiotic or anthelmintic properties. Without further analysis however, this information remains anecdotal. Analyzing the metabolomic content and antimicrobial properties of whole forage samples and specific plants could lead to the development of new seed mixes used to sow improved leys and conservation grasslands and the discovery of lead molecules to create new antibiotics and anthelmintics. Additional research is also needed to determine whether the presence, abundance, or chemical potency of these plants changes over time.

[7] For example, the majority of the rare plants monitored by conservation groups at SSSIs around Oxfordshire have medicinal properties (e.g. *Marrubium vulgare;* French 2014) yet these were not seen during fieldwork.

Table 9 Plants with medicinal properties found on species-rich grasslands. The table provides their Latin name, common name, some of the phytochemicals they contain, and whether it contained plant secondary metabolites with antibiotic and/or anthelmintic metabolites.

Species	Common name	Example phytochemicals	Antibiotic/ anthelmintic	Source
***Achillea millefolium* L.**	yarrow	flavonoids, phenolic acids, coumarins, terpenoids	antimicrobial	Bessada et al. 2015; Saeidnia et al. 2011; Stojanovic et al. 2005
***Agrimonia eupatoria* L.**	agrimony	Polyphenols	antibacterial	Ivanova et al. 2005; Vogl et al. 2013; Watkins et al. 2011
***Filipendula ulmaria* (L.) Maxim**	meadowsweet	avicularin, coumarin, rutin, salicin, vanillin	antibiotic, antiviral, antifungal	Cwikla et al. 2010; Dadakova et al. 2010; Fecka 2009
***Lotus corniculatus* L.**	bird's foot trefoil	Tannins	anthelmintic	Beynon 2012, Girardi et al. 2014; MacAdam et al. 2015
***Medicago sativa* L.**	lucerne	adenine, ascorbic-acid, genistein, lutein, malic-acid, tannins	anthelmintic, antifungal	Duke 1992, Piluzza et al. 2014; Vieira et al. 2001
***Mentha longifolia* (L.) Huds.**	wild mint	menthone, isomenthone, menthol, 1, 8-cineole, borneol, and piperitenone	antimicrobial	Gulluce et al. 2007; Hajlaoui et al. 2008; Hussain et al. 2015; Sarac and Ugur 2009
***Origanum vulgare* L.**	wild marjoram	apigenin, carvone, geraniol, linoleic-acid, luteolin, rosmarinic-acid, rutin, tannins, vanillic-acid	antibacterial, antiviral, anthelmintic	Kozlowska 2015, Nabavi 2015; Sesterhenn et al. 2015
***Plantago lanceolata* L.**	plantain	apigenin, linoleic-acid, salicylic-acid	antibacterial	Dalar et al. 2012; Ferrazzano et al. 2015; Karakas et al. 2012
***Rumex acetosa* L.**	dock	ascorbic-acid, hyperin, rutin, tannins	antibacterial, antifungal	Duke 1992; Johann et al. 2008; Wegiera et al. 2011
***Stachys officinalis* (L.) Trevis. ex Briq.**	betony	rosmarinic-acid, tannins	antibacterial, anthelmintic	Grujic-Jovanovic et al. 2004; Lazarevic et al. 2013; Stamatis et al. 2003
***Thymus praecox* Opiz**	wild thyme	carvacrol, carvone, geranial, linoleic-acid, luteolin	antibacterial, nematicide	Herman et al. 2016; Ozen et al. 2011; Ozkan et al. 2016
***Urtica dioica* L.**	nettle	linoleic-acid, P-coumaric-acid, scopoletin	antibacterial	Duke 1992; Ceyhan et al. 2012; Samoilova et al. 2014

5.3 Implications for Agriculture and Conservation

Highlighting the agricultural and economic value of grassland biodiversity may help create more sustainable livestock production systems. For example, exploiting grassland biodiversity could help reduce antibiotic and anthelmintic use. Currently, many farms rely on these as prophylactics or to promote livestock growth in addition to treating ill animals. Exploitation of grassland medicinal plants could become increasingly important given recent EU regulations that now limit the number and type of antibiotics farmers may give to their livestock and increased concern about antibiotic and anthelmintic resistance (Brooker and Acamovic 2005; Gallagher 2015; Makkar et al. 2009). In addition, emphasizing the connection between grassland biodiversity and the quality of local meat and dairy products could tap into increasing interest in locally-produced food and serve as one way of marketing these lower-input, and often more expensive, products. For example, previous studies indicate that cattle raised on grassland produce higher grade meat with less saturated fat and cheese with higher Omega-3 and linoleic acid levels compared to livestock fed on cereals (De Noni and Battelli 2008; MacAdams and Villalba 2015; Martin 2005). Grazing species-rich grasslands may also affect the aroma of dairy products (Carpino et al. 2004).

These shifts would have wider ecological ramifications. Increased agricultural use of species-rich grasslands could reduce reliance on high input feeds like cereals, sugar beets, and soybeans, which require high levels of fertilizers, pesticides and herbicides. They have a negative effect on biodiversity by displacing local native vegetation and by decreasing landscape heterogeneity. At the same time, it could promote the reversion of intensively cultivated arable fields currently used to grow commercial livestock feeds to grassland (Bruinenberg et al. 2002; Tallowin and Jefferson 1999). Although previous studies have stated that species-rich grasslands cannot produce enough forage to sustain large-scale livestock production systems (Peeters 2009), other research suggests that grassland

biodiversity can have a positive effect on productivity, particularly under periods of environmental stress (e.g. drought) (Bomford 2012; Bullock et al. 2007; Hector et al. 1999; Isselstein et al. 2005; Isbell et al. 2015: Tilman et al. 2006; Wrage et al. 2011). While it may not be feasible for global livestock production systems to rely solely on grassland-based feeding systems, increasing the number of livestock raised on grasslands would still have a positive environmental impact.

However, current agro-environmental and conservation schemes may serve as a temporary barrier to increasing the agricultural use of species-rich grasslands. In Oxfordshire, farmers believed conservation guidelines restricting grazing and hay-cutting reduced forage quality by increasing coarse vegetation and invasive plants. In some cases, ecological research lends support to these claims. For example, a number of studies have found that low levels of grazing can allow Tor grass to spread, while heavy grazing, particularly early in spring, can help control this grass (Bobbinka and Willlems 1993; Buckland et al. 2001; Wilson et al. 1995).[8] Due to their effect on forage quality, these guidelines were unpopular among the study participants. A large number of participants who managed grasslands under agroenvironmental schemes stated that they would leave these schemes in order to maintain their livelihoods rather than accept payments to conserve local biodiversity. In addition, several farms with SSSIs where conservation guidelines had severely impacted how they used their land (e.g. for grazing) have turned over their SSSIs to conservation groups (because landowners with SSSIs cannot 'opt out' of the conservation guidelines they must follow). This conflicts with previous research which argues that agroenvironmental payments are the main motivation for farmers to maintain grassland biodiversity (Jitea and Arion 2015; Stenseke 2006). In part, variation in farmer acceptance and adherence to agroenvironmental policies among different regions may rely on how well these policies fit into current land management regimes (Batáry et al. 2015) and those who have managed the same area for generations may be less open to change (Catsadorakis 2007).

[8] Tor grass is palatable to sheep only early in the spring when it is still green and supple. Once the grass matures later into the summer sheep avoid it, which partly accounts for its spread.

Increased collaboration with farmers to revise current conservation guidelines could help balance the agricultural use of species-rich grasslands with biodiversity conservation. This study found that farmers and graziers had a great deal of local ecological knowledge about the local vegetation and changes in grassland biodiversity and composition. This knowledge was based on farmers' daily interaction with the landscape: walking through fields, monitoring livestock, removing unwanted (e.g. poisonous, invasive) plants, cutting hay, and maintaining watercourses (in the case of flood meadows). This knowledge could be used to modify current guidelines and workshops could also be held to promote the exchange of local ecological knowledge with more formal 'academic' ecological knowledge among farmers. This could become increasingly important as new farmers restore or recreate species-rich grasslands who have not managed these systems before. Similar grassland biodiversity conservation initiatives in Europe and the Middle East have involved farmers and graziers the conservation process, relying on local ecological knowledge to maintain rangeland biodiversity and control invasive plants (Al-Tabini et al. 2012; Babaí and Molnár 2014; Rowe 1999). Taking steps in this direction may be the best way forward to achieving a greater balance in the agricultural and conservation value of these highly diverse, and often historic, pastoral landscapes.

6. Conclusion

Research on the nutritional value of forage from species-rich grasslands is currently lacking and may help decrease reliance on improved leys and commercial feeds and reduce the negative environmental impacts of intensive livestock production systems. This study provides field-based quantitative and qualitative research on how biodiversity affects forage quality. Unimproved grasslands had higher levels of species richness and forage DM, ADF and P content compared to improved leys and conservation grasslands and contained medicinal plants with pre-established antibiotic and

anthelmintic properties. Improved leys and other commercial feedstocks like oilseed meal may be higher in protein, but these feeds lack the diversity in mineral and phytochemical composition of species-rich grasslands. These results suggest that increasing species-rich grassland use could help reduce reliance on commercial feeds, limit the current use of antibiotics and anthelmintics, increase the marketability of pastoral products from livestock raised on species-rich grasslands, and support the maintenance, and even recreation of, species-rich grasslands. However, creating more flexible agro-environmental guidelines around species-rich grassland use is necessary to balance the agricultural as well as ecological value of these landscapes and ensure farmer participation in these schemes. Although based on ecological and ethnographic work with farmers and graziers from one particular region in the UK, this research helps further our understanding of the relationship between biodiversity and forage quality, and ultimately, the socio-economic value of this natural resource.

Appendix I

Table 1 Location and description of grassland sites involved in the present study. Sites with a * indicate fields were forage samples were taken.

Name	Grassland Type[9]	Livestock[10]	Lat	Long
***Wolfson Meadow**	U	C	51.77112	-1.2525
Port Meadow	U	C, H	51.77299	-1.284
***Park Farm Meadow**	U	C	51.76879	-1.25203
***Trinity Meadow**	U	n/a	51.76096	-1.2414
***Iffley Meadow A**	U	C	51.7347	-1.24438
***Iffley Meadow B**	U	C	51.7339	-1.24389
Iffley Meadow C	C	C	51.73195	-1.24353
***Oxey Mead**	U	C,S	51.79269	-1.3066
***Cassington Meadow**	C	C	51.78821	-1.3285
***Riverside Meadows-Field B**	C	C	51.59971	-1.1185
Riverside Meadows-Field C	C	C	51.59804	-1.1192
***King's Meadow**	C	C	51.60433	-1.11811
Blenheim	U	S	51.8526	-1.36296
Berins Hill SSSI	U	S	51.56145	-1.0576
Berins Hill A	C	S	51.56113	-1.05942
Berins Hill B	C	n/a	51.56211	-1.05761
Berins Hill C	I	H	51.56243	-1.0584
***Alison's Meadow**	C	C,S	51.78567	-1.33148
***The Eye**	C	C,S	51.78754	-1.3204
Somerford West	C	C,S	51.78426	-1.33697
***FAI-lucerne ley**	I	n/a	51.7845	-1.31939
Step Farm-lucerne ley	I	n/a	51.66844	-1.6027
Step Farm-2yr ley	I	n/a	51.66719	-1.6036
***Step Farm- legume ley**	I	n/a	51.66953	-1.6113
Rushy Meadow	U	C	51.82341	-1.3016
Warren Bank	U	S	51.56715	-1.05865
***Park Farm-Rush Meadows**	U	C	51.75997	-1.24451
Johnston-Houghton	U	S	51.5493	-1.2272
Lyons	U	S	51.54934	-1.2183
Allen Farm field A	C	S	51.54372	-1.22136

[9] U=unimproved, C= conservation, I= improved.
[10] C=cattle, S=sheep, H=horses

Table 2 Comparison of macro and micro nutrient composition between Oxfordshire forage samples. Protein, lignin, Ca, Na, and micro minerals varied considerably among samples.

Site Name	WM	PF_M	TM	IM_A	IM_B	OM	PF_R	CM	RM_B	KM	AM	FAI_L	SF_PB
Site Type	U	U	U	U	U	U	U	C	C	C	C	I	I
Macro-nutrients													
Protein (CP) (% DMC)	13.9	12.53	11.21	10.01	11.48	10.33	11.41	10.68	9.81	11.96	9.21	24.54	16.23
Neutral Detergent Fiber (NDF) (% DMC)	54.91	55.08	57.6	61.59	54.14	57.78	60.4	55.34	65.15	57.5	59.76	29.57	53.97
Acid Detergent Fiber (ADF) (%DMC)	30.33	35.3	36.4	35.73	34.18	34.64	34.65	35.09	35.29	31.99	36.5	23.49	32.04
Lignin (ADL) (% DMC)	4.3	7.3	11.4	7.3	10.8	6.2	25.2	9.4	7.9	15.1	17.7	0.1	2.6
Sugar (WSC) (% DMC)	8.77	9.97	8.51	9.04	10.7	11.72	5.17	11.63	9.85	10.4	9.67	2.66	8.28
Macro-minerals													
Phosphorus (P) (% DMC)	0.33	0.29	0.42	0.31	0.45	0.4	0.42	0.41	0.32	0.44	0.31	0.27	0.2
Magnesium (Mg) (% DMC)	0.2	0.11	0.24	0.12	0.22	0.17	0.13	0.15	0.18	0.15	0.16	0.17	0.12
Calcium (Ca) (% DMC)	0.98	0.54	2.19	0.61	0.62	0.53	0.2	0.35	0.64	0.42	0.42	0.69	0.28
Sodium (Na) (% DMC)	0.24	0.06	0.02	0.04	0.25	0.08	0.01	0.1	0.06	0.16	0.28	0.27	0.09
Potassium (K) (% DMC)	1.55	2.67	3.47	2.37	2.62	3.55	0.04	2.97	2.58	3.25	1.73	1.55	1.35
Chloride (Cl) (% DMC)	0.56	0.4	0.43	0.28	0.37	0.27	0.51	0.35	0.52	0.77	0.35	0.4	0.94
CAB (mEq/kg) (% DMC)	376	593	985	522	606	784	302	585	487	577	440	408	81
Micro-nutrients													

Manganese (Mn) (mg/kg)	57	36.4	18.2	35	122.4	65.9	51.7	72.6	115.8	51.8	38.6	30.1	235.3
Copper (Cu) (mg/kg)	8.7	4.5	12.1	5.2	10	6.3	11.1	6	8.3	7	4.5	6.1	4.5
Zinc (Zn) (mg/kg)	33.7	19.7	29.4	17.7	41.6	23.9	100.7	28	32.4	25.2	17.4	28.8	20.3
Selenium (Se) (mg/kg)	0.06	0.01	0.02	0.21	0.1	0.09	0.06	0.03	0.06	0.2	0.18	0.53	0.02
Cobalt (Co) (mg/kg)	0.09	0.06	0.04	0.01	0.05	0.02	0.05	0.04	0.01	0.05	0.01	0.06	0.01
Iodine (I) (mg/kg)	0.7	0.58	0.49	0.55	0.47	0.62	0.62	0.63	0.62	0.64	0.57	1.89	1.25
Antagonists													
Iron (Fe) (mg/kg)	84	101	79	70	129	124	300	227	96	114	98	62	114
Aluminium (Al) (mg/kg)	16	29	17	17	52	49	54	63	25	49	39	19	32
Molybdenum (Mo) (mg/kg)	0.89	0.66	1.95	1.32	1.71	1.41	1.27	1.16	1.69	2.1	0.95	1.9	0.55
Sulfer (S) (% DMC)	0.24	0.1	0.34	0.17	0.25	0.25	0.29	0.23	0.3	0.21	0.11	0.22	0.14
Lead (Pb) (mg/kg)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0

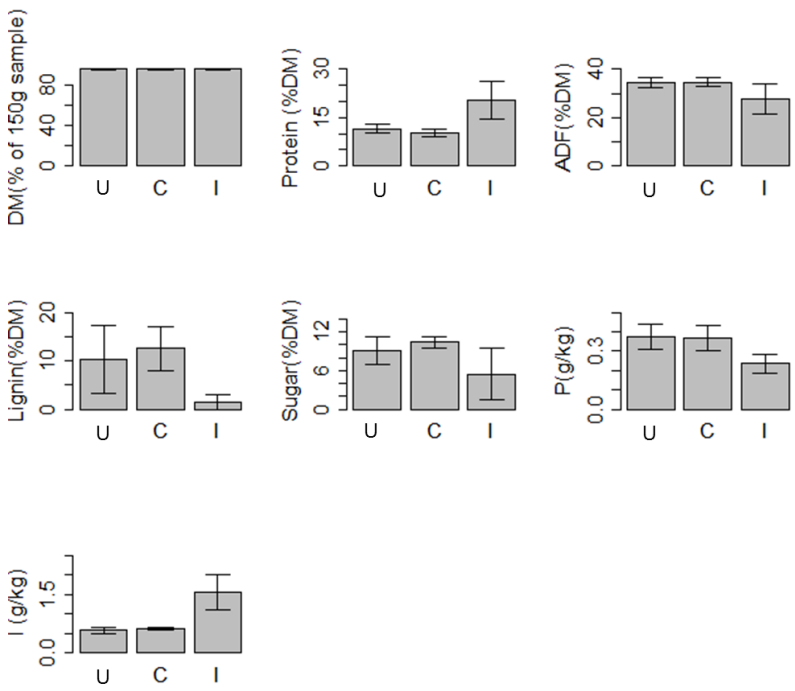


Figure 1 Comparison of nutrient content of grass sample from Oxfordshire according to grassland type (conservation, improved and unimproved). Unimproved and conservation grasslands were high in DM, ADF, NDF, and sugar while improved grasslands were high in protein content. C=Conservation grassland; I=Improved grassland; U=Unimproved grassland. Plots show means ±1 standard error.





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Survey on Grassland Biodiversity & Management			
Participant Name		Age	
Site Name			
Location			
Land Use	Dairy		☐
	Arable		☐
	Non-arable		☐
	Other (e.g. SSSI)		☐
Site History	History of grassland use	1-5 years	☐
		6-10 years	☐
		11-20 years	☐
		over 20 years	☐
Livestock	Stocking rates		
	Type of stock & breed	Cattle	☐
		Sheep	☐
		Horses	☐
Management	Management type	Grazed	☐
		Mown	☐
		Mown & Grazed	☐
		Conservation Grazed	☐
		Conservation Mown	☐
		Seeded	☐
	Timing/rate of application of manure/lime (if applicable)		
	Seed sowing (mix type)		
	Seasonality of grassland use	Year round	☐
		Autumn	☐
		Winter	☐
		Spring	☐
		Summer	☐

Open response questions	Please answer the questions below in as much detail as possible. If some questions are not relevant, feel free to leave these blank.
Agricultural Value of Grassland Biodiversity	What is the agricultural value of your botanically-rich grasslands compared to improved leys?
Forage Quality	What plants grow naturally in your fields?
	What plants (if any) do you intentionally sow?
	Are there any weeds/invasive plants that are removed to prevent livestock poisoning or to maintain the productivity of the fields?
	What species are most palatable to livestock?

Agro-environmental and Conservation Management	What challenges (policy, financial, practical, etc.) have you faced in managing your land under current agro-environmental schemes?
	In your opinion, what are the benefits of these schemes?

References

Anon, Feedipedia: An on-line encyclopedia of animal feeds | Feedipedia. Available at: http://www.feedipedia.org/ [Accessed October 19, 2015].

Babaí, D. & Molnár, Z., 2014. Small-scale traditional management of highly species-rich grasslands in the Carpathians. *Agriculture, Ecosystems & Environment*, 182, pp.123–130.

Barnes, R., 2003. *Forages* 6th ed., Ames: Iowa State Press.

Barry, T.N. & McNabb, W.C., 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *The British Journal of Nutrition*, 81(4), pp.263–272.

Berkes, F., Colding, J. & Folke, C., 2000. Rediscovery of traditional ecological knowledge as adaptive management. *Ecological Applications*, 10(5), pp.1251–1262.

Batáry, P. et al., 2015. The role of agri-environment schemes in conservation and environmental management. *Conservation Biology*, 29(4), pp.1006–1016.

Bessada, S.M.P., Barreira, J.C.M. & Oliveira, M.B.P.P., 2015. Asteraceae species with most prominent bioactivity and their potential applications: A review. *Industrial Crops and Products*, 76, pp.604–615.

Bezák, P. & Halada, L., 2010. Sustainable management recommendations to reduce the loss of agricultural biodiversity in the mountain regions of NE Slovakia. *Mountain Research and Development*, 30(3), pp.192–204.

Biasi, R. et al., 2015. Linking traditional tree-crop landscapes and agro-biodiversity in central Italy using a database of typical and traditional products: a multiple risk assessment through a data mining analysis. *Biodiversity and Conservation*, 24(12), pp.3009–3031.

Birge, T. & Herzon, I., 2014. Motivations and experiences in managing rare semi-natural biotopes: A case from Finland. *Land Use Policy*, 41, pp.128–137.

Blackstock et al., 1999. The extent of semi-natural grassland communities in lowland England and Wales: a review of conservation surveys 1978–96. *Grass and Forage Science*, 54(1), pp.1–18.

Bomford, M.K., 2012. Grassland productivity and ecosystem services. *Choice*, 49(11), pp.49–6269.

Brooker, J.D. & Acamovic, T., 2005. Phytochemicals in livestock production systems. *Animal Feed Science and Technology*, 121(1), pp.1–4.

Bruinenberg, M.H. et al., 2002. Factors affecting digestibility of temperate forages from semi-natural grasslands: a review. *Grass and Forage Science*, 57(3), pp.292–301.

Buchin, S. et al., 1999. Influence of the composition of Alpine highland pasture on the chemical, rheological and sensory properties of cheese. *Journal of Dairy Research*, 66(04), pp.579–588.

Bullock, J.M. et al., 2001. Restoration of biodiversity enhances agricultural production. *Ecology Letters*, 4(3), pp.185–189.

Bullock, J.M., Pywell, R.F. & Walker, K.J., 2007. Long-term enhancement of agricultural production by restoration of biodiversity. *Journal of Applied Ecology*, 44(1), pp.6–12.

Carpino, S. et al., 2004. Composition and aroma compounds of Ragusano cheese: Native pasture and total mixed rations. *Journal of Dairy Science*, 87(4), pp.816–830.

Catsadorakis, G., 2007. The conservation of natural and cultural heritage in Europe and the Mediterranean: A Gordian Knot? *International Journal of Heritage Studies*, 13(4-5), pp.308–320.

Ceyhan, N., Keskin, D. & Ugur, A., 2012. Antimicrobial activities of different extracts of eight plant species from four different families against some pathogenic microoorganisms. *Journal of Food Agriculture & Environment*, 10(1), pp.193–197.

Cooper, E.A., 1998. *Summary descriptions of National Vegetation Classification grassland and montane communities*, Defra.

Cornara, L., Xiao, J. & Burlando, B., 2015. Therapeutic Potential of Temperate Forage Legumes: A Review. *Critical Reviews in Food Science and Nutrition*, 0(ja), pp.00–00.

Csergő, A.M. & Demeter, L., 2009. *Plant species diversity and traditional management in Eastern Carpathian grasslands*, County Durham: European Forum on Nature Conservation and Pastoralism (EFNCP).

Critchley, C.N.R., Burke, M.J.W. & Stevens, D.P., 2004. Conservation of lowland semi-natural grasslands in the UK: a review of botanical monitoring results from agri-environment schemes. *Biological Conservation*, 115(2), pp.263–278.

Csergő, A.M., Demeter, L. & Turkington, R., 2013. Declining Diversity in Abandoned Grasslands of the Carpathian Mountains: Do Dominant Species Matter? *PLoS ONE*, 8(8), p.e73533.

Cwikla, C. et al., 2010. Investigations into the Antibacterial Activities of Phytotherapeutics against Helicobacter pylori and Campylobacter jejuni. *Phytotherapy Research*, 24(5), pp.649–656.

Dadakova, E., Vrchotova, N. & Triska, J., 2010. Content of selected biologically active compounds in tea infusions of widely used European medicinal plants. *Journal of Agrobiology*, 27(1), pp.27–34.

Dalar, A., Turker, M. & Konczak, I., 2012. Antioxidant capacity and phenolic constituents of Malva neglecta Wallr. and Plantago lanceolata L. from Eastern Anatolia Region of Turkey. *Journal of Herbal Medicine*, 2(2), pp.42–51.

Daly, L. et al., 2016. Integrating Ontology into Ethnobotanical Research. *Journal of Ethnobiology*, 36(1), pp.1–9.

Darwish, R.M. & Aburjai, T.A., 2010. Effect of ethnomedicinal plants used in folklore medicine in Jordan as antibiotic resistant inhibitors on Escherichia coli. *Bmc Complementary and Alternative Medicine*, 10, p.9.

Dawson, L.E.R. et al., 2011. Grassland systems of red meat production: integration between biodiversity, plant nutrient utilisation, greenhouse gas emissions and meat nutritional quality. *animal*, 5(09), pp.1432–1441.

Defra, 2011. Biodiversity 2020: A strategy for England's wildlife and ecosystem services - Publications - GOV.UK. Available at: https://www.gov.uk/government/publications/biodiversity-2020-a-strategy-for-england-s-wildlife-and-ecosystem-services [Accessed October 23, 2015].

Donath, T.W. et al., 2004. Perspectives for incorporating biomass from non-intensively managed temperate flood-meadows into farming systems. *Agriculture, Ecosystems & Environment*, 104(3), pp.439–451.

Duke, J.A., 1992. *Handbook of Phytochemical Constituent Grass, Herbs and Other Economic Plants*, CRC Press.

Durmic, Z. & Blache, D., 2012. Bioactive plants and plant products: Effects on animal function, health and welfare. *Animal Feed Science and Technology*, 176(1–4), pp.150–162.

Fernandez-Gimenez, M.E., 2000. The role of Mongolian nomadic pastoralists' ecological knowledge in rangeland management. *Ecological Applications*, 10(5), pp.1318–1326.

Ferrazzano, G.F. et al., 2015. Determination of the in vitro and in vivo antimicrobial activity on salivary Streptococci and Lactobacilli and chemical characterisation of the phenolic content of a Plantago lanceolata infusion. *BioMed research international*, 2015, p.286817.

Fitter, R., et al., 1984. *Collins guide to the grasses, sedges, rushes, and ferns of Britain and Northern Europe*, London: Collins.

Foley, J.A. et al., 2011. Solutions for a cultivated planet. *Nature*, 478(7369), pp.337–342.

French, K., 2014. The Medicinal Value of Some Oxfordshire Rare Plants: an Ethnobotanical Perspective. A talk delivered to the Ashmolean Natural History Society. University of Oxford. March 16, 2014.

Gallagher, J., Antibiotic resistance: World on cusp of "post-antibiotic era." *BBC News*. Available at: http://www.bbc.co.uk/news/health-34857015 [Accessed November 20, 2015].

Gardarin, A. et al., 2014. Plant trait–digestibility relationships across management and climate gradients in permanent grasslands. *Journal of Applied Ecology*, 51(5), pp.1207–1217.
Garg, N. & Chandel, S., 2010. Arbuscular mycorrhizal networks: process and functions. A review. *Agronomy for Sustainable Development*, 30(3), pp.581–599.
Gassmann, A. et al., 2006. The potential for biological control of invasive alien aquatic weeds in Europe: a review. In J. M. Caffrey et al., eds. *Macrophytes in Aquatic Ecosystems: From Biology to Management*. Developments in hydrobiology. Springer Netherlands, pp. 217–222.
Giagourta, P. et al., 2015. Phenotypic variation of *Lotus corniculatus* L. Natural populations in relation to habitat type and management regimes. *Archives of Biological Sciences*, 67(3), pp.841–848.
Githiori, J.B., Athanasiadou, S. & Thamsborg, S.M., 2006. Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. *Veterinary Parasitology*, 139(4), pp.308–320.
Girardi, F.A. et al., 2014. Phytochemical profile and antimicrobial properties of Lotus spp. (Fabaceae). *Anais Da Academia Brasileira De Ciencias*, 86(3), pp.1295–1302.
Githiori, J.B., Athanasiadou, S. & Thamsborg, S.M., 2006. Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. *Veterinary Parasitology*, 139(4), pp.308–320.
Godfray, H.C.J. et al., 2010. Food Security: The challenge of feeding 9 billion people. *Science*, 327(5967), pp.812–818.
González, J.A., García-Barriuso, M. & Amich, F., 2011. Ethnoveterinary medicine in the Arribes del Duero, western Spain. *Veterinary Research Communications*, 35(5), pp.283–310.
Grujic-Jovanovic, S. et al., 2004. Composition and antibacterial activity of the essential oil of six *Stachys* species from Serbia. *Flavour and Fragrance Journal*, 19(2), pp.139–144.
Gulluce, M. et al., 2007. Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp *longifolia*. *Food Chemistry*, 103(4), pp.1449–1456.
Hajlaoui, H. et al., 2008. Comparison of chemical composition and antimicrobial activities of *Mentha longifolia* L. ssp *longifolia* essential oil from two Tunisian localities (Gabes and Sidi Bouzid). *Annals of Microbiology*, 58(3), pp.513–520.
Hector, A. et al., 1999. Plant diversity and productivity experiments in European grasslands. *science*, 286(5442), pp.1123–1127.
Herman, A., Tambor, K. & Herman, A., 2016. Linalool Affects the Antimicrobial Efficacy of Essential Oils. *Current Microbiology*, 72(2), pp.165–172.
Hodgson, J.G., Montserrat-Martí, G., et al., 2005a. How much will it cost to save grassland diversity? *Biological Conservation*, 122(2), pp.263–273.
Hodgson, J.G., Grime, J.P., et al., 2005b. The impacts of agricultural change (1963–2003) on the grassland flora of Central England: processes and prospects. *Basic and Applied Ecology*, 6(2), pp.107–118.
Hofmann, M. & Isselstein, J., 2005. Species enrichment in an agriculturally improved grassland and its effects on botanical composition, yield and forage quality. *Grass and Forage Science*, 60(2), pp.136–145.
Huffman, M.A., 2003. Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. *The Proceedings of the Nutrition Society*, 62(2), pp.371–381.
Isbell, F. et al., 2015. Biodiversity increases the resistance of ecosystem productivity to climate extremes. *Nature*, 526(7574), pp.574–577.
Isselstein, J., 2005. Enhancing grassland biodiversity and its consequences for grassland management and utilization. In D. A. McGilloway, ed. *Grassland: a Global Resource*. Netherlands: Wageningen Academic Publishers, pp. 305–321.
Ivanova, D. et al., 2005. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethnopharmacology*, 96(1-2), pp.145–150.

Jaric, S. et al., 2015. An ethnobotanical survey of traditionally used plants on Suva planina mountain (south-eastern Serbia). *Journal of Ethnopharmacology*, 175, pp.93–108.

Jitea, M.I. & Arion, F.H., 2015. The role of agri-environment schemes in farm economic sustainability from high natural value Transylvanian areas. *Environmental Engineering and Management Journal*, 14(4), pp.943–953.

Johann, S. et al., 2008. Inhibitory effect of extracts from Brazilian medicinal plants on the adhesion of *Candida albicans* to buccal epithelial cells. *World Journal of Microbiology & Biotechnology*, 24(11), pp.2459–2464.

Karakas, F.P., Yildirim, A. & Turker, A., 2012. Biological screening of various medicinal plant extracts for antibacterial and antitumor activities. *Turkish Journal of Biology*, 36(6), pp.641–652.

Karlovsky, P., 2008. Secondary metabolites in soil ecology. In P. D. P. Karlovsky, ed. *Secondary Metabolites in Soil Ecology*. Soil Biology. Springer Berlin Heidelberg, pp. 1–19.

Kemp, P.D. et al., 2001. The forage value of poplars and willows. In *Proceedings of the Conference-New Zealand Grassland Association*. pp. 115–120.

Killick, J., 1998. *The flora of Oxfordshire*, Newbury: Pisces.

Knaus, W., 2013. Food security and dairy cow feeding: The necessity for a paradigm shift. *Agriculturae Conspectus Scientificus (ACS)*, 78(3), pp.149–152.

Kozlowska, M. et al., 2015. Chemical composition and antibacterial activity of some medicinal plants from Lamiaceae family. *Acta Poloniae Pharmaceutica*, 72(4), pp.757–767.

Lazarevic, J.S. et al., 2013. Chemical composition and antimicrobial activity of the essential oil of *Stachys officinalis* (L.) Trevis. (Lamiaceae). *Chemistry & Biodiversity*, 10(7), pp.1335–1349.

Loehr, G. et al., 2009. Antiadhesive effects of natural extracts out of *Myrothamnus flabellifolia* Welw. and *Rumex acetosa* L. against *Porphyromonas gingivalis*. *Planta Medica*, 75(9), pp.897–898.

Losvik, M.H., 2001. Variations in species richness in hay-meadow vegetation at Voss, west Norway, in relation to long-term management practices. *Norsk Geografisk Tidsskrift - Norwegian Journal of Geography*, 55(2), pp.77–84.

Lüscher, A. et al., 2014. Potential of legume-based grassland–livestock systems in Europe: a review. *Grass and Forage Science*, 69(2), pp.206–228.

Ma, J.K.-C. et al., 2005. Plant-derived pharmaceuticals – the road forward. *Trends in Plant Science*, 10(12), pp.580–585.

MacAdam, J.W. & Villalba, J.J., 2015. Beneficial effects of temperate forage legumes that contain condensed tannins. *Agriculture*, 5(3), pp.475–491.

Maddock, A., 2011. UK Biodiversity Action Plan priority habitat descriptions. Available at: http://www.jncc.gov.uk/page-5155.

de Macedo, M., et al. 2015. "Anti-infective agents: The example of antibacterial drug leads." In *Ethnopharmacology*. Heinrich, Michael and Anna K. Jäger, eds. Wiley Blackwell. Pp. 111-123.

Makkar, H.P.S., Francis, G. & Becker, K., 2007. Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. *Animal: An International Journal of Animal Bioscience*, 1(9), pp.1371–1391.

Martin, B. et al., 2005. How do the nature of forages and pasture diversity influence the sensory quality of dairy livestock products? *Animal Science*, 81(02), pp.205–212.

Matesanz, S., Gianoli, E. & Valladares, F., 2010. Global change and the evolution of phenotypic plasticity in plants. *Annals of the New York Academy of Sciences*, 1206, pp.35–55.

Mikaili, P. et al., 2013. Pharmacological and therapeutic effects of Mentha Longifolia L. and its main constituent, menthol. *Ancient Science of Life*, 33(2), pp.131–138.

Miraglia, M. et al., 2009. Climate change and food safety: an emerging issue with special focus on Europe. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*, 47(5), pp.1009–1021.
Misra, M., 2009. Mobile pastoralists in Iran's arid lands. *International Journal of Environmental Studies*, 66(3), pp.357–370.
Morris, R.K.A. et al., 2006. The creation of compensatory habitat—Can it secure sustainable development? *Journal for Nature Conservation*, 14(2), pp.106–116.
Muller, S., 2002. Appropriate agricultural management practices required to ensure conservation and biodiversity of environmentally sensitive grassland sites designated under Natura 2000. *Agriculture, Ecosystems & Environment*, 89(3), pp.261–266.
Natural England, 1999. Lowland Grassland Management Handbook (Second edition). *Natural England - Access to Evidence*. Available at: http://publications.naturalengland.org.uk/publication/35034
Natural England, 2012. Higher Level Stewardship: Environmental Stewardship Handbook, Fourth Edition. *Natural England - Access to Evidence*. Available at: http://publications.naturalengland.org.uk/publication/2827091 [Accessed October 15, 2015].
Naylor, R. et al., 2005. Losing the Links between Livestock and Land. *Science*, 310 (5754), pp.1621–1622.
Noni, I.D. & Battelli, G., 2008. Terpenes and fatty acid profiles of milk fat and "Bitto" cheese as affected by transhumance of cows on different mountain pastures. *Food Chemistry*, 109(2), pp.299–309.
Noor Alhamad, M., 2006. Ecological and species diversity of arid Mediterranean grazing land vegetation. *Journal of Arid Environments*, 66(4), pp.698–715.
Noor, Y., 2014. Food vs feed: The livestock equation in food security. Presented at the "Ethics, Efficiency and Food Security" The Crawford Fund 2014 Annual Parliamentary Conference, Parliament House Canberra ACT, Australia, August 26–28.
O'Mara, F.P., 2012. The role of grasslands in food security and climate change. *Annals of Botany*, 110(6), pp.1263–1270.
Oxfordshire County Council, Oxfordshire's Biodiversity Action Plan. *http://www.oxfordshire.gov.uk*. Available at: https://www.oxfordshire.gov.uk/cms/content/oxfordshires-biodiversity-action-plan [Accessed October 23, 2015].
Ozkan, O. et al., 2016. Determination of antibacterial activities of different Thymus praecox subsp. grossheimii var. grossheimii extracts. *Indian Journal of Animal Research*, 50(1), pp.85–88.
Ozen, T., Demirtas, I. & Aksit, H., 2011. Determination of antioxidant activities of various extracts and essential oil compositions of *Thymus praecox* subsp. *skorpilii* var. *skorpilii*. *Food Chemistry*, 124(1), pp.58–64.
Parniske, M., 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology*, 6(10), pp.763–775.
Pe'er, G. et al., 2014. EU agricultural reform fails on biodiversity. *Science*, 344(6188), pp.1090–1092.
Peeters, A., 2009. Importance, evolution, environmental impact and future challenges of grasslands and grassland-based systems in Europe. *Grassland Science*, 55(3), pp.113–125.
Pesavento, G. et al., 2015. Antibacterial activity of *Oregano*, *Rosmarinus* and *Thymus* essential oils against *Staphylococcus aureus* and *Listeria monocytogenes* in beef meatballs. *Food Control*, 54, pp.188–199.
Petrovic, N.V. et al., 2016. Chemical composition, antioxidant and antimicrobial activity of *Thymus praecox* supercritical extracts. *Journal of Supercritical Fluids*, 110, pp.117–125.
Piluzza, G., Sulas, L. & Bullitta, S., 2014. Tannins in forage plants and their role in animal husbandry and environmental sustainability: a review. *Grass and Forage Science*, 69(1), pp.32–48.

Provenza, F.D. & Villalba, J.J., 2010. The role of natural plant products in modulating the immune system: An adaptable approach for combating disease in grazing animals. *Small Ruminant Research*, 89(2–3), pp.131–139.

Radulovic, N.S. et al., 2013. Antimicrobial plant metabolites: Structural diversity and mechanism of action. *Current Medicinal Chemistry*, 20(7), pp.932–952.

Rath, M. & Peel, S., 2005. Grassland in Ireland and the UK. In D. A. McGilloway, ed. *Grassland: a Global Resource*. Netherlands: Wageningen Academic Publishers, pp. 13–28.

Reiné, R. et al., 2014. Pyrenean meadows in Natura 2000 network: grass production and plant biodiversity conservation. *Spanish Journal of Agricultural Research*, 12(1), pp.61–77.

Ridding, L.E., Redhead, J.W. & Pywell, R.F., 2015. Fate of semi-natural grassland in England between 1960 and 2013: A test of national conservation policy. *Global Ecology and Conservation*, 4, pp.516–525.

Robinson, R.A. & Sutherland, W.J., 2002. Post-war changes in arable farming and biodiversity in Great Britain. *Journal of Applied Ecology*, 39(1), pp.157–176.

Rodwell, J.S., ed., 1992. *British Plant Communities. Volume 3. Grassland and montane communities*, Cambridge: Cambridge University Press.

Rodwell, J.S. ed., 1995. *British Plant Communities. Volume 4. Aquatic communities, swamps and tall-herb fens*, Cambridge: Cambridge University Press.

Rose, F., 1989. *Color identification guide to the grasses, sedges, rushes and ferns of the British Isles and north-western Europe*, London: Viking.

Rowe, A.G., 1999. The exploitation of an arid landscape by a pastoral society: the contemporary eastern Badia of Jordan. *Applied Geography*, 19(4), pp.345–361.

Sala, O.E. et al., 2000. Global Biodiversity Scenarios for the Year 2100. *Science*, 287(5459), pp.1770–1774.

Samoilova, Z. et al., 2014. Medicinal plant extracts can variously modify biofilm formation in *Escherichia coli*. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, 105(4), pp.709–722.

Sarac, N. & Ugur, A., 2009. The in-vitro antimicrobial activities of the essential oils of some Lamiaceae species from Turkey. *Journal of Medicinal Food*, 12(4), pp.902–907.

Sanderson, M.A. et al., 2005. Forage mixture productivity and botanical composition in pastures grazed by dairy cattle. *Agronomy Journal*, 97(5), p.1465.

Sesterhenn, R. et al., 2015. In vitro evaluation of antimicrobial activities of *Eugenia caryophyllata* and *Origanum vulgare* against avian *Escherichia coli* isolates. *Acta Scientiae Veterinariae*, 43, p.1312.

Scotton, M., Sicher, L. & Kasal, A., 2014. Semi-natural grasslands of the Non Valley (Eastern Italian Alps): Agronomic and environmental value of traditional and new Alpine hay-meadow types. *Agriculture, Ecosystems & Environment*, 197, pp.243–254.

Stamatis, G. et al., 2003. In vitro anti-Helicobacter pylori activity of Greek herbal medicines. *Journal of Ethnopharmacology*, 88(2–3), pp.175–179.

Stojanović, G. et al., 2005. In vitro antimicrobial activity of extracts of four *Achillea* species: the composition of *Achillea clavennae* L. (Asteraceae) extract. *Journal of Ethnopharmacology*, 101(1-3), pp.185–190.

Tabini, R. Al-, Khalidi, K. Al- & Shudiefat, M. Al-, 2012. Livestock, medicinal plants and rangeland viability in Jordan's Badia: through the lens of traditional and local knowledge. *Pastoralism: Research, Policy and Practice*, 2(1), p.4.

Tallowin & Jefferson, 1999. Hay production from lowland semi-natural grasslands: a review of implications for ruminant livestock systems. *Grass and Forage Science*, 54(2), pp.99–115.

Taube, F. et al., 2014. Grassland and globalization – challenges for north-west European grass and forage research. *Grass and Forage Science*, 69(1), pp.2–16.

Tilman, D., Hill, J. & Lehman, C., 2006. Carbon-negative biofuels from low-input high-diversity grassland biomass. *Science*, 314(5805), pp.1598–1600.

Tilman, D., Isbell, F. & Cowles, J.M., 2014. Biodiversity and ecosystem functioning. *Annual Review of Ecology, Evolution, and Systematics*, 45(1), pp.471–493.

Toro-Mujica, P.M. et al., 2015. Changes in the pastoral sheep systems of semi-arid Mediterranean areas: association with common agricultural policy reform and implications for sustainability. *Spanish Journal of Agricultural Research*, 13(2), p.0102.

Tscharntke, T. et al., 2012. Global food security, biodiversity conservation and the future of agricultural intensification. *Biological Conservation*, 151(1), pp.53–59.

Uzun, F., Donmez, H.B. & Ocak, N., 2015. Genetic potential of wild birdsfoot trefoil (*Lotus corniculatus* L.) seeds collected from different geographical locations regarding to nutrient composition and nutritive value. *Agroforestry Systems*, 89(6), pp.963–972.

Vasta, V. et al., 2008. Alternative feed resources and their effects on the quality of meat and milk from small ruminants. *Animal Feed Science and Technology*, 147(1–3), pp.223–246.

Villalba, J.J. & Provenza, F.D., 2009. Learning and dietary choice in herbivores. *Rangeland Ecology & Management*, 62(5), pp.399–406.

Vogl, S. et al., 2013. Ethnopharmacological in vitro studies on Austria's folk medicine—An unexplored lore in vitro anti-inflammatory activities of 71 Austrian traditional herbal drugs. *Journal of Ethnopharmacology*, 149(3), pp.750–771.

Waghorn, G.C. & McNabb, W.C., 2003. Consequences of plant phenolic compounds for productivity and health of ruminants. *The Proceedings of the Nutrition Society*, 62(2), pp.383–392.

Waller, P.J. et al., 2001. Plants as de-worming agents of livestock in the Nordic countries: Historical perspective, popular beliefs and prospects for the future. *Acta Veterinaria Scandinavica*, 42(1), p.31.

Watkins, F. et al., 2012. Antimicrobial assays of three native British plants used in Anglo-Saxon medicine for wound healing formulations in 10th century England. *Journal of Ethnopharmacology*, 144(2), pp.408–415.

Wegiera, M., Kosikowska, U., Malm, A. & Smolarz, H.D., 2011. Antimicrobial activity of the extracts from fruits of *Rumex* L. species. *Central European Journal of Biology*, 6(6), pp.1036–1043.

Yee, T.W. & Mitchell, N.D., 1991. Generalized additive models in plant ecology. *Journal of Vegetation Science*, 2(5), pp.587–602.

Zent, S., 2009. Traditional Ecological Knowledge (TEK) and biocultural diversity: A close-up look at linkages, learning trends & changing patterns of transmission. In P. Bates et al., eds. *Learning and Knowing in Indigenous Societies Today*. Paris: UNESCO, pp. 102–121.

CHAPTER 3

CALCULATING THE BIOENERGY POTENTIAL OF SPECIES-RICH GRASSLANDS IN LOWLAND ENGLAND BASED ON LIGNOCELLULOSIC COMPOSITION AND LAND COVER AREA

Abstract

Producing bioenergy from species-rich grasslands may be a sustainable alternative to generating electricity and gas from nutrient-hungry crops such as maize, cereals, and rapeseed while also promoting biodiversity conservation. However, the bioenergy potential of grasslands is widely debated: detractors argue high levels of lignin in grass reduce bioenergy yields and that the bioenergy yield of grasslands cannot compete with other bioenergy crops. This paper aims to (1) establish whether the lignocellulosic composition of species-rich grasslands serves as a barrier to bioenergy production, (2) determine the effect of biodiversity (in terms of both species richness and composition) on biogas yield, and (3) establish whether grassland biogas yields can compete with those of other bioenergy crops. I examined the lignocellulosic composition and biogas yields of biomass samples from 13 grasslands in Oxfordshire, England and compared these values to 73 other biofuel feedstocks. I then estimated and compared the biogas yield of species-rich grasslands (SSSIs and grasslands currently managed under agro-environmental schemes) and Miscanthus fields in the county. Species richness, hemicellulose content, lignin content, and the abundance of specific grassland species were associated with increased biogas yield. The lignin content of species-rich grass was up to 50% less than other bioenergy crops and was comparable to that of crop waste and switchgrass. Species-rich grasslands yielded up to 160% more biogas per ton dry matter than cereals or crop waste and only slightly less than Miscanthus. SSSIs and grasslands managed under agro-environmental schemes were larger (20.57 ha and 8 ha respectively) than Miscanthus fields (5.95 ha) and produced up to 117% more biogas per average field. Producing bioenergy from species-rich grasslands can thu contribute to biodiversity conservation and local economies, and ultimately, to meeting UK national bioenergy goals.

1. Introduction

Primary bioenergy crops like maize and jatropha were once hailed as an environmentally friendly and economically attractive alternative to fossil fuels (Openshaw 2000). Bioenergy can be produced from plants by breaking down cellulose, sugars and starch to produce ethanol or by anaerobic digestion of plant material to produce biogas (Halford 2015). However, we now know that expanded cultivation of primary bioenergy crops has led to the loss of local biodiversity, destruction of soil microbial communities, and increased competition between food and fuel production (Blanchard et al. 2015; Prochnow et al. 2009; Hellmand and Verburg 2010; Brown and Li 2012).

Bioenergy production from lignocellulosic biomass, and biomass from species-rich grasslands in particular, may serve as a more sustainable alternative to bioenergy crops such as wheat and maize. Lignocellulosic biomass refers to substrates with high levels of cellulose and hemicellulose bound together by lignin. Microbial digestion of this biomass is required to break down this material to produce biogas, ethanol and electricity. Recent advances in anaerobic digestion technology and the use of specific pretreatments (e.g. steam explosion) have been shown to double biogas yields (Bauer et al. 2014; Teghammer et al. 2010), meaning that the lignin content of these feedstocks is no longer an insurmountable obstacle to biogas production (Himmel et al. 2007; Liao et al. 2016). Examples of lignocellulosic biomass include crop wastes, paper, yard clippings, Miscanthus (*Micanthus* x *giganteus* Keng), Switchgrass (*Panicum virgatum* L.) and Short Rotation Coppice (*Salix* spp. and *Populus* spp.) (Karp and Shield 2008; Sharma et al. 2011; Streffer 2014). These are all sources of extraneous, residual biomass that could be converted to bioenergy.

However, previous studies provide conflicting results on the viability of producing bioenergy from species-rich grasslands. Research on experimental grasslands and prairies in the US have estimated that species-rich grasslands produce ethanol and biogas yields competitive with those of Switchgrass and Miscanthus (Adler et al. 2009; Sanderson and Adler 2008; Thumm et al. 2014). In addition, Tilman et al. (2006) and Fischer et al. (2009) have argued that grasslands could produce more bioenergy than current bioenergy crops like maize and wheat on a national and global scale because they cover larger geographic areas. In contrast, several studies have reported that grass lignin levels are too high and that their bioenergy yield cannot compete with that of other bioenergy crops like Miscanthus (Frigon and Giuiot 2010; Triolo et al. 2012). In addition, Russelle et al. (2007) and Steubing et al. (2010) have argued that studies promoting bioenergy production from grasslands are overly optimistic and may overestimate the potential land available for bioenergy production. A more detailed study comparing the lignocellulosic composition and bioenergy yield of species-rich grassland

to other bioenergy feedstocks is thus needed. In addition, a regional scale assessment comparing the potential biogas yield of grasslands and fields currently sown with bioenergy crops would help determine the agricultural and economic value of grasslands for bioenergy production.

The present analysis will focus on the bioenergy potential of species-rich grasslands in lowland England and specifically the county of Oxfordshire as a case study. Specifically, I aim to (1) establish whether the lignocellulosic composition of species-rich grasslands serves as a barrier to bioenergy production, (2) determine the effect of biodiversity (in terms of both species richness and composition) on biogas yield, and (3) establish whether grassland biogas yields can compete with those of other bioenergy crops. This analysis is motivated by increased interest and investment in bioenergy production in the UK since 2004 with the launch of Defra's strategy for the production and use of non-food crops in 2004 (Defra 2006, 2007). The core aim of this strategy is to expand the area used to cultivate bioenergy crops up to 1 million hectares (17% of UK arable land) by 2020, and ultimately, to reduce carbon emissions by 60% by 2050, a goal shared by other EU countries and the US (Defra 2007; Lynd et al. 2011). At the same time, there is local interest in alternative uses of grassland biomass (in part, due to a decrease in the agricultural value of unimproved grassland for livestock production) and in increasing reliance on renewable energy. Producing bioenergy from species-rich grasslands would provide a low-cost, low-maintenance alternative to bioenergy production from other crops such as wheat, maize and Miscanthus and would help maintain the ecosystem services these landscapes provide.

To better understand the bioenergy potential of local grasslands, I examine the effect of species richness on the lignocellulosic content and biogas yield of biomass samples from species-rich grasslands and identify the species associated with increased biogas yields. I then compare the lignocellulosic composition and biogas yield of these samples to 73 current bioenergy feedstocks. To

assess the potential value of grassland biomass as a bioenergy crop in Oxfordshire, I estimated and compared the potential bioenergy yield of land covered by species-rich grasslands (SSSIs and agroenvironmental schemes) and land currently used for growing Miscanthus. The overall goal of this analysis is to test whether producing bioenergy from excess grassland biomass is a feasible future use for grasslands currently managed for conservation in the UK and northern Europe where bioenergy production from non-food crops is increasingly prioritized.

2. Methods

2.1 Study Area and Selection of Fields

The county of Oxfordshire is located in south-east England. It has a maritime temperate climate with an annual rainfall of between 570 -750 mm (depending on elevation) (Killick *et al.* 1998). Local farms engage in arable and livestock production. The primary crops are wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and rapeseed (*Brassica napus* L.). Miscanthus and Short Rotation Coppice are currently grown as bioenergy crops. While there are currently six anaerobic digesters in the region, none process biomass from local grasslands (**Table 1**) (The National Non-Food Crops Center (NNFCC) database, http://www.nnfcc.co.uk/). However, they do process other forms of lignocellulosic biomass, specifically, crop waste, cereals, maize, and ryegrass. Throughout England, there are 258 anaerobic digesters, 32 of which use grass silage and/or ryegrass as one of their feedstocks.

Table 1 Anaerobic digestion facilities in Oxfordshire, England. Most digesters convert biomass to electricity. Several process crop and food waste and one processes grass silage. In the chart, kWe = Kilowatt-electric (One thousand watts of electric capacity) and Nm^3 biogas = Normal meters cubed of biogas. Data source: The National Non-Food Crops Center (NNFCC).

Site	Date of Completion	Capacity (kWe)	Capacity (Nm^3 biogas/ hr)	Feedstock	Feed Demand (tons per annum)
Battle Farm	2013	2,400		Food waste, crop silage	45,000
Cassington AD	2010	2,100		Food waste	50,000
Stuchbury Manor Farm	2012	500		Animal slurry, maize, grass silage	12,800
Kraft Food AD	2008	250		Coffee waste	10,000
Grange Farm	2014	500		Maize, sugar beet, poultry litter, slurry	14, 125
Icknield Farm	2014	250	330	Cereal crops, maize , pig slurry	34,000
				Total capacity	**165, 925**

From June-July 2015, I collected biomass samples from 13 grasslands shown in **Figure 1**. These sites consisted of nine species-rich hay meadows (seven unimproved meadows, four conservation (restored) meadows), and two improved grasslands. Initially, the research design was balanced and sought to collect samples from five unimproved, five conservation and five improved grasslands. However, during fieldwork it became clear that three sites initially identified as conservation grasslands were unimproved and three identified as improved had recently undergone arable reversion. In addition, upon visiting two of the conservation grassland study sites, volunteers had cut the hay before the site was visited, excluding these sites from the analysis.

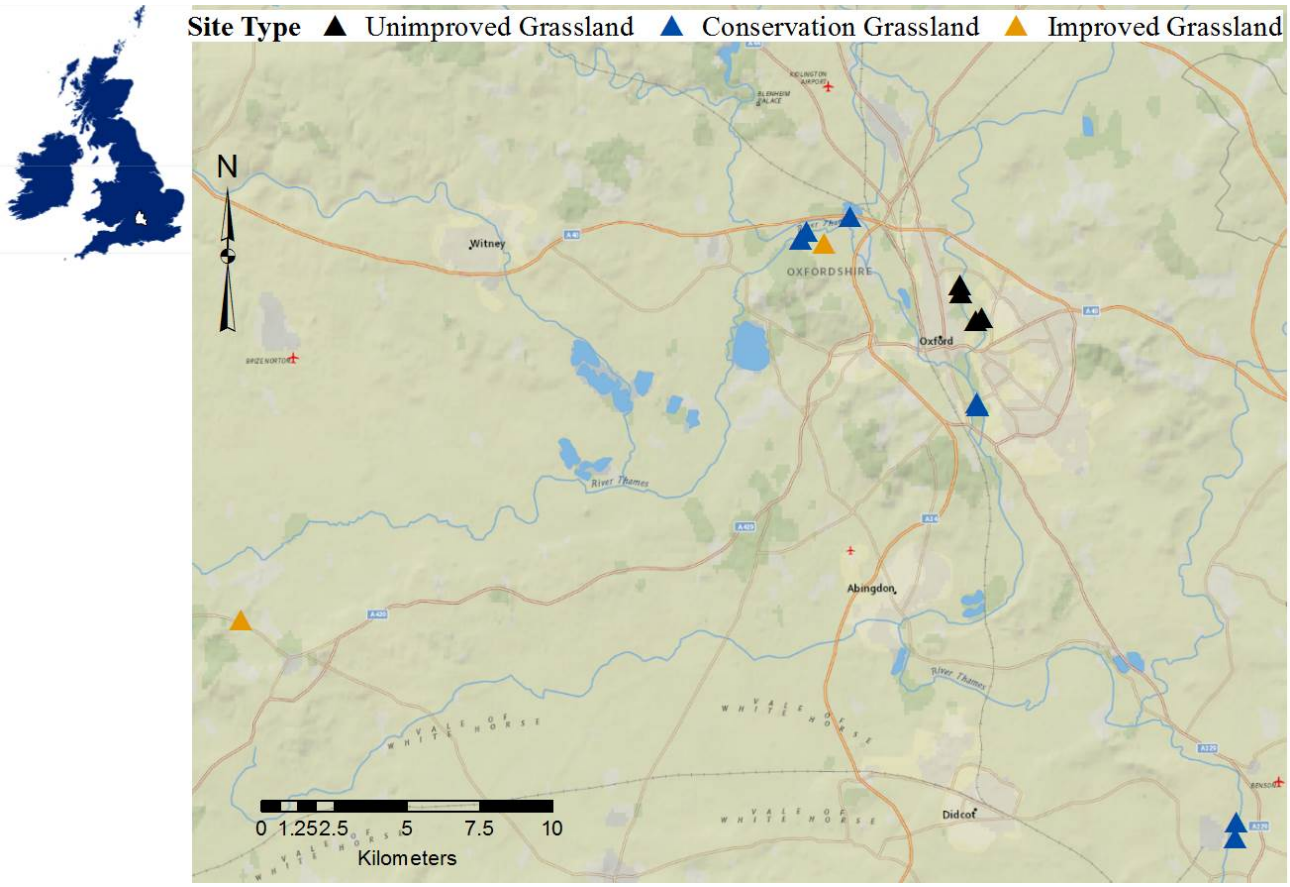


Figure 1 Map of Oxfordshire, England showing sites where biomass samples were taken. Sites (triangles) are color coded by grassland type. Black = unimproved grasslands, Blue = conservation grasslands, Yellow = improved grasslands.

Unimproved grasslands are fields which have not been used for arable production in living memory. Many (over 100) are considered Sites of Special Scientific Interest and protected for their rare flora (Aitchison et al. 2001). 'Conservation' grasslands are sites that have been reverted from arable cultivation (e.g. of barley, wheat etc.) to species-rich grassland. Sites under arable reversion have been re-sown with commercial seed mixtures that mimic the species composition of unimproved grasslands. Conservation groups manage these sites around the ecology of particular rare charismatic species like fritillaries (*Fritillaria meleagris* L.) and orchids (*Orchis militaris* L., *Orchis anthropophora* (L.) All.). Both unimproved and conservation grasslands are cut between late June-late August, depending on what type of conservation and/or agro-environmental agreement they are managed under and

subsequently grazed by livestock. Improved grasslands are sown annually or biannually with short term ley mixes composed of ryegrass (*Lolium perenne* L.), and red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), and sometimes Lucerne (*Medicago sativa* L.). Farmers apply nitrogen, phosphorus, potash, and sometimes livestock manure to these fields.

2.2 Vegetation Surveys

At each site, vegetation surveys were conducted to determine the species composition and richness. To ensure these surveys were representative of the overall vegetation at each site, I excluded a 1 m margin around each field (which can reflect 'disturbed' vegetation as opposed to the vegetation character of the main field. To determine species composition, five $1m^2$ quadrats were randomly placed at each field and the species present in each quadrat were recorded. The abundance of each species was based on the number of quadrats it occurred in. Quadrats were not selected beforehand because farmers decided which grasslands could be sampled on the day of the site visit.

2.3 Biomass Sample Collection and Analysis

At each site, I collected biomass samples of ca. 150 grams based on guidelines provided by the Agri-Food and Biosciences Institute (AFBI) (Belfast, Northern Ireland) (http://www.afbini.gov.uk/). I cut handfuls of grass ca. 10 cm from the ground with shears at ca. 20 different locations within each field to capture the overall variation in biomass composition of each field. During collection, grass was placed in a canvas bag to prevent sweating (and the start of any 'ensiling' processes, which can change the chemical composition of the sample). After each day of collection, I brought samples to the lab and recorded their species content. To test how representative the biomass sample was for each field, I compared the botanical content to the list of species recorded from the $1m^2$ quadrats. In each case, the

species found in the biomass samples and in the quadrats were the same. I then mailed the fresh samples to the Agri-Food and Biosciences Institute (AFBI) agricultural research center in Hillsborough. Upon receiving the samples, the Agri-Food and Biosciences Institute dried and milled the samples. Wet chemistry was used to establish the dry matter content (DM), acid detergent fiber content (ADF), neutral detergent fiber content (NDF) and lignin content (ADL) of each sample. I calculated the lignocellulosic content of each sample using the data received from AFBI using the following equations based on previous protocols used by Adler et al. (2009):

$$\text{Cellulose content} = \text{ADL} - \text{ADF} \quad (1)$$

$$\text{Hemicellulose content} = \text{NDF} - \text{ADF} \quad (2)$$

The lignin content of each sample was based on the ADL values provided by AFBI.

2.4 Collection of Comparative Data on Bioenergy Feedstocks

To compare the lignocellulosic composition of species-rich grass to other bioenergy feedstocks, I collected data on 73 contemporary bioenergy crops from several databases (**Table 2**). The Energy Research Center of the Netherland's Phyllis2 database (https://www.ecn.nl/phyllis2/) provides the chemical composition of major bioenergy feedstocks. In addition, I also collected data from the multi-national project *Feedapedia*, a forage database run jointly by INRA (Institut National de la Recherche Agronomique, French National Institute for Agricultural Research), CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement, French Agricultural Research Center for International Development, AFZ (Association Française de Zootechnie, French Association for Animal Production) and the FAO (Food and Agriculture Organization of the United Nations) (http://www.feedipedia.org/). Only samples with data on cellulose, hemicellulose and lignin content

were included in the analysis. An attempt was made to include at least three examples of each feedstock but unfortunately this was not possible for all crops.

Table 2 List of contemporary bioenergy feedstocks that were compared to biomass from species-rich grasslands. The lignocellulosic composition of biomass from species-rich grasslands was compared to all feeds listed in Table 1. The biogas yield of biomass from species-rich grasslands was only compared to those feedstocks marked with a *.

Feedstock	No.	Species (if applicable)
Agave	1	*Agave* sp.
Bamboo	1	*Bambuseae* sp. Kunth ex Dumort
***Cereals**	12	wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), and maize (*Zea mays* L.)
***Crop waste**	8	corn stover (maize stalks) and straw from wheat, barley and oats
***Grass**	15	Timothy (*Phleum pratense* L.), orchard grass (*Dactylis glomerata* L.), Bromegrass (*Bromus* sp.), Big Bluestem (*Andropogon gerardi* Vitman), Tall Fescue (*Festuca arundinacea* Schreb.), Reed Canary Grass (*Phalaris arundinacea* L.), and verge grass
***Hemp**	2	*Cannabis sativa* L.
***Kenaf**	2	*Hibiscus cannabinus* L.
***Legumes**	12	lucerne (*Medicago sativa* L.), clover (*Trifolium* spp), and soybean (*Glycine max* (L.) Merr.)
***Miscanthus**	3	*Miscanthus* x *giganteus* Keng
Newsprint/Paper	6	recycled paper, newsprint, and domestic paper waste
***Rapeseed**	3	*Brassica napus* L.
***Sisal**	2	*Agave sisalana* Perrine
Sugarcane	2	*Saccharum* sp. L.
***Switchgrass**	4	*Panicum virgatum* L
Total	**73**	

I compared the biogas yield and methane content of the species-rich grass samples from Oxfordshire to a subset of these bioenergy feedstocks (cereals, crop wastes, grass, Miscanthus, legumes, rapeseed, and switchgrass). Unfortunately, I could not calculate the bioenergy yield for five feedstocks (newsprint, agave, bamboo, hemp, and kenaf) due to absence of dry matter content (DM) data.

2.5 Estimated Bioenergy Output

There are several methods of calculating biogas yield: by physically measuring the methane output of the substrate using Biochemical Methane Potential (BMP) tests; by using the chemical composition of

the substrate; and by multiplying biomass yield by a fixed ratio of ethanol (in liters (L)) or biogas (Nm^3) per dry matter (DM) unit (kg or tons) (Capareda 2013; Triolo et al. 2011). In this analysis, I used cellulose, hemicellulose, and lignin content to calculate the potential bioenergy yield of each feedstock based on recent advances demonstrating the degradability of these materials.[11] It is also more precise than calculating bioenergy yield based on biomass alone, as it accounts for variation in bioenergy yield based on organic composition.

First, I established the biogas yield of lignin, cellulose, and hemicellulose using the Buswell formula ($C_cH_hO_oN_nS_s + \{(4c - h - 2o + 3n + 2s)/4\} H_2O \rightarrow \{(4c - h + 2o + 3n + 2s)/8\} CO_2 + \{(4c + h - 2o - 3n - 2s)/8\} CH_4 + nNH_3 + sH_2S$) (Symons and Buswell 1933; Teghammer 2013; Triolo et al. 2012). In this formula, the subscripts $_{c, h, o, n}$ and $_s$ (in blue in the equation) refer to the number of molecules of C, H, O, N and S in each substrate. I used this formula to calculate the H_20, CO_2 and CH_4 content for 1 kg of cellulose, hemicellulose, and lignin. As there are three chemical formulas for lignin ($C_9H_{10}O_2$, $C_{10}H_{12}O_3$, and $C_{11}H_{14}O_4$), I calculated the molar mass, carbon yield and methane yield for each and used the average of the three.

To calculate the carbon and methane yields of cellulose, hemicellulose and lignin, I used the formulae CO_2yield = V/molar mass and CH_4yield=V/molar mass, where V= nRT/p. In V = nRT/p, n = amount of substance (mol), R = gas constant (L atm K^{-1} mol^{-1}), T = absolute temperature (K), and p = absolute pressure of the gas (atm). In this analysis, R was set at 0.08205747 L atm K^{-1} mol^{-1}, T was set at 273.15 K, and p was set at 1 atm according to previously established protocols for estimating biogas

[11] Previous studies have estimated biogas yield based on the fat/lipid, protein and/or carbohydrate content of the substrate. In this analysis, biogas yield was based on cellulose, hemicellulose because the bioenergy yield from lignocellulosic feedstocks (e.g. species-rich grass, Miscanthus, corn stover, etc.) is derived from the carbon dioxide and methane produced from the digestion of these plant cell components. In addition, fat/lipid and protein contents were not available for all feedstocks, limiting the number of possible comparisons had these values been included. The values of protein and fats/lipids are very low for lignocellulosic materials so this should make little difference to the total bioenergy yield.

yield (Teghammer 2013; Richards et al. 2001). The total biogas yield was calculated as CO_2yield + CH_4yield. To obtain the % of methane in the gas, the ratio of CO_2 to CH_4 was calculated.

Table 3 lists the total biogas yield and methane yield of cellulose, hemicellulose, and lignin. Lignin has the highest biogas and methane yield followed by hemicellulose, in part, due to their molecular weight. The potential bioenergy from lignocellulosic substrates is comparable to the other components usually used to infer potential methane output (namely, fats/lipids, protein and carbohydrate). The biogas yield of lignin and hemicellulose is similar to fat/lipid ($C_{57}H_{104}O_6$) (1.4 Nm^3/kg) although the methane concentration of fats/lipids is much higher (70%). The biogas yields of protein ($C_5H_7O_2N$) (1.0 Nm^3/kg) and carbohydrate ($C_6H_{12}O_6$ Nm^3/kg) (0.8 Nm^3/kg) are lower than cellulose, hemicellulose, and lignin although their methane output is similar (50%).

Table 3 Biogas yield from Cellulose, Hemicellulose and Lignin based on the Buswell Formula. Lignin and hemicellulose produce more biogas per kg than cellulose.

	Cellulose ($C_6H_{10}O_5$)	Hemicellulose ($C_{31}H_{34}O_{11}$)	Lignin (Average)
H_2O	1.75	17.75	15.66667
CO_2	4.5	18.5	5.75
CH_4	4.75	16.75	6
Molar mass (g/mol)	**176.1473**	**596.6018**	**180.2005**
Carbon dioxide volume (V = nRT/p)	100.863	414.659	128.8805
Carbon yield (Nm^3/ kg)	**0.572606**	**0.695035**	**0.718163**
Methane volume (V = nRT/p)	106.4665	375.4345	134.484
Methane yield (Nm^3/ kg)	**0.604417**	**0.629288**	**0.746298**
Total Biogas Yield (per kg)	**1.177023**	**1.324323**	**1.464462**
Ratio CO_2 toCH_4	1.13:1.19	9.25:8.38	5.75:6
% that is methane	**51.35**	**47.52**	**50.97**

To calculate the biogas output of the biomass samples from Oxfordshire and other bioenergy feedstocks, I calculated the biogas yields of cellulose, hemicellulose and lignin for 1 ton dry matter of each sample based on previous established protocols (see Hafner et al. 2015; Teghammer 2013;

Rittmann et al. 2001). First, I calculated the biogas yield of cellulose, hemicellulose and lignin as follows based on the percent of each component in each sample:

Biogas yield of Cellulose (**BYC**) = 1000 kg (wet weight) x % DM (%TS of wet weight) x 0.9 (% VS of TS) x % cellulose x 1.18 (Nm^3 biogas/ t cellulose) (3)

Biogas yield of Hemicellulose (**BYH**) = 1000 kg (wet weight) x % DM (%TS of wet weight) x 0.9 (% VS of TS) x % hemicellulose x 1.32 (Nm^3 biogas/ t hemicellulose) (4)

Biogas yield of Lignin (**BYL**) = 1000 kg (wet weight) x % DM (%TS of wet weight) x 0.9 (% VS of TS) x % lignin x 1.46 (Nm^3 biogas/ t lignin) (5)

In equations 3-5, the DM of each sample is used as the total solids (TS) of the wet sample (e.g. fresh grass). 0.9 is the percent volatile solids (VS) of the total solids. This refers to the amount of substrate which is actively converted into biogas. It allows for up to 10% of the substrate to be consumed by bacteria during the anaerobic digestion process. The percent cellulose, hemicellulose and lignin refers to the amount (% DM) of each component in each sample. All values in blue are values unique to each sample (e.g. a sample may be composed of 90% DM, 30% cellulose, 20% hemicellulose and 15% lignin). This is then multiplied by the biogas yield per kg of cellulose (1.18 Nm^3), hemicellulose (1.32 Nm^3), and lignin (1.46 Nm^3). These calculations result in the biogas yield of the cellulose, hemicellulose and lignin in each sample.

To obtain the total biogas yield, methane yield and methane concentration per sample, I used the following equations (again, modified from pre-established protocols):

Sum biogas yield per sample (**SB**) = BYC + BYH + BYL = biogas yield of sample (Nm^3/t) (6)

Sum methane yield per sample (**SM**) = BYC (0.51) + BYH (0.48) + BYL (0.51) = methane yield

(Nm^3/t) (7)

Methane concentration = **SM**/**SB** (8)

In equation 7 to establish total methane (SM), 0.51 refers to the methane concentration of cellulose, 0.48 refers to the methane concentration of hemicellulose and 0.51 refers to the methane concentration of lignin (see **Table 3**). I used equations 6-8 to obtain the total biogas yield, methane yield and methane concentration for each sample. The advantage of this method is that the biogas yield of any feedstock can be calculated as long as the DM, cellulose, hemicellulose, and lignin content of the feedstock is known and the results are easily reproducible.

2.6 Comparison of Grassland and Energy Crop Area and Yield

To compare the area covered by species-rich grassland to that used for cultivating bioenergy crops in Oxfordshire, data on the total area of SSSIs, grasslands under agro-environmental schemes, and fields sown with Miscanthus and Short Rotation Coppice in the UK was obtained from Natural England (http://www.geostore.com/environment-agency/WebStore?xml=environment-agency/xml/ogcDataDownload.xml). This data is public sector information licensed under the Open Government License v1.0. All maps were created using using ArcGIS® software by Esri.

To calculate the potential biomass yield of species-rich grassland and of cropland dedicated to bioenergy production in the county, I used the average low and average high biomass yield per hectare (ha) of high diversity grasslands and Miscanthus from northern Europe. The potential yield of grassland biomass was estimated at 8-10 tons dry matter (t/DM) per ha based on previous estimates of average grassland biomass yields in the UK and northern Europe (Amon et al. 2006; Rösch et al. 2009;

Seppälä et al. 2009).[12] The potential yield of Miscanthus was estimated at 10-14 t/ha based on previous research by the Biomass Energy Center and the UK Forestry Commission (Biomass Energy Center 2008).

To estimate the biogas yield per field for SSSIs and grasslands managed under agroenvironmental schemes, I calculated the average yield per ha for grasslands based on the lowest average biomass yield for grasslands (8 t/ha) and the average biogas yield of species-rich grasslands estimated in section 3.3. To estimate the biogas yield of local fields sown with Miscanthus, I calculated the yield per ha for Miscanthus based on the lowest average biomass yield for Miscanthus (10t/ha) and the average biogas yield of Miscanthus estimated in section 3.3. These predicted yields are based on actual field sizes (ha). These estimates are used for heuristic purposes and I acknowledge that actual biomass yields per hectare may vary from field to field and from year to year given variation in species composition (and in the case of Miscanthus, genotype) and annual rainfall (Clifton-Brown et al. 2001).

2.7 Statistical Analysis

I used One-way analysis of variance (ANOVA) to determine whether there were differences in lignin, hemicellulose, and cellulose contents of the forage samples from Oxfordshire. To determine which species were associated high levels of lignin, hemicellulose and cellulose content, I used indirect gradient analysis using Redundancy Analysis (RDA) followed by a Monte Carlo permutation test with 499 permutations on log-transformed data (Canoco version 4.5, Lepš and Šmilauer 2003). Based on

[12] Previous studies report yields from semi-natural grasslands that range from ca. 3-25 t/ha (De Hann et al. 2009; Seppälä et al. 2009; Thumm et al. 2014; Tilman et al. 2006). For example, the yield of semi-natural grassland in the American prairies is 3.7 t/ha while the yield of *Phragmites australis* dominated wetlands in Sweden is 10 t/ha (Lin 2012; Sanderson and Adler 2010). The same is true for Miscanthus, with biomass yields ranging from 8-27 t/ha (Bauen et al. 2010; Christian et al. 2008; Fletcher et al. 2011; Himken et al. 1997; Jørgensen 1997; Kahle et al. 2001; Richter et al. 2008). Given the wide variation in biomass yields for grasslands and Miscanthus, I decided to use the more conservative yield estimates for both crops based on UK and European sources.

this data, I created General Additive Models (GAMs) for cellulose, hemicellulose, and lignin to determine which species were associated with increased yields of each material. Only species with a response variable of $P < 0.05$ were included. To determine the relationship between species-richness and bioenergy yield, correlation analysis (Pearson's Correlation Coefficient) was used followed by a *t*-test to establish significance of the r values (Crawley 2011). The same test was performed to determine whether cellulose, hemicellulose and lignin were correlated with biogas yield. To compare the lignocellulosic composition of species-rich grass and other bioenergy feedstocks, I used correspondence analysis (CA). The data was log-transformed and in the diagrams samples are coded by feedstock type. To determine whether there were differences in lignin, hemicellulose, cellulose content, and bioenergy yield of species-rich grasslands and other bioenergy crops, I used ANOVA. Correlation analysis and ANOVA were performed in R version 3.2.2 ("Fire Safety") and RDA and CA was performed in Canoco (version 4.5, Lepš and Šmilauer 2003).

3. Results

3.1 Lignocellulosic Composition and Biogas Yield of Species-Rich Grass

Table 4 shows the average cellulose, hemicellulose, lignin and biogas yields for unimproved, conservation and improved grasslands in Oxfordshire. Individual results for each grassland can be found in **Appendix II Table 1**. Cellulose ($F_{2, 10} = 0.333$, $P = 0.725$) and lignin content ($F_{2, 10} = 2.408$, $P = 0.14$) did not vary significantly among the three grassland types. However, there was a marginally significant difference in hemicellulose content among grassland types ($F_{2, 10} = 3.775$, $P = 0.06$). Unimproved grasslands had the highest average cellulose content while conservation grasslands had the highest hemicellulose content. Biogas yields varied significantly among grassland types ($F_{2, 10} = 6.243$, $P = 0.017$). Conservation grasslands had the highest average biogas yield followed by

unimproved grasslands, although there was no significant difference between the two (t = 0.699, P = 0.50). The average biogas yield of improved grasslands was 143% lower than that of unimproved grasslands.

Table 4 Comparison on the cellulose, hemicellulose, lignin and biogas yields of unimproved, conservation and improved grasslands. The lignocellulosic composition and biogas yields of unimproved grasslands and conservation grasslands were highly similar. Improved grasslands had the lowest average biogas yield per ton DM. Data in the table shows the mean ± one standard error.

	Cellulose (% DM)	Hemicellulose (% DM)	Lignin (% DM)	Biogas Yield (Nm3 / ton)
Unimproved Grassland	24.1 ± 2.298	22.9 ± 1.76	10.36 ± 2.27	625.13 ± 28.33
Conservation Grassland	22.19 ± 3.81	24.72 ± 2.9	12.53 ± 3.77	657.9 ± 46.98
Improved Grassland	26.42 ± 2.3	14.01 ± 3.73	1.35 ± 4.82	437.5 ± 60.09

3.2 Effect of Species Richness on Lignocellulosic Composition and Biogas Yield

Species-richness showed a strong positive correlation with lignin content (r = 0.71, t = 3.30, df = 11, P = 0.008) was not significantly correlated with either hemicellulose content (r = 0.29, t = 0.999, df = 11, P = 0.34) or cellulose content (r = 0.46, t = -1.73, df = 11, P = 0.11). Species richness and lignocellulosic composition both affected the biogas yield of the grass samples from Oxfordshire. There was a marginally significant positive correlation between vegetation species and biogas yield (r = 0.50, t = 1.92, df =11, P = 0.08) (**Figure 2)**. Hemicellulose (r = 0.94, t = 9.456, df = 11, P < 0.001) and lignin (r = 0.655, t = 2.877, df = 11, P = 0.015) were strongly positively correlated with biogas yield. There was no correlation between biogas yield and cellulose content (r = -0. 237, t = -0.989, df = 11, P = 0.456).

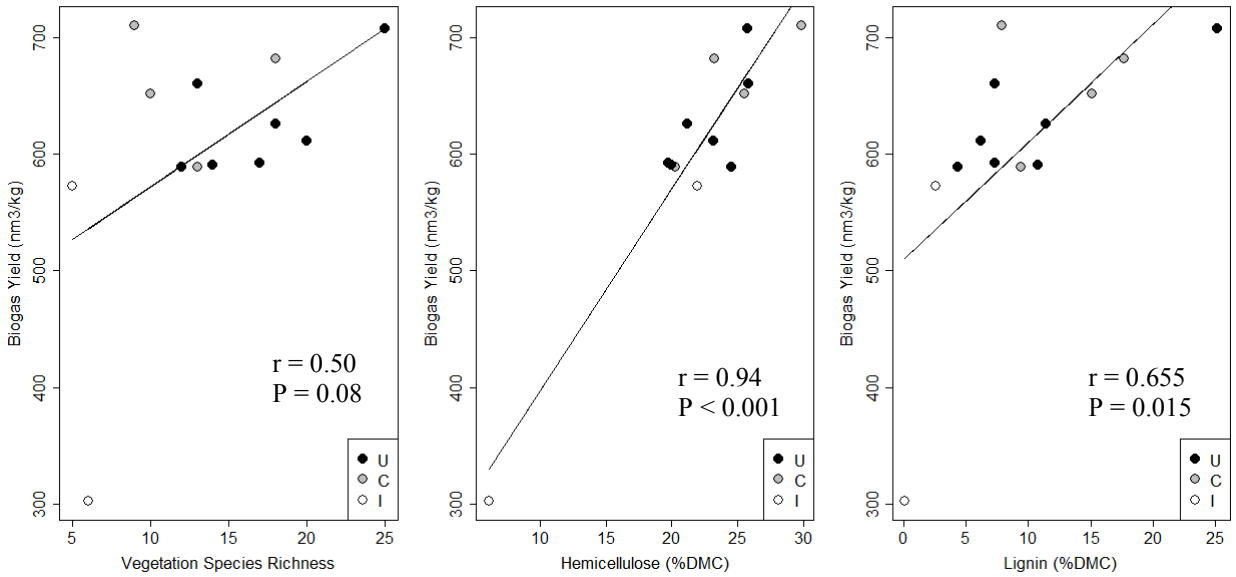


Figure 2 Correlation analysis showing the relationship between vegetation species richness and lignocellulosic composition on biogas yield of Oxfordshire grassland biomass samples. Vegetation species richness, hemicellulose content and lignin content were correlated with increased biogas yield.

RDA analysis indicates that biogas yield was not correlated with any particular species. Instead, grass samples with high biogas yields contained a variety of plants (**Figure 3 A**). For example, site RM_B and site PF_RM both had high biogas yields, yet their vegetation was very different. RM_B is a grassland under arable reversion and the vegation at the site was dominated by ryegrass (*Lolium perenne* L.), orchard grass, yellow oat grass (*Trisetum flavescens* (L.) Beauv.), and false oat grass (*Arrhenatherum elatius* L.). In contrast, PF_RM is an unimproved grassland and the vegetation at the site consists of reeds (*Phragmites australis*), sedges (*Carex* spp.), orchardgrass (*Dactylis glomerata*) and giant fescue (*Festuca gigantea* (L.) Vill.). Species response curves for hemicellulose and lignin indicate specific species are associated with increased levels of cellulose, hemicellulose and lignin. For example, Yellow oat grass ($F = 6.07$, P = 0.019), nettle (*Urtica dioica* L.) ($F = 10.72$, P = 0.003) and ryegrass ($F = 8.19$, P = 0.008) were associated with increases in hemicellulose while the abundance of plantain (*Plantago lanceolata* L.), red clover (*Trifolium pratense* L.) and lucerne (*Medicago sativa* L.)

decline (**Figure 3 B**). Increased lignin levels were associated with increased abundances of orchardgrass ($F = 6.69$, P = 0.01), Yorkshire Fog (*Holcus lanatus* L.) ($F = 9.22$, P = 0.005), common reed ($F = 33.78$, P < 0.001), and Floating Sweet Grass (*Glyceria fluitans* (L.) R. Br.) ($F = 33.78$, P < 0.001) (**Figure 3 C**).

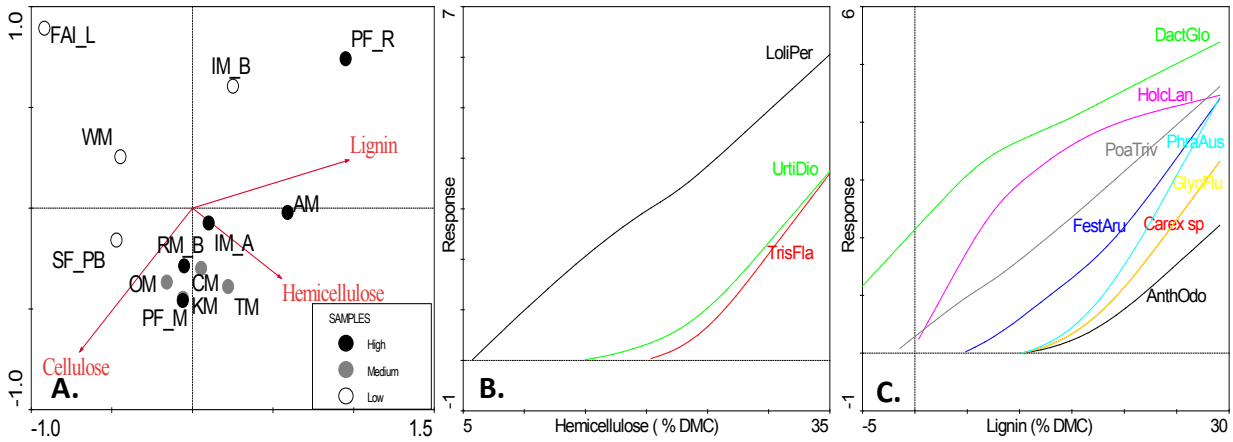


Figure 3 Effect of species composition on lignocellulosic composition of grass samples from Oxfordshire. A. RDA analysis showing lignocellulosic composition of grass samples from Oxfordshire. Samples are coded based on biogas yield (high, medium, low). RDA explains 45% of the variation in species among samples and 100% of the correlation between species and the lignocellulosic components ($F = 1.957$, P = 0.026). B and C. Generalized Additive Models (GAMs) showing the association of particular species with hemicellulose and lignin. Species are labeled by the first four letters of the genus and first three letters of the species. Only species with P < 0.05 were included in each model.

3.3 Comparison of Lignocellulosic Composition and Biogas Yields of Species-rich Grasslands to other Bioenergy Feedstocks

The average cellulose, hemicellulose and lignin content of all feedstocks are listed in **Table 5** (see also **Appendix II Figure 1**). Cellulose content varied significantly among crop types ($F_{14, 69} = 6.642$, P < 0.001). Hemp, sisal and kenaf had the highest cellulose content. Cereals, biomass from species-rich grasslands, and legumes had the lowest cellulose content. Hemicellulose content varied significantly among crop types ($F_{14, 69} = 6.789$, P < 0.001). Switchgrass, Miscanthus, and crop waste had the highest average hemicellulose levels and kenaf, hemp and legumes had the lowest. Lignin content also varied significantly among bioenergy feedstocks ($F_{14, 69} = 4.028$, P < 0.001). Bamboo, Miscanthus,

and rapeseed had the highest average lignin levels while agave, cereals and hemp had the lowest. Correspondence analysis indicates that the lignocellulosic composition of biomass from species-rich grasslands is most comparable to crop waste, grass, and switchgrass (**Figure 4)**.

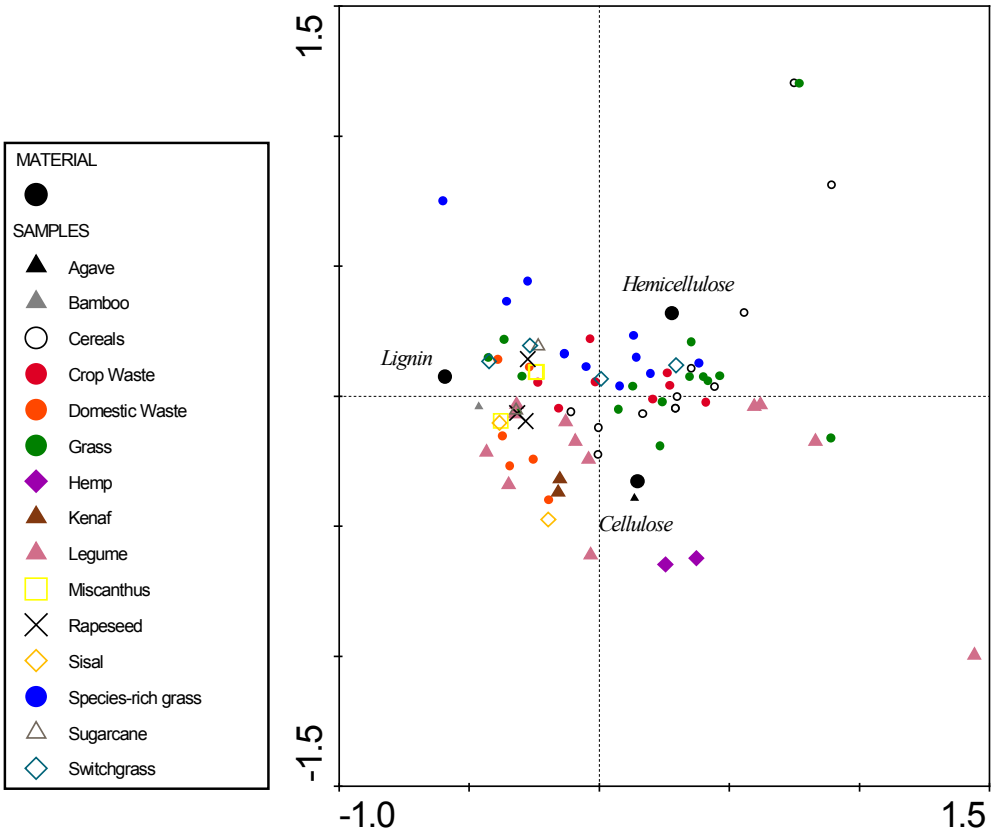


Figure 4 Correspondence analysis demonstrates species-rich grass is closest in lignocellulosic composition to crop waste, grass, cereals and Switchgrass. Correspondence analysis explains 100% of the variation among bioenergy feedstocks.

The average biogas yield of each feed stock is listed in **Table 5** (**Appendix II Table 2** contains the results for each sample). Biogas yield varied significantly among bioenergy feedstock types ($F_{7, 35} = 5.33$, $P < 0.001$). The average biogas yield of species-rich grass was up to three times higher than that of cereals, legumes, grass, and rapeseed. There was no significant difference between the biogas yield of biomass from species rich grasslands and crop waste ($t = -1.573$, $P = 0.12$), Switchgrass ($t = -1.534$,

P = 0.134), and Miscanthus (t = 1.470, P = 0.15). Despite differences in biogas and methane yield, methane concentrations of all feedstocks were similar (around 50%).

Table 5 Comparison of lignocellulosic composition and biogas yield of biomass from species-rich grasslands to other bioenergy feedstocks. Biomass from species-rich grasslands was most similar in lignocellulosic composition to cereals and grass. Miscanthus had the highest biogas yield followed by biomass from species-rich grasslands. In the table, averages for each feedstock are represented as the mean ± one standard error. Cellulose, hemicellulose and lignin contents are represented as % DM. Biogas and methane yields are represented as Nm^3. DM content was not available for bioenergy feedstocks with * so biogas yield could not be calculated.

Crop	Cellulose	Hemi-cellulose	Lignin	DM (kg/t wet weight)	Biogas yield	Methane yield
Species-rich Grass	23.41 ± 3.06	23.56 ± 1.6	11.15 ± 1.77	949.9 ± 48.22	637.1 ± 54.79	316.9 ± 27.34
Agave	55.79 ±10.6	15.31 ±5.55	6.8 ± 6.13	*	*	*
Bamboo	39.5 ± 10.6	17.6 ±5.55	25.2 ± 6.13	*	*	*
Cereals	24.86 ± 4.24	18.01 ± 2.22	4.933 ±2.45	679 ± 104.16	222.9 ± 118.36	109.9± 59.09
Crop Waste	38.16 ± 4.72	26.67 ± 2.47	9. 78 ± 2.73	775.6 ± 77.32	498.8 ± 87.86	247.5 ± 43.85
Domestic Waste	38.83 ± 5.15	15.88 ± 2.64	16.02 ± 2.72	*	*	*
Grass	20.68 ± 4.03	22.92 ± 2.10	8.919 ±2.48	880.8 ± 86.26	439.7 ± 98.01	218 ± 48.92
Hemp	68.5 ± 7.8	12.5 ± 4.08	4.5 ± 2.33	*	*	*
Kenaf	53.95 ± 7.8	14 ± 4.08	12.05 ± 4.51	*	*	*
Legumes	22.74 ± 4.24	10.07 ± 2.22	7.117 ± 4.51	829.3 ± 71.88	285.2 ± 81.68	142.7 ± 40.76
Miscanthus	44.6 ± 6.61	25.8 ± 2.21	21.2 ± 2.45	768 ± 104.16	811.1 ± 118.36	406.6 ± 59.07
Rapeseed	42 ± 6.61	22 ± 3.46	19.33 ± 3.82	903.5 ± 122.93	276.5 ± 139.69	138.1 ± 69.72
Sisal	58.9 ± 7.8	15.35 ± 3.46	17.95 ± 4.51	*	*	*
Sugar Beet	34.8 ± 7.8	23 ± 4.08	17.75 ± 4.51	*	*	*
Switchgrass	37.12 ± 5.93	29.17 ± 4.08	15.7 ± 3.93	470 ± 104.16	244.5 ± 118.36	227 ± 59.07

3.4 Comparison of the Estimated Bioenergy Yields of Grassland and Miscanthus Fields in Oxfordshire

The area, average field size, and total biomass yield varied significantly among SSSIs, fields managed under agroenvironmental schemes, and Miscanthus ($F_{20, 7585}$ = 45.49, P < 0.001) (**Appendix II Table 3**). In Oxfordshire, there are 107 SSSI grasslands occupying 2,201.53 ha. The average site size is 20.57 ± 0.06 ha^2. Using the minimum estimate of 8 t/DM per ha, the minimum potential biomass yield from these areas would be 17,612.17 t/DM while using the maximum estimate of 10 t/ DM ha gives a total of 22,015.34 t/DM. There are 7,479 grasslands managed under 19 different agro-environmental

schemes (Entry and High Level Landscape Stewardship) in the county. Most sites are managed under schemes that maintain permanent grassland under Article 13 (3,187 sites) and permanent grasslands with low (1,471 sites) and very low inputs (1,330 sites) that are not part of Severely Disadvantaged Areas (SDA) which make farming challenging and the Moorland Line (ML) (areas used for extensive rough grazing) (Defra 2012). Areas managed under agro-environmental schemes cover a total of 30, 331.13 ha. Overall, the average field size is 8.31 ± 0.92 ha and the largest fields are managed to protect the habitat of breeding wetland birds. Using the minimum estimate of 8 t/DM per ha, the minimum potential biomass yield from these areas would be 242,649 t/DM while using the maximum estimate of 10 t/ DM ha gives a total of 303,311.3 t/DM. In comparison, the average area used for bioenergy crop production under Defra's bioenergy crops scheme from 2003-2013 was 174.18 ha, with 119.06 ha planted with Miscanthus.[13] Miscanthus fields were 5.95 ± 1.58 ha on average. Using the minimum estimate of 10 t/DM per ha, the minimum potential biomass yield from these areas would be 1,190.6 t/DM while using the maximum estimate of 14 t/ DM ha gives a total of 1,664.84 t/DM.

The average biogas yield per field varied significantly among SSSIs, Miscanthus, and the fields managed under the 19 different agroenvironmental schemes ($F_{20, 17585} = 45.54$, $P < 0.001$) (**Figure 5**). SSSIs had the highest yield (104, 867 ± 3, 191Nm^3), followed by wetlands maintained for breeding waders (73, 415 ± 6, 174 Nm^3), and Miscanthus (48, 283 ± 8, 041Nm^3). Permanent grasslands managed with low and very low inputs (18, 723 ± 3, 305 Nm^3 and 15, 605 ± 3, 317 Nm^3 respectively) and newly created heathlands had the lowest average yields per field (3, 568 ± 33, 161 Nm^3). In order to produce yields competitive to Miscanthus fields producing on average 8, 110.72 Nm^3/ha, grasslands would need to be at least 9.5 ha large. This would include roughly 43% (46) of grassland SSSIs in the county and 8% (616) of grasslands under agro-environmental schemes.

[13] The remaining area was planted with Short Rotation Coppice.

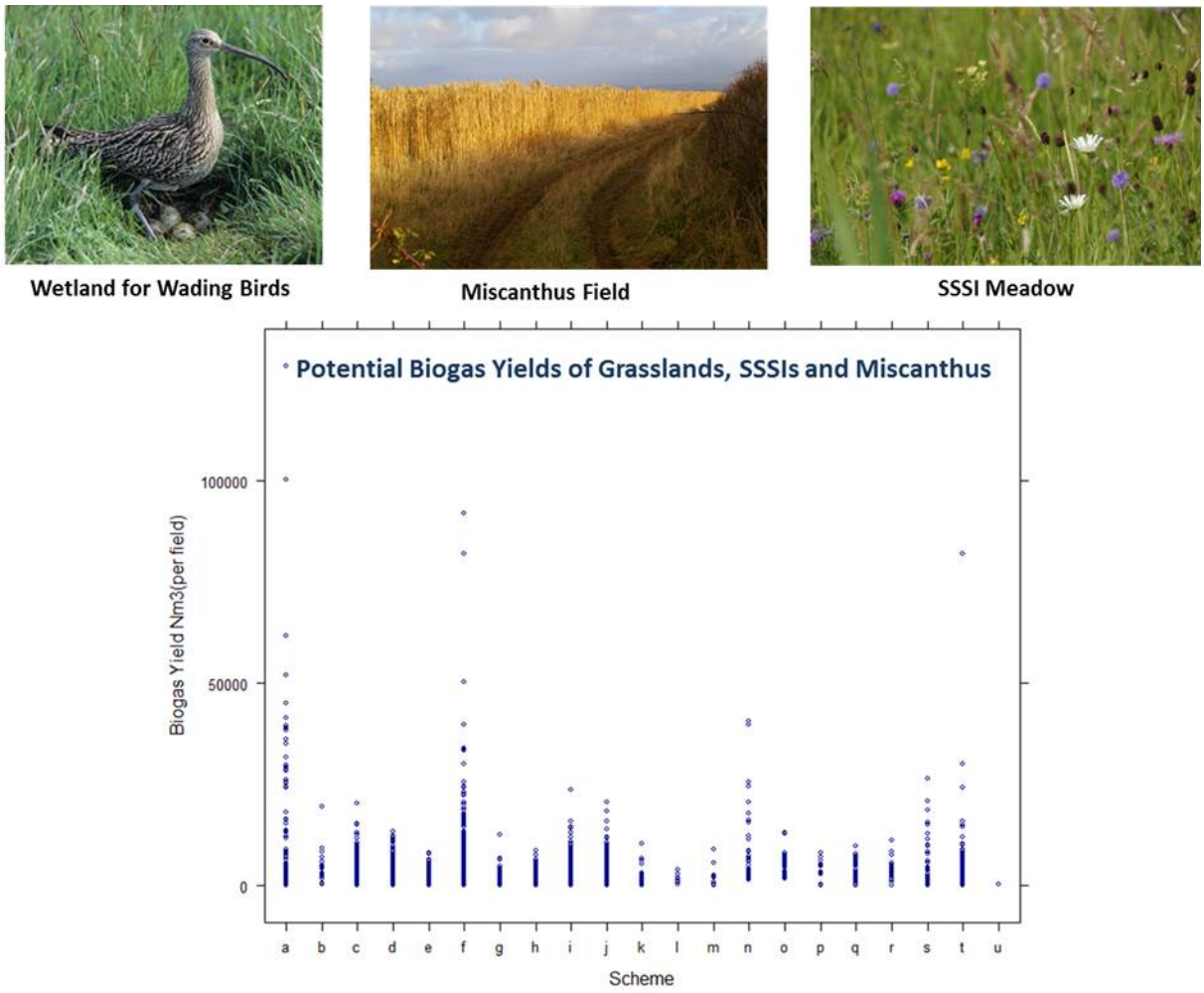


Figure 5 Potential biogas yield (per area ha) of grasslands under agro-environmental Schemes, SSSIs, and Miscanthus. The yield per ha for grasslands is calculated as 8 t/ha x 603.36 Nm^3 per ton dry matter. The yield per ha for Miscanthus is calculated as 10t/ha x 811.07 Nm^3 biogas per ton dry matter. Forecasted yields are based on actual field sizes (ha). Although Miscanthus produces more biogas per ton and per hectare, grasslands produce more biogas per area due to larger field sizes. Schemes are identified by alphabetical letter in Appendix I, Table I. Select schemes mentioned in the results: a = SSSIs, b = Miscanthus, c = Maintenance of species-rich, semi-natural grassland; f = Permanent grassland (unpaid under Article 13), n = I Maintenance of wet grassland for breeding waders; s = Creation of grassland for target features; t = Maintenance of grassland for target features. Image Credits: Nesting Curlew © BBOWT; Miscanthus field, Devon © Ben Harris; Yarnton Mead © BBOWT.

The total biogas yield per scheme (according to total biomass yield per ha) also varied significantly among grasslands protected as SSSIs, managed under agroenvironmental schemes, and sown with Miscanthus (**Figure 6**). The schemes with the highest total biogas yield were permanent grasslands maintained under Article 13 (69, 879, 544 Nm^3), permanent grasslands with low inputs (outside SDA

and ML) (24,598,946 Nm^3), permanent grasslands with very low inputs (outside SDA and ML) (20, 756,514 Nm^3), SSSIs (11,223,459 Nm^3), and maintained species-rich, semi-natural grasslands (9,490,812 Nm^3). Miscanthus fields produced 965, 209 Nm^3. The schemes with the lowest potential total biogas yield were unfertilized grasslands created to prevent erosion (184,619 Nm^3), permanent grasslands managed with low inputs (128,892 Nm^3) and newly created heathlands (3,379 Nm^3).

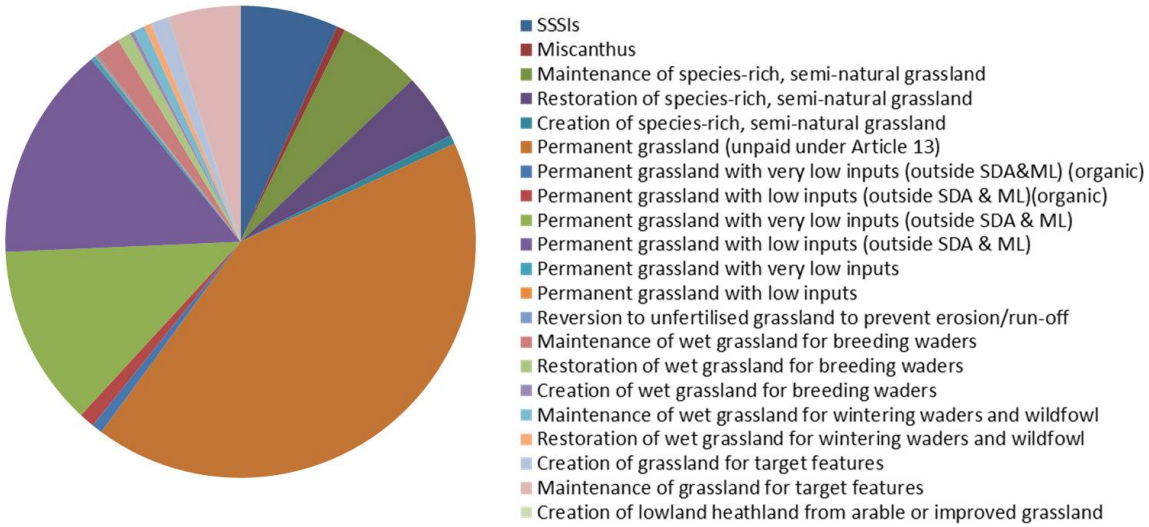


Figure 6 Total Biogas Yield by Scheme. Permanent grasslands set aside under Article 13, permanent grasslands under very low inputs (outside SDA & ML), and permanent grasslands under low inputs (outside SDA & ML) produced the most biogas due to the large number of fields enrolled in these agroenvironmental schemes.

4. Discussion

My analysis demonstrated that species richness and species composition both impact grassland biogas yield. At the same time, it highlights that species-rich grass is comparable to other bioenergy feedstocks in terms of lignocellulosic composition and biogas yield. Calculating the potential biogas yields of SSSIs and grasslands managed under agro-environmental schemes illustrates that grassland biomass could be used for biogas production and would produce more bioenergy than an average Miscanthus field of 5.95 ha if the grassland is at least 9.5 ha large. These results have significant

ramifications for viability of using species-rich grasslands for biogas production and how species-rich grasslands are managed under current agro-environmental and conservation schemes.

4.1 The Effect of Biodiversity on Grassland Biogas Yield

For species-rich grasslands, both species-richness and lignocellulosic composition strongly affected biogas yield. Grass samples high in hemicellulose and lignin produced higher biogas yields than samples high in cellulose and specific species (reeds (*P. australis*), sedges (*Carex* spp.), Orchard grass (*D. glomerata*), Yellow Oat-Grass (*T. flavescens*), and Giant Fescue (*F. gigantea*)) were also associated with increased yields. In a grassland biodiversity experiment in Minnesota, Tilman et al. (2006) also found that increased species richness was associated with increased bioenergy (in this case, ethanol) yield. Plots were sown with 1, 2, 4, 8 or 16 perennial grassland species and left unfertilized. Plots with the highest species richness produced 238% more bioenergy than plots sown with just one species. These results could be due to two factors: (1) increased biomass production (t/DM ha) due to the presence of legumes in the species-rich swards (which increase soil fertility and grassland productivity) and (2) increased chance of tall species (which again, result in higher biomass yields). These same properties may help explain why species-rich grasslands in Oxfordshire produced more biogas than species-poor improved grasslands. The former were dominated by a variety of legumes as well as very tall grasses. The latter also contained an abundance of legumes, however, the sward height was much shorter (data not shown). The observed positive effect of biodiversity on biogas yield may simply be due to the increased chance of tall grasses/forbs on species-rich grasslands compared to species-poor grasslands.

Previous studies have also found that species composition strongly affects the bioenergy potential of grasslands. For example, Adler et al. (2009) found that fields dominated by native, tall C_4 prairie

grasses had higher ethanol yields than grasses with shorter forbs (even if these were higher in species richness). Although there is little research on the biogas yield of most grassland species, previous studies in Germany and Sweden have shown that reeds produce more biogas than other grasses (e.g. ryegrass) (Lin 2012; Melts et al. 2013, 2014; Prochnow et al. 2009). The high biogas yield of reeds is rarely explained but it may be due to the high levels of lignin in this plant and the fact that lignin produces more biogas and methane than cellulose and hemicellulose, as shown in section 2. In Lithuania, Butkute et al. (2014) also found that local and commercial varieties of *Dactylis glomerata*, *Festuca arundinacea*, and *Phalaris arundinacea* also differed in biogas yield. Wild ecotypes from the local region produced significantly more biogas than improved cultivars and ecotypes from other countries. These results have important ramifications for assessing the suitability of grasslands for bioenergy production, in that, they emphasize that locally adapted plants might yield more bioenergy than commercial varieties. This may be due to the fact that modern breeding has reduced the lignocellulosic composition of most grasses (e.g. lignin) in an attempt to increase the protein and sugar content (and palatability) of forage grasses for livestock.

The critical role of species-composition on biogas yield has two important implications for the use of grasslands for bioenergy production. First, the biomass from conservation grasslands could be used for biogas production (**Figure 7**). These grasslands often contain an abundance of reeds, rushes and tall grasses and forbs which are often cut and burned by volunteers to maintain the openness of the vegetation (and in some cases, to mimic traditional hay cuts). Second, grasslands created for bioenergy production could be sown with specific species suitable for biogas production. Since late 2015, commercial seed companies began to produce grass mixes to sow as short-term leys specifically for biogas production. However, these mixes do not contain species associated with increased biogas yields. Instead, seed mixes for biogas leys contain ryegrass (*Lolium perenne*), clover (*Trifolium pratense* and *Trifolium repens*) and festulolium (*Festuca* x *Lolium*). These plants, especially ryegrass

and clover, require high levels of inputs and lack persistence and are not the most suitable plants for biogas production using anaerobic digestion. Instead, seed companies could use local genetic resources (e.g. of *Dactylis glomerata*, *Festuca arundinacea* etc.) to develop new varieties with increased lignocellulosic composition. Alternatively, seed companies could work more closely with local communities to harvest seed from local meadows to use for bioenergy production. This could ensure both the maintenance of local biodiversity as well as the promotion of biodiversity on newly created grasslands.



Figure 7 Biomass from conservation grasslands could serve as a source of bioenergy. Volunteers cutting back reeds in the Lye Valley. Photo credit: J. Webb.

4.2 Can Grass Compete with other Bioenergy Crops?

The lignocellulosic composition of species-rich grass was similar to, and in some cases even lower than, other bioenergy crops suggesting that there should be no technological barrier to producing biogas from grass. This conflicts with previous studies which propose producing bioenergy (biogas or ethanol) from grasslands is technically difficult due to the recalcitrance of lignocellulosic material

(Frigon and Giuiot 2010; Mack *pers. comm*.). A number of studies report that the lignin content of grass reduces biogas yields. For example, Triolo et al. (2012) compared the biomethane potential of different herbaceous and non-herbaceous feedstocks (e.g. yard waste, grassland forbs, crops, hedge clippings and wood cuttings), and found a negative correlation between biomethane output and degree of lignification. Butkute et al. (2014) also found a negative correlation between lignin content and biogas yield in cultivar and wild types of *Dactylis glomerata*, *Festuca arundinacea* and *Phalaris arundinacea*. Finally, when Amon et al. (2007) compared the biogas yields of different Austrian bioenergy feedstocks using batch scale digesters, they found that maize produced the highest methane yields (7,500 - 10,200 m^3N CH4 ha^{-1}) while Alpine grasslands produced the lowest (2,700-3,500 m^3 N CH4 ha^{-1}), citing the lignin content of the latter as responsible. However, none of these studies pre-treated their biomass samples before addition to the batch scale digesters nor was the bacteria used in the digesters optimized for lignocellulosic material. Previous research has demonstrated that steam (Bauer et al. 2014; Hendriks et al. 2009; Johnson et al. 2008; Mosier et al. 2005; Taherzadeh et al. 2008), chemical (Teghammer et al. 2010; Teghammer et al. 2012; Teghammer 2013), fungal (Mackuľak et al. 2012; Montgomery and Bochmann 2014; Zhao 2013), and bacterial pretreatments (Sun 2015) can double biogas yields. This highlights the importance of optimizing anaerobic digestion to process lignocellulosic materials to realize the true bioenergy potential of biomass from grasslands.

In contrast to these studies, I found that lignin, along with hemicellulose, actually contribute to biogas yield and that species-rich grasslands produce biogas yields comparable to other feedstocks like Miscanthus. Several other studies have also reported high biogas/ethanol yields from grasslands in Europe and the US. For example, Steubing et al. (2010) found that biogas yields from meadows and pastures (17.4 GJ / t DM) was slightly higher than the yield from forest wood (15.8 GJ / t DM), waste paper and cardboard (17 GJ / t DM), and current bioenergy crops (17.3 GJ / t DM). Based on biomass yield and estimating ethanol output at 0.255 L per kg^{-1} dry matter, Tilman et al. (2006) found that low-

input high diversity grasslands in the US produced three times more bioenergy than Switchgrass. Research on German and American species-rich grasslands and Estonian fields of reed canary grass suggest that there is a strong correlation between late grassland harvest dates and biogas yields (Amon et al. 2005; Blokhina et al. 2011; Heinsoo et al. 2011; Herrmann et al. 2013; Jungers et al. 2013). This could be due to the increased amount of cellulose, hemicellulose and lignin content of grasses as the summer continues and overall increased dry matter content (which greatly affects biogas yield).

4.3 Bioenergy Production from Grasslands under Agroenvironmental Schemes

Using Oxfordshire as a case study demonstrates that the average field size of grasslands currently managed under agro-environmental schemes is larger than the average size of fields sown with the local bioenergy crop, Miscanthus, and that there are significantly more species-rich grasslands compared to Miscanthus fields. Thus, although Miscanthus may produce slightly more biogas per ton of dry matter, species-rich grasslands would produce more bioenergy due to larger average field sizes and total land cover area. This agrees with Tilman et al. (2006) and Fischer et al. (2009) who argue that grasslands cover a larger area than field sown with bioenergy crops. For example, Tilman et al. (2006) argue that grasslands cover 5×10^8 ha of land globally and could produce 13% of the world's petrol and 19% of the world's electricity needs. The data from Oxfordshire confirms that even on a local scale the number and average size of grasslands means that more biomass, and thus biogas, can be produced from grassland compared to other feedstocks.

My analysis also demonstrated that the three best candidates for bioenergy production are SSSIs, wetlands maintained for breeding birds, and permanent grasslands. All three grassland types cover large areas and produced some of the highest biogas yields by field and in total ha^2. In many cases, these fields are not used for livestock production (for hay or grazing) and produce excess biomass that

could be used for bioenergy production. This could potentially provide farmers with an additional source of income, particularly as they do not receive payments for maintaining certain grasslands (e.g. those maintained under Article 13).[14] Other studies have also proposed that unproductive waste land (Smolker 2008), prairies (Adler et al. 2009), wetlands around waste water plants (Lin 2009), and European meadows (Melts et al. 2013, 2014) could be used for biogas production, but this is the first analysis to suggest SSSIs and grasslands managed under current agroenvironmental schemes in the UK are a viable, sustainable source of biomass for biogas production.

This study also found, inadvertently, that previous Defra schemes to grow bioenergy crops, specifically Miscanthus and Short Rotation Coppice, have historically failed in the region. For example, between 2003 and 2015 thirty local farms entered the program but as of 2015 only two fields sown with Miscanthus are still enrolled in the scheme. In part, this was due to the fact that the machinery required to plant and cut Short Rotation Coppice was expensive (Dobermann *pers. comm*.). The inability to harvest both crops annually may have also decreased the economic viability of these crops. This agrees with previous studies in the UK and Europe which have found that machinery and transportation costs associated with Short Rotation Coppice and Miscanthus, as well as the risk involved with planting these new crops, decreases the economic viability of these crops for farmers (Krasuska et al. 2008; Sherrington et al. 2008, 2010; Styles et al. 2008).

Given the failure of Miscanthus and Short Rotation Coppice in Oxfordshire and the EU in general, producing bioenergy from grasslands may be an attractive, and feasible, alternative. Farmers are familiar with managing grasslands, do not need specialized machinery to harvest hay, and harvesting

[14] In 2014, Article 13 of the Common Agricultural Policy mandated that farmers with permanent grassland could no longer plough up these fields and convert them to arable (Jowit 2012; Parliament 2012). In order to be eligible for other payment schemes (e.g. Higher Level Stewardship) farmers must register and maintain these lands. However, farmers do not receive payments specifically for maintaining these permanent grasslands.

grass for bioenergy fits into current arable time cycles.[15] In Oxfordshire (and the UK more broadly), there are currently large, commercial scale anaerobic digesters in the region which already process cereals and crop waste and could potentially process grassland biomass as well. Alternatively, on-farm anaerobic digestion of grassland biomass could provide farms with electricity and heating (which could be used or sold) [16] and the digestate from the digester could be used as a source of nutrient-rich fertilizer. Although on-farm anaerobic digestion is currently rare in the UK, farms in Germany and Sweden have adopted this new technology, leading to economic growth as well as reduced greenhouse gas emissions (**Figure 8**) (Jones and Salter 2013; Parmlind 2014; Tranter et al. 2011).



Figure 8 Small-scale biogas facility in Linköping, Sweden. The plant produces biogas for local buses. Image credit: Swedish Biogas (Held et al. 2008).

4.4 Environmental Benefits and Social Challenges of Producing Bioenergy from Grasslands

In addition to the economic competitiveness and agricultural feasibility of producing bioenergy from grasslands, there are three main *environmental* benefits to using grasslands for bioenergy production

[15] Cutting hay in late August or September avoids conflicts over the availability and use of combine harvesters. These are often used for harvesting arable crops (cereals and rapeseed) earlier into the summer (July-early August).

[16] Biogas produced on farms can be sold and connected to local gas or electricity grids (e.g. by working with gas companies such as the Northern Gas Network). See http://biomethane.northerngasnetworks.co.uk/.

compared to other bioenergy feedstocks. First, maintaining pre-existing species-rich grasslands would maintain habitat for rare botanical and zoological species which live within these ecosystems (Prochnow et al. 2009). For example, previous studies have demonstrated that harvesting biomass from meadows and wetlands late in the summer or early autumn helps preserve breeding grounds and habitat for local bird populations (Robertson et al. 2011; Upham et al. 2009; Werling et al. 2013). These populations would disappear if the land was under cultivation for wheat or maize (Fletcher et al. 2011). Second, maintaining, restoring and even creating new species-rich grasslands would help sustain the ecosystem services these landscapes provide. For example, maintaining or planting new species-rich grasslands for bioenergy production would benefit pollinators, as many species-rich meadows contain flowers used as bee forage (Gardiner et al. 2010; Holzschuh et al. 2011; Martins et al. 2015; Verdade et al. 2015). Finally, producing bioenergy from species-rich grasslands would reduce the amount of inputs (e.g. artificial fertilizers, pesticides) to the wider arable landscape and use of natural resources needed to grow these crops. Other bioenergy feedstocks, including wheat, rapeseed, and lucerne, require high levels of nitrogen and phosphorus. Even Miscanthus and Short Rotation require inputs, in the form of water, to produce adequate yields. In contrast, species-rich grasslands are dominated by plants with low nutrient requirements and contain legumes which naturally boost soil fertility. In addition, these grasslands perform well under drought conditions (Tilman and Downing 1994).

However, like any other bioenergy crop producing bioenergy from grasslands is not without controversy. Production of biogas from grasslands could also compete with forage production. For example, Steubing et al. (2010) found that bioenergy production in Switzerland using extensive meadowland and mountain pastures would not be sustainable because pastoralists already use the land for grazing livestock. Similarly, Ciello (2009) argues we should be promoting the use of grassland biomass for feeding livestock as opposed to producing bioenergy. In the UK however, currently very

few commercial livestock production enterprises use species-rich grasslands for forage production, although a number of small to mid-sized beef and dairy farms do still graze livestock on permanent pastures (many of which are common land) and harvest hay from historic meadows. These grasslands provide low maintenance (and often 'free') forage that is valued for its nutritional composition and medicinal properties (Lowry *pers. comm*.; French *unpublished data*). Potentially, the best way around this may be to reserve the oldest species-rich grasslands (especially SSSIs) for livestock while using grasslands under arable reversion and specially planted grasslands for biogas production.

4.5 Future directions

As I have shown, bioenergy production from species-rich grasslands is a viable option to help reduce reliance on more nutrient-intensive bioenergy feedstocks. However, more research is needed to fine-tune estimates for the bioenergy yield from species-rich grasslands and these enterprises require greater socio-economic support. Future studies could collect additional biomass samples from a larger number of species-rich grasslands and complement the present estimated biogas yields with actual biomethane potential tests. These tests would need to use at least one form of pretreatment aimed at breaking down lignocellulosic biomass or otherwise the 'yield' will be artificially low. In addition, future research could focus on identifying new microbial communities with a strong potential for breaking down lignocellulosic material quickly and efficiently. Communicating these results to engineers and chemists who design anaerobic digestion facilities would ensure the translation of these advances into real-world situations. Finally, increasing monetary incentives and knowledge exchange among researchers, engineers and farmers could help facilitate the use of species-rich grasslands for bioenergy production. For example, Defra could modify Entry Level and Higher Level Landscape Stewardship agreements to give farmers and landowners payments for managing species-rich grassland for bioenergy production. In addition, farms in the UK which have successfully used

anaerobic digesters and/or have started harvesting grass for biogas production could offer support and advice to farmers looking at entering this sector of agricultural production.

5. Conclusion

In this study, I assessed biomass samples taken from semi-natural, species-rich grasslands from Oxfordshire, England in terms of their lignocellulosic content and potential bioenergy yield. I demonstrated that species richness was positively correlated with increased biogas yield and that species high in hemicellulose and lignin contributed to increased biogas yields. The biogas yield of species-rich grasslands was slightly lower than that of Miscanthus but still exceeded that of cereals and crop waste. Using Oxfordshire as an example demonstrated that species-rich, semi-natural grasslands (SSSIs) and grasslands created under agroenvironmental schemes could potentially produce more biogas than fields sown with Miscanthus due to larger average field size and the sheer number of grasslands in the region. The excess biomass from these sites could be used as opposed to going to waste and could serve as a new source of income for farmers. Biogas production from species-rich grasslands can thus contribute to reducing reliance on fossil fuels, decreasing cultivation of primary bioenergy crops, and achieving national goals of reducing carbon emissions by 2050, all while conserving native biodiversity.

Appendix II

Table 1 Species Richness and Lignocellulosic Content of Forage Samples from Oxfordshire. The lignocellulosic composition is in the form % DM.

Site	Type	Species Richness	Cellulose	Hemicellulose	Lignin
WM	Unimproved	12	26.03	24.58	4.3
PF_M	Unimproved	17	28	19.78	7.3
TM	Unimproved	18	25	21.20	11.4
IM_A	Unimproved	13	28.43	25.86	7.3
IM_B	Unimproved	14	23.38	19.96	10.8
OM	Unimproved	20	28.44	23.14	6.2
CM	Conservation	13	25.69	20.25	9.4
RM_B	Conservation	9	27.39	29.86	7.9
KM	Conservation	10	16.89	25.51	15.1
AM	Conservation	18	18.8	23.26	17.7
FAI_L	Improved	6	23.39	6.08	0.1
SF_PB	Improved	5	29.44	21.93	2.6
PF_R	Unimproved	25	9.45	25.75	25.2

Table 2 Potential biogas yield, methane yield and methane concentration for bioenergy feedstocks based on lignocellulosic composition. Species-rich grass samples are in bold.

Sample	DMC(%)	DMC (Kg)	VS (at 90%)	Cellulose (%DMC)	Hemicellulose (%DMC)	Lignin (%DMC)	Total biogas yield	Total methane yield	Methane concentration
Mscnth1	76.9	769	692.1	45	30	21	850.660	425.615	0.500
Mscnth2	76.9	769	692.1	44.7	29.6	21	844.577	422.622	0.500
HordStw	90.6	906	815.4	42.2	31.3	6.6	818.058	407.103	0.498
TritStw	91	910	819	42.8	27.5	7.2	793.513	395.773	0.499
AvnStw	89.6	896	806.4	38	31.4	6.6	770.467	382.911	0.497
Mscnth3	76.9	769	692.1	44.1	17.8	21.6	737.979	371.491	0.503
RM_B	**95.14**	**951.4**	**856.26**	**27.39**	**29.86**	**7.9**	**709.583**	**351.715**	**0.496**
PF_R	**96.65**	**966.5**	**869.85**	**9.45**	**25.75**	**25.2**	**707.623**	**351.932**	**0.497**
AM	**96**	**960**	**864**	**18.8**	**23.26**	**17.7**	**681.437**	**339.663**	**0.498**
IM_A	**94.89**	**948.9**	**854.01**	**28.43**	**25.86**	**7.3**	**660.150**	**327.883**	**0.497**
KM	**95.17**	**951.7**	**856.53**	**16.89**	**25.51**	**15.1**	**651.905**	**323.653**	**0.496**
PhlHy	88	880	792	33.4	27.6	4.4	648.917	322.292	0.497
DactHy	89.1	891	801.9	32	27.2	4.5	640.830	318.186	0.497
TM	**95.18**	**951.8**	**856.62**	**25**	**21.20**	**11.4**	**625.590**	**311.927**	**0.499**
AvnHy	89.2	892	802.8	33.9	23.6	4.2	617.731	307.540	0.498
OM	**94.49**	**944.9**	**850.41**	**28.44**	**23.14**	**6.2**	**611.275**	**304.005**	**0.497**
PF_M	**94.8**	**948**	**853.2**	**28**	**19.78**	**7.3**	**591.950**	**295.137**	**0.499**
IM_B	**94.64**	**946.4**	**851.76**	**23.38**	**19.96**	**10.8**	**590.866**	**294.596**	**0.499**
CM	**93.52**	**935.2**	**841.68**	**25.69**	**20.25**	**9.4**	**588.839**	**293.642**	**0.499**
WM	**94.4**	**944**	**849.6**	**26.03**	**24.58**	**4.3**	**588.433**	**291.690**	**0.496**
SF_PB	94.4	944	849.6	29.44	21.93	2.6	572.206	284.423	0.497
DrdMze	87.3	873	785.7	21.9	33	1.7	563.072	276.899	0.492
SwtchG3	47	470	423	37	31.6	21.6	492.956	246.114	0.499

SwtchG4	47	470	423	44.9	31.4	12	471.649	235.281	0.499
LucHy	89.4	894	804.6	25.8	11.4	7.6	453.231	227.516	0.502
LtsHy	91.2	912	820.8	24.3	7.2	9.6	426.414	215.131	0.505
SwtchG2	47	470	423	36.6	32.7	6.2	402.011	199.548	0.496
AvnCr	87.9	879	791.1	13.7	19.3	2.5	357.221	176.137	0.493
FAI_L	96.18	961.8	865.62	23.39	6.08	0.1	302.759	152.351	0.503
PisSil	84.4	844	759.6	18.8	8.7	4.2	300.893	150.838	0.501
Rpsd	90.1	901	810.9	10.9	8.1	7.9	283.645	142.058	0.501
LinM	90.6	906	815.4	9.1	10.1	6.2	269.335	134.099	0.498
HordCr	87.1	871	783.9	5.3	15.3	1.1	219.516	107.203	0.488
HordSIl	36.3	363	326.7	25.2	18	4.4	194.935	97.088	0.498
Wht	29.5	295	265.5	27.3	24.5	4.6	188.497	93.558	0.496
Trtcle	87.1	871	783.9	2.6	10.9	1.1	149.223	72.720	0.487
TritCr	87	870	783	2.5	10.3	1.1	141.934	69.193	0.487
Pisum	86.5	865	778.5	6.6	7.2	0.4	138.651	68.492	0.494
Soyb	88.8	888	799.2	6.8	5.5	1.2	135.608	67.420	0.497
SoyM	87.9	879	791.1	7.5	5.4	0.8	135.049	67.183	0.497
MzSil	61	610	549	8.2	9.1	1.7	132.243	65.466	0.495
Mze	86.3	863	776.7	2.4	9.2	0.6	122.936	59.868	0.487
LtsF	23.1	231	207.9	18.3	10.1	9.9	102.281	51.332	0.502
SgrBt	16.3	163	146.7	6.1	6.2	1	24.618	12.195	0.495

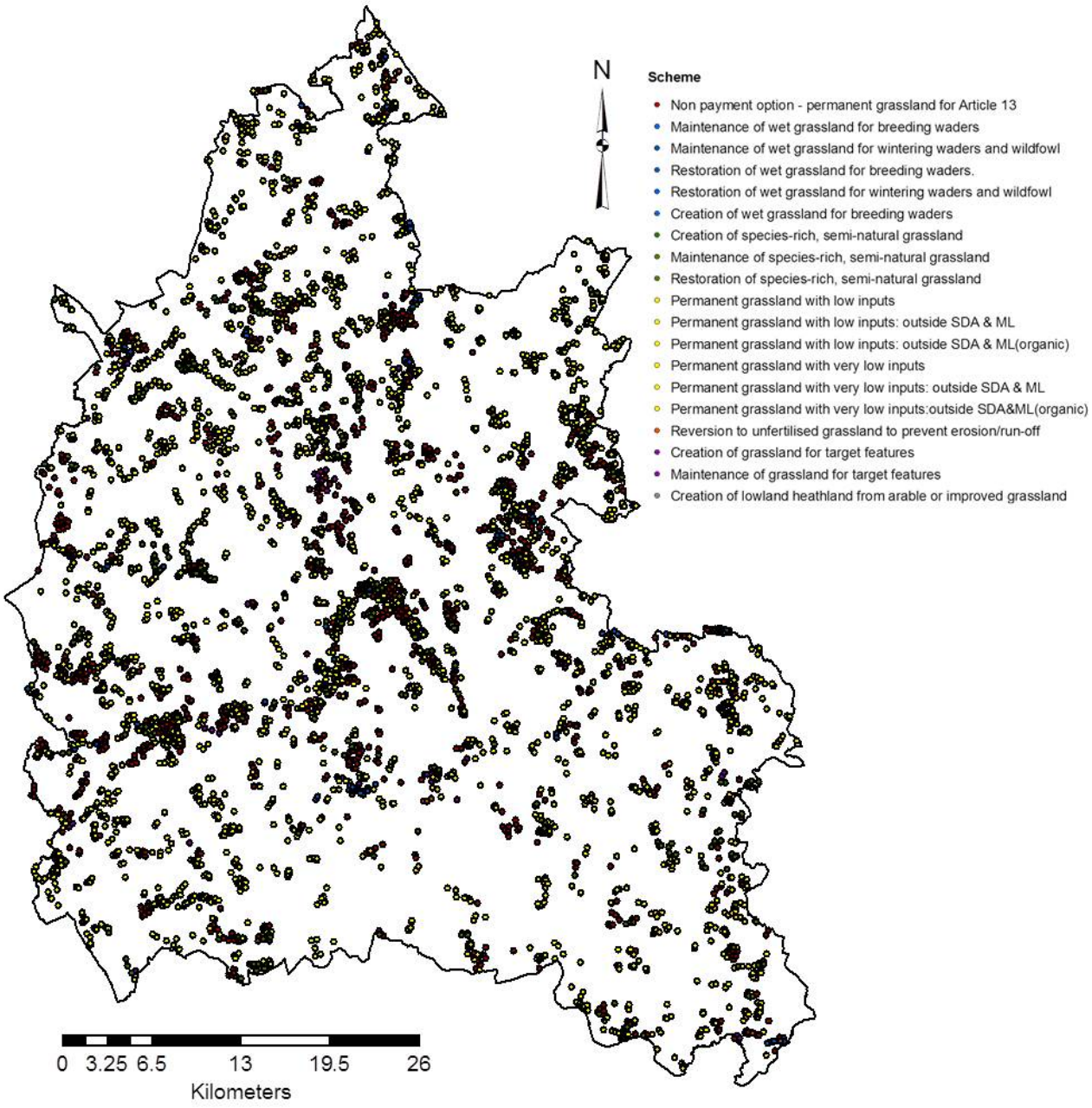


Figure 1 Location of grasslands managed under agro-environmental schemes in Oxfordshire. Sites are coded by scheme. Schemes relating to particular grassland types (e.g. wetland for wading birds, species-rich grasslands etc.) are coded the same color.

Table 3 Average biogas yield per hectare of grasslands under current agro-environmental schemes, SSSIs and Miscanthus. The average biogas yield per field for grasslands under current agro-environmental schemes and SSSIs was based on average biogas yield for species-rich grasslands from Oxfordshire (637. 1 Nm^3) producing 8 tons dry matter per hectare. The average yield per field of Miscanthus was based on the average yield of Miscanthus (811.07 Nm^3) producing 10 tons dry matter per hectare. For all sites, the actual size (in hectares) of each site was used to determine average biogas yield. The average for each field type was then calculated. The total biomass yield and total bioenergy yield for each scheme was calculated as the sum of all the individual field values within each scheme. All data on the number of farms in each agro-enviornmental scheme and the size of each parcel of land was obtained from Natural England (see Chapter 3 Methods, section 2.6).

	Scheme	Number of Fields	Average Area (ha)	Average Yield per Field (Nm^3)	Total Biomass Yield (tDM/ ha)	Total Biogas Yield
a	SSSIs	107	20.58± 0.63	104, 867 ± 3, 191	17, 616.48	11, 223, 459
b	Miscanthus	20	5.95±1.58	48, 283 ± 8, 041	1, 190	965, 209
c	Maintenance of species-rich, semi-natural grassland	408	4.56 ± 0.7	23, 264 ± 3, 585	14, 896.9	9, 490, 812
d	Restoration of species-rich, semi-natural grassland	320	4.67± 0.72	23, 787 ± 3, 585	11, 947.52	7, 611, 765
e	Creation of species-rich, semi-natural grassland	44	4.61 ± 1.16	23, 516 ± 5, 911	1, 624.13	1, 034, 732
f	Permanent grassland (unpaid under Article 13)	3187	4.30 ± 0.64	21, 924 ± 3, 244	109, 683.79	69, 879, 544
g	Permanent grassland with very low inputs (outside SDA&ML) (organic)	72	3.34 ± 0.99	17, 034 ± 5, 031	1, 924.99	12, 26, 412
h	Permanent grassland with low inputs (outside SDA & ML)(organic)	91	3.79 ± 0.92	19, 296 ± 4, 707	2, 756.21	1, 755, 980
i	Permanent grassland with very low inputs (outside SDA & ML)	1330	3.06± 0.65	15, 605 ± 3, 317	32, 579.68	20, 756, 514
j	Permanent grassland with low inputs (outside SDA & ML)	1471	3.28 ± 0.65	18, 723 ± 3, 305	38, 610.81	24, 598, 946
k	Permanent grassland with very low inputs	22	4.63 ± 1.51	23, 614 ± 7, 727	815.41	519, 496
l	Permanent grassland with low inputs	9	2.97 ± 2.25	15, 121 ± 11, 456	213.62	136, 100
m	Reversion to unfertilised grassland to prevent erosion/run-off	8	4.78 ± 2.37	24, 369 ± 12, 098	305.98	194, 942
n	Maintenance of wet grassland for breeding waders	39	14.4 ± 1.21	73, 415 ± 6, 174	4, 492.8	2, 862, 363
o	Restoration of wet grassland for breeding waders	34	8.83 ± 1.27	44, 994 ± 6, 498	2, 401.22	1, 529, 815
p	Creation of wet grassland for breeding waders	13	7.14 ± 1.89	36, 391 ± 9, 695	742.56	473, 085
q	Maintenance of wet	42	6.13 ± 1.18	31, 234 ± 6, 010	2, 059.01	1, 311, 794

	grassland for wintering waders and wildfowl					
r	Restoration of wet grassland for wintering waders and wildfowl	27	6.18 ± 1.39	31, 489 ± 7, 109	1, 334.45	850, 177
s	Creation of grassland for target features	54	7.56 ± 1.08	38, 513 ± 5, 510	3, 264.19	2, 079, 617
t	Maintenance of grassland for target features	307	5.29 ± 0.73	26, 978 ± 3, 706	12, 999.61	8, 282, 050
u	Creation of lowland heathland from arable or improved grassland	1	0.7 ± 6.5	3, 568 ± 33, 161	5.6	3, 568

References

Adler, P.R. et al., 2009. Plant species composition and biofuel yields of conservation grasslands. *Ecological Applications*, 19(8), pp.2202–2209.

Amon, T. et al., 2005. Biogas production from grassland biomass in the Alpine region. *Landtechnik*, 60(6), pp.336–337.

Amon, T. et al., 2007. Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. *Bioresource Technology*, 98(17), pp.3204–3212.

Anon, AD map. Anaerobic Digestion & Bioresources Association. *ADBA | Anaerobic Digestion & Bioresources Association*. Available at: http://adbioresources.org/about-ad/ad-map [Accessed October 30, 2015].

Anon, Miscanthus (*Miscanthus* x *giganteus*) for biofuel production - eXtension. Available at: http://www.extension.org/pages/26625/miscanthus-miscanthus-x-giganteus-for-biofuel-production [Accessed October 30, 2015].

Ariza-Montobbio, P. & Lele, S., 2010. Jatropha plantations for biodiesel in Tamil Nadu, India: Viability, livelihood trade-offs, and latent conflict. *Ecological Economics*, 70(2), pp.189–195.

Bauen, A.W. et al., 2010. Modelling supply and demand of bioenergy from short rotation coppice and Miscanthus in the UK. *Bioresource Technology*, 101(21), pp.8132–8143.

Berndes, G., Hoogwijk, M. & van den Broek, R., 2003. The contribution of biomass in the future global energy supply: a review of 17 studies. *Biomass and Bioenergy*, 25(1), pp.1–28

Biomass Energy Center (BEC), 2008, Miscanthus. *Sources of Biomass*. Available at: http://www.biomassenergycentre.org.uk/.

Blackstock et al., 1999. The extent of semi-natural grassland communities in lowland England and Wales: a review of conservation surveys 1978–96. *Grass and Forage Science*, 54(1), pp.1–18.

Blanchard, R., O'Farrell, P.J. & Richardson, D.M., 2015. Anticipating potential biodiversity conflicts for future biofuel crops in South Africa: incorporating spatial filters with species distribution models. *GCB Bioenergy*, 7(2), pp.273–287.

Blokhina, Y.N. et al., 2011. Concepts and profitability of biogas production from landscape management grass. *Bioresource Technology*, 102(2), pp.2086–2092.

Butkutė, B. et al., 2014. Cocksfoot, tall fescue and reed canary grass: Dry matter yield, chemical composition and biomass convertibility to methane. *Biomass and Bioenergy*, 66, pp.1–11.

Campbell, A. et al., 2009. Biofuels and biodiversity: An assessment of the impacts of biofuels on biodiversity and the appropriate policy responses. *IOP Conference Series: Earth and Environmental Science*, 6(18), p.182.

Capareda, S., 2013. *Introduction to Biomass Energy Conversions*, CRC Press.

Ceotto, E., 2009. Grasslands for bioenergy production: A review. In E. Lichtfouse et al., eds. *Sustainable Agriculture*. Springer Netherlands, pp. 141–151.

Christian, D.G., Riche, A.B. & Yates, N.E., 2008. Growth, yield and mineral content of *Miscanthus* × *giganteus* grown as a biofuel for 14 successive harvests. *Industrial Crops and Products*, 28(3), pp.320–327.

Chynoweth, D.P., Owens, J.M. & Legrand, R., 2001. Renewable methane from anaerobic digestion of biomass. *Renewable Energy*, 22(1–3), pp.1–8.

Clifton-Brown, J.C. et al., 2001. Performance of 15 genotypes at five sites in Europe. *Agronomy Journal*, 93(5), p.1013.

Cornara, L., Xiao, J. & Burlando, B., 2015. Therapeutic potential of temperate forage legumes: A review. *Critical Reviews in Food Science and Nutrition*, 0(ja), pp.00–00.

Crawley, M.J., 2011. *Statistics: an introduction using R.*, Hoboken: John Wiley & Sons.

Defra, Spotlight on SSSIs – Working towards the goals of Biodiversity 2020: Issue 6 – June 2015 –

NE610. *Natural England - Access to Evidence*. Available at: http://publications.naturalengland.org.uk/publication/6071981091848192 [Accessed November 2, 2015].
Defra, SSSI condition assessment: a guide for owners and occupiers - NE61. *Natural England - Access to Evidence*. Available at: http://publications.naturalengland.org.uk/publication/50008 [Accessed November 2, 2015].
Defra, Sites of Special Scientific Interest: A brief guide for land owners and occupiers - NE322. *Natural England - Access to Evidence*. Available at: http://publications.naturalengland.org.uk/publication/1019788 [Accessed November 2, 2015].
Dhillon, R.S. & von Wuehlisch, G., 2013. Mitigation of global warming through renewable biomass. *Biomass and Bioenergy*, 48, pp.75–89.
Eggers, J. et al., 2009. Is biofuel policy harming biodiversity in Europe? *GCB Bioenergy*, 1(1), pp.18–34.
Energy Research Centre of the Netherlands, Phyllis2, database for biomass and waste. Available at: https://www.ecn.nl/phyllis2.
Fischer, G. et al., 2010. Biofuel production potentials in Europe: Sustainable use of cultivated land and pastures, Part II: Land use scenarios. *Biomass and Bioenergy*, 34(2), pp.173–187.
Fletcher, R.J. et al., 2011. Biodiversity conservation in the era of biofuels: risks and opportunities. *Frontiers in Ecology and the Environment*, 9(3), pp.161–168.
Florine, S.E. et al., 2006. Yield and composition of herbaceous biomass harvested from naturalized grassland in southern Iowa. *Biomass and Bioenergy*, 30(6), pp.522–528.
Foley, J.A. et al., 2011. Solutions for a cultivated planet. *Nature*, 478(7369), pp.337–342.
Frigon, J.-C. & Guiot, S.R., 2010. Biomethane production from starch and lignocellulosic crops: a comparative review. *Biofuels, Bioproducts and Biorefining*, 4(4), pp.447–458.
Gardiner, M.A. et al., 2010. Implications of three biofuel crops for beneficial rrthropods in agricultural landscapes. *Bioenergy Research*, 3(1), pp.6–19.
Green, D.I.G. et al., 2015. Maximizing alcohol yields from wheat and maize and their co-products for distilling or bioethanol production. *Journal of the Institute of Brewing*, 121(3), pp.332–337.
Godfray, H.C.J. et al., 2010. Food Security: The Challenge of Feeding 9 Billion People. *Science*, 327(5967), pp.812–818.
Gunnarsson, C., Vågström, L. & Hansson, P.-A., 2008. Logistics for forage harvest to biogas production—Timeliness, capacities and costs in a Swedish case study. *Biomass and Bioenergy*, 32(12), pp.1263–1273.
Gützloe, A., Thumm, U. & Lewandowski, I., 2014. Influence of climate parameters and management of permanent grassland on biogas yield and GHG emission substitution potential. *Biomass and Bioenergy*, 64, pp.175–189.
Halford, N.G., 2015. *An Introduction to Bioenergy*, Imperial College Press.
Hartwich, J., Bölscher, J. & Schulte, A., 2014. Impact of short-rotation coppice on water and land resources. *Water International*, 39(6), pp.813–825.
Harris, Z.M., Spake, R. & Taylor, G., 2015. Land use change to bioenergy: A meta-analysis of soil carbon and GHG emissions. *Biomass and Bioenergy*, 82, pp.27–39.
Heinsoo, K. et al., 2011. Reed canary grass yield and fuel quality in Estonian farmers' fields. *Biomass and Bioenergy*, 35(1), pp.617–625.
Held, J., Mathiasson, A. & Nylander, A., 2008. *Biogas from manure and waste products - Swedish case studies*, Sweden: Swedish Gas Association.
Hellmann, F. & Verburg, P.H., 2010. Impact assessment of the European biofuel directive on land use and biodiversity. *Journal of Environmental Management*, 91(6), pp.1389–1396.
Herrmann, C. et al., 2014. Biomass from landscape management of grassland used for biogas production: effects of harvest date and silage additives on feedstock quality and methane yield. *Grass and Forage Science*, 69(4), pp.549–566.

Hertel, T.W. et al., 2010. Effects of US maize ethanol on global land use and greenhouse gas emissions: Estimating Market-Mediated Responses. *BioScience*, 60(3), pp.223–231.
Himken, M. et al., 1997. Cultivation of Miscanthus under West European conditions: Seasonal changes in dry matter production, nutrient uptake and remobilization. *Plant and Soil*, 189(1), pp.117–126.
Himmel, M.E. et al., 2007. Biomass recalcitrance: Engineering plants and enzymes for biofuels production. *Science*, 315(5813), pp.804–807.
Holzschuh, A. et al., 2011. Expansion of mass-flowering crops leads to transient pollinator dilution and reduced wild plant pollination. *Proceedings of the Royal Society B-Biological Sciences*, 278(1723), pp.3444–3451.
Institute National de la Recherche Agronomique et al., Feedipedia: Animal Feed Resources Information System. Available at: http://www.feedipedia.org/content/about-feedipedia.
Jarchow, M.E. & Liebman, M., 2012. Nutrient enrichment reduces complementarity and increases priority effects in prairies managed for bioenergy. *Biomass and Bioenergy*, 36, pp.381–389.
Johnson, D.K. & Elander, R.T., 2008. Pretreatments for Enhanced Digestibility of Feedstocks. In M. E. H. P. Scientist, ed. *Biomass Recalcitrance*. Blackwell Publishing Ltd., pp. 436–453.
Jones, P. & Salter, A., 2013. Modelling the economics of farm-based anaerobic digestion in a UK whole-farm context. *Energy Policy*, 62, pp.215–225.
Jowit, J., 2012. EU rules "encouraging farmers to plough up grasslands." *The Guardian*. Available at: http://www.theguardian.com/environment/2012/feb/03/eu-farmers-plough-grasslands [Accessed December 8, 2015].
Jungers, J.M. et al., 2013. Energy potential of biomass from conservation grasslands in Minnesota, USA. *PLOS ONE*, 8(4), p.e61209.
Kahle, P. et al., 2001. Cropping of *Miscanthus* in Central Europe: biomass production and influence on nutrients and soil organic matter. *European Journal of Agronomy*, 15(3), pp.171–184.
Kirkham, F.W. et al., 2013. Effects of spreading species-rich green hay on the botanical composition of an agriculturally improved hay meadow in northern England. *Grass and Forage Science*, 68(2), pp.260–270.
Kullander, S., 2009. Energy from biomass. *The European Physical Journal Special Topics*, 176(1), pp.115–125.
Krasuska, E. & Rosenqvist, H., 2012. Economics of energy crops in Poland today and in the future. *Biomass & Bioenergy*, 38, pp.23–33.
Lepš, J. & Šmilauer, P., 2003. *Multivariate analysis of ecological data using CANOCO*, Cambridge: Cambridge University Press.
Liao, J.C. et al., 2016. Fuelling the future: microbial engineering for the production of sustainable biofuels. *Nature Reviews Microbiology*, 14(5), pp.288–304.
Lin, S., 2012. *Wetland biomass - Chemical benefits and problems with biogas usage*, Available at: http://www.diva-portal.org/smash/record.jsf?pid=diva2%3A535260&dswid=-7907 [Accessed October 30, 2015].
Lynd, L.R. et al., 2011. A global conversation about energy from biomass: the continental conventions of the global sustainable bioenergy project. *Interface Focus*, 1(2), pp.271–279.
Mackuľak, T. et al., 2012. Increase of biogas production from pretreated hay and leaves using wood-rotting fungi. *Chemical Papers*, 66(7), pp.649–653.
Martins, K.T., Gonzalez, A. & Lechowicz, M.J., 2015. Pollination services are mediated by bee functional diversity and landscape context. *Agriculture Ecosystems & Environment*, 200, pp.12–20.
Melts, I. et al., 2013. Comparison of two different bioenergy production options from late harvested biomass of Estonian semi-natural grasslands. *Energy*, 61, pp.6–12.
Melts, I., Heinsoo, K. & Ivask, M., 2014. Herbage production and chemical characteristics for bioenergy

production by plant functional groups from semi-natural grasslands. *Biomass and Bioenergy*, 67, pp.160–166.
Morris, R.K.A. et al., 2006. The creation of compensatory habitat—Can it secure sustainable development? *Journal for Nature Conservation*, 14(2), pp.106–116.
Mosier, N. et al., 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, 96(6), pp.673–686.
Muir, J.P., Pitman, W.D. & Foster, J.L., 2011. Sustainable, low-input, warm-season, grass–legume grassland mixtures: mission (nearly) impossible? *Grass and Forage Science*, 66(3), pp.301–315.
The National Non-Food Crop Center (NNFCC), Feedstocks. *The National Non-Food Crop Center*. Available at: http://www.nnfcc.co.uk/.
Openshaw, K., 2000. A review of Jatropha curcas: an oil plant of unfulfilled promise. *Biomass & Bioenergy*, 19(1), pp.1–15.
Peeters, A., 2009. Importance, evolution, environmental impact and future challenges of grasslands and grassland-based systems in Europe. *Grassland Science*, 55(3), pp.113–125.
Parliament, 2012. *Greening the Common Agricultural Policy: First Report of Session 2012-13, Report, Together with Formal Minutes, Oral and Written Evidence*. Great Britain Parliament House of Commons Environment, Food and Rural Affairs. The Stationery Office.
Parmlind, E., 2014. *Energy analysis of farm-based biogas plants in Sweden*. Uppsala: SLU, Dept. of Energy and Technology. Available at: http://stud.epsilon.slu.se/6765/.
Pedroli, B. et al., 2013. Is energy cropping in Europe compatible with biodiversity? – Opportunities and threats to biodiversity from land-based production of biomass for bioenergy purposes. *Biomass and Bioenergy*, 55, pp.73–86.
Piepenschneider, M. et al., 2016. Energy recovery from grass of urban roadside verges by anaerobic digestion and combustion after pre-processing. *Biomass and Bioenergy*, 85, pp.278–287.
Prochnow, A. et al., 2009. Bioenergy from permanent grassland – A review: 1. Biogas. *Bioresource Technology*, 100(21), pp.4931–4944.
Richards, B.K. et al., 1991. Methods for kinetic analysis of methane fermentation in high solids biomass digesters. *Biomass and Bioenergy*, 1(2), pp.65–73.
Richter, G.M. et al., 2008. Is UK biofuel supply from *Miscanthus* water-limited? *Soil Use and Management*, 24(3), pp.235–245.
Rittmann, B.E., 2001. *Environmental biotechnology: principles and applications*, Boston: McGraw-Hill.
Robertson, B.A. et al., 2011. Perennial biomass feedstocks enhance avian diversity. *Global Change Biology Bioenergy*, 3(3), pp.235–246.
Robinson, R.A. & Sutherland, W.J., 2002. Post-war changes in arable farming and biodiversity in Great Britain. *Journal of Applied Ecology*, 39(1), pp.157–176.
Rösch, C. et al., 2009. Energy production from grassland – Assessing the sustainability of different process chains under German conditions. *Biomass and Bioenergy*, 33(4), pp.689–700.
Rowe, R.L., Street, N.R. & Taylor, G., 2009. Identifying potential environmental impacts of large-scale deployment of dedicated bioenergy crops in the UK. *Renewable and Sustainable Energy Reviews*, 13(1), pp.271–290.
Russelle, M.P. et al., 2007. Comment on "Carbon-negative biofuels from low-input high-diversity grassland biomass". *Science (New York, N.Y.)*, 316(5831), p.1567; author reply 1567.
Sanderson, M.A. & Adler, P.R., 2008. Perennial Forages as Second Generation Bioenergy Crops. *International Journal of Molecular Sciences*, 9(5), pp.768–788.
Sasha D. Hafner, C.R., 2015. Validation of a simple gravimetric method for measuring biogas production in laboratory experiments. *Biomass and Bioenergy*, 83, pp.297–301.
Schädel, C. et al., 2010. Quantification and monosaccharide composition of hemicelluloses from different plant functional types. *Plant Physiology and Biochemistry*, 48(1), pp.1–8.
Schläpfer, F., Tucker, M. & Seidl, I., 2002. Returns from hay cultivation in fertilized low diversity and

non-fertilized high diversity grassland. *Environmental and Resource Economics*, 21(1), pp.89–100.
Seppälä, M. et al., 2009. Biogas production from boreal herbaceous grasses – Specific methane yield and methane yield per hectare. *Bioresource Technology*, 100(12), pp.2952–2958.
Sherrington, C., Bartley, J. & Moran, D., 2008. Farm-level constraints on the domestic supply of perennial energy crops in the UK. *Energy Policy*, 36(7), pp.2504–2512.
Sherrington, C. & Moran, D., 2010. Modelling farmer uptake of perennial energy crops in the UK. *Energy Policy*, 38(7), pp.3567–3578.
Skenhall, S.A., Berndes, G. & Woods, J., 2013. Integration of bioenergy systems into UK agriculture–New options for management of nitrogen flows. *Biomass and Bioenergy*, 54, pp.219–226.
Steubing, B. et al., 2010. Bioenergy in Switzerland: Assessing the domestic sustainable biomass potential. *Renewable and Sustainable Energy Reviews*, 14(8), pp.2256–2265.
Streffer, F., 2014. Lignocellulose to Biogas and other Products. *ResearchGate*, 2(1), p.1023. Available at: http://www.researchgate.net/publication/263661808_Lignocellulose_to_Biogas_and_other_Products [Accessed November 6, 2015].
Styles, D., Thorne, F. & Jones, M.B., 2008. Energy crops in Ireland: An economic comparison of willow and Miscanthus production with conventional farming systems. *Biomass & Bioenergy*, 32(5), pp.407–421.
Sturludottir, E. et al., 2014. Benefits of mixing grasses and legumes for herbage yield and nutritive value in Northern Europe and Canada. *Grass and Forage Science*, 69(2), pp.229–240.
Sun, L., Müller, B. & Schnürer, A., 2013. Biogas production from wheat straw: community structure of cellulose-degrading bacteria. *Energy, Sustainability and Society*, 3(1), p.15.
Sun, L., 2015. Biogas production from lignocellulosic materials microbial community structure.
Teghammer, A. et al., 2012. Enhanced biogas production from rice straw, triticale straw and softwood spruce by NMMO pretreatment. *Biomass and Bioenergy*, (36), pp.116–120.
Teghammer, A., 2013. *Biogas Production from Lignocelluloses: Pretreatment, Substrate Characterization, Co-digestion, and Economic Evaluation*. PhD thesis. Göteborg, Sweden: Doktorsavhandlingar vid Chalmers Tekniska Högskola.
Thumm, U., Raufer, B. & Lewandowski, I., 2014. Novel products from grassland (bioenergy & biorefinery). In A. Hopkins et al., eds. *EGF at 50: The Future of European Grasslands Proceedings of the 25th General Meeting of the European Grassland Federation Aberystwyth, Wales 7-11 September 2014*. Ceredigion, Wales: Gomer Press Ltd., pp. 429–437.
Tilman, D., Hill, J. & Lehman, C., 2006. Carbon-Negative Biofuels from Low-Input High-Diversity Grassland Biomass. *Science*, 314(5805), pp.1598–1600.
Tilman, D. & Downing, J.A., 1994. Biodiversity and stability in grasslands. *Nature*, 367(6461), pp.363–365.
Tonn, B., Thumm, U. & Claupein, W., 2010. Semi-natural grassland biomass for combustion: influence of botanical composition, harvest date and site conditions on fuel composition. *Grass and Forage Science*, 65(4), pp.383–397.
Tranter, R.B. et al., 2011. Assessing the potential for the uptake of on-farm anaerobic digestion for energy production in England. *Energy Policy*, 39(5), pp.2424–2430.
Triolo, J.M. et al., 2011. A new algorithm to characterize biodegradability of biomass during anaerobic digestion: Influence of lignin concentration on methane production potential. *Bioresource Technology*, 102(20), pp.9395–9402.
Triolo, J.M. et al., 2012. Biochemical methane potential and anaerobic biodegradability of non-herbaceous and herbaceous phytomass in biogas production. *Bioresource Technology*, 125, pp.226–232.
Tscharntke, T. et al., 2012. Global food security, biodiversity conservation and the future of agricultural intensification. *Biological Conservation*, 151(1), pp.53–59.

Upham, P. et al., 2009. Substitutable biodiesel feedstocks for the UK: a review of sustainability issues with reference to the UK RTFO. *Journal of Cleaner Production*, 17, Supplement 1, pp.S37–S45.
Verdade, L.M., Piña, C.I. & Rosalino, L.M., 2015. Biofuels and biodiversity: Challenges and opportunities. *Environmental Development*, 15, pp.64–78.
Ward, A.J. et al., 2008. Optimization of the anaerobic digestion of agricultural resources. *Bioresource Technology*, 99(17), pp.7928–7940.
Werling, B.P. et al., 2014. Perennial grasslands enhance biodiversity and multiple ecosystem services in bioenergy landscapes. *Proceedings of the National Academy of Sciences of the United States of America*, 111(4), pp.1652–1657.

CHAPTER 4

EFFECT OF AGRICULTURAL INTENSIFICATION AND ARABLE REVERSION ON MICROBIAL DIVERSITY AND COMMUNITY COMPOSITION

Abstract

The rapid global conversion of biodiverse landscapes to intensively managed arable fields may decrease microbial diversity and threaten the long-term fertility of native soils. However, previous laboratory and experimental studies provide conflicting results: some record declines in overall microbial diversity and particular taxa under intensified cultivation while others report no change (or even increased) diversity. More research based on actual agroecological systems under active farmer management and analysis of changes in a wider range are needed. I analysed the soil metagenomic communities of 36 current and former grasslands in Oxfordshire, England using Illumina sequencing of the bacterial 16S and fungal ITS1 regions to examine how agricultural intensification alters native microbial communities and whether arable reversion can reverse these processes. I demonstrate that land use change did not affect bacterial OTU diversity or specific beneficial OTUs (nitrogen-fixing bacteria and mycorrhizas). However, fungal OTU diversity declined and OTUs identified as potential plant, human and livestock 'pathogens' were significantly more abundant on agriculturally improved fields. On sites where arable reversion took place, microbial communities were similar to those from unimproved grasslands although overall vegetation diversity and fungal OTU richness was lower. Maintaining species-rich grasslands could ensure the maintenance of native soil microbial diversity. This diversity can serve as a source of inoculum to restore fields under arable reversion and as a biofertilizer to naturally boost the productivity of arable fields, thus contributing to global food security by increasing local agricultural sustainability.

1. Introduction

Globally, agricultural intensification may pose a threat to microbial diversity and long-term soil fertility (Garbeva et al. 2004; Lee and Lee 2013; Salles et al. 2006). Agricultural intensification refers to the conversion of land to arable production and the increased use of inputs (e.g. fertilizers, pesticides) on these fields to boost productivity. Replacing highly biodiverse semi-natural landscapes with intensively cultivated arable fields may irreversibly alter or decrease native microbial communities. The loss of this diversity has important ramifications for agriculture and food security. In one gram of soil there are over 50,000 bacterial and fungal species which play a key role in plant health, soil fertility, and wider ecosystem functioning (Chaparro et al. 2012; Mendes et al. 2013).

Maintaining microbial diversity can contribute to creating more sustainable agricultural production systems and may help lower overall dependence on agricultural inputs in the future (Brussaard et al. 2007; Chaparro et al. 2012; Mendes et al. 2013).

In northern Europe, and in the UK in particular, agricultural intensification poses the greatest threat to species-rich grasslands. Species-rich 'unimproved' grasslands have developed over thousands of years of human activity (e.g. grazing, meadow management) and are characterized by high levels of botanical and zoological diversity. Douds and Millner (1999), Gosling et al. (2006), and van der Heijden et al. (2006) also suggest that unimproved grasslands contain high levels of microbial diversity, particularly in regards to fungi. However, since the 1950s farmers began to plough up unimproved grasslands, replacing them with (ostensibly more productive and financially profitable) improved grasslands or arable crops, a trend which has accelerated in recent years. In lowland England alone, over 97% of unimproved grasslands have been lost to these processes (Blackstock et al. 1999).

Currently, how agricultural intensification may affect grassland microbial diversity is unknown. Previous laboratory and experimental research on arable fields suggests that microbial diversity and specific beneficial microbial communities, like arbuscular mycorrhizal fungi (AMF) which provide their plant hosts with phosphorus (P), decline under increased fertilization (Gosling et al. 2006; Kiers et al. 2002; Oehl et al. 2010; Verbruggen et al. 2013). Addition of increased nutrients to the soil may also reduce the effectiveness of microbiota associated with plant-defence and nitrogen (N) fixation (Salles et al. 2006; Fox et al. 2007). On the other hand, several studies indicate that certain beneficial microbiota like Plant-Growth-Promoting Rhizobia (PGPR) and mycorrhizas are resilient to increased inputs and may even perform better under increased soil fertility (Saia et al. 2015; Shafiani and Malik 2003).

Yet how applicable these results are to grasslands is unclear. Previous research on the effect of agricultural intensification on soil microbial diversity is largely based on lab experiments or on field-scale experiments conducted under controlled conditions. Results from such studies may not capture the dynamic nature of soil, plant, and ecosystem interactions found in actual agroecological systems. In addition, previous research on how agricultural intensification affects microbial diversity has only looked at a limited number of taxa. More research is needed which looks at the effect of intensification on wider microbial community composition, particularly in regards to 'beneficial' and 'detrimental' microbiota.

This paper has two main aims: (1) to determine how reduction of vegetation diversity and increased soil fertility under agricultural intensification affects microbial diversity and the abundance of 'beneficial' and potentially 'pathogenic' microbiota, and (2) whether arable reversion can reverse these processes. The goal of this analysis is to inform current agricultural practices as well as grassland conservation. Establishing how these changes will affect microbial diversity is particularly critical in order to plan for, and potentially counteract, the effects of agricultural intensification on the landscape.

To examine whether agricultural intensification threatens native microbial diversity, I conducted vegetation surveys, soil analysis, and metagenomic research at 36 current and former grasslands in Oxfordshire, England. All sites are part of active, working farms. Specifically, I sampled unimproved grasslands which had never been ploughed or used for arable cultivation; improved grasslands which receive high levels of inorganic inputs and are sown with commercial ryegrass (*Lolium perenne* L.) and clover (*Trifolium pratense* L. and *T. repens* L.) based seed mixes; 'conservation grasslands' which are former arable fields which have undergone arable reversion; and two types of arable fields (rapeseed (*Brassica napus* L.) and poppy (*Papaver somniferum* L.)). I sampled these field types because they reflect real changes affecting the local landscape and environment within the study area.

I also intentionally sampled rapeseed and poppy fields because both crops do not form symbioses with mycorrhizas and there has been some concern regarding the long term effect of cultivating non-mycorrhizal crops on soil fungal diversity. I determined the soil metagenomic profiles of each site using Illumina sequencing of the bacterial 16S and fungal ITS1 regions and I compared differences in bacterial and fungal Operational Taxonomic Unit (OTU) diversity among the land use types. I also determined whether there were significant differences in two groups of beneficial microbiota with known functions, nitrogen-fixing bacteria and mycorrhizas. Although tentative, I examined differences in other potentially 'beneficial' and 'pathogenic' OTUs. Changes in the abundances of these OTUs can contribute to our understanding of how agricultural intensification affects soil fertility and soil health. Finally, throughout these analyses I compared the microbial diversity and community composition of unimproved and conservation grasslands to determine whether arable reversion can restore microbial diversity.

2. Methods

2.1 Study Area and Site Selection

The county of Oxfordshire is located in south-east England, UK. It has a maritime temperate climate with an annual rainfall of between 570-750 mm (depending on elevation) (Killick et al. 1998) (**Figure 1**). The daily average temperature in winter (November - February) ranges from 4.9 - 7.6 $^{\circ}$C and the daily average temperature in summer (May – August) ranges from 12.5 - 17.6 $^{\circ}$ C. The three main soil types are chalk, alluvial, and sandstone. The primary crops are wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), and rapeseed. Farmers raise livestock for meat and dairy production.

Between June-July 2015, I conducted fieldwork at 36 sites in the region. These sites fell under five major land use categories (**Table 1**).

Table 1 Description of Land Use Categories. The study sites are divided into five land use categories: unimproved grasslands, conservation grasslands, improved grasslands, poppy fields, and rapeseed fields. Each category is characterized by type of land use (grassland or arable), function (agriculture or conservation), vegetation (unsown (i.e. natural) or sown), seed source (local or commercial), application of inorganic inputs (N, P, and K), use of rotation, and whether the field is grazed, mown, mown and grazed, or cut.

Description	Unimproved grassland (n=14)	Conservation grassland (n=10)	Improved grassland (n=6)	Poppy field (n=3)	Rapeseed field (n=3)
Land Use	Grassland	Grassland	Grassland	Arable	Arable
Function	Agricultural	Conservation	Agricultural	Agricultural	Agricultural
Vegetation	Unsown	Sown	Sown	Sown	Sown
Seed source	Local	Local, commercial	Commercial	Commercial	Commercial
Application of inorganic N, P, K	No	No	Yes	Yes	Yes
Rotation	No	No	Yes	Yes	Yes
Grazed, Mown, or Cut	G, M, MG	G, MG	Cut	Cut	Cut

Unimproved grasslands are pastures and meadows managed by farmers (for fodder) or by conservation groups (to maintain rare plant species located there). All unimproved sites, as far as I know, have never been ploughed. Conservation grasslands are former intensively cultivated arable fields that have undergone arable reversion. This is achieved by (1) soil amendment (where soil is stripped to reduce soil nutrients) and (2) re-sowing former arable fields with plants characteristic of unimproved grasslands. Improved grasslands are leys sown with legume-based seed mixtures and treated with fertilizers (inorganic and organic). These fields are ploughed and re-sown every year or every other year depending on the yield of the first season and if a rotation system is used.

The rapeseed and poppy fields were converted from grassland to arable within the past 30 years. Farmers increasingly replace grassland with rapeseed because they can sell it to different markets (e.g.

for culinary oil, bioenergy) so it is more profitable than pastureland or meadows. Since 2008, farmers in the region also began to grow opium poppy due to national efforts to localize the production of morphine and its derivatives in the UK. Farmers consider it a good break crop and incorporate it into their current rotation systems. All agriculturally improved arable fields receive inorganic N and P (and in one case, digestate from an anaerobic digester). None of these fields are tilled and all undergo rotation. In north Oxfordshire, farmers rotate rapeseed with wheat, barley, oats and peas (*Pisum sativum* L.). In south Oxfordshire, farmers rotate the rapeseed and poppy fields together along with wheat and barley.

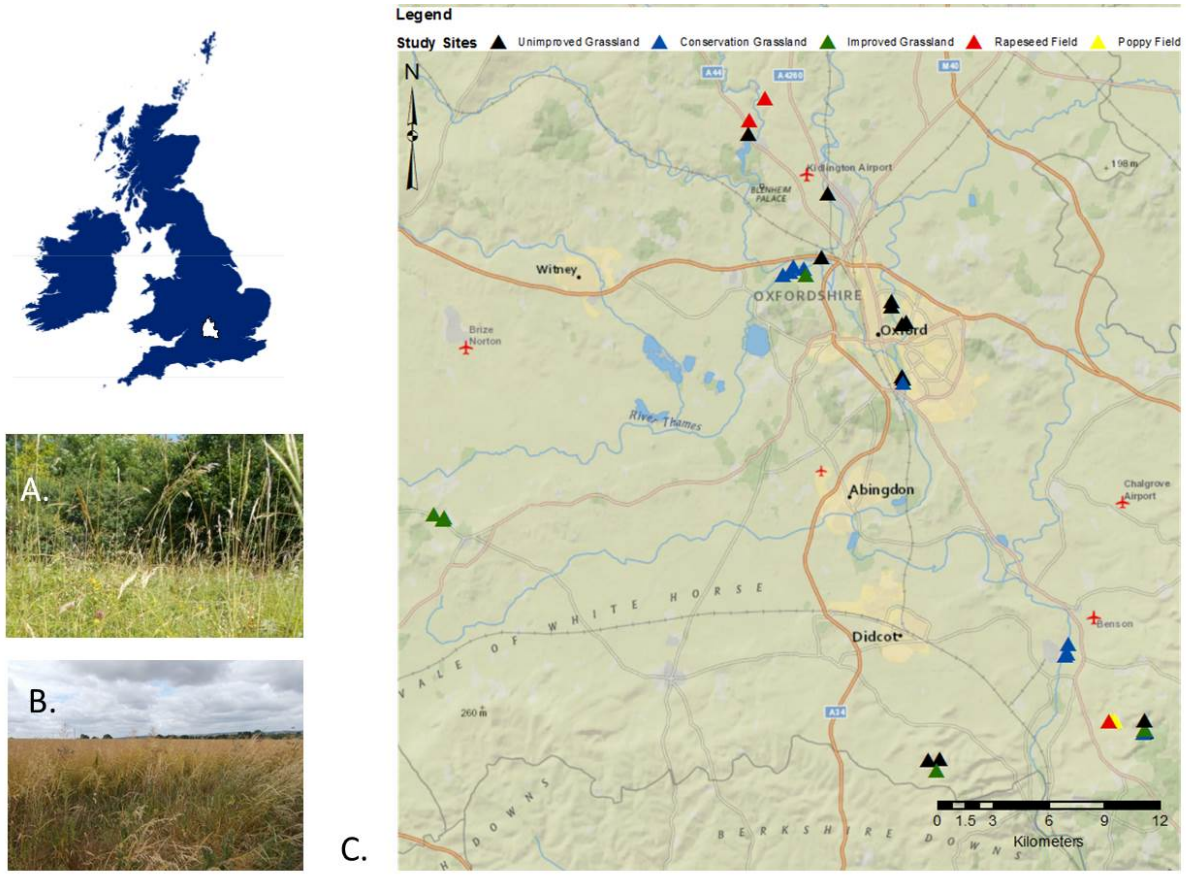


Figure 1 Location of study sites in Oxfordshire, England. A. A species-rich, unimproved chalk grassland in south Oxfordshire. B. A rapeseed field from south Oxfordshire 2km from the unimproved chalk grassland in figure 1A. C. Map of Oxfordshire, England showing the location of study sites where soil samples were taken.

2.2 Vegetation Surveys and Soil Sample Collection

At each site, I conducted vegetation surveys by placing five $1m^2$ quadrats randomly in each field and recording which species were present. I avoided the edges of sites (which may be subject to disturbance) by excluding a 1 m margin around each field. I collected soil samples at each site at the same time of day (early morning), season (early summer), and weather (no rain) in order to ensure comparability between sampling units. This is particularly important because microbial abundance can decline during certain seasons (particularly winter) and increase under certain environmental conditions (e.g. rainfall) or management practices (e.g. fertilizer application) (Barni and Siniscalco 2000; Eriksson 2001; Schneider *et al.* 2010).

To ensure the soil samples reflected local grassland vegetation and soil diversity, I took twenty 5 x 13 cm cores from each field. Contamination was avoided by using different corers for each field. I then combined and homogenized the remaining soil from each core and removed a small subsample (ca. 100 g). This subsample contained both roots and soil to capture the variation in the rhizosphere communities at each site (Berg and Smalla 2009; Berendson et al. 2012; Chaparro et al. 2010). I then sent the rest of the sample to Anglian Soil Analysis to establish Total N, P, K, Mg, and pH (Anglian Soil Analysis, Boston, Lincolnshire).

2.4 DNA Extraction and PCR

I extracted DNA from the soil samples using the protocols for the ZR Soil Microbe DNA Miniprep kit (Zymo Research). To identify both bacterial and fungal communities in the samples, I selected PCR primers targeting the V4 region of 16S rRNA for bacteria and the ITS1 region for fungi. The forward primer used for the 16S region was F515 5'-GTGCCAGCMGCCGCGGTAA-3' and the reverse primer

was R806 5'-ATTAGAWACCCBDGTAGTCC-3' (Caporaso et al. 2011). Degenerate primers were used for the 16S region to account for the potential changes in base pairs (A and C) in bacterial sequences. The forward primer used for the fungal ITS1 region was ITS1F 5'-CTTGGTCATTTAGAGGAAGTAA-3' and the reverse primer was ITS2 5'-GCTGCGTTCTTCATCGATGC-3' (Buée et al. 2009; White 1999). Barcoding was used to mark each sample so that samples could be combined after PCR and separated after sequencing.

I performed PCR twice (once for bacteria and once for fungi) using a Phusion High-Fidelity PCR kit (New England Biolabs). Each 20µl reaction mixture contained 4 µl Phusion, 0.4 µl dNTPs, 1 µl forward primer (10 pmol final concentration), 1 µl reverse primer (10 pmol final concentration), 1 µl template DNA, and 0.2 µl Phusion DNA polymerase according to standard protocols. Initial denaturation of the DNA was run for one cycle at 98° degrees for 3 minutes. Denaturation, annealing and extension were run for 30 cycles at 98° C (15 seconds), 55° C (15 seconds) and 72° C (30 seconds) respectively. The final extension was run for 7 minutes at 72° C.

To establish the quality of the DNA, I assessed 5µl of the PCR product from each sample by electrophoresis in 1.5% agarose gels. For samples where the quality of the DNA was low, I performed PCR a second time, ran an agarose gel to check DNA quality, and if the quality was higher than the first round of PCR, I used the second sample. I then combined 10 µl of PCR product for each of the fungal and bacterial samples. To quantify the final PCR product before sending the combined sample for sequencing, Qubit™ fluorometric quantitation was performed using a Qubit™ Quantification Assay Kit (Thermo Fisher Scientific) following standard procedures. The final concentration of the DNA was 6.8 ng/µl in 200µl of product.

2.5 Sequencing and Analysis of Microbial Data

The PCR product was sent to MR DNA in Texas for MiSeq Illumina sequencing. Once the sequences were received, FLASh (**F**ast **L**ength **A**djustment of **SH**ort reads) was used to join pair-ended reads and to remove low-quality sequences (Magoc and Salzberg 2011). Sequences with a phred score below 33 were cut to improve the overall accuracy of the analysis (Walshaw et al. 2011). To assign OTUs to the sequences that passed this quality benchmark, local alignment was set to a minimum of a 50 bp (base pair) match with a 10% threshold for base pair mismatch. To remove chimeras and to bin similar sequences together, Usearch was used (Edgar 2010). All OTUs were matched at the 97% similarity level. The barcoded samples were then separated using Bash, resulting in one bacterial and one fungal sample representative of the microbial community for each of the 36 sites.

To assign taxonomic identities to the OTUs in the samples, I used the SILVA small-subunit rRNA (SSU) database (version 126) to predict rRNA genes (Quast et al. 2013). The maximum e-value cut off was set to 1^{e-5}, the minimum alignment identity was set to 100%, and the minimum alignment length cut-off was set to 15bp in order to achieve a high rate of certainty in OTU identifications. I used the NCBI database (http://blast.ncbi.nlm.nih.gov/Blast) to annotate fungal samples. I optimized the nucleotide database (nr/nt) query for highly similar sequences (megablast). I used MEGAN 5 (MEtaGenome ANalyzer, Center for Bioinformatics, Tübingen, Germany, http://ab.inf.uni-tuebingen.de/software/megan5/) to determine the taxonomic profile and abundance of bacterial and fungal OTUs for each sample (Huson et al. 2011; Oulas et al. 2015). I determined the bacterial and fungal OTU richness based upon the number of OTUs for each sample. To calculate evenness, I used the Shannon Weaver Index.

2.6 Analysis of Microbial OTUs

In this study, I compared taxonomic differences and OTU abundances of two microbial groups known to affect plant growth and health: N-fixing bacteria and mycorrhizas. For heuristic purposes, I also divided OTUs with known ecological functions relating to soil fertility, agricultural productivity, and plant, livestock and human health into nine additional functional groups. Taxa were assigned to each functional group at the species level when possible, as certain genera (e.g. *Pseudomonas*, *Stenotrophomonas*) contain OTUs that are either beneficial or pathogenic to plants (Alavi et al. 2014; Zeilinger et al. 2015). These additional functional groups were: Plant-Growth-Promoting Rhizobia (PGPR), P-solubilizing bacteria, pathogen-supressing fungi, saprotrophic fungi, bacterial plant pathogens, bacterial human and livestock pathogens, fungal plant pathogens, and fungal human and livestock pathogens. As I have not tested for pathogenicity (e.g. by immunofluorescence assay, etc.) (Cahill 1999), I emphasize that I refer to OTUs which reflect *potential* soil pathogens.

This system of classification builds upon previous studies which have sought to determine how different land management practices positively or negatively affect soil quality by comparing the functional genes (Delmont et al. 2011; Fierer et al. 2012; Langille et al. 2013; Simon and Daniel 2011; Torsvik and Øvreås 2002) and chemical activity profiles (e.g. using substrate-induced respiration, community level physiological profiling, and extracellular enzyme assays) of soil microbial communities (Balser et al. 2010; Nannipieri et al. 2003). As these tests cannot identify the taxa responsible for these processes, I hope that my speculative division of OTUs into functional groups can add to this research and can serve to generate new hypotheses regarding how changes in land use affect microbial diversity and soil fertility.

2.7 Statistical Analysis

I used One-way analysis of variance (ANOVA) to determine whether land use had a significant effect on vegetation diversity, soil fertility, microbial diversity, and specific microbial taxa. *F* tests were used to determine whether there were significant differences among land-use types and a limited number of *t* tests were performed to specifically establish whether the mean values of the other land use types differed significantly from that of the unimproved grasslands. I analysed the differences in microbial community composition among sites using Correspondence Analysis (CA). To determine the effect of soil fertility on microbial communities, I used Redundancy Analysis (RDA) (Canoco version 4.5). I used Pearson Correlation analysis to determine whether there was a correlation between vegetation, bacterial and fungal OTU richness. I performed ANOVA and correlation analysis in R (version 3.2.2, "Fire Safety") and CA and RDA in Canoco (version 4.5, Lepš and Šmilauer 2003).

3. Results

3.1 Variation in Vegetation Species Richness and Soil Fertility

Vegetation species-richness varied significantly among sites ($F_{4,31} = 16.61$, $P < 0.001$; **Appendix III Table 1 and Figure 1**). Unimproved grasslands had the highest species-richness (16.42 ± 1.1 species) and were dominated by a wide variety of grasses (e.g. *Festuca gigantea* (L.) Vill., *Festuca pratensis* Huds., *Dactylis glomerata* L., *Glyceria fluitans* (L.) R. Br.) and legumes (e.g. *Lathyrus pratensis* L., *Vicia cracca* L.). Although the botanical composition of conservation grasslands closely mirrored that of unimproved grasslands, the species richness of conservation grasslands was on average lower by 5.23 ± 1.716 species ($t = -3.047$, $P = 0.005$). Species richness was lowest on the rapeseed and poppy fields.

Soil N ($F_{4,31}$ = 5.701, P = 0.001), P ($F_{4,31}$ = 5.405, P = 0.002), and K ($F_{4,31}$ = 4.944, P = 0.003) varied significantly among the study sites (**Appendix III Table 1 and Figure 2**). Improved grasslands had the highest level of soil P (73.5 ± 12.48 mg/L) and K (342.33 ± 44.31 mg/L). Unimproved grasslands had the highest levels of total N (6.625± 0.513 mg/L). There was no significant difference between unimproved and conservation grasslands in either soil N (t = -1.104, P = 0.278) or K (t = 0.958, P = 0.346). However, soil P was significantly higher in fields under arable reversion compared to unimproved grasslands (t = 2.324, P = 0.027). Neither soil Mg ($F_{4,31}$= 0.907, P = 0.472) nor pH ($F_{4,33}$ = 2.251, P = 0.086) differed significantly among land-use types.

3.2 Soil Microbial Diversity

Bacterial OTU richness did not vary among sites ($F_{4,31}$ = 0.906, P= 0.472) and was highest in the samples from the rapeseed fields (1749 ± 334.049 OTUs) and lowest in samples from the poppy fields (1155 ± 334.049 OTUs) (**Appendix III Table 1 and Figure 1)**. The most frequent bacteria OTUs were *Gaiella*, *Bacillus, Solirubrobacter, Defluviicoccus,* and *Pseudomonas,* which were found across all site types at relatively similar frequencies. 28% of bacteria OTUs were site specific (found only at one site). 19% of bacterial OTUs were unique to species-rich grasslands (unimproved and/or conservation grasslands) and 11% were unique to agriculturally improved fields.

Fungal OTU richness varied significantly among sites ($F_{4,31}$ = 7.442, P < 0.001) and was highest in unimproved grasslands (178.786 ± 8.65 OTUs) and lowest in the rapeseed (108 ± 20.599 OTUs) and poppy fields (111.67 ± 20.599 OTUs). Fungal OTU richness was also significantly lower (on average by 45.886 ± 13.406 OTUs) on conservation grasslands compared with unimproved grasslands (t = -3.423, P = 0.002). The abundance of the most frequent fungal taxa, *Mortierella*, *Fusarium,*

Cladosporium, *Preussia*, and *Plectosphaerella* varied substantially among sites. 40% of fungal OTUs were site specific. 56% of fungal OTUs were unique to species-rich grasslands while only 9% of OTUs were unique to agriculturally improved fields. There was no correlation between bacterial and fungal OTU diversity (r = 0.163, P = 0.341). This suggests that decrease in the diversity of one community does not impact the other.

In terms of overall community composition, correspondence analysis demonstrates the close relationship between unimproved and conservation grasslands (**Figure 2**) while samples from agriculturally improved fields are similar to one another.

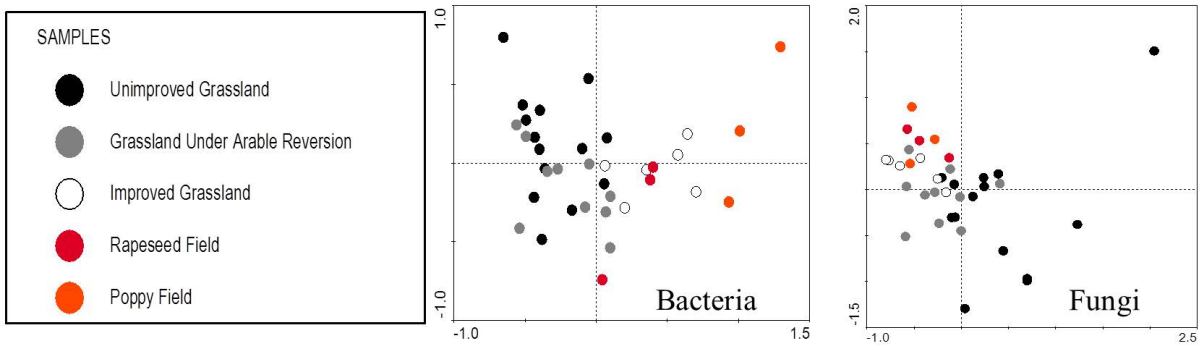


Figure 2 Correspondence analysis showing the relationship among bacterial (A) and fungal (B) communities among sites. A. Bacterial community composition was similar between unimproved grasslands and fields under arable reversion while the communities from improved grasslands, rapeseed fields and poppy fields were similar. Correspondence analysis explains 26.6 % of the variation in bacterial OTUs. B. Fungal communities between unimproved grasslands and fields under arable reversion were similar. Fungal community composition among rapeseed fields, poppy fields and improved grasslands was similar. The fungal communities of two improved grasslands showed some similarity to those from unimproved grasslands. Correspondence analysis explains 24.5 % of the variation in OTUs.

There was no correlation between vegetation species richness and bacterial OTU diversity (r = 0.135, P = 0.432) but fungal OTU diversity declined with decreased vegetation species richness (r = 0.552, P < 0.001). Redundancy (RDA) analysis of the bacteria samples and soil variable explains 29 % of the variation in OTU data and 96 % of OTU-environment correlations ($F_{4, 31}$ = 2.601, P = 0.002) and 18.5 % of the variation among OTUs and 87.6 % of OTU-environment correlations of the fungal samples ($F_{4, 31}$ = 1.6, P = 0.002). The Generalized Additive Models (GAMs) in **Figure 3** show the sensitivity

of bacterial and fungal OTU diversity to increased soil fertility. Bacterial OTU diversity did not decrease with increased soil N ($F_{4, 31} = 2.69$, P = 0.069) or P ($F_{4, 31} = 1$, P = 0.378) but declined under increased K ($F_{4, 31} = 6.19$, P = 0.005). Fungal OTU diversity was not affected by increased N ($F_{4, 31} = 0.968$, P = 0.39) or K ($F_{4, 31} = 1.06$, P = 0.17) but declined under increased P ($F_{4, 31} = 6.29$, P = 0.004). Increased soil pH did not affect bacterial OTU diversity ($F_{4, 31} = 0.96$, P = 0.39) or fungal OTU diversity ($F_{4, 31} = 0.631$, P = 0.461).

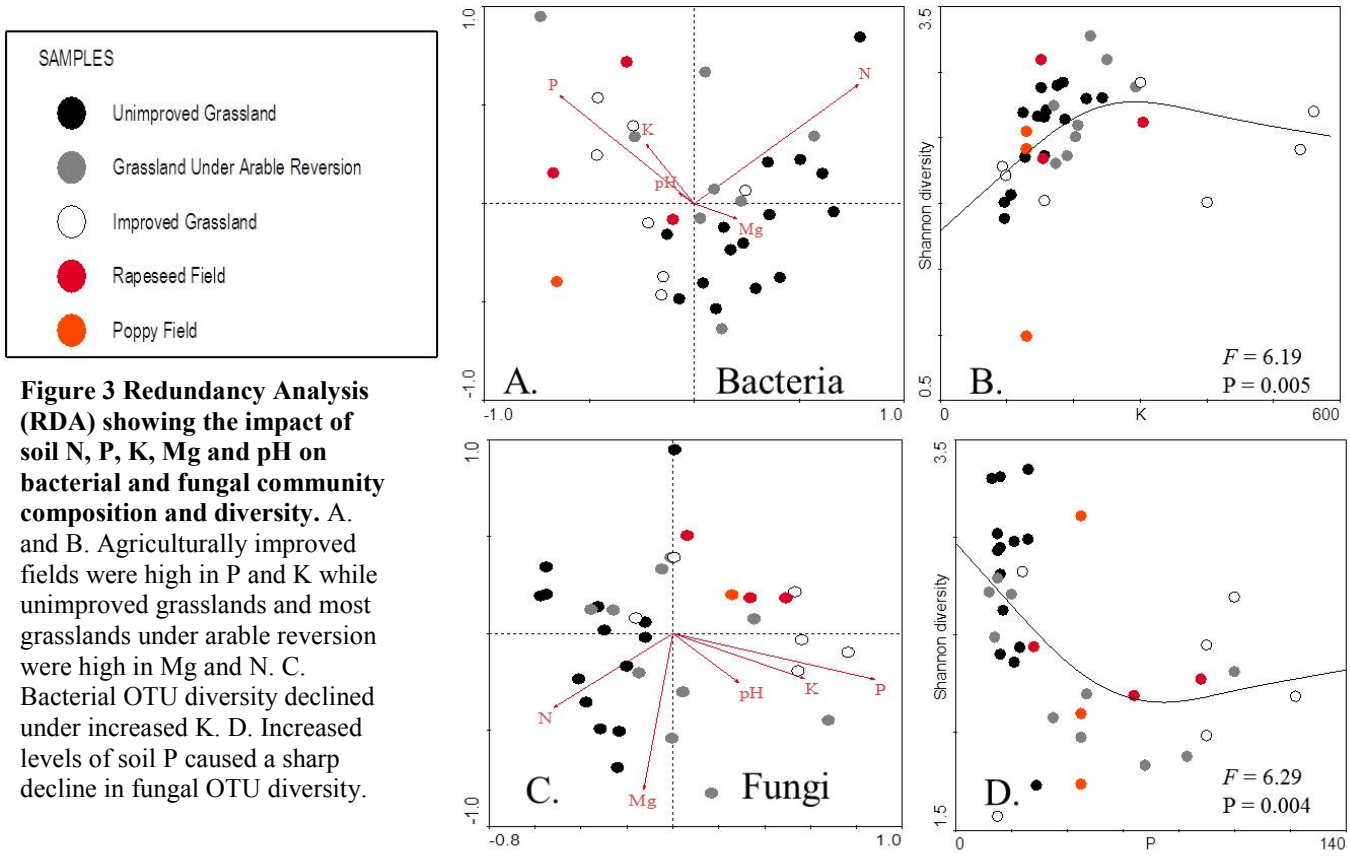


Figure 3 Redundancy Analysis (RDA) showing the impact of soil N, P, K, Mg and pH on bacterial and fungal community composition and diversity. A. and B. Agriculturally improved fields were high in P and K while unimproved grasslands and most grasslands under arable reversion were high in Mg and N. C. Bacterial OTU diversity declined under increased K. D. Increased levels of soil P caused a sharp decline in fungal OTU diversity.

3.3 Variability in Microbial OTUs with Agroecological Significance

There was no significant difference in the abundance of N-fixing bacteria among sites ($F_{4, 31} = 1.395$, $P = 0.263$) (**Appendix III Figure 3**). However, certain OTUs were associated with particular land-use types. *Bradyrhyzobium* spp., *Sinorhizobium* spp., *Frankia* spp., *Heliobacter* spp., *Nitrosomonas* spp.,

and *Paenibacillus polymyxa* were abundant on unimproved and conservation grasslands (**Figure 4**). *Rhizobium spp., Cyanobacteria* sp., and *Azotobacter* spp. were abundant in the samples from the poppy and rapeseed fields. Although the primers used in this analysis were not mycorrhiza-specific, several OTUs were picked up by sequencing. The abundance of identified mycorrhizas did not vary significantly among sites ($F_{4, 31} = 1.327$, P = 0.282). *Glomus* spp. were found in low abundances across the study sites. However, some OTUs, namely *Paraglomus* sp. and *Acaulospora* sp., were found only in samples from unimproved and conservation grasslands (**Figure 4**).

Other OTUs with potential positive effects on plant growth and health were ubiquitous across the study sites although many of these OTUs were associated to particular land uses (**Appendix III Table 1, Appendix III Figure 3**). There was no significant difference in the abundance of Plant Growth-Promoting Rhizobia (PGPR) among the different site types ($F_{4, 31} = 0.489$, P = 0.744). *Streptomyces africanus* and *S. virginiae* were abundant in the samples from the unimproved and conservation grasslands while *S. carpaticus, S. clavuligens* and *S. platensis* were abundant in samples from the improved grasslands, rapeseed and poppy fields. Bacteria that solubilize phosphate into an accessible form that can be taken up by plants were also ubiquitous ($F_{4, 31} = 2.263$, P = 0.09), yet were more abundant in samples from the rapeseed (25.33 ± 8.903 OTUs; $t = 2.162$, P = 0.04) and poppy fields (26.33 ± 8.903 OTUs; $t = 2.274$, P = 0.0314) compared to the unimproved grasslands (6.083± 3.982 OTUs). *Azospirillium* spp. and *Burkholderia* spp. were found across the different land uses. *Micrococcus* spp. was more abundant on unimproved and conservation grasslands while *Flavobacterium* spp. were abundant on the improved grasslands and rapeseed fields. *Achromobacter* sp., *Chryseobacterium* spp., and *Stenotrophomanas rhizophilia* were only found in the samples from the poppy fields.

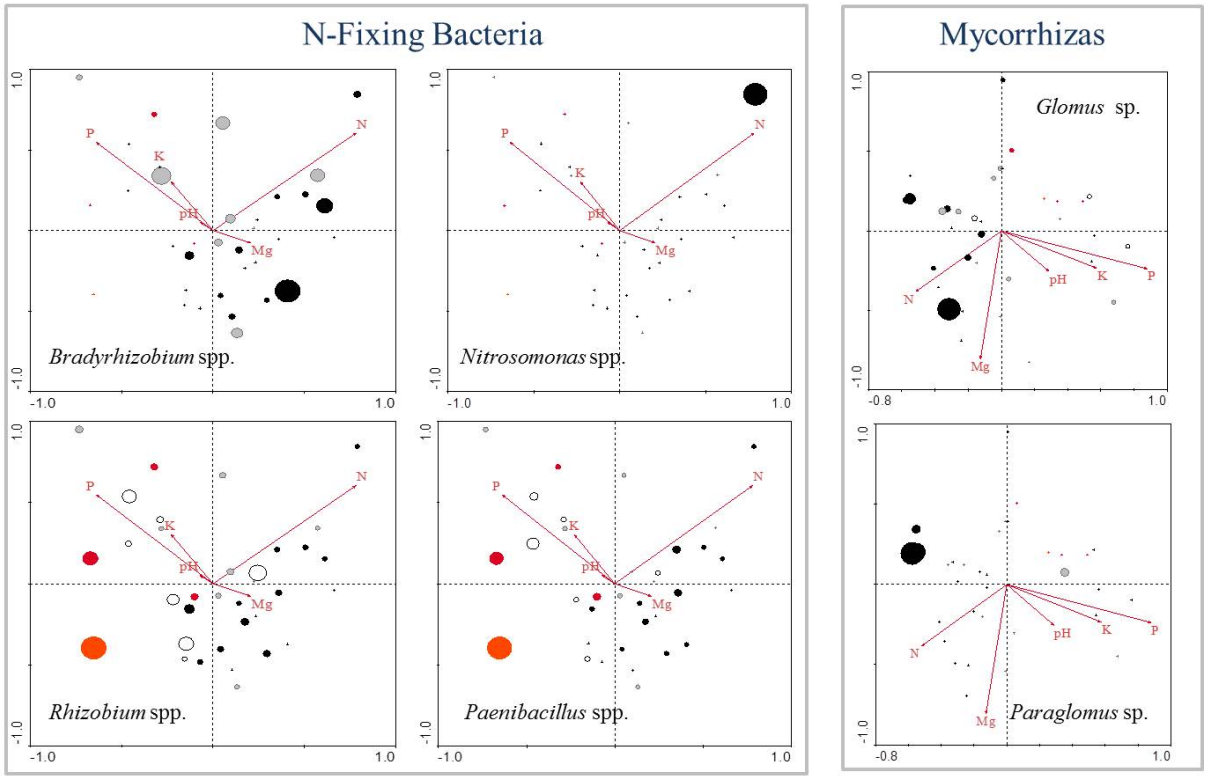


Figure 4 RDA showing differences in abundances of N-fixing bacteria and mycorrhizas among sites. *Bradyrhizobium* spp. was associated with unimproved grasslands and fields under arable reversion while *Rhizobium* spp. and *Paenibacillus* spp. were assocaited with agriculturelly improved fields. *Nitrosomonas* spp. were only found on unimproved grasslands. *Glomus* spp. were found across the study sites and while *Paraglomus* sp. was found only on unimproved grasslands and grasslands under arable reversion. In the figures, samples are represented by the abundance of each N-fixing bacteria or mycorrhiza (absence is symbolized by a '+') and are color-coded by land-use type. The figures are based on the RDAs in Figure 3.

The abundance of beneficial fungal OTUs associated with pathogen suppression ($F_{4, 31} = 2.671$, P = 0.05) varied among sites and were most abundant on rapeseed fields (75.667 ± 18.239 OTUs) and conservation grasslands (55.6 ± 11.870 OTUs) compared to unimproved grasslands (27± 7.662 OTUs). There was no significant variation in the abundance of fungal OTUs associated with the decomposition of organic matter among field types ($F_{4, 31} = 1.589$, P = 0.202), although they were significantly less abundant on agriculturally improved leys ($t = -2.105$, P = 0.043) compared to unimproved grasslands. Specific beneficial fungal OTUs were also associated with particular land uses. For example, samples from unimproved and conservation grasslands were dominated by

Penicillum and *Preussia* while the poppy and rapeseed fields were dominated by *Cadophora*, *Clonostachys*, and *Minimedusa polyspora*. Only one OTU, *Mortierella,* was found across the study sites. Of the saprotrophic fungi, *Clavaria*, *Gandoderma* and *Stereum* were found primarily on unimproved and conservation grasslands.

Although their actual pathogenicity is unknown, OTUs which may represent potential plant, human and livestock pathogens were more abundant on agriculturally improved fields. The abundance of potential bacterial plant pathogens varied significantly among site types ($F_{4, 31} = 12.71$, $P < 0.001$) and were almost twenty times more abundant in samples from the poppy fields (23.67 ± 3.24 OTUs) compared to the unimproved grasslands (1.50 ± 1.45 OTUs). Similarly, the abundance of potential plant fungal pathogens varied significantly among sites ($F_{4, 31} = 3.449$, $P = 0.019$) and were significantly higher on the rapeseed (71.37 ± 17.522 OTUs) and poppy fields (61 ± 17.522 OTUs) compared to the unimproved grasslands (16.786 ± 7.361 OTUs). Potential fungal pathogens affecting humans and livestock also varied significantly among sites ($F_{4, 31} = 12.56$, $P < 0.001$) and were more abundant on the rapeseed fields (61.667 ± 8.497 OTUs) compared to unimproved grasslands (5.429 ± 3.569 OTUs). *Erwinia* spp, *Fusarium* sp., *Olpidium brassicae*, *Microdochium* sp., *Pseudomonas viridiflava*, *Pyrenochaeta lycopersici* and *Verticillium dahliae* were also all five to ten times more abundant on improved leys, rapeseed and poppy fields compared to unimproved and conservation grasslands (**Figure 5, Appendix III Figure 4**). The only abundant potentially pathogenic human and livestock OTU on unimproved grasslands was *Clostridium botulinum*. Several pathogens affecting humans and livestock (particularly *Chaetomium* spp., *Cryptococcus* spp., *Enterobacter amnigenus*, *Stenotrophomonas maltophilia* and *Yersinia* spp.) were only found in samples from the rapeseed and poppy fields.

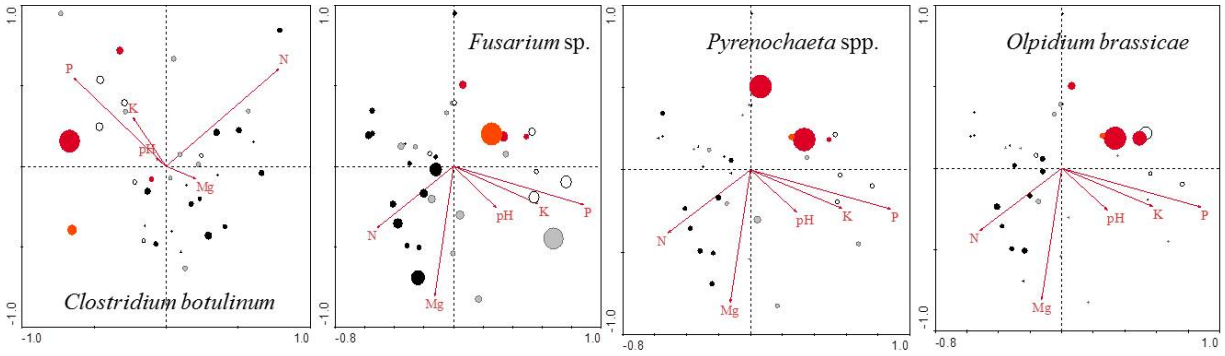


Figure 5 RDA showing differences in potentially 'pathogenic' bacterial and fungal OTUs among the study sites. *Clostridium botulinum* and *Fusarium spp.* were found across the sites. *Pyrenochaeta* spp. and *Olpidium brassicae* were most abundant on agriculturally improved fields. In the figures, samples are represented by the abundance of each bacteria or fungus (absence is symbolized by a '+') and are color-coded by land-use type. The figures are based on the RDAs in Figure 3.

4. Discussion

The microbial samples analysed here indicate that agricultural intensification and land-use change do not decrease overall bacterial OTU diversity but do cause fungal OTU diversity to decline. Changes in land use also led to more subtle shifts in microbial OTUs with agroecological significance. OTUs known for their generally positive effect on plant growth, namely N-fixing bacteria and mycorrhizas, were found across the study sites, yet specific taxa were associated with particular land uses. Other OTUs I identified as having particular agricultural functions (e.g. P-solubilizing bacteria) were also ubiquitous across the sites. Although somewhat contentious, I found that potentially pathogenic OTUs were significantly more abundant on agriculturally improved sites (improved grasslands, poppy fields, and rapeseed fields). Although the arable reversion process was not able to fully recreate vegetation species-richness or fungal OTU richness, there was no significant difference in bacterial OTU diversity between unimproved and conservation grasslands.

4.1 Resilience of Microbial Diversity to Agricultural Intensification

Bacterial OTU diversity did not vary among the study sites, yet fungal OTU diversity decreased on agriculturally improved fields. Bacterial OTU diversity declined slightly under increased K while fungal OTU diversity declined under increased P, which agrees with previous research suggesting microbial diversity declines under increased inputs (Hansen et al. 2000; Raun and Johnson 1999). However, neither bacterial nor fungal diversity declined under increased N, contrary to previous claims (Aeron et al. 2011; Chaparro et al. 2012; Johnson et al. 2005; Seneviratne and Kulasooriya 2012; Tilston et al. 2010). In part, this may be because sites with increased soil N were all unimproved grasslands where N levels were naturally high (e.g. from manure and legumes). In contrast, agriculturally improved sites had low levels of N despite receiving N in the form of mineral N (or in one case, human waste from an anaerobic digester).

The increased use of inputs (e.g. artificial fertilizers, pesticides) on agriculturally improved fields also had little effect on the presence of 'beneficial' microbial taxa. For example, although previous research suggests that N-fixing bacteria decline with the addition of mineral N, in my study N-fixing bacteria were found in abundance on agriculturally improved sites (Berthrong et al. 2014; Fox et al. 2007). This agrees with recent research which has shown that some N-fixing bacteria (*Pseudomonas*, *Rhizobium*, and *Azotobacter*) are resilient to crop pesticides (e.g. endosulfan, carbofuran, and malathion) (Shafiani and Malik 2003). Mycorrhizas were also found at all sites although previous studies have suggested that increased soil N and the application of agrochemicals can reduce nodule formation (Fox et al. 2007; Kiers et al. 2002; Treseder 2004). In this study, *Glomus* sp. WU M3 was found in samples from one poppy field and one rapeseed field despite the fact that these are non-mycorrhizal species. These results agree with research by Saia et al. (2015), who found that inoculation of wheat (*Triticum durum* Desf.) with mycorrhizas (*Scutellospora*, *Gigaspora*, *Glomus*, *Acaulospora*, *Funneliformis,* and

Rhizophagus) increased N and P uptake, as well as plant biomass, under unfertilized and fertilized conditions. In the future, using specific primers targeting specific mycorrhizas could provide a more nuanced picture of how agricultural intensification affects mycorrhizal communities (Lumini et al. 2011; Redecker 2000; Robinson et al. 2005).

The rapid evolution of bacterial communities may explain their resilience to changes in land use and increased soil fertility. The lifecycles of bacteria are short and 100-300 generations can occur each year, leading to high rates of competition between bacteria and as a result new communities adapted to new environmental constraints (Pennisi 2013; Wiser et al. 2013). In contrast, the sensitivity of fungi to changes in agricultural practices may be linked to their slower reproductive cycle and reliance on specific plant hosts to survive.

The use of crop rotation, no-till agriculture, and arable margins may also explain the high abundance of beneficial microbiota despite increased inputs (Barni and Siniscalco 2000; Lupwayi et al. 1998). Rotation followed by no-tillage can promote the resilience of mycorrhizas in the soil year to year despite a lack of mycorrhizal hosts. Roots infected from previous years may also auto-inoculate the soil the following year. In addition, no-till regimes can protect mycorrhizal hyphal networks and thus mycorrhizal abundance (compared to tilled fields). The use of arable field margins around fields may also serve as reserves for 'beneficial' fungi and bacteria. In Oxfordshire, field margins were composed of a variety of plants usually associated with grasslands and may have microbial communities similar to unimproved grasslands. Kivlin et al. (2011) have shown that wind, water, and animals can move the spores of these communities up to 10 m from their origin. This suggests that field margins could potentially serve to 'restore' the microbial communities within the arable fields the border. Future studies could compare the microbial communities of arable margins and the arable fields they border to definitively establish the restorative potential of these borders. More quantitative research looking at

the effect of these practices on microbial communities could make these practices more mainstream. Ultimately, this could offset the loss of microbial diversity within intensively managed agricultural systems, not only within the UK, but globally.

4.2 Loss of Vegetation Diversity and Use of Digestate May Increase Soil Pathogens

Although speculative, I found that agriculturally improved fields contained more potentially pathogenic OTUs. These OTUs reduced plant growth and development (e.g. *Agrobacterium tumefaciens, Olpidium brassicae, Verticillium dahliae*) by causing stem lesions and root rot (Berg and Smalla 2009; Carrieri et al. 2014; D'Amico et al. 2008; Garibaldi 2013; Goodner et al. 2001; Mansfield et al. 2012; Sarris et al. 2012; Waipara et al. 1996). Rapeseed and poppy fields also contained a high abundance of potentially pathogenic OTUs affecting human and livestock health (e.g. *Chaetomium* spp., *Listeria monocytogenes*, *Stenotrophomonas maltophilia, Yersinia seeligeri*) (Barron et al. 2003; Berg et al. 2005; Correia et al. 2014; Mendes et al. 2013; Kozak et al. 1996; Strydom and Witthuhn 2015). Other metagenomic studies have also found that soil pathogens increase on agriculturally improved fields. For example, Hartmann et al. (2014) also found that *Fusarium* was more abundant on conventionally managed arable fields in Switzerland compared to organically managed fields.

Decreased vegetation diversity may partially contribute to increased soil pathogen levels. Host-specific pathogens can rapidly multiply on arable fields sown with monocultures (Knops et al. 1999; Mitchell et al. 2002; Stuckenbrock and McDonald 2008). For example, Dick (1992) demonstrated that soil pathogens like *Fusarium* can build up in monoculture fields over time and increase the abundance of other pathogenic bacteria (e.g. certain *Pseudomonas* species) due to decreased host diversity. Similarly, Perez-Brandan et al. (2014) found that two soybean fungal pathogens, *Fusarium*

crassistipitatum and *Macrophomina phaseolina*, increased in fields under increased application of inorganic inputs and monoculture cropping.

Application of digestate from anaerobic digesters as a fertilizer on arable fields may also increase the abundance and diversity of soil pathogens. In this study, only one field received digestate as a fertilizer, so statistically determining whether digestate had an effect on soil pathogen abundance sites is not possible. However, this site had the highest level of potentially pathogenic OTUs out of all the study sites and contained many potentially pathogenic OTUs not found on the other sites. Nkoa (2014) and Sahlström (2003) have also found that applying digestate from anaerobic digesters as a fertilizer on arable fields increases levels of pathogenic bacteria in the soil. Bonetta et al. (2014) have shown that spores from pathogens like *Salmonella* and *Listeria* can survive the pasteurization process while Neuhaus et al. (2015) illustrate that soil pathogens common in natural soils (e.g. *C. botulinum*) are more concentrated in digestate and can shift the microbial community composition of agricultural fields. Eikmeyer et al. (2013) have contested the validity of such claims, but these results indicate that more research is necessary understand the potential agroecological ramifications of applying digestate on arable fields. This is particularly important as current bioenergy initiative increasingly promote digestate as a nutrient-rich fertilizer to local farmers.

Combining metagenomic analysis of soil microbial communities with metatranscriptomics and metaproteomics could determine more fully the potential effect of agricultural intensification on soil pathogens. Although several studies have proposed metagenomic analysis as a way to quickly identify pathogens in soil and water (e.g. Cahill 1999; Delmont et al. 2011; Deng et al. 2015; Pettengill et al. 2012; Pongor et al. 2014, Zhu et al. 2015), metagenomic data alone cannot differentiate between expressed and non-expressed genes, and thus, whether a 'potential' pathogen is 'active.' Complementing metagenomic analysis with metatranscriptomics could highlight differences in

expressed genes among sites while metaproteomics could be used to assess the catalytic potential of the different microbial communities at each field (Bastida et al. 2008; Gilbert and Hughes 2011; Simon and Daniel 2010; Wilmes and Bond 2010). Although perhaps more expensive and time consuming, pursuing these additional analyses could be used as a way to interrogate preliminary metagenomic data providing evidence for increased soil pathogens associated with particular land uses, crops, or fertilizers.

4.3 The Conservation and Agricultural Importance of Grassland Microbial Diversity

Maintaining the microbial diversity of species-rich grasslands is highly important given the incomplete ability of arable reversion to restore microbial diversity. My analysis shows that arable reversion was only partially successful in restoring microbial diversity. Although correspondence analysis illustrated the close similarly in microbial community composition between conservation and unimproved grasslands, fungal diversity was still significantly lower. In part, this could be due to the fact that the seed mixtures often used to restore grasslands do not always contain species characteristic of older unimproved grasslands. In many cases, these are grasses, forbs and legumes which are often left out of the seed mixes used to sow fields under arable reversion, as many restoration projects focus on increasing the abundance of rare herbs or plants historically associated with old hay meadows (French, unpublished data). In addition, conservation grasslands under arable reversion still had high levels of soil P.

These results disagree with Plassart et al (2008), who compared the differences in microbial biomass, diversity, and C sequestration among intensively cultivated arable fields, leys, permanent grasslands, and fields under arable reversion in the Haute-Normandie region and found that arable reversion quickly restored fungal biomass and diversity. Smith et al.'s (2003) research at a series of experimental

plots at a UK National Nature Reserve also found that arable reversion could restore grassland vegetation diversity and increased soil microbial biomass. However, in both cases only microbial diversity was examined. Microbial community composition and specific OTUs could have differed among sites but this was not examined. Instead, my results agree more closely with Jangid et al.'s (2003) research at the Long-Term Ecological Research site at the Konza Prairie Biological Station (KPBS) (Kansas, USA), who found that arable reversion did not successfully 'recreate' prairie microbial communities on land formerly used to grow soybean, wheat, and sorghum. By combining PFLA analysis with metagenomic analysis of the 16S rRNA region, they demonstrated that while the functionality (e.g. in terms of biomass, C sequestration) of grassland soil microbiota may be 'restored' over the long term, restored sites had different abundances of *Bacteroidetes*, *β-Proteobacteria*, and *Verrucomicrobia* and had OTUs not found on native prairielands. This highlights the importance of looking at changes in specific OTUs in order to truly understand how agricultural intensification affects microbial diversity.

The difficulty in fully restoring microbial diversity highlights the key importance of the microbial diversity of species-rich grasslands in conservation. Unimproved grasslands contain 'unique' microbial communities that can be used as a source of new inoculum to aid bioremediation efforts. As previously noted, unimproved grasslands contained beneficial microbiota (e.g. specific N-fixing bacteria and mycorrhizas) not found on other sites. Previous studies have demonstrated that inoculum created from native soils can be used to effectively restore microbial diversity on degraded land. For example, Richter and Stutz's (2002) research on the efficacy of native inoculum on restoring microbial diversity of land degraded through mining activities found that soil inoculum from local *Sporobolus wrightii* Munro ex Scribn. grassland communities increased the productivity of degraded grasslands. Similarly, experimental research by Torez et al. (2016) on restoring a former military site in Belgium back into a species-rich grassland and Emam (2016) on reclamation of a mercury mining site in California also

found that using inoculum created from native grasslands boosts the success of grassland restoration by increasing establishment of seeded plots and vegetation diversity on degraded land compared to treatments using commercial inoculum or no inoculum.

In addition, the high level of microbial diversity of soil samples from unimproved grasslands could also be exploited as a potential new source of biofertilizers. I found that specific microbiota associated with crop productivity (e.g. *Bradyrhyzobium* spp., *Sinorhizobium* spp., *Nitrosomonas* spp., *Paenibacillus polymyxa*, *Paraglomus* sp. and *Acaulospora* sp) were only present on species-rich grasslands and not on arable fields. Creating inoculum from these grasslands could increase the number and diversity of beneficial microbiota in intensively cultivated fields, potentially boosting soil fertility and decreasing reliance on chemical fertilizers. A number of previous laboratory experiments have demonstrated the positive effects of inoculating crops such as soybean, maize and rice with specific rhizobia and/or mycorrhizas, but few have examined whether inoculum from native soil could have a similar, or even increased, effect (Garg and Chandel 2010; Suzuki et al. 2005). Although inoculum is available commercially, it usually contains a very limited number of taxa (e.g. *Bacillis* spp. or *Glomus* spp.). In contrast, the heterogeneity of microbiota in inoculum from native grasslands may provide a greater range of 'beneficial' microbiota to the soil (Gosling et al. 2006; Howeler et al. 1987; Shtark et al. 2010; Stahl et al. 1988; van der Heijden 2006; Veresoglou and Rillig 2014). As such, the microbial diversity found on species-rich grasslands potentially has a strong economic and agricultural value, lending further support to calls to conserve these landscapes.

5. Conclusion

Based on field research in Oxfordshire, this study suggests that agricultural intensification and land-use change do not decrease bacterial OTU diversity but do cause fungal OTU diversity to decline.

Bacterial OTU diversity was particularly sensitive to increases in soil K while fungal OTU diversity declined under increased soil P. Surprisingly, changes in land use and soil fertility did not cause beneficial bacteria and fungi OTUs like N-fixing bacteria and mycorrhizas to decline. Other functional groups of beneficial microbiota identified in the samples also did not decline under increased soil fertility. The ubiquity of beneficial microbiota may be due to the use of crop rotation, species-rich field margins, and a lack of tilling at the sites under more intensive, conventional agricultural management. However, I found that potentially pathogenic OTUs did increase under agricultural intensification. My results indicate arable reversion can create microbial communities similar to unimproved grasslands, suggesting the potential efficacy of activities such as sowing species-rich seed mixtures on restoring microbial community composition. As such, agricultural intensification should not be viewed necessarily as a threat to microbial diversity *per se.* However, over the long term it could lead to increases in soil pathogens. At the same time, it could lead to the loss of the microbial genetic resources specific to unimproved grasslands which could be used as a source of inoculum to naturally increase soil fertility of arable fields or to restore fields where microbial diversity has declined due to anthropogenic causes. My results illustrate the complicated, and sometimes unexpected, effect of anthropogenic impacts on soil microbial diversity while highlighting avenues of future research.

Data accessibility:

I have deposited the raw sequence data and the metadata associated with each sample with the European Nucleotide Archive (ENA). It can be accessed at http://www.ebi.ac.uk/ena/data/view/PRJEB13388.

Appendix III

Table 1 Study sites where soil samples were taken. The table provides the soil nutrients (N, P, K, Mg, and pH), bacterial, fungal and vegetation species richness for each site.

Site Name	Land Use[17]	N (g/kg)	P (mg/L)	K (mg/L)	Mg (mg/L)	pH	BSR[18]	FSR[19]	VSR[20]
WM	U	11.55	26	175	111	6.45	1949	184	12
PM	U	8.4	21	243	144	7.4	1716	122	15
PFM	U	8.4	16	187	132	6.35	1780	180	17
TM	U	3.36	21	151	28	7.15	2009	178	18
IFA	U	5.67	23	146	103	7.65	2000	182	13
IFB	U	8.26	17	159	86	7.55	1915	148	14
OM	U	6.3	16	184	167	7.15	1772	220	20
BP	U	4.06	16	156	154	6.55	406	167	10
BHS	U	4.2	15	124	102	7.6	1371	142	14
RM	U	7.35	16	106	137	5.6	1691	189	15
WB	U	6.3	26	156	166	7.34	724	215	26
PMRM	U	5.95	29	219	196	7.35	2136	158	25
JH	U	7.35	15	96	142	7.7	2020	206	21
L	U	5.6	13	96	112	7.75	1879	212	10
IFC	C	8.96	20	173	90	7.45	1786	187	8
CM	C	3.71	15	190	140	6.8	1907	190	13
RMB	C	5.25	68	250	91	7.6	1826	108	9
RMC	C	5.6	45	225	138	7.6	1837	117	6
KM	C	8.75	83	293	186	7.35	1811	125	10
BHA	C	3.15	14	127	77	7.6	411	77	12
BHB	C	2.8	12	93	63	7.7	756	147	17
AM	C	6.65	47	206	156	7.6	1960	105	18
TE	C	5.95	100	170	114	7.65	1779	136	13
SW	C	6.65	35	203	137	7.3	205	137	6
BHC	I	3.15	15	98	61	7.7	1850	131	5
FAIL	I	4.2	122	560	150	7.3	1942	110	4
SFL	I	4.2	100	540	160	7.5	996	140	5
SF2L	I	5.25	90	300	100	7.6	1596	120	7
SFPB	I	5.2	90	400	90	7.6	1684	101	5

[17] Refers to how the site is used. U= Unimproved grassland. C= Conservation grassland. I= Improved grassland. R = Rapeseed field. P = Poppy field.

[18] BSR= Bacterial Species Richness

[19] FSR= Fungal Species Richness

[20] VSR= Vegetation Species Richness

AFL	I	6.65	24	156	92	7.6	1889	72	12
IPP1	P	1.05	45	129	118	6.74	1451	157	1
IPP2	P	1.1	47	131	120	6.70	1340	101	1
IPP3	P	1.1	45	127	117	6.72	674	77	1
BRA	R	4.2	28	154	52	7.6	1829	158	1
BRB	R	6.3	64	151	59	7.65	1703	76	1
IPR	R	2.8	88	304	135	6.4	1715	90	1

Table 2 Microbial OTUs with established agroecological functions present in the soil samples from Oxfordshire. This list of OTUs accounts for 10% (203 species) of the bacteria OTUs and 17% (26 species) of the fungal OTUs found in the samples. [21]

Functional Group	Genus
Plant-Growth-Promoting-Rhizobia	*Alcaligenes faecalis, Arthrobacter agilis, Arthrobacter arilaitensis, Arthrobacter aurescens, Arthrobacter citreus, Arthrobacter globiformis, Arthrobacter oxydans, Arthrobacter pascens, Arthrobacter protophormiae, Enterobacter turicensis, Lactobacillus sp., Serratia sp., Streptomyces aculeolatus, Streptomyces africanus, Streptomyces albogriseolus, Streptomyces almquistii, Streptomyces argenteolus, Streptomyces aureofaciens, Streptomyces aureus, Streptomyces bikiniensis, Streptomyces caeruleus, Streptomyces californicus, Streptomyces carpaticus, Streptomyces cattleya, Streptomyces chattanoogensis, Streptomyces cinereoruber, Streptomyces clavifer, Streptomyces clavuligerus, Streptomyces coelicolor, Streptomyces cyaneus, Streptomyces filamentosus, Streptomyces flavovirens, Streptomyces fulvorobeus, Streptomyces glaucescens, Streptomyces griseus, Streptomyces hiroshimensis, Streptomyces hygroscopicus, Streptomyces kanamyceticus, Streptomyces lavendulae, Streptomyces lienomycini, Streptomyces luridus, Streptomyces luteoverticillatus, Streptomyces maritimus, Streptomyces megasporus, Streptomyces netropsis, Streptomyces olivaceus, Streptomyces olivoverticillatus,Streptomyces phaeochromogenes, Streptomyces platensis, Streptomyces pulveraceus, Streptomyces rameus, Streptomyces ramulosus, Streptomyces ribosidificus, Streptomyces roseoverticillatus, Streptomyces seoulensis, Streptomyces sp., Streptomyces sp. A00076, Streptomyces spectabilis, Streptomyces sulfonofaciens, Streptomyces tendae, Streptomyces thermoatroviridis, Streptomyces torulosus, Streptomyces toxytricini, Streptomyces tricolor, Streptomyces turgidiscabies, Streptomyces umbrinus, Streptomyces vinaceus, Streptomyces virginiae, Streptomyces vitaminophilus, Streptomyces xylophagus, uncultured Streptomyces sp.*
Nitrogen-Fixing bacteria	*Azotobacter chroococcum, Azotobacter vinelandii, Bradyrhizobium canariense, Bradyrhizobium elkanii, Bradyrhizobium japonicum, Bradyrhizobium liaoningense, Bradyrhizobium sp. Crj-1, Bradyrhizobium sp. ISLU207, Bradyrhizobium sp. Pter17, Bradyrhizobium sp. onl92.6, uncultured Bradyrhizobium sp., Burkholderia phymatum, Cyanobacteria, Frankia sp., Frankia sp. BMG5.11, Frankia sp. BMG5.6, Frankia sp. Cc13, Frankia symbiont of Datisca glomerata, Gluconacetobacter hansenii, Heliobacterium chlorum, Heliobacterium modesticaldum, Nitrosomonas sp. NL7, Nitrosomonas sp. Nm148, Nitrosomonas ureae, Paenibacillus polymyxa, Rhizobium etli, Rhizobium leguminosarum, Rhizobium sp. CCBAU 23084, Rhizobium sp. LXD30, Rhizobium sp. NCHA22, Rhizobium sp. TJ171, Rhizobium sp. YAS34, Rhizobium sphaerophysae, Rhizobium tropici,*

[21] Sources: Aeron et al. 2011:3; Alavi et al. 2014; Berendson et al. 2012; Chaparro et al. 2012; Deacon et al. 2006; Gale et al. 2009; Gianinazzi et al. 2010; Gosling et al. 2006; Hartmann et al. 2014; Hurek and Reinhold-Hurek 2003; Kumar et al. 2008; Kumar et al. 2011; Melo et al. 2014; Mendes et al. 2013; Moricca and Ragazzi 2008; Neuhaus et al. 2015; Nkoa 2014; Oehl et al. 2003; Pinzari et al. 2014; Sachdev and Chameotra 2013; Sahlstrom 2003; Sarris et al. 2012; Saraf et al.2011; Schmidt et al. 2012; Shtark et al. 2010; Suckstorff and Berg 2003; Tilak et al. 2005; Vandenkoornhuyse et al. 2002; Moricca and Ragazzi 2008.

	Rhizobium vallis, uncultured Rhizobium sp., Sinorhizobium fredii, Sinorhizobium sp. CCBAU 53044B, Sinorhizobium sp. TJ170, Sinorhizobium xinjiangense
Phosphorus-Solubilizing Bacteria	*Achromobacter, Azospirillum amazonense, Azospirillum lipoferum, Burkholderia cepacia, Burkholderia gladioli, Burkholderia glathei, Burkholderia kururiensis, Burkholderia multivorans, Burkholderia pseudomallei, Burkholderia thailandensis, Chryseobacterium balustinum, Chryseobacterium formosense, Chryseobacterium haifense, Chryseobacterium indologenes, Chryseobacterium soldanellicola, Chryseobacterium soli, Flavobacterium aquatile, Flavobacterium columnare, Flavobacterium denitrificans, Flavobacterium johnsoniae, Flavobacterium psychrophilum, Flavobacterium sp., Flavobacterium sp. MSY2, Flavobacterium sp. S1, Flavobacterium sp. SOC A4(12), Micrococcus luteus, Micrococcus lylae, Stenotrophomonas rhizophila*
Pathogen-Suppressing Bacteria	*Bacillus thuringiensis, Bacillus alcalophilus, Bacillus amyloliquefaciens, Bacillus anthracis, Bacillus aquimaris, Bacillus badius, Bacillus caldotenax, Bacillus cereus, Bacillus clausii, Bacillus coagulans, Bacillus coahuilensis, Bacillus firmus, Bacillus flexus, Bacillus halodurans, Bacillus horikoshii, Bacillus infernus, Bacillus litoralis, Bacillus massiliensis, Bacillus megaterium, Bacillus niacini, Bacillus pseudofirmus, Bacillus psychrodurans, Bacillus pumilus, Bacillus simplex, Bacillus smithii, Bacillus thermoamylovoransBacillus subtilis, Pseudomonas alcaligenes, Pseudomonas brassicacearum, Pseudomonas coronafaciens, Pseudomonas fluorescens, Pseudomonas mandelii, Pseudomonas migulae, Pseudomonas oryzihabitans, Pseudomonas plecoglossicida, Pseudomonas putida, Pseudomonas rhodesiae, Pseudomonas stutzeri, Pseudomonas thermotolerans, Pseudomonas tolaasii, Pseudomonas vancouverensis, Pseudomonas sp. AF32, Pseudomonas sp. B06, Pseudomonas sp. GD200, Pseudomonas sp. KBOS 03, Pseudomonas sp. MFY71, Pseudomonas sp. PB1, Pseudomonas sp. PTB2093, Pseudomonas sp. QD03, Pseudomonas sp. R21-1, Pseudomonas sp. RW10S2, Pseudomonas sp. SK-1-3-4*
'Beneficial' Fungi	*Cadophora*, *Cladosporium*, *Clonostachys*, *Minimedusa polyspora*, *Penicillium* sp., *Preussia* sp., *Racocetra fulgida*
Mycorrhizas	*Acaulospora koskei*, *Glomus invermaium, Glomus sp. N10.2, Glomus sp. WUM3, Glomus compressum, Glomus macrocarpum, Glomus versiforme, Glomus mycorrhizal symbiont of Paecilomyces marquandii, Paecilomyces carneus*, *Paraglomus laccatum*,
Saprotrophic Fungi	*Clavaria falcata, Clavaria incarnata, Clavaria flavipes, Clavaria fuscata*, *Ganoderma*, *Mortierella elongata, Mortierella pseudozygospora*, *Stereum ostrea*
Bacterial Plant Pathogens	*Agrobacterium tumefaciens*, *Erwinia persicina, Erwinia rhapontici, Pseudomonas cichorii, Pseudomonas viridiflava, Stenotrophomonas maltophilia*
Bacterial Human and Livestock Pathogens	*Clostridium botulinum*, *Clostridium chauvoei, Escherichia* sp., *Listeria monocytogenes*, *Stenotrophomonas maltophilia, Streptococcus suis, Yersinia seeligeri*
Fungal Plant Pathogens	*Acremonium alcalophilum, Acremonium cereale, Acremonium longisporum, Acremonium psammosporum, Acremonium sp. DWS6m3, Acremonium persicinum*, *Olpidium brassicae*, *Fusarium* sp., *Microdochium*, *Plectosphaerella* sp., *Plectosphaerella cucumerina, Verticillium dahliae*, *Waitea circinata*
Fungal Human and Livestock Pathogens	*Chaetomium*, *Cryptococcus podzolicus, Cryptococcus dimennae, Cryptococcus terricola*, *Pyrenochaeta sp. MUT 4378, Pyrenochaeta sp. 14009, Pyrenochaeta sp. GMG_PPb7, Pyrenochaeta sp. shylm24, Pyrenochaeta lycopersici, Pyrenochaeta sp. 112_114B*

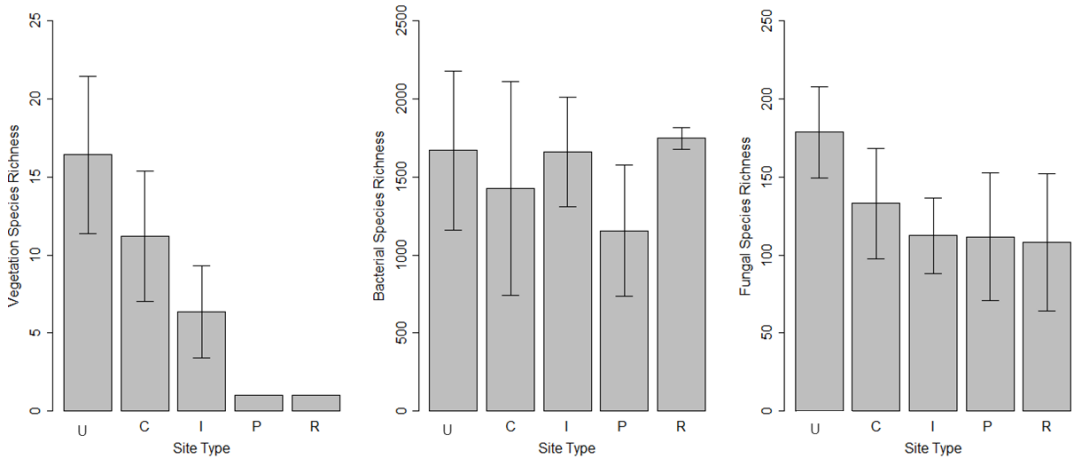


Figure 1. Variation in vegetation species-richness and microbial OTU richness among land-use types. Vegetation diversity was highest on unimproved grasslands and lowest on the rapeseed and poppy fields (which were dominated by their respective crops). Rapeseed fields and unimproved grasslands had the highest bacterial OTU richness. The bacterial OTU richness of conservation grasslands had the greatest variation among sites. Unimproved grasslands and conservation grasslands had the highest fungal OTU richness. Changes in land use decreased fungal OTU richness up to 50%. U= Unimproved grassland; C=Conservation grassland; I=Improved grassland; P=Poppy field; R=Rapeseed field. Plots show means ±1 standard error.

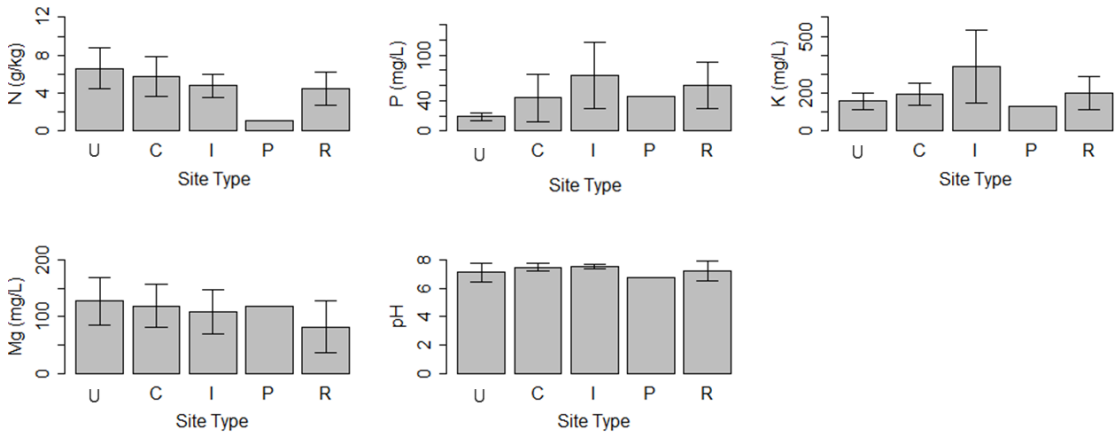


Figure 2 Comparison of soil fertility among land-use types. Unimproved and conservation grasslands were characterized by high levels of N and Mg, while agriculturally improved fields (improved grasslands, poppy and rapeseed fields) were high in P and K. U = Unimproved grassland; C = Conservation grassland; I = Improved grassland; P = Poppy field; R = Rapeseed field. Plots show means ±1 standard error.

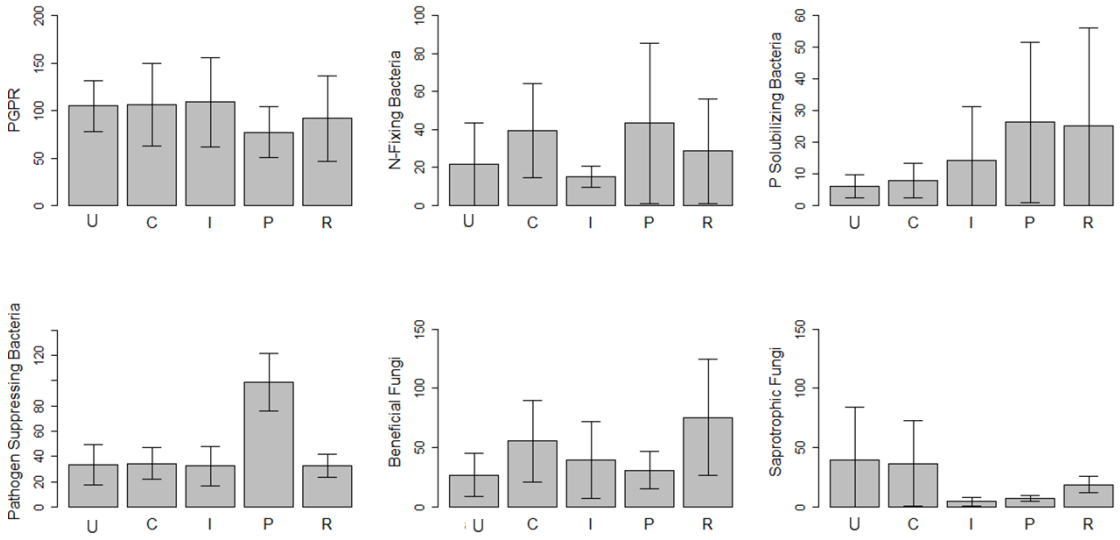


Figure 3 Comparison of 'beneficial' microbiota among sites by functional category. Plant-Growth-Promoting Rhizobia (PGPR), N-fixing bacteria, P-solubilizing bacteria, 'beneficial fungi,' and saprotrophic fungi were ubiquitous and there was no significant variation in OTU abundance among sites. The only significant difference in beneficial microbiota among sites was in relation to pathogen-suppressing bacteria ($F_{4, 32} = 12.43$, P<0.001), which were most abundant in samples from the poppy fields. U = Unimproved grassland; C = Conservation grassland; I=Improved grassland; P = Poppy field; R = Rapeseed field. Plots show means ±1 standard error.

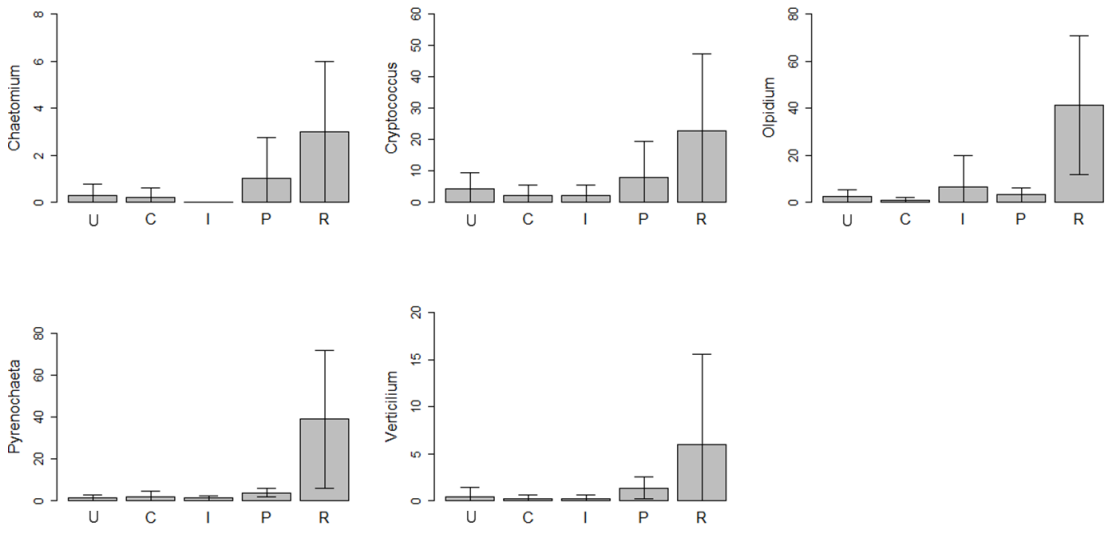


Figure 4 Comparison of the abundance of select potential plant, human and livestock soil pathogens. All abundances of these OTUs varied significantly among sites (*Chaetomium* $F_{4, 31} = 6.273$, P < 0.001; *Cryptococcus* $F_{4, 31} = 4.191$, P = 0.007; *Olpidium* $F_{4, 31} = 11.61$, P<0.001; *Pyrenochaeta* $F_{4, 31} = 13.14$, P < 0.001; *Verticillium* $F_{4, 31} = 3.565$, P = 0.017) and all were more abundant on rapeseed and/or poppy fields. U = Unimproved grassland; C = Conservation grassland; I = Improved grassland; P = Poppy field; R = Rapeseed field. Plots show means ±1 standard error.

References

Aeron, A. et al., 2011. Emerging role of Plant Growth Promoting Rhizobacteria in agrobiology. In D. K. Maheshwari, ed. *Bacteria in Agrobiology: Crop Ecosystems*. Berlin: Springer-Verlag, pp. 1–36.

Alavi, P. et al., 2014. *Stenotrophomonas* comparative genomics reveals genes and functions that differentiate beneficial and pathogenic bacteria. *BMC Genomics*, 15(1), p.482.

Balser, T.C. et al., 2010. The microbiology of natural soils. In G. R. Dixon & E. L. Tilston, eds. *Soil Microbiology and Sustainable Crop Production*. Springer Netherlands, pp. 27–57.

Barni, E. & Siniscalco, C., 2000. Vegetation dynamics and arbuscular mycorrhiza in old-field successions of the western Italian Alps. *Mycorrhiza*, 10(2), pp.63–72.

Barron, M.A. et al., 2003. Invasive mycotic infections caused by *Chaetomium perlucidum*, a new agent of Cerebral Phaeohyphomycosis. *Journal of Clinical Microbiology*, 41(11), pp.5302–5307.

Bastida, F. et al., 2008. Past, present and future of soil quality indices: A biological perspective. *Geoderma*, 147(3-4), pp.159–171.

Berg, G. & Smalla, K., 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS microbiology ecology*, 68(1), pp.1–13.

Berendsen, R.L., Pieterse, C.M.J. & Bakker, P.A.H.M., 2012. The rhizosphere microbiome and plant health. *Trends in Plant Science*, 17(8), pp.478–486.

Berthrong, S.T. et al., 2014. Nitrogen fertilization has a stronger effect on soil Nitrogen-Fixing bacterial communities than elevated atmospheric CO^2. *Applied and Environmental Microbiology*, 80(10), pp.3103–3112.

Blackstock et al., 1999. The extent of semi-natural grassland communities in lowland England and Wales: a review of conservation surveys 1978–96. *Grass and Forage Science*, 54(1), pp.1–18.

Bonetta, S., S. Bonetta, E. Ferretti, G. Fezia, G. Gilli, and E. Carraro. 2014. Agricultural reuse of the digestate from anaerobic co-digestion of organic waste: Microbiological contamination, metal hazards and fertilizing performance. *Water Air Soil Pollution* 225:2046.

Brussaard, L., de Ruiter, P.C. & Brown, G.G., 2007. Soil biodiversity for agricultural sustainability. *Agriculture, Ecosystems & Environment*, 121(3), pp.233–244.

Buée, M. et al., 2009. 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytologist*, 184(2), pp.449–456.

Cahill, D.M., 1999. Detection, identification and disease diagnosis of soilborne pathogens. *Australasian Plant Pathology*, 28(1), pp.34–44.

Caporaso, J.G. et al., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences*, 108(Supplement 1), pp.4516–4522.

Carrieri, R. et al., 2014. First report of necrotic leaf spot caused by *Plectosphaerella cucumerina* on lamb's lettuce in Southern Italy. *Plant Disease*, 98(7), pp.998–998.

Celestino, J. dos R. et al., 2014. Bioprospecting of Amazon soil fungi with the potential for pigment production. *Process Biochemistry*, 49(4), pp.569–575.

Chaparro, J.M. et al., 2012. Manipulating the soil microbiome to increase soil health and plant fertility. *Biology and Fertility of Soils*, 48(5), pp.489–499.

Correia, C.R., 2014. Stenotrophomonas maltophilia: Rare cause of meningitis. *Pediatrics International*, 56(4), p.e21.

D'Amico, M., Frisullo, S. & Cirulli, M., 2008. Endophytic fungi occurring in fennel, lettuce, chicory, and celery--commercial crops in southern Italy. *Mycological Research*, 112(Pt 1), pp.100–107.

Deacon, L.J. et al., 2006. Diversity and function of decomposer fungi from a grassland soil. *Soil Biology and Biochemistry*, 38(1), pp.7–20.

Delmont, T.O. et al., 2011. Metagenomic mining for microbiologists. *The ISME Journal*, 5(12), pp.1837–1843.
Delmont, T. et al., 2012. Structure, fluctuation and magnitude of a natural grassland soil metagenome. *The ISME Journal*, 6, pp.1677–1687.
Deng, X., 2015. An ensemble strategy that significantly improves de novo assembly of microbial genomes from metagenomic next-generation sequencing data. *Nucleic acids research*, 43(7), p.e46.
Dick, R.P., 1992. Biotic Diversity in Agroecosystems A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. *Agriculture, Ecosystems & Environment*, 40(1), pp.25–36.
Dixon, G.R. & Tilston, E.L., 2010. Soil-Borne Pathogens and Their Interactions with the Soil Environment. In G. R. Dixon & E. L. Tilston, eds. *Soil Microbiology and Sustainable Crop Production*. Springer Netherlands, pp. 197–271.
Douds, D. and P. Millner. 1999. Biodiversity of arbuscular mycorrhizal fungi in agroecosystems. *Agriculture, Ecosystems and Environment* 74: 77–93.
Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26(19), pp.2460–2461.
Eikmeyer, F.G. et al., 2013. Detailed analysis of metagenome datasets obtained from biogas-producing microbial communities residing in biogas reactors does not indicate the presence of putative pathogenic microorganisms. *Biotechnology for Biofuels*, 6, p.49.
Emam, T., 2016. Local soil, but not commercial AMF inoculum, increases native and non-native grass growth at a mine restoration site. *Restoration Ecology*, 24(1), pp.35–44.
Eriksson, Å., 2001. Arbuscular mycorrhiza in relation to management history, soil nutrients and plant species diversity. *Plant Ecology*, 155(2), pp.129–137.
Fierer, N. et al., 2012. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *PNAS*, 109(52), pp.1–6.
Fox, J.E. et al., 2007. Pesticides reduce symbiotic efficiency of nitrogen-fixing rhizobia and host plants. *Proceedings of the National Academy of Sciences*, 104(24), pp.10282–10287.
Garbeva, P., van Veen, J.A. & van Elsas, J.D., 2004. Microbial Diversity in Soil: Selection of Microbial Populations by Plant and Soil Type and Implications for Disease Suppressiveness. *Annual Review of Phytopathology*, 42(1), pp.243–270.
Garg, N. & Chandel, S., 2010. Arbuscular mycorrhizal networks: process and functions. A review. *Agronomy for Sustainable Development*, 30(3), pp.581–599.
Garibaldi, A. et al., 2013. First report of a new leaf spot caused by *Plectosphaerella cucumerina* on field grown endive (*Cichorium endivia*) in Italy. *Plant Disease*, 97(6), pp.848–848.
Gilbert, J.A. & Hughes, M., 2011. Gene expression profiling: metatranscriptomics. *Methods in Molecular Biology (Clifton, N.J.)*, 733, pp.195–205.
González, A.J., Tello, J.C. & Rodicio, M.R., 2007. *Erwinia persicina* causing chlorosis and necrotic spots in leaves and tendrils of *Pisum sativum* in Southeastern Spain. *Plant Disease*, 91(4), pp.460–460.
Goodner, B. et al., 2001. Genome sequence of the plant pathogen and biotechnology agent *Agrobacterium tumefaciens* C58. *Science*, 294(5550), pp.2323–2328.
Gosling, P. et al., 2006. Arbuscular mycorrhizal fungi and organic farming. *Agriculture, Ecosystems and Environment*, 113, pp.17–35.
Hansen, B. et al., 2000. Nitrogen leaching from conventional versus organic farming systems — a systems modelling approach. *European Journal of Agronomy*, 13(1), pp.65–82.
Hartmann, M., B. Frey, J. Mayer, P. Mäder, and F. Widmer. 2014. Distinct soil microbial diversity under long-term organic and conventional farming. *The ISME Journal* 2014: 1-18.

Howeler, R.H., E. Sieverding, and S. Saif. 1987. Practical aspects of mycorrhizal technology in some tropical crops and pastures. *Plant and Soil* 100: 249-283.

Huson, D.H. et al., 2011. Integrative analysis of environmental sequences using MEGAN4. *Genome Research*, 21(9), pp.1552–1560.

Jangid, K. et al., 2010. Development of soil microbial communities during tallgrass prairie restoration. *Soil Biology and Biochemistry*, 42(2), pp.302–312.

Johnson, D., Leake, J.R. & Read, D.J., 2005. Liming and nitrogen fertilization affects phosphatase activities, microbial biomass and mycorrhizal colonisation in upland grassland. *Plant and Soil*, 271(1-2), pp.157–164.

Kiers, E.T., West, S.A. & Denison, R.F., 2002. Mediating Mutualisms: Farm Management Practices and Evolutionary Changes in Symbiont Co-operation. *Journal of Applied Ecology*, 39(5), pp.745–754.

Kivlin, S.N., Hawkes, C.V. & Treseder, K.K., 2011. Global diversity and distribution of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry*, 43(11), pp.2294–2303.

Knops, J. m. h. et al., 1999. Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. *Ecology Letters*, 2(5), pp.286–293.

Kozak, J. et al., 1996. Prevalence of Listeria monocytogenes in foods: Incidence in dairy products. *Food Control*, 7(4), pp.215–221.

Langille, M.G.I. et al., 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology*, 31(9), pp.814–821.

Lee, M.H. & Lee, S.-W., 2013. Bioprospecting Potential of the Soil Metagenome: Novel Enzymes and Bioactivities. *Genomics & Informatics*, 11(3), pp.114–120.

Lepš, J., and Šmilauer, P., 2003. *Multivariate analysis of ecological data using CANOCO*, Cambridge: Cambridge University Press.

Lumini, E. et al., 2011. Metagenomics applied to arbuscular mycorrhizal fungal communities. In M. Diana, ed. Metagenomics: Current Innovations and Future Trends. Norwich, England: Caister Academic Press. 159-176.

Lupwayi, N.Z., Rice, W.A. & Clayton, G.W., 1998. Soil microbial diversity and community structure under wheat as influenced by tillage and crop rotation. *Soil Biology and Biochemistry*, 30(13), pp.1733–1741.

Magoč, T. & Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics (Oxford, England)*, 27(21), pp.2957–2963.

Mansfield, J. et al., 2012. Top 10 plant pathogenic bacteria in molecular plant pathology. *Molecular Plant Pathology*, 13(6), pp.614–629.

Mendes, R. et al., 2011. Deciphering the Rhizosphere microbiome for disease-suppressive bacteria. *Science*, 332(6033), pp.1097–1100.

Mendes, R., Garbeva, P. & Raaijmakers, J.M., 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiology Reviews*, 37(5), pp.634–663.

Meyer, F. et al., 2008. The metagenomics RAST server – a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics*, 9, p.386.

Mitchell, C., Tilman, D. & Groth, J., 2002. Effects of grassland plant species diversity, abundance, and composition on foliar fungal disease. *Ecology*, 83(6), pp.1713–1726.

Nannipieri, P. et al., 2003. Microbial diversity and soil functions. *European Journal of Soil Science*, 54(4), pp.655–670.

Neuhaus, J., W. Schrödel, A. Shehata, and M. Krüger. 2015. Detection of *Clostridium botulinum* in liquid manure and biogas plant wastes. *Folia Microbiologia* 60:451–456.

Nkoa, R. 2014. Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. *Agron. Sustain. Dev.* 34:473–492.

Oehl, F. et al., 2010. Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. *Soil Biology & Biochemistry* , 42, pp.724–738.
Oulas, A., Pavloudi, C., Polymenakou, P., Pavlopoulos, G.A., et al., 2015. Metagenomics: Tools and insights for analyzing Next-Generation Sequencing data derived from biodiversity studies. *Bioinformatics and Biology Insights*, 9, pp.75–88.
Perez-Brandan, C. et al., 2014. Soybean fungal soil-borne diseases: a parameter for measuring the effect of agricultural intensification on soil health. *Canadian Journal of Microbiology*, 60(2), pp.73–84.
Pettengill James B et al., 2012. Using metagenomic analyses to estimate the consequences of enrichment bias for pathogen detection. *BMC Research Notes*, 5(1), p.378.
Pennisi, E., 2013. The Man Who Bottled Evolution. *Science*, 342(6160), pp.790–793.
Pinzari, F. et al., 2014. Metabolic profiling of *Minimedusa polyspora* (Hotson) Weresub & P.M. LeClair, a cellulolytic fungus isolated from Mediterranean maquis, in southern Italy. *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology*, 148(2), pp.333–341.
Plassart, P. et al., 2008. Molecular and functional responses of soil microbial communities under grassland restoration. *Agriculture, Ecosystems & Environment*, 127(3–4), pp.286–293.
Pongor, L.S., Vera, R. & Ligeti, B., 2014. Fast and sensitive alignment of microbial whole genome sequencing reads to large sequence datasets on a desktop PC: Application to metagenomic datasets and pathogen identification. *PLoS ONE*, 9(7).
Quast, C. et al., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41(Database issue), pp.D590–596.
Raun, W.R. & Johnson, G.V., 1999. Improving Nitrogen Use Efficiency for Cereal Production. *Agronomy Journal*, 91(3), p.357.
Redecker, D., 2000. Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. *Mycorrhiza*, 10(2), pp.73–80.
Richter, B.S. & Stutz, J.C., 2002. Mycorrhizal inoculation of Big Sacaton: Implications for grassland restoration of abandoned agricultural fields. *Restoration Ecology*, 10(4), pp.607–616.
Robinson, C.H., Miller, E.J.P. & Deacon, L.J., 2005. Biodiversity of saprotrophic fungi in relation to their function: do fungi obey the rules? In *Biological Diversity and Function in Soils*. Ecological Reviews. Cambridge University Press.
Saia, S. et al., 2015. Soil inoculation with symbiotic microorganisms promotes plant growth and nutrient transporter genes expression in durum wheat. *Frontiers in Plant Science*, 6.
Salles, J.F., van Elsas, J.D. & van Veen, J.A., 2006. Effect of agricultural management regime on Burkholderia community structure in soil. *Microbial Ecology*, 52(2), pp.267–279.
Salström, L. 2003. A review of survival of pathogenic bacteria in organic waste used in biogas plants. *Bioresource Technology* 87: 161–166.
Sarris, P.F. et al., 2012. *Pseudomonas viridiflava*, a multi-host plant pathogen with significant genetic variation at the molecular level. *PLoS ONE*, 7(4), p.e36090.
Schmidt, C.S. et al., 2012. *Stenotrophomonas rhizophila* DSM14405T promotes plant growth probably by altering fungal communities in the rhizosphere. *Biology and Fertility of Soils*, 48(8), pp.947–960.
Schneider, S., M. Hartmann, J. Enkerli, and F. Widmer. 2010. Fungal community structure in soils of conventional and organic farming systems. *Fungal Ecology* 3: 215 – 224.
Seneviratne, G. & Kulasooriya, S.A., 2013. Reinstating soil microbial diversity in agroecosystems: The need of the hour for sustainability and health. *Agriculture, Ecosystems and Environment*, 164, pp.181–182.

Shtark, O.Y. et al., 2010. Intimate associations of beneficial soil microbes with host plants. In G. R. Dixon & E. L. Tilston, eds. *Soil Microbiology and Sustainable Crop Production*. Springer Netherlands, pp. 119–196.

Simon, C. & Daniell, R., 2011. Metagenomic analyses: Past and future trends. *Applied and Environmental Microbiology*, 77(4), pp.1153–1161.

Smith, R.S. et al., 2003. Soil microbial community, fertility, vegetation and diversity as targets in the restoration management of a meadow grassland. *Journal of Applied Ecology*, 40(1), pp.51–64.

Stukenbrock, E.H. & McDonald, B.A., 2008. The origins of plant pathogens in agro-ecosystems. *Annual Review of Phytopathology*, 46(1), pp.75–100.

Suzuki, C. et al., 2005. Microbial indices of soil fertility. *Journal of Applied Microbiology*, 98(5), pp.1062–1074.

Van Der Heijden, M.G.A. et al., 2006. The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. *New Phytologist*, 172(4), pp.739–752.

Tilston, E.L. et al., 2010. The impact of land-use practices on soil microbes. In G. R. Dixon & E. L. Tilston, eds. *Soil Microbiology and Sustainable Crop Production*. Springer Netherlands, pp. 273–295.

Torrez, V. et al., 2016. Effects of adding an arbuscular mycorrhizal fungi inoculum and of distance to donor sites on plant species recolonization following topsoil removal. *Applied Vegetation Science*, 19(1), pp.7–19.

Torsvik, V. & Øvreås, L., 2002. Microbial diversity and function in soil: from genes to ecosystems. *Current Opinion in Microbiology* , 5, pp.240–245.

Treseder, K.K., 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO2 in field studies. *New Phytologist*, 164(2), pp.347–355.

Verbruggen, E. et al., 2013. Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. *New Phytologist*, 197(4), pp.1104–1109.

Walshaw, J., Etherington, G. & MacLean, D., 2011. Next-Generation Sequencing approaches to metagenomics. In *Metagenomics: Current Innovations and Future Trends*. Norfolk, UK: Caister Academic Press, pp. 63–88.

White, T.J., et al. 1999. Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. , pp.315–322.

Wilmes, P. & Bond, P.L., 2006. Metaproteomics: studying functional gene expression in microbial ecosystems. *Trends in Microbiology*, 14(2), pp.92–97.

Wiser, M.J., Ribeck, N. & Lenski, R.E., 2013. Long-term dynamics of adaptation in asexual populations. *Science*, 342(6164), pp.1364–1367.

Zeilinger, S. et al., 2015. Friends or foes? Emerging insights from fungal interactions with plants. *FEMS Microbiology Reviews*, p.fuv045.

CHAPTER 5

VEGETATION CHANGE AND BIODIVERSITY HOTSPOTS THROUGH TIME: IMPLICATIONS FOR GRASSLAND RESTORATION AND PLANT GENETIC RESOURCE MANAGEMENT

Abstract

Palaeoecological research can shed light on long term changes in biodiversity, the biotic and abiotic processes that drive these changes, and the resilience of landscapes to environmental and anthropogenic disturbance. These results can guide conservation activities and can inform the sustainable management of ecosystem services and natural resources. Here I show how combining palaeoecological data with modern vegetation survey data can contribute to the conservation of grassland biodiversity. These landscapes are quickly disappearing in Europe and the long term ramifications of these changes are unknown. This paper aims to determine: (1) the stability of grassland biodiversity and the role of anthropogenic disturbance in this process, (2) whether there is continuity between historic and contemporary sites of biodiversity, and (3) whether plant genetic resource, specifically crop wild relatives of forage crops, are linked to areas of historic biodiversity. Focusing on species-rich grasslands in Oxfordshire, England, I demonstrate that species-rich grasslands only developed around 2,000 years ago due to anthropogenic intervention and would revert to oscillating between open grassland and woodland without human management. Temporally and spatially, high levels of biodiversity are associated with the creation of species-rich grasslands. These areas are still hotspots of vegetation diversity today and wild relatives of forage grasses and legumes are closely linked to these areas. I argue conservation activities could focus their attention on species-rich grasslands located in areas of long-term biodiversity, allowing other sites to revert, and future research could focus on monitoring, characterizing and collecting plant genetic resources from these sites.

1. Introduction

The rapid loss of botanical diversity due to anthropogenic activity has made understanding the stability of biodiversity over time a priority within palaeoecology and conservation since the early 21st century (Magurran et al. 2010). Palaeoecology uses palynological (fossilized pollen and spores) and macrobotanical (carbonized and waterlogged plants) remains to determine long-term changes in vegetation. Historically, paleoecology was often descriptive and focused on determining how species distributions and communities have changed over time in response to environmental change and anthropogenic disturbance. More recently however, Poudevigne and Baudry (2003), Weng et al. (2005), Willis et al. (2007), Froyd and Willis (2008), and Birks (2012) have argued that palaeoecology

can play an important role in conservation, specifically by providing a yardstick of how biodiversity has changed over time, what biotic and abiotic factors drove these changes, and the resilience of vegetation to disturbance. In addition, Colombaroli and Tinner (2013) and Jeffers et al. (2015) have argued that paleoecology can inform the provision of ecosystem services and the sustainable use of natural resources by highlighting specific points in the landscape with long-term provisioning ability.

In this paper, I examine how palaeoecology can inform grassland biodiversity conservation. Globally, species-rich grasslands are quickly disappearing and with them the biological diversity they harbor. Species-rich grasslands refer to unimproved pastures and meadows. Although previous palaeoecological studies in Europe suggest that they developed out of long term human agricultural and pastoral activities (Cousins and Eriksson 2002; Poschlod and Wallis De Vries 2003), palaeoecological research in other parts of the world indicates that grassland development is often due to a variety of local factors, including geography, elevation, hydrology, and climate. For example, several studies in Africa suggest that although savannah-type grassland are often considered recent additions to the landscape, a reflection of human deforestation and overgrazing, these ecosystems developed over 13,000 years ago before human impact on the environment (Finch and Marchant 2011).

However, to date few have used palaeoecological data to look at whether grassland biodiversity is stable over time, whether it requires human management to persist, and how the local landscape might revert if these grasslands were no longer managed. Previous research on grassland origin and development in the UK has primarily used long term environmental data to inform grassland conservation and management. Chambers et al. (2007) used paleoecology to identify the long-term factors driving mire degradation in Wales in order to aid conservation management strategies. They found that increased atmospheric pollution and grazing pressure were the main causes of mire decline

and suggested reducing grazing by sheep, reintroducing light grazing by cattle, restoring earlier hydrological systems, and reducing burning to restore mire ecosystems. Similarly, in order to understand the decline of lowland heathlands in southern England in recent years, Groves et al. (2012) examined palynological samples from the region and found that anthropogenic management, and specifically the use of fire, played a strong role in the origin and expansion of these landscapes. They suggested that increasing the use of fire to manage heathlands could help restore these ecosystems. However, none of these studies have specifically looked at how the biodiversity of these areas has changed over time and whether this would decrease if these landscapes disappeared.

Looking at how grassland biodiversity has changed over time can also inform our understanding of how specific ecosystem services attached to this biodiversity may be affected by the loss of these landscapes. Here, I refer to the plant genetic resources attached to species-rich grasslands, and specifically, wild relatives of fodder grasses and legumes. Crop wild relatives are plants genetically closely related to crops (either in gene pools 1 and 2 or taxon groups 1-4) (Heywood et al. 2007). Previous research on the domestication of crops such as cereals and legumes suggests that crop wild relatives are linked to long-term centers of human activity (Altieri et al. 1987; Fuller 2007; Burger et al. 2008). Whether this is also true for forage crop wild relatives is unclear, in part, due to lack of archaeobotanical and phylogenetic research on the origin and domestication of forage grasses and legumes. Jarvis et al.'s (2015) recent research on hotspots of crop wild relatives in the UK suggests that most of these occur on grasslands and are related to fodder crops, yet whether these resources are specifically linked to historic, anthropogenic-created pastures and meadows is not clear. If they are linked, this would imply maintaining species-rich grasslands is necessary to manage these genetic resources. These resources are an important source of genetic variability and can be used in crop breeding to increase crop resilience to low nutrient conditions, environmental stress and pathogens (Breed et al. 2013; Esquinas-Alcazar 2005; Gepts 2006; Helgadóttir et al. 2000). For example, to

improve yield in white clover (*Trifolium repens* L.), researchers identified genes relating to flowering and resistance to clover cyst nematodes in one of its crop wild relatives, *Trifolium nigrescens,* which were then introgressed into *Trifolium repens* (Marshall et al. 2010; Pollock et al. 2005). Despite the economic value of these resources and the need to prioritize conservation efforts (Maxted et al. 2007; Fielder et al. 2015a, 2015b; Castaneda-Alvarez et al. 2016), few studies have looked at whether crop wild relatives of forage crops may be linked to areas of long-term grassland biodiversity.

This paper aims to determine: (1) the stability of grassland biodiversity and the role of anthropogenic disturbance in this process, (2) whether there is continuity between historic and contemporary sites of biodiversity, and (3) whether plant genetic resource, specifically crop wild relatives of forage crops, are linked to areas of historic biodiversity. This analysis focuses on species-rich grasslands in Oxfordshire, England due to the availability of vegetation survey data and palaeoecological data in the region. In this analysis, I identify the origin and development of species-rich grasslands in Oxfordshire using palaeoecological data from the region. I then determine temporal and spatial changes in biodiversity using palaeoecological data and modern vegetation surveys. Finally, I compare historic hotspots of biodiversity and modern hotspots of biodiversity to establish whether the two are connected. As part of this analysis, I compare both hotspots of modern species richness and hotspots of crop wild relatives to the historic data. Although experimental, this research provides a new framework of inquiry and evaluation which other studies can replicate. These results can be used to inform grassland conservation and sustainable land use in the region and to identify long term hotspots of species-richness in order to prioritize areas for plant genetic resource collection and management.

2. Methods

2.1 Collection of palaeoecological data

To establish whether species-rich grasslands are a permanent, natural part of the landscape in the region or whether they developed due to anthropogenic disturbance, I analyzed palaeoecological data from Oxfordshire. Pollen cores from two current species-rich grassland SSSIs, Cothill Fen and Sidlings Copse, were re-analyzed (**Figures 1 and 2**). Today, Cothill Fen is characterized by broad-leafed deciduous woodland (*Alnus* sp., *Fraxinus* sp., *Crataegus* sp.), wet calcareous fen vegetation (*Molinia caerulea* (L.) Moench , *Parnassia palustris* L., *Schoenus nigricans* L., *Juncus subnodulosus* Schrank,), and wet grassland (*Filipendula ulmaria* L., *Potentilla erecta* Uspenski ex Ledeb, *Briza media* L., *Lysimachia vulgaris* L. *and Brachypodium pinnatum* (L.) P. Beauv.).[22] The modern vegetation of Sidlings Copse is characterized by broad leafed deciduous woodland (*Quercus* sp., *Fagus sylvatica* L.), limestone grassland (*Viola hirta* L., *Primula veris* L., *Centaurea* sp., *Origanum majorana* L., *Rhinanthus minor* L.), and scrubland (*Ulex europaeus* L., *Calluna vulgaris* (L.) Hull).[23] Cothill Fen is grazed by cattle and horses and Sidlings Copse is grazed by sheep.

[22] "Cothill National Nature Reserve." http://www.ocv.org.uk/sites.php?id=42. Last accessed 26 Dec. 2014.

[23] "Sydlings Copse, North-East Oxford." http://www.ocv.org.uk/sites.php?id=154. Last accessed 26 Dec. 2014.



Figure 1 Location of pollen cores used in this study. Cothill Fen (left; Photo credit: BBOWT) and Sidlings Copse (right; Photo Credit: B. Clarke).

Petra Dark (neé Day) analyzed the palynological data from Cothill Fen and Sidlings Copse as part of her doctoral research on the historic environment of lowland Britain in the 1980s (Day 1991, 1993). [24] These cores provide information on changes in particular arboreal, herb, and Pteridophyte taxa through time. I re-analyzed the original data files using Psimpoll, a software package that analyzes and visualizes pollen data (http://www.chrono.qub.ac.uk/psimpoll/psimpoll.html). In the re-analysis, samples were grouped according to time period based on the ^{14}C dates for each layer. These groups are as follows: Early Mesolithic, mid-Mesolithic, Late Mesolithic, Neolithic, early Bronze Age, mid-Bronze Age, Late Bronze Age, Iron Age, Roman period, and Medieval period (**Table 1**).

[24] Dark kindly shared the raw pollen abundance data (%) for Cothill Fen and Sidlings Copse. Any inaccuracies in the data analysis presented in this paper are thus my own and readers are encouraged to also refer to Day 1992 for the original analysis of the pollen cores.

Table 1 Chronology at Cothill Fen and Sidlings Copse. Period refers to cultural period. Core depth refers to how far down in cm a sample was taken. Date BP is the date of each ^{14}C sample before present. Sample number refers to the number given to each sample from the pollen core. Dark's zones refers to the initial zones (temporal periods) Dark proposed in Day 1991.

Site	Period	Core Depth (cm)	Sample Numbers	Date BP (^{14}C)	Dark's Zones
Cothill Fen **Total samples: 49** **Total taxa: 47**	Early Mesolithic	230-392	1-19	9070 ± 100	CF1
	Mid-Mesolithic	144-224	20-31	8350 ± 100	CF2-3a
	Late Mesolithic	56-136	32-44	7530 ± 120	CF3b
	Neolithic	32-52	45-49	6790 ± 100	CF4
Sidlings Copse **Total samples: 70** **Total taxa: 63**	Early Bronze Age	256-274	1-6		SC1-2
	Mid-Bronze Age	144-252	7-31	3820 ± 70	SC3-4
	Late Bronze Age	116-142	32-42	3820 ± 100	SC5
	Iron Age	64-112	43-55	2350 ± 70	SC6
	Roman Period	40-60	56-61	1820 ± 70	SC7
	Medieval	0-31	62-70	855 ± 70	SC8

I collected macrobotanical data from over 200 published and over 50 unpublished ('grey literature') excavation reports from archaeological excavations of Neolithic to Medieval settlements (3000 BCE – 1500 CE) in Oxfordshire from the mid-20th century onwards (Tomlinson and Hall 1996; http://archaeologydataservice.ac.uk/). To ensure reliability of the data, I only included reports where samples were ^{14}C dated or dated to a given century based on stratigraphy (instead of a broad range, i.e. Iron Age-Medieval). Of these sites, 43 contained adequate archaeobotanical data for analysis (**Figure 2; Appendix Table 1**).[25] All reports provided a table with the presence and/or abundance data of plant taxa recovered from the site. This data was either reported on a site or sample by sample basis. This data was entered into a database and 'cleaned' to update species identifications to current nomenclature. Each sample was categorized as either environmental (e.g. ditches, pits, river terraces, and wells) or anthropogenic (e.g. abandoned occupation floors, fire pit). Each taxon was categorized into four broad ecological system (grassland, arable, wetland, woodland) based on Grime et al. 1990. These 43 sites represented a total of 117 phases of occupation (Neolithic (n = 7), Bronze Age (n = 8),

[25] If a site was occupied for more than one period, i.e. Bronze Age, Anglo-Saxon, etc., it is still counted as one 'site.'

Iron Age (n = 14), Roman (n = 25), early Medieval (n = 34) and late medieval (n = 28)). In total, the macrobotanical record for the region consisted of 490 samples representing 498 taxa and were largely environmental (77%) (the rest came from anthropogenic contexts (e.g. living floors, fire pits)).

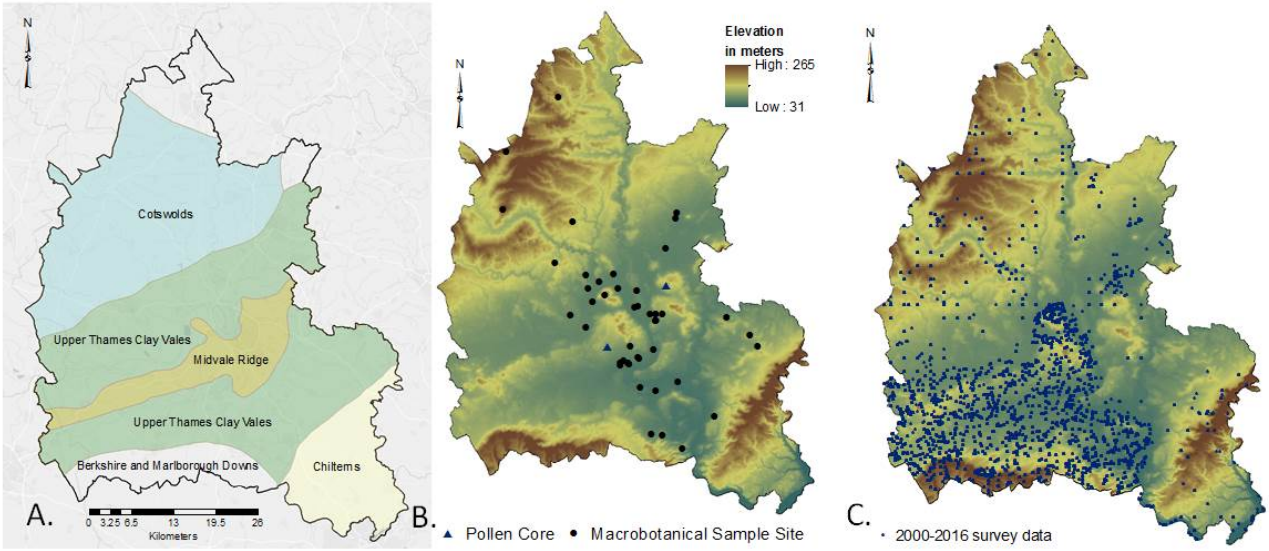


Figure 2 Location of study area and study sites. A. Major National Character Areas (NCAs) in Oxfordshire. B. Location of sites with palynological and macrobotanical remains. C. Location of sites with modern vegetation surveys.

2.2 Identification of Species-Rich Grasslands in the Palaeoecological Record

The use of particular taxa as indicators of particular landscapes or as proxies for environmental change and/or anthropogenic activity on the landscape is common in palaeoecology (Behre 1981; Hodgson et al. 1999). Previous palynological studies have used proxies to study eutrophication due to climate and human land use (Brush 2001) and the effect of agriculture on vegetation change and regeneration (Goman and Byrne 1998; Lomas-Clarke and Barber 2004; Court-Picon 2006). In this study, specific taxa (and in some cases, species) were used as indicators to identify the development of anthropogenic grasslands (pastures and meadows) in the palaeoecological record (**Table 2**). I selected palynological indicators based on previously published reports and macrobotanical indicators were based on previous reports and on species currently found in UK grasslands. To determine whether

anthropogenic grasslands developed during periods of agricultural intensification, I used taxa indicative of arable cultivation (specifically, arable weeds) as proxies (Charles 1997; Jones et al. 2009). To determine whether fire played a role in grassland development, I used the abundance of charcoal in the pollen cores from Cothill Fen and Sidlings Copse as an indicator (Kershaw et al. 1997; Lindbladh et al. 2003; Patterson et al. 1987). As recurrent 'natural' fires rarely occur in northern Europe, when charcoal fragments are found in pollen cores they are often interpreted as indication of anthropogenic use of fire for land clearance in the local vicinity (c.f. Caldararo 2002; Salzmann 2000; Moore 2000). Previous experimental studies suggest that charcoal fragments rarely travel more than 1km and thus reflect localized fire use (Lindbladh 1999).

Table 2 Indicators of grassland expansion, agricultural intensification, and fire use. Proxies are based on taxa used in other palaeoecological studies.

Landscape Change	Palynological Indicator	Macrobotanical Indicator	Source
Pasture/Meadow	Asteraceae, *Filipendula*, *Plantago lanceolata,* Poaceae, *Ranunculus*-type, *Rhinanthus*-type, *Rumex acetosa*, *Trifolium*-type	*Anthriscus sylvestris, Bromus* sp., *Bromus hordaceus*, *Cynosurus cristatus*, *Trifolium* spp., *Lolium* spp., *Medicago* spp., Poaceae, *Poa* spp., *Phleum* spp., *Vicia* spp.	Behre 1981; Court- Picon 2006; Derreumaux 2005; Hejcman et al. 2013; Hodgson et al. 1999; Overland and Hjelle 2008; Robinson 1992; Robinson 2011; Trondman et al. 2015
Agricultural Intensification	Brassicaceae, Cerealia	*Chenopodium album* L., *Fallopia convolvulus* (L.) Love, *Papaver rhoeas* L., *Polygonum aviculare* L., *Sinapis arvensis* L., *Stellaria media* L.	Behre 1981; Goman and Byrne 1998; Grime et al. 1990; Lomas-Clarke and Barber 2004; Trondman et al. 2015
Fire Use	charcoal particles in pollen cores	n/a	Brush 2001; Kershaw et al. 1997; Lindbladh et al. 2003; Patterson et al. 1987

Analysis of palaeoecological data can only provide a partial picture of vegetation change and the limitations (and potential biases) of the data should be acknowledged. Although palynological data provides a continuous record of vegetation change (because pollen is released by plants annually) not all plants are represented equally. For example, cereals are often self pollinated and smaller herbs are

often insect pollinated (and so produce less pollen than trees) (Eriksson et al. 2002). In addition, because pollen from different grass species is morphologically very similar, distinguishing differences in grass species over time is difficult and in most cases grass pollen is grouped together as 'Poaceae' (Needham et al. 2015). Taphonomic processes can also bias the available data and this is particularly problematic with macrobotanical remains. Many grassland species have small, delicate seeds that are often not preserved archaeobotanically. The seeds of grassland flora, including fodder grasses and legumes, will only be preserved if they end up in waterlogged contexts like pits and wells or if they are carbonized by fire (e.g. in corn driers (special ovens used to dry cereals)). These factors limit the resolution of the palynological and archaeobotanical data, but are often the only yardsticks we have to chart long term changes in vegetation composition and diversity through time.

2.3 Calculation of Palaeoecological Species Richness

To determine long term changes in biodiversity, species richness was calculated using the palynological and macrobotanical samples. For the palynological samples, the number of taxonomic units for each sample was counted. Taxonomic units refer to families and genera of plants (and on the rare occasion, species). While some taxa can be identified to the species level based on the morphology of their pollen grains (e.g. *Rumex acetosa*, *Plantago lanceolata*, and *Secale cereale*), others can only be identified to family (e.g. Poaceae, Cyperaceae, Asteraceae, Apiaceae) or genus (e.g. *Trifolium*, *Rhinanthus*) level and are referred to as 'types' (Bennett 1994). Undoubtedly this underestimates historic species richness, particularly regarding grasses. Nevertheless, palynological data allows for a relatively reliable comparison of species richness over time. This is based on the fact that pollen levels are relatively stable from year to year and provide an incremental measurement of

vegetation change similar to the way in which dendrochronology can provide a reliable indicator of changes in rainfall based on the width of each tree ring.[26]

Calculation of species richness for the macrobotanical remains was performed in a similar manner. First, the species richness for each macrobotanical sample was calculated. All species (woodland, grassland, cultivated species, etc.) were included in the count. For sites with more than one sample the average species richness was calculated. Macrobotanical remains provide a less accurate way of assessing change in species richness over time due to differences in sampling strategy and preservation at each site. However, analyzing both palynological and macrobotanical data can help 'check' one data set against the other.

2.4 Collection of Modern Vegetation Survey Data

I collected vegetation survey data for the county using the Botanical Society of the British Isles (BSBI) database. The BSBI manages a repository of past vegetation surveys conducted across the UK and is freely available online (http://bsbi.org/). I collected records from between 2000-2016 in this analysis (**Figure 2**). These surveys reflect the most recent data on local species distributions. This data set included a total of 25,765 records. This data was entered into an Excel database. To identify whether there were crop wild relatives among these taxa, the PGR Forum Crop Wild Relative Information System was used (http://www.pgrforum.org/cwris.htm) (PGR Forum 2005). I then checked to see whether any of these species were present in the database of species found in Oxfordshire. There were 208 records of crop wild relatives in the region.

[26] When the number of pollen spores in each sample are known, rarefaction analysis can be used to take into account fluctuations in pollen rain in relation to the number of taxa identified in each sample (Birks and Line 1992; Berglund *et al.* 2008; Meltsov *et al*. 2013). Unfortunately, the total pollen sums used in the original analysis of the data were unknown so rarefaction analysis could not be performed.

2.5 GIS analysis of Biodiversity Hotspots

To establish whether 'hotspots' of biodiversity changed over time, I mapped the species richness of the macrobotanical sites and the species richness of contemporary sites using ArcGis. To determine whether historic and cotemporary biodiversity hotspots were statistically significant, I performed Nearest Neighbor Analysis and Hot Spot Analysis in ArcGis (Mitchell 2005). Nearest Neighbor Analysis computes the average distance between all data points in a layer and determines whether the data is clustered, random or dispersed. Hot Spot Analysis calculates the Getis-Ord Gi* statistic for a set of data points with weighted values to identify clusters of high and low values. The Gi* is the value of a given data point based on the value of the neighboring points within a specified distance, which is then divided by the sum of all data points. A high Gi* score indicates that a data point with a high value is surrounded by other data points with high values and thus may statistically be considered a 'hot spot.' A data point with a low Gi* score indicates it is also surrounded by sites with low scores and thus be considered 'cold spots.'

In Hot Spot Analysis, the input field was 'species richness' the spatial features were conceptualized as fixed distance bands, and distances between neighboring data points was calculated using Euclidean distances. For each analysis, the search parameter ('distance band') was set to the average Euclidean distance between neighboring points for the palaeoecological and modern data sets respectively. To calculate the average Euclidean distance for each data set, the Distance Band from Neighbor Count tool was used. The number of neighbors was set to three. After Hot Spot Analysis was performed, Interpolation using Inverse Distance Weighting (IDW) was used to create a hot-spot map modeling larger areas of species richness in the county for the palaeoecological and modern data sets. Inverse Distance Weighting is a multivariate interpolation method that uses the weighted averages of known data points to give values to surrounding points. This method of interpolation is based on the idea that

objects closer together are more similar than those that are farther apart. For each data set, I created two interpolation models. The first model used the z-score for each sample as the z-values (the magnitude of each point) to get a sense of where hotspots in the region were located. I then ran the models again using the Gi* values as the z-values to identify where the most statistically significant hotspots.

2.6 Statistical Analysis

ANOVA analysis was used to determine whether there were significant changes in vegetation, land cover and species-richness among the palynological and macrobotanical samples (which were grouped according to cultural period). To determine whether historic species richness was related to expansion of grassland cover, correlation analysis followed by a *t*-test to determine the statistical significance of the correlation was used (Crawley 2011). Correlation analysis was also used to determine whether fire was used to reduce tree cover or to maintain grasslands. ANOVA and correlation analysis were performed in R (R version 3.2.2, "Fire Safety").

3. Results

3.1 *Palaeoecological evidence for origins and stability of species-rich grasslands*

At Cothill Fen and Sidlings Copse, open grassland oscillated with woodland cover (**Table 3, Appendix Figures 2 and 3**). At Cothill Fen, herb pollen varied significantly among cultural periods ($F_{3, 45} = 13.2$, $P < 0.001$) and was highest in the early Mesolithic (0.60 ± 0.05) and decreased by up to 70% over the course of the mid to late Mesolithic before returning to previous levels in the Neolithic (0.501 ± 0.11). At Sidlings Copse, total herb pollen also varied significantly among cultural periods (*F*

$_{5, 64}$ = 41.1, P < 0.001) and was highest in the early Bronze Age (0.44 ± 0.06), Iron Age (0.38 ± 0.07), Roman period (0.74 ± 0.08), and medieval period (0.60 ± 0.02). During the late mid to late Bronze Age herb pollen decreased by up to 80% (0.09 ± 0.06 and 0.07 ± 0.02 respectively). Periods of low grassland cover were characterized by increased total tree pollen.

Table 3 Change in vegetation composition at Cothill Fen and Sidlings Copse. The chart shows the ten most abundant species (in order of abundance) and a general interpretation of the vegetation for each cultural period. Taxa indicative of species-rich grasslands increase significantly after the Iron Age. The pollen diagrams in **Appendix 5 Figures 1-2** provide a more detailed picture of vegetation change at each site.

Cultural phase	Ten most abundant species	Interpretation of vegetation
Early Mesolithic	*Filicales,* Cyperaceae*, Pinus sylvestris* L.*, Corylus avellana* L.*, Thelypteris, Ulmus,* Poaceae*, Betula, Quercus, Typha latifolia* L.*, Artemisia*	Wet grassland
Mid-Mesolithic	*Pinus sylvestris, Corylus avellana, Quercus, Filicales , Ulmus,* Cyperaceae*,* Poaceae*, Thelypteris, Polypodium, Pteridium aquilinum* (L.) Kuhn*, Betula*	Woodland
Late Mesolithic	*Corylus avellana, Quercus, Pinus sylvestris,* Cyperaceae*, Ulmus, Filicales, Polypodium, Alnus glutinosa,* Poaceae*,* Brassicaceae*, Pteridium aquilinum*	Woodland
Neolithic	Cyperaceae*, Corylus avellana, Quercus, Tilia cordata* Mill.*, Alnus glutinosa* (L.) Gaertn.*, Filicales, Ulmus, Polypodium, Pinus sylvestris,* Poaceae*, Pteridium aquilinum*	Wet grassland with pockets of woodland
Early Bronze Age	*Corylus avellana,* Poaceae*, Pinus sylvestris, Bidens, Asteraceae, Betula, Salix,* Cyperaceae*, Filipendula, Filicales*	Wet grassland
Mid-Bronze Age	*Corylus avellana, Ulmus, Tilia cordata, Bidens, Quercus, Salix, Alnus glutinosa, Filicales ,* Brassicaceae*,* Poaceae	Woodland
Late-Bronze Age	*Corylus avellana, Alnus glutinosa, Tilia cordata, Filicales, Quercus, Ulmus,* Poaceae*,* Brassicaceae*, Umbelliferae, Polypodes*	Woodland
Iron Age	*Filicales, Pteridium aquilinum,* Cyperaceae*, Corylus avellana,* Poaceae*, Alnus glutinosa,* Asteraceae*, Quercus, Filipendula, Tilia cordata*	Arable fields and pastures
Roman	*Poaceae,* Cyperaceae*,* Brassicaceae*, Salix,* Asteraceae*, Corylus avellana, Pteridium aquilinum, Plantago lanceolata, Quercus, Equisetum*	Arable fields, pastures and meadows
Early Medieval	Poaceae*,* Cyperaceae*, Quercus,* Brassicaceae*, Salix,* Asteraceae*, Pteridium aquilinum, Corylus avellana, Pinus sylvestris, Plantago lanceolata*	Arable fields, meadows, wood pasture, rush-pasture

At Cothill Fen, there are few indicators of cultivated pastures or meadows (**Figure 3, Appendix Table 2)**. Grassland was largely 'natural' and was dominated by taxa indicative of wet grassland. Periods of

increased grassland cover in the early Mesolithic and Neolithic were associated with increases in Cyperaceae, *Typha*, and Poaceae pollen. Asteraceae pollen was also found in very low abundance during this period. There was a slight positive association between charcoal abundance and total tree pollen (r = 0.268, t = -1.909, df = 47, P = 0.06) and a weak negative association between charcoal abundance and total herb pollen (r = -0.242, t = -1.709, df = 42, P = 0.09), potentially indicating the use of fire to control vegetation cover.

At Sidlings Copse, grassland expansion in the early Bronze Age was initially associated with taxa indicative of dry grassland (e.g. Poaceae) and indicators of anthropogenic grasslands (e.g. pastures and meadows) increased during the Iron Age. The abundances of pollen from *Plantago lanceolata* ($F_{5, 64}$ = 21.68, P < 0.001), *Ranunculus* type ($F_{5, 64}$ = 14.42, P < 0.001), *Trifolium* type ($F_{5, 64}$ = 4.107, P = 0.003), *Filipendula* type ($F_{5, 64}$ = 4.782, P < 0.001), and Asteraceae type ($F_{5, 64}$ = 13.11, P < 0.001) varied significantly among cultural periods and were all more abundant after the end of the late Bronze Age (**Figure 3, Appendix Table 3**). *Plantago lanceolata* and *Ranunculus* increased steadily over time. Asteraceae was most abundant during the Iron Age and Medieval periods. *Trifolium* was most abundant in the Roman and medieval periods. *Rhinanthus* was only found in Iron Age and Medieval samples in very low amounts. Grassland expansion after the Iron Age was associated with increased arable cultivation. Levels of Ceralia and Brassicaceae pollen increase after the Iron Age and are highest during the Roman period. There was no significant correlation between charcoal area and total tree pollen (r = -0.146, t = -1.2199, df = 68, P = 0.227) or total herb pollen (r = 0.149, t = -1.243, df = 68, P = 0.218), suggesting fire was not used to clear woodland or manage grassland.

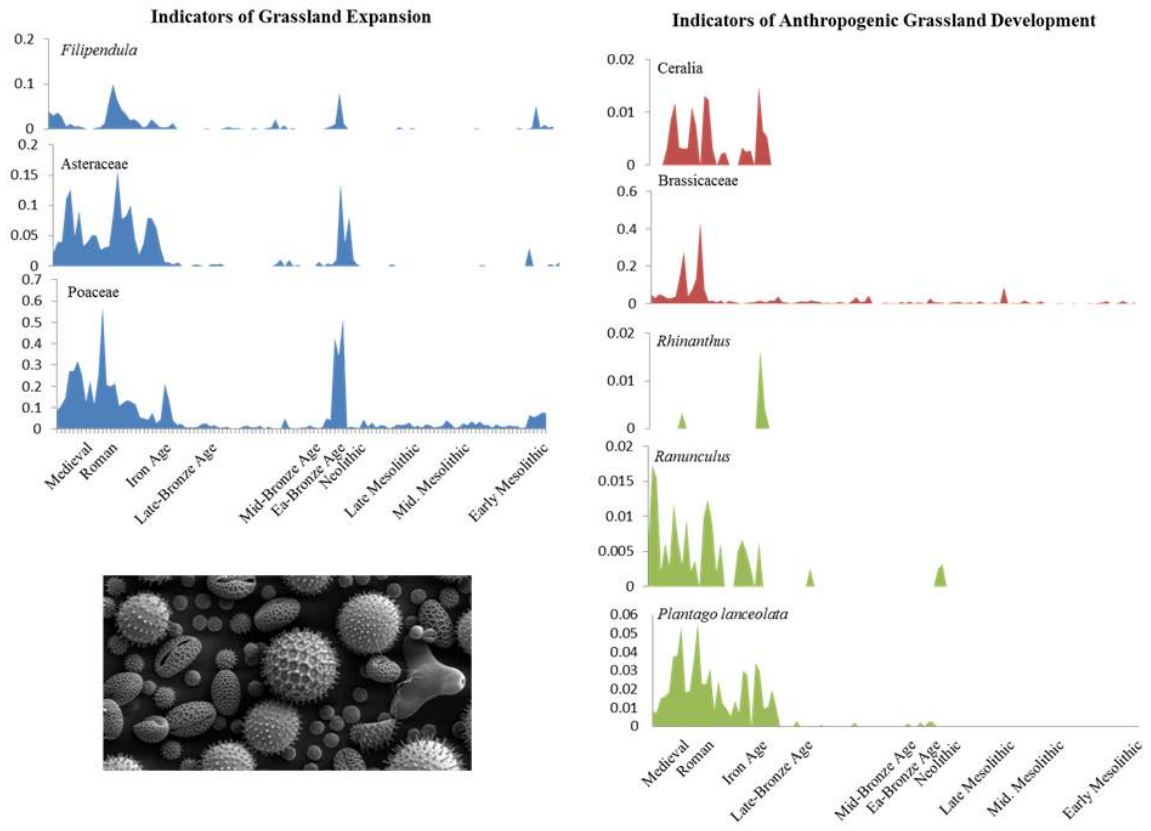


Figure 3 Taxonomic indicators of grassland expansion and anthropogenic grassland development. Periods of grassland expansion were characterized by increased pollen abundance from Poaceae, Asteraceae, and *Filipendula*. Periods of anthropogenic grassland development were characterized by increased abundances of *Plantago lanceolata*, *Ranunculus*, and *Rhinanthus*. Indicators of agricultural intensification (Ceralia and Brassicaceae) also increase at the same time. All values are in represented as the abundance (%) of pollen in each sample. For full palynological sequences, see **Appendix Figures 1-2.**

The macrobotanical evidence also indicates that species-rich grasslands developed in the Iron Age (**Table 4**). **Appendix Table 3** provides an overview of the vegetation species characteristic of each cultural period. Samples from the Neolithic and Bronze Age are dominated by arboreal species. Five taxa (*Lathyrus* sp., *Plantago lanceolata*, Poaceae, *Trifolium pratense*, and *Vicia* sp.) occur across the samples and are associated with grassland expansion. 18 taxa characteristic of intensively managed grasslands appear during or after the Iron Age and are most abundant in samples from the Roman and early medieval periods. For example, *Medicago lupulina* appears only after the Iron Age while *Trifolium arvense* and *Cynosurus cristatus* appear only after the Roman period. *Lolium perenne*, *Poa*

pratensis, *Poa annua*, and *Phleum pratense* were associated with samples from the early medieval period. There was little evidence for crop wild relatives, apart from *Medicago lupulina*, *Trifolium arvense*, and *Vicia cracca*, all of which appear after the Iron Age.

Table 4 Macrobotanical evidence for anthropogenic grasslands. Five taxa occurred across multiple cultural periods and are indicative of grassland expansion. 18 taxa found on contemporary pastures and meadows only occur after the Iron Age and are good indicators of the creation of special purpose anthropogenic grasslands.

Grassland Type	Indicator Species	Neolithic	Bronze Age	Iron Age	Roman	Early Medieval	Late Medieval
Grassland Expansion	*Lathyrus* sp.	x	x	x	x	x	x
	Plantago lanceolata		x	x	x	x	x
	Poaceae	x	x	x	x	x	x
	Trifolium pratense		x	x	x	x	
	Vicia sp.	x	x	x	x	x	x
Meadow	*Bromus hordaceus*			x	x		
	Bromus sp.			x	x	x	x
	Phleum sp.					x	
	Sanguisorba officinalis			x	x	x	
	Vicia cracca			x	x		
Pasture/ Meadow	*Anthriscus sylvestris*				x		x
	Cynosurus cristatus				x		
	Lolium perenne					x	
	Lolium sp.				x		
	Medicago lupalina			x	x	x	x
	Medicago sp.			x		x	
	Ranunculus sp.			x	x		
	Trifolium arvense					x	
	Trifolium sp.			x	x	x	x
	Urtica dioica			x	x	x	x
Wet pasture/ meadow	*Carex* sp.			x	x	x	x
	Eleocharis palustris			x	x	x	x
	Juncus sp.			x	x	x	x

3.2 Long-Term Changes in Species Richness

Species-richness varied significantly among cultural periods at Cothill Fen ($F_{3, 45}$= 6.431, P < 0.001) and Sidlings Copse ($F_{5, 64}$= 339.7, P < 0.001) and was strongly associated with grassland expansion during and after the Iron Age (**Figure 4**). At Cothill Fen, species-richness was lowest during the early Mesolithic (15.68 ± 0.56 species) and slowly increased over time, reaching its highest level in the Neolithic (20 ± 1.22 species). Species richness was not correlated with tree (r = 0.218, t =1.532, df = 47, P = 0.132) or grassland cover (r = -0.207, t = -1.449, df= 47, P = 0.154). At Sidlings Copse, species-richness declined by up to 25% after the early Bronze Age and increased by 47 % by the Iron Age (28.62 ± 0.1 species). Species richness was highest during the Roman (32.67 ± 0.1 species, t = 25.42, P < 0.001) and medieval periods (32.89 ± 0.1 species). Species-richness was strongly positively correlated with grassland cover (r = 0.759, t = 9.604, df = 68, P < 0.001) and strongly negatively correlated with tree cover (r = -0.818, t = -11.724, df = 68, P < 0.001). Macrobotanical remains also indicate that species-richness varied significantly among cultural periods ($F_{5, 111}$ = 8.22, P < 0.001) (**Figure 4**). Species-richness was lowest during the Bronze Age (4.44 ± 11.38 species) and was significantly higher during the Iron Age (33.07 ± 10.45 species) and Roman (44.76 ± 9.65 species) periods. However, species-richness declined during the early medieval (21.06 ± 9.37 species) and late medieval (12.93 ± 9.54 species) periods.

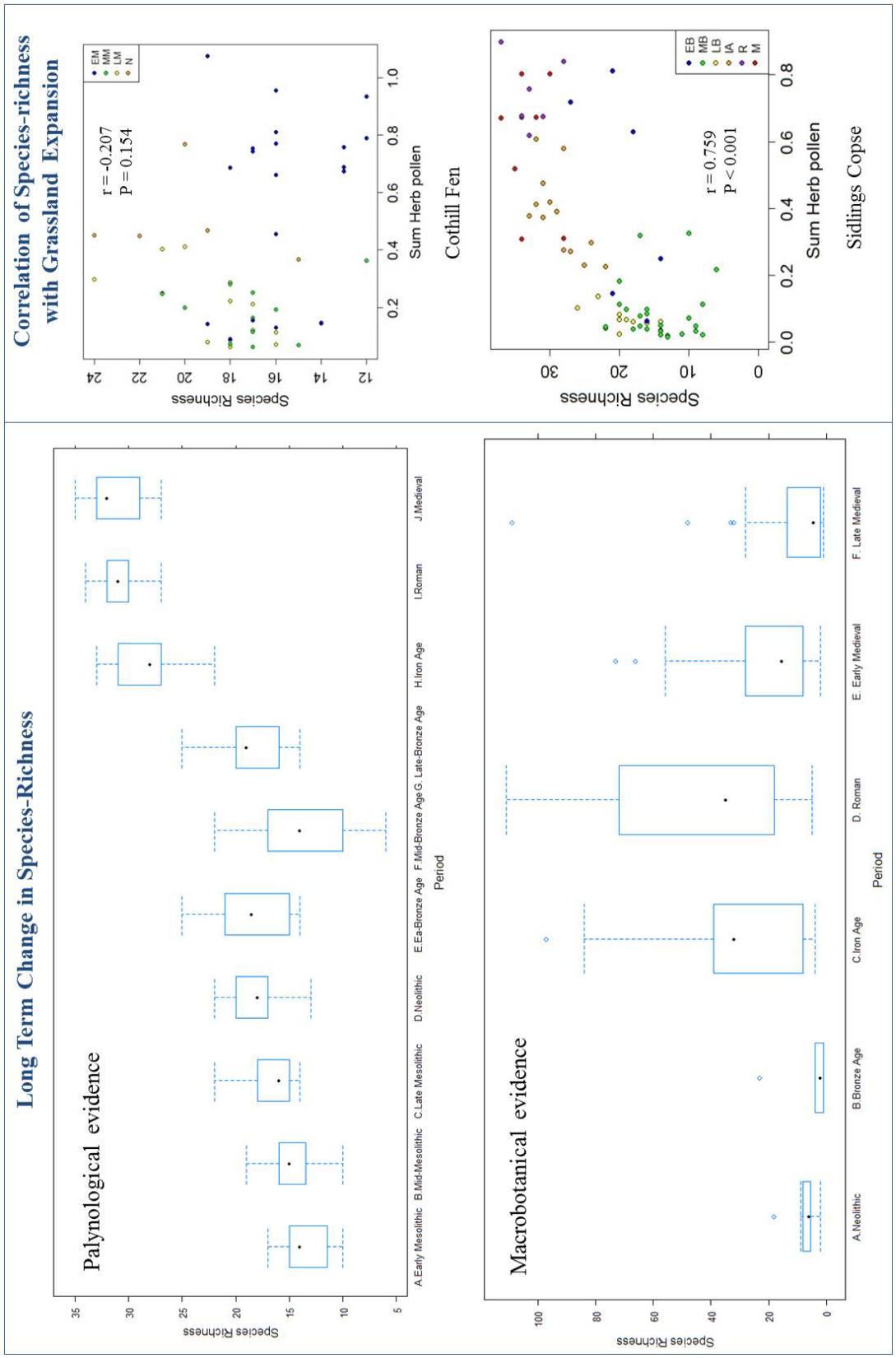


Figure 4 Palynological and macrobotanical data indicates species-richness increased dramatically after the Iron Age. While the palynological data suggests species richness continued to increase after the Roman period, macrobotanical evidence suggests it declined. Species-richness was not correlated with land cover from the Mesolithic to Neolithic period at Cothill Fen but was positively correlated with grassland cover after the late Bronze Age at Sidlings Copse.

3.3 GIS Identification of Continuity and Change in Biodiversity Hotspots through Time

Average Nearest Neighbor analysis indicates that the palaeoecological data is clustered (Nearest Neighbor Ratio: -0.36, z = -12.90, P < 0.001). The same was true for the modern vegetation data (Nearest Neighbor Ratio: 0.026, z = -299.218, P < 0.001) and the data specifically on crop wild relatives (Nearest Neighbor Ratio: 0.310, z = -34.030, P < 0.001). Hot spot analysis indicates past sites of botanical diversity are located in the Upper Thames Clay Vales and Midvale Ridge (**Figure 5**). Modern hotspots of biodiversity are located in the same area but have also shifted north over time. Hotspots of crop wild relatives have specifically persisted in the Midvale Ridge Region. These hotspots contained 36 wild relatives of fodder crops in total (**Appendix III Table 4**). 23 of these were wild relatives of seven forage legumes, *Hippocrepis*, *Lathyrus*, *Lotus*, *Medicago*, *Melilotus*, *Trifolium*, and *Vicia*. 13 were wild relatives of the forage grasses *Aloperecus*, *Festuca*, *Lolium*, *Phleum*, and *Poa*. The five most frequent species were *Trifolium dubium* Sibth., *Vicia cracca* L., *Medicago arabica* (L.) Huds., *Alopecurus geniculatus* L., and *Lathyrus latifolius* L..

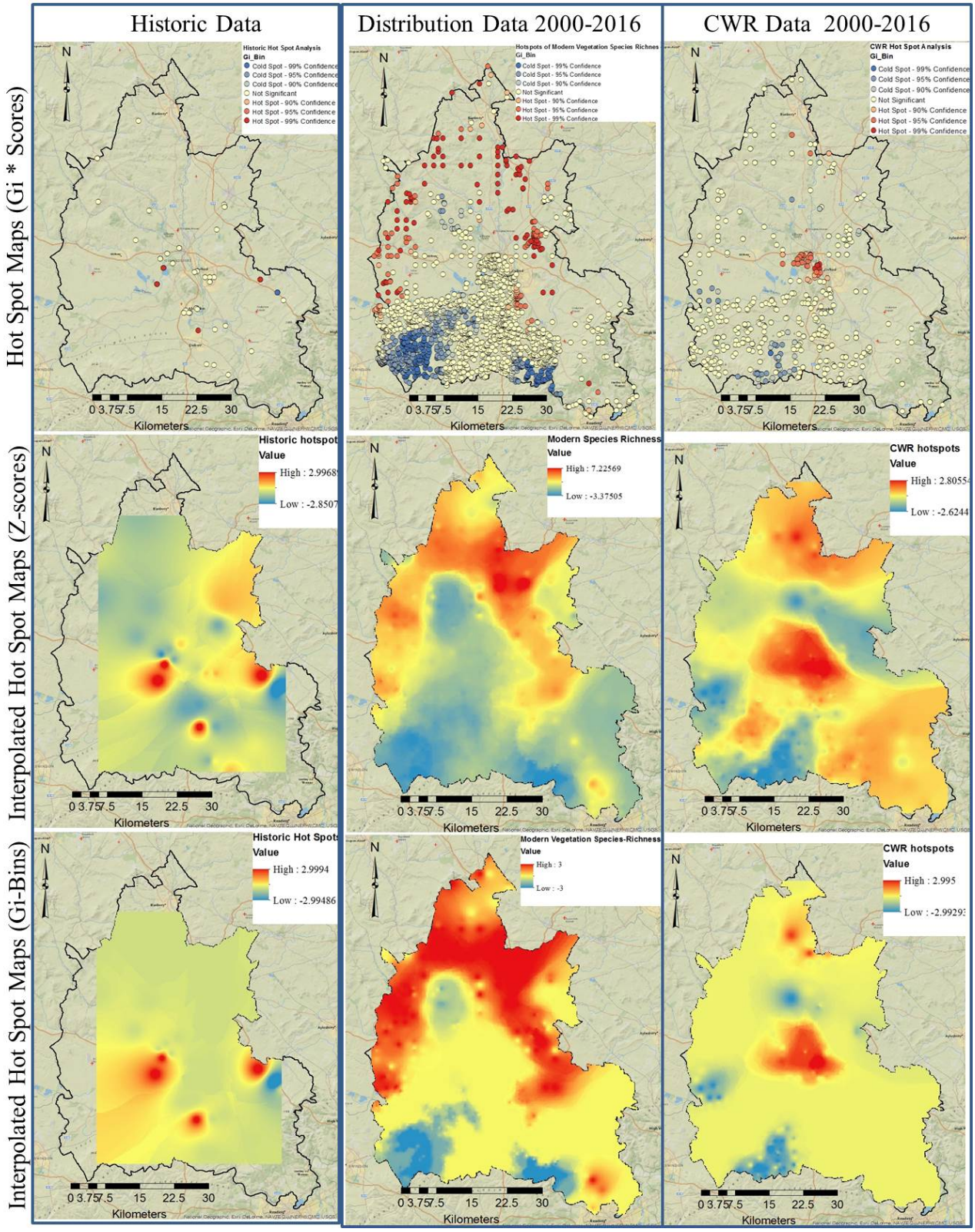


Figure 5 Change in 'hotspots' of diversity over time. Top row: Hot spot maps show the Gi-scores for each site. Gi-scores represent how statistically significant a hot spot is: the higher the score, the more significant. Middle

Row: Interpolated hot spot maps created using the Z-scores from the Hot Spot Analysis. How warm a site is is based on the entire data set for each site. Bottom row: Interpolated hot spot maps using Gi-bins. The Gi-bins show the statistically significant hots spots. In general, species-richness shifted northward over time. However, hot spots of crop wild relatives remained near areas of historic species richness. In all charts, the Gi-scores range from -3 (significantly 'cold') to 3 (significantly 'hot'). These scores are a combination of the z-scores and p-values for each site.

4. Discussion

In this analysis, I show that grassland cover oscillated with woodland cover over the past 7,000 years and that intensively managed anthropogenic, species-rich grasslands only developed around 2,000 years ago during the Iron Age. Historic periods of increased species richness were associated with the development of species-rich grasslands and agricultural intensification and were highest in the Roman and medieval periods. GIS analysis of historic and contemporary vegetation data indicates that hotspots of modern biodiversity overlap with historic areas of biodiversity, although new hotspots have also shifted northward over time.

4.1 Grassland Biodiversity and Anthropogenic Management

Palaeoecological data from Oxfordshire indicates that species-rich grasslands developed in the region between the Iron Age and medieval period during periods of agricultural intensification and would likely disappear without human management. Beginning in the Iron Age, modern forage crops begin to appear in macrobotanical samples (e.g. *Lolium perenne*, *Phleum pratense*). It is unlikely that farmers domesticated these crops at this time (e.g. because domestication can take 500 to 4,000 years; Fuller 2007). However, it is possible that the creation of hay meadows and pastures 'semi-domesticated' these crops. Specifically, creating highly anthropogenic grasslands that were cut annually and/or grazed by livestock could have unintentionally selected for these crops (and their wild relatives) and the wider phytosocial community they were a part of (e.g. specific herbs, grasses, and

forbs). These activities would have exerted selective pressure absent during the Mesolithic, Neolithic and Bronze Age, when a more transhumant pastoral lifestyle was the norm. Although there was some evidence that natural and/or anthropogenic factors may have contributed to the development of grasslands in the Mesolithic (for clearing trees or rushes is uncertain), there was no evidence to suggest fire was used to manage species-rich grasslands, highlighting the close connection between the development of these landscapes and human agricultural activities. The close association of grassland development with agricultural intensification agrees with archaeological evidence from the region (e.g. linear ditches, crop marks, small enclosed plots located adjacent to settlements) which suggest a more intensified agricultural system developed in the late Iron Age (ca. 100 BCE – 0 CE) and increased in the Roman period (1st - late 4th century CE) (Allen 2006; Booth 2009; Robinson 1992).

Other palynological and archaeobotanical studies in the UK and northern Europe also demonstrate that taxa indicative of pastures and meadows increase between the Iron Age and medieval periods (roughly 50 CE - 1200 CE) (Hjelle et al. 2012; Johansson et al. 2008; Lindbladh 1999; Peglar 1993). For example, Berglund et al.'s research on two Holocene palynological sequences from Färskesjön, and Krageholmssjön in southern Sweden demonstrates that over a 6,000 year period, peaks in species-richness correspond to deforestation and expansion of open-landscape by humans, and was at its peak ca. 1000 years ago (Berglund et al. 2008). This suggests that the development of species-rich grasslands in northern Europe may have a strong cultural component, specifically, shared traditions of land management and land use related to forage production.

The increased biodiversity associated with historic anthropogenic, species-rich grasslands may be due to (1) the specific cultivation of particular grassland plants, (2) increased land fragmentation caused by human land use, and (3) grazing. Historical sources from this period suggest farmers began to actively select specific species to cultivate in meadows (Fowler 1997; Fowler 2002; Harlan 1992; Murphy

2007). Two recent meta-analyses of the effect of contemporary land fragmentation on different global ecosystems also suggest that, overall, land fragmentation produces multiple habitats within a given landscape, providing the resources as well as refuge from predators/pathogens required by high densities of diverse animal and plant species to coexist (Benton et al. 2011; Fahrig 2003). In addition, numerous ecological studies have demonstrated that grazing promotes vegetation diversity by selecting specific species (at the patch, community and landscape scale) and by spreading their seeds (Adler et al. 2011; Metera et al. 2010; Olff et al. 1999).

Without human management, these grasslands would likely revert to oscillating between wet open grassland and woodland. This historic oscillation was seen at Cothill Fen and Sidlings Copse and is mirrored in other parts of northern Europe over the course of the Holocene (Dark 2006; McNeely 1994). This agrees with other ecological studies which point to the role of anthropogenic disturbance, climate change and even microbial communities in directing cycles of grassland expansion and contraction (Hijmans and Graham 2006; Leake et al. 2004; Lin et al. 2015; van der Heijden et al. 1998; van der Heijden et al. 2008; Walther et al. 2002; Vera 2002). This oscillation between grassland and woodland also occurs outside of Europe. For example, in Africa, savannah landscapes oscillate with woodland based on rainfall patterns, tree competition, as well as natural and anthropogenic fires (Wiegand et al. 2006; Meyer et al. 2009). This suggests that if conservation of species-rich grasslands in Oxfordshire (and in Europe more broadly) is a priority, they will require long-term human management.

4.2 Stability and Change in Spatial 'Hotspots' of Biodiversity

GIS analysis of the spatial distribution of historic and modern species richness indicates that, historically, the Midvale Ridge and Upper Thames Clay Vales were sites of 'hotspots' of past

vegetation diversity for several thousand years. I also found that hotspots of crop wild relatives occurred specifically in the Midvale Ridge and Upper Thames Clay Vales. Areas of historic biodiversity contained 36 crop wild relatives of fodder grasses and legumes. Continuity in biodiversity over time in the Midvale Ridge and Upper Thames Clay Vales may be due to both biotic as well as anthropogenic factors. The historic and modern vegetation survey data indicates that grasslands have dominated these areas for the past 2,000 years. Long-term grazing in these regions may have helped to sustain local populations by spreading seed from one place to another (as discussed above in section 4.1). In addition, in many areas farmers and graziers have kept the land as grassland for livestock production (e.g. grazing or for hay) due to the fact that these areas flood and are unsuitable for arable production (French, unpublished data). In regards to the specific association between hotspots of crop wild relatives and historic land use, my analysis agrees with research by Altieri et al. (1987), Erskine et al. (1994), and Brown et al. (2008) which has demonstrated that wild relatives tend to be found around domesticated plants and are partially evolved to agricultural ecosystems and human management. Although there was little macrobotanical evidence for crop wild relatives (apart from *Medicago lupulina*, *Trifolium arvense*, and *Vicia cracca*), this could be due to two reasons: the seeds of these plants were not preserved or archaeobotanists wrongly identified crop wild relatives as domesticated forage plants (e.g. *Trifolium* spp., etc.). The latter is more probable given the fact that preservation of charred remains is often poor and it is difficult to identify plants down to the species level. In addition, few reference collections contain crop wild relatives. Overall, these results suggest that anthropogenic land management may lend a degree of stability to grassland biodiversity over time.

My analysis agrees with other studies in northern Europe which have also found that many modern hotspots of biodiversity are found within the vicinity of historic sites of human land use. For example, research by Dupouey et al. (2002) and Dambrine et al. (2007) note that high levels of soil fertility and species richness have been found in areas of old Roman settlements within the Tronçais forest in

Central France and suggest this may be due to increased soil fertility at these sites. Similarly, Lindbladh's palynological research in southern Sweden demonstrated that a grassland area in Osaby was a direct descent of a medieval meadow that developed ca. 1,000 years ago (Lindbladh 1999). Outside of Europe, a number of recent studies have also demonstrated that the present day diversity of tropical forests in Brazil and Borneo are the result of thousands of years of human activities (Heckenberger et al. 2007; Hunt and Rabett 2014; Maloney 1998).

However, I also found that new 'hotspots' of biodiversity also developed over time. These hotspots appear in the north-west and south-west of the county where elevation is slightly higher and there is a greater annual rainfall. Whether these locations were also 'hot' in the past is unclear due to lack of palaeoecological data in these regions. If these are new hotspots, this would agree with other studies which have found that biodiversity hotspots can shift over time because of increased temperature and/or anthropogenic disturbance over the past century (Jiang et al. 2013; Pearson and Dawson 2013; Thomas et al. 2004; Thuiller et al. 2005). For example, Thuiller et al. (2005) used bioclimatic envelope models to determine how the distributions of 2,294 European vascular plants might change and found that more than half moved northwards or to higher elevations. The biodiversity of ecosystems with less anthropogenic management may thus be more susceptible to changes in climate and other biotic/abiotic factors.

4.3 Applied Paleoecology: Landscape Re-creation and Plant Genetic Resource Management

The palaeoecological evidence for grassland development can inform conservation objectives in the region. Currently, although a number of species-rich grasslands are actively maintained by farmers, land managers, and conservation groups, many grasslands are actively 'reverting' to more 'wild,' hybrid forms of grassland, such as rush pasture or wood pasture. The palaeoecological evidence

suggests this is a natural process. However, conservation groups often halt these transitions (e.g. by clearing woodland and scrubland to keep grassland open, cutting back wetland vegetation (sedges and rushes) in areas that are becoming increasingly wet) in an effort to maintain historic landscapes (**Figure 6**). Allowing grasslands to revert to a more natural state would provide three key ecological and economic benefits to local communities. First, promoting the re-creation and maintenance of rush pasture and wood pasture would help to conserve these equally species-rich, and often over looked, landscapes (Critchley et al. 2004; Kirby et al. 1995; Plieninger et al. 2015). Second, increasing the area covered by wetland and woodland could help stem local incidences of flooding, which have increased in recent years. Third, increased availability of rush pasture and wood pasture could be used as a source of low-maintenance forage for livestock and/or as biomass for bioenergy (French, this thesis). Working with the natural landscape instead of manipulating it into a certain form might also lead to a more sustainable use of local natural resources.

A.  B. 

Figure 6 Current conservation activities control vegetation change and biodiversity dynamics. A. Volunteers coppicing trees may help promote a more sustainable use of local natural resources (Photo credit: Rushbeds Wood, BBOWT). B. Clearing natural vegetation (e.g. reeds) to promote the growth of rare plants or to keep species-rich meadows from reverting to wetland vegetation often relies heavily on volunteers and might be less sustainable in the long term (Photo Credit: Lye Valley, J. Webb).

The long-term evidence for persistence in biodiversity through time (temporally and spatially) can be used to prioritize the conservation of plant genetic resources. In addition to crop wild relatives, these centers of long-term diversity may also harbor landraces of forage grasses and legumes well adapted to

local conditions that could be used in national forage breeding programs. Although genetic analysis of forage grasses and legumes was not conducted in this study, Fischer et al. (2008) and Treuren et al. (2005) have shown that grasses from areas of long term pastoralism mature earlier and display a higher rate of genetic diversity compared to modern commercial cultivars. These genetic resources may contribute to creating forage crops with lower nutrient requirements and increased resilience to environmental stress. For western Europe, including the UK, the forecasted changes in climate are primarily increased summer drought and winter flooding (Miraglia et al. 2009; Rath and Peel 2005). Currently, few forage breeding programs make use of local germplasm but this may become more prevalent in the future with advances in high-throughput sequencing and genomic selection (Humphreys 2003). Promoting the in-situ conservation, monitoring, and seed collection of forage landraces and crop wild relatives found on species-rich grasslands would help ensure that these economically important species continue to adapt to changing environmental conditions, thus serving as a continual bank of genetic diversity for plant breeders to draw upon (Joshi et al. 2001; Hodgkin and Bordoni 2012). This is particularly important, as few conservation groups monitor these resources (Fielder et al. 2015a; Maxted 2003; Maxted et al. 2007).[27]

5. Conclusion

In this analysis I have shown how combining paleoecology with modern vegetation surveys can help direct the conservation of grassland biodiversity. Palaeoecological data from the region indicates that species-rich grasslands developed roughly 2,000 years ago and would disappear without human management. Species richness fluctuated over time and was strongly linked to the development of

[27] For example, in Oxfordshire local conservation groups currently monitor 29 rare plants listed on the UK Biodiversity Action Plan (1994) but only one of the crop wild relatives (*Apium repens*) found in the region. In part, this may be due to a lack of awareness, among conservation groups as well as the farmers who manage these conservation areas, of the identity and importance of crop wild relatives.

these highly anthropogenic landscapes. GIS comparison of historic with modern biodiversity hotspots indicates that historic hotspots persisted over time due in the Midvale Ridge and Upper Thames Clay Vales due to a variety of environmental and cultural factors (e.g. continuity in land use). Areas of long term biodiversity contained economically valuable plant genetic resources, specifically, 36 wild relatives of forage grasses and legumes, which may be closely linked to historic, highly anthropogenic pastures and meadows. Areas of long-term biodiversity may thus serve as an untapped source of plant genetic resources for forage grass and legume breeding and more attention should be given to monitoring specific resources (e.g. crop wild relatives, ecotypes) with economic potential.

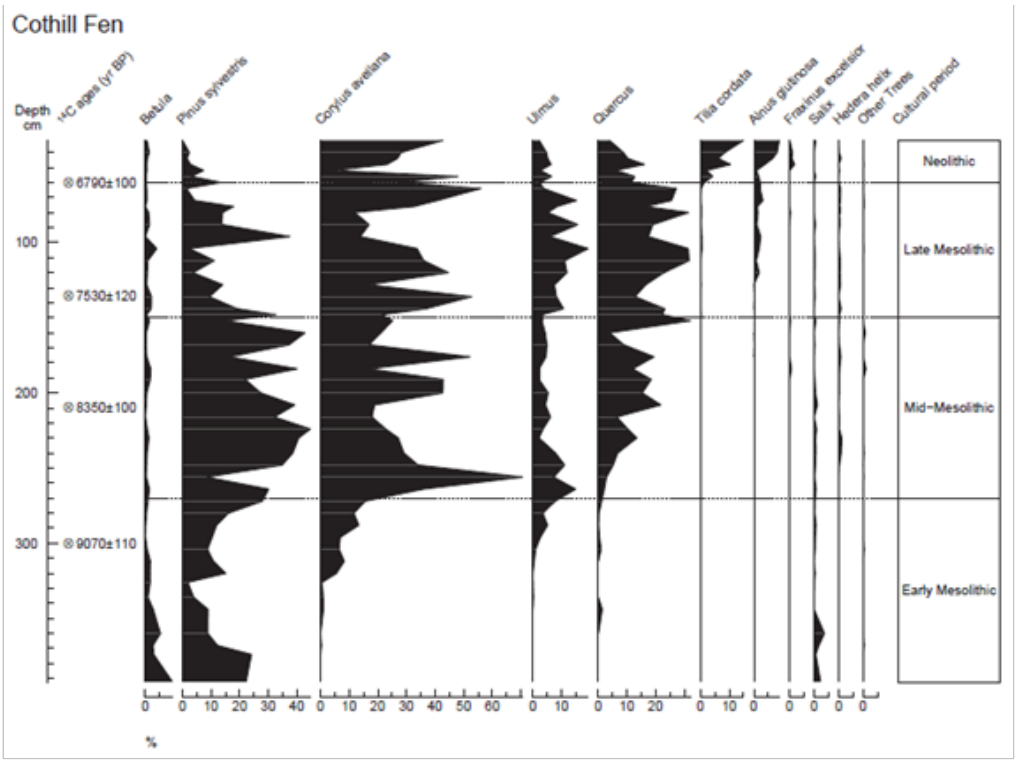
Palaeoecology thus provides important insight into the impact of past human activities on vegetation change and biodiversity through time. Perhaps most importantly, it highlights how cultural landscapes like grasslands are always in the making: they represent an ongoing relationship between people and plants. When human social structures, economic activities, and cultural values change, their relationship with the environment and natural resource use also changes. Although conservation will always be a very subjective, and very human, activity, such long term perspectives on human-environment interactions can contribute to developing more sustainable land management practices in the 21st century.

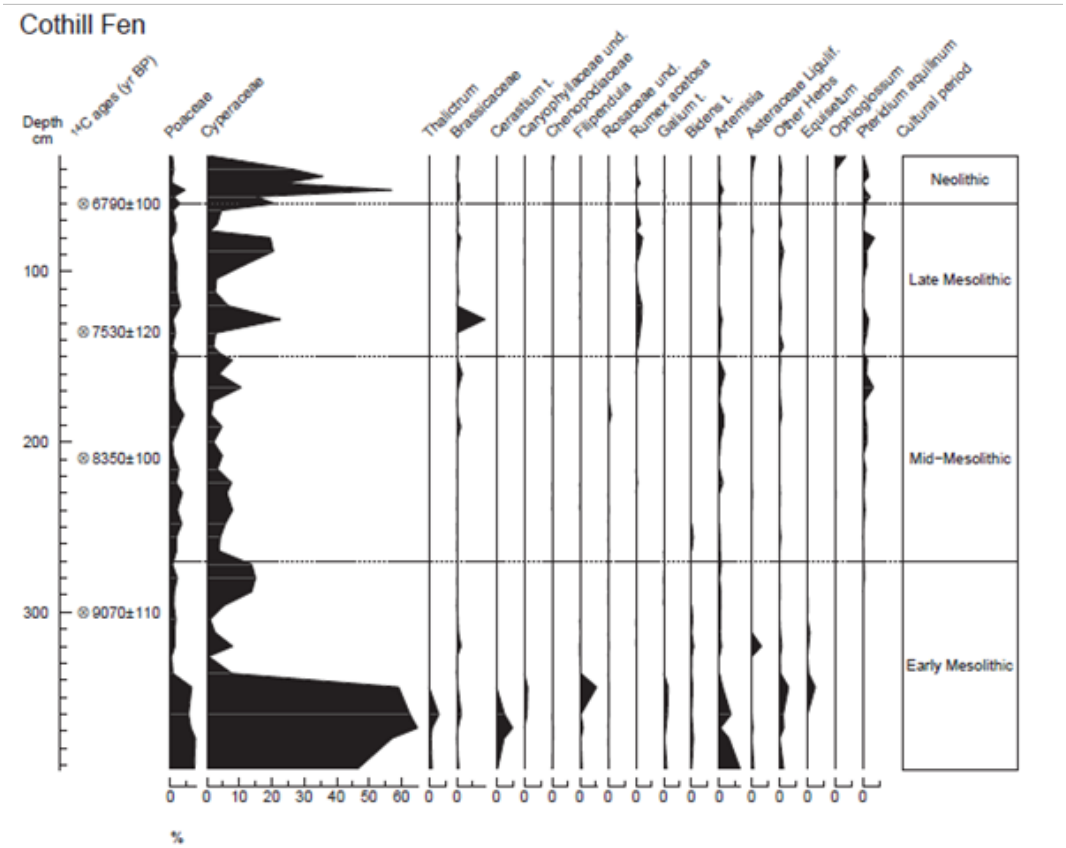
Appendix IV

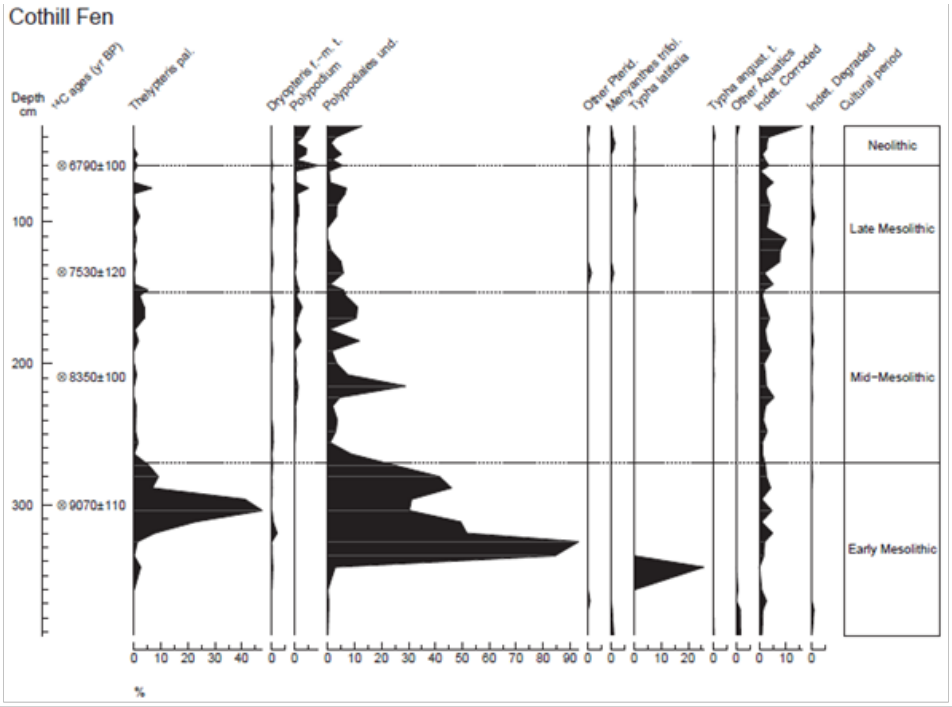
Table 1 Archaeological Sites in Oxfordshire with Palaeoecological Remains. The table lists the name, location and reference for each site. Macrobotanical data from each site was used to calculate historic changes in vegetation and species richness.

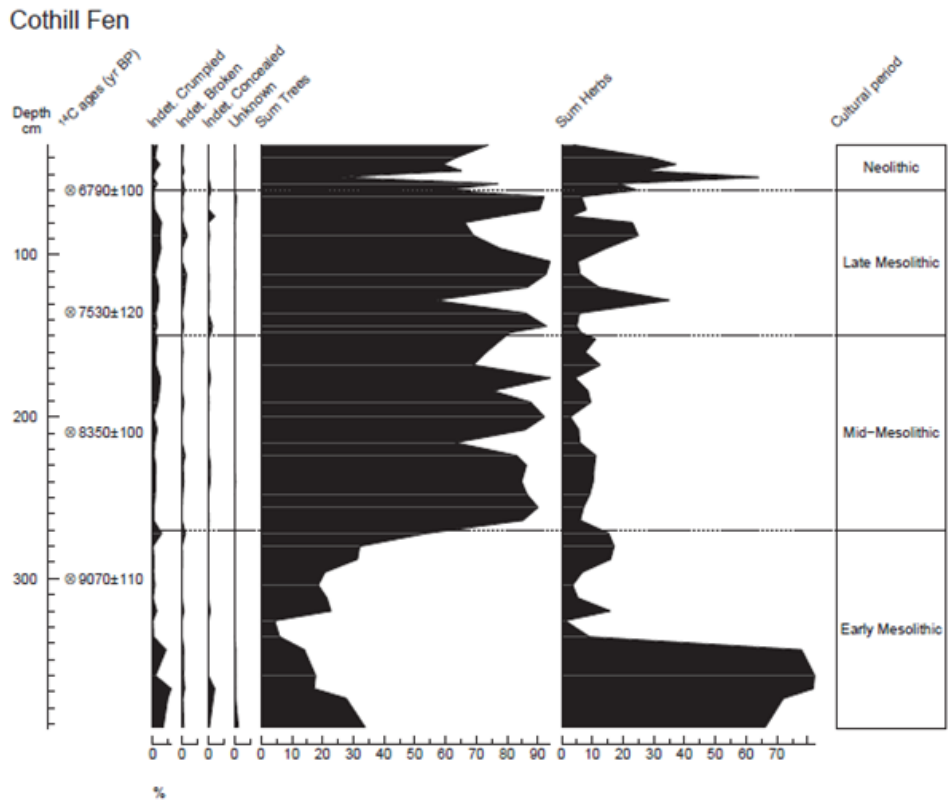
Site Name	Location	Latitude	Longitude	Reference
MG Abingdon	Abingdon	51.680967	-1.2624534	Moffet et al. 1989
Mount Farm	Dorchester	51.667761	-1.1571125	Jones 1980
Barrow Hills	Radley, nr Abingdon	51.67933	-1.25944	Moffet et al. 1989
Blewbury	nr Didcot	51.547823	-1.2511955	Moffet et al. 1989
Oddington	nr Oxford	51.834101	-1.195809	Sims 2009
Radley Barrow Hills 83-5	Radley, nr Abingdon	51.67933	-1.25944	Moffet 1991
Summertown	Banbury	51.774399	-1.2632999	Duigan 1955
Long Wittenham	nr Dorchester	51.638954	-1.2037436	Gray 1971
City Farm 64	Church Hanborough, nr Oxford	51.813129	-1.38311	Dimbleby 1966
Ashville Estate 74-6	Abingdon	51.672394	-1.3014927	Jones 1978
Cassington	nr Oxford	51.787771	-1.3519051	Chalk 1946
Stanton Harcourt 61	nr Oxford	51.749591	-1.409235	Pilcher 1968
Rollright Stones 82-6	nr Chipping Norton	51.974879	-1.5690374	Straker 1988; Robinson 1988
Ashville Estate 74-6	Abingdon	51.672394	-1.3014927	Jones 1978
Cassington	nr Oxford	51.787771	-1.3519051	Chalk 1946; Western 1982
Chinnor	nr Princes Risborough	51.698908	-0.8908702	Richardson and Young 1951
Halfpenny Lane 89	Cleeve-Didcote pipeline (nr Goring)	51.551006	-1.163454	Carruthers 1990
Madmarston Camp 57-8	Swalcliffe, nr Banbury	52.04716	-1.43857	Dimbleby 1960
Watkins Farm	Northmoor, nr Oxford	51.728139	-1.383139	Robinson 1990
Farmoor 74-6	Farmoor, Upper Thames	51.75996	-1.36389	Jones and Robinson 1979
Alchester	Bicester	51.87567	-1.17342	Robinson 1976
Appleford	nr Abingdon	51.63796	-1.25723	Robinson 1981
Broad St Abingdon 73	Abingdon	51.67686	-1.29564	Jones 1975
Callow Hill	nr Woodstock	51.87167	-1.404694	Brown 1957

Cold Harbour Farm, Wallingford	nr Woodstock	51.59583	-1.088944	Vitolo 2009
Farmoor 74-6	Farmoor, Upper Thames	51.75996	-1.36389	Jones 1979
Madmarston Camp 57-8	Swalcliffe, nr Banbury	52.04716	-1.43857	Dimbleby 1960
North Leigh 52	nr Witney	51.814346	-1.450167	Morrison 1960
Tiddington	nr Thame	51.73545	-1.060133	Robinson 1988
Watkins Farm	Northmoor, nr Oxford	51.728139	-1.383139	Robinson 1990
Ashville	Oxfordshire	51.672394	-1.3014927	Jones 1978
Faccenda Farm	Oxfordshire	51.883281	-1.1696515	Giorgi and Robinson 1984
Aston Tirrold 89	Goring/Didcot	51.561222	-1.1840398	Carruthers 1990
Barrow Hills	Radley, nr Abingdon	51.67933	-1.25944	Moffet et al. 1989
Cornmarket St 70	Oxford	51.75306	-1.25969	Brown 1971
New Inn Court 72	Oxford	51.73212	-1.219484	Robinson 1983
Queen St (11-18) 68-80	Oxford	51.73212	-1.219484	Robinson 1983
St Aldates (33)	Oxford	51.73212	-1.219484	Robinson 1984
St Aldates (65)	Oxford	51.73212	-1.219484	Robinson 1984
Tiddington	nr Thame	51.72832	-1.06752	Robinson 1988
Chapel st, Bicester		51.89669	-1.33501	
Alchester	SE (Oxfordshire)	51.87789	-1.1699	Robinson 1976
Eynsham Abbey	SE, Oxfordshire	51.77861	-1.37389	Hardy et al. 1983
Yarnton	SE (Oxfordshire)	51.798346	-1.31405	Hey 2004
Church St Oxford 68	Oxford (Domus Mirifield)	51.74111	-1.21933	Brown 1969
Copt Hay 72	Tetsworth, nr Thame	51.70984	-1.00711	Robinson 1979
Seacourt 58-9	nr Oxford	51.768794	-1.334787	Arthur 1962; Dimbleby 1962
Old Gaol Abingdon 73	Abingdon	51.672299	-1.285587	Jones 1975
Dominican Priory Oxford 76-83	Oxford	51.73212	-1.21948	Robinson 1985
Frewin Hall Oxford	Oxford	51.74101	-1.20485	Robinson 1978
St Helen's Passage 74-5	Hertford College Oxford	51.741206	-1.233811	Robinson 1976
Stert St 75-6	Abingdon	51.67189	-1.28163	Jones 1979
The Hamel 75-6	Oxford	51.751308	-1.266958	Robinson 1980


Cothill Fen
Depth cm
14C ages (yr BP)
Betula
Pinus sylvestris
Corylus avellana
Ulmus
Quercus
Tilia cordata
Alnus glutinosa
Fraxinus excelsior
Salix
Hedera helix
Other Trees
Cultural period
6790±100
7530±120
8350±100
9070±110
Neolithic
Late Mesolithic
Mid-Mesolithic
Early Mesolithic
%


Cothill Fen
Depth cm
14C ages (yr BP)
Poaceae
Cyperaceae
Thalictrum
Brassicaceae
Cerastium t.
Caryophyllaceae und.
Chenopodiaceae
Filipendula
Rosaceae und.
Rumex acetosa
Galium t.
Bidens t.
Artemisia
Asteraceae Liguliflorae
Other Herbs
Equisetum
Ophioglossum
Pteridium aquilinum
Cultural period
6790±100
7530±120
8350±100
9070±110
Neolithic
Late Mesolithic
Mid-Mesolithic
Early Mesolithic
%


Cothill Fen
Depth cm
14C ages (yr BP)
Thelypteris pal.
Dryopteris f.-m. t.
Polypodium
Polypodiales und.
Other Pterid.
Menyanthes trifol.
Typha latifolia
Typha angust. t.
Other Aquatics
Indet. Corroded
Indet. Degraded
Cultural period
⊗6790±100
⊗7530±120
⊗8350±100
⊗9070±110
Neolithic
Late Mesolithic
Mid-Mesolithic
Early Mesolithic
100
200
300
0 10 20 30 40
0 0
0 10 20 30 40 50 60 70 80 90
0 0 0 10 20
0 0 0 10 0
%


Cothill Fen
Depth cm
14C ages (yr BP)
Indet. Crumpled
Indet. Broken
Indet. Concealed
Unknown
Sum Trees
Sum Herbs
Cultural period
⊗6790±100
⊗7530±120
⊗8350±100
⊗9070±110
Neolithic
Late Mesolithic
Mid-Mesolithic
Early Mesolithic
100
200
300
0 0 0 0 0 10 20 30 40 50 60 70 80 90
0 10 20 30 40 50 60 70
%

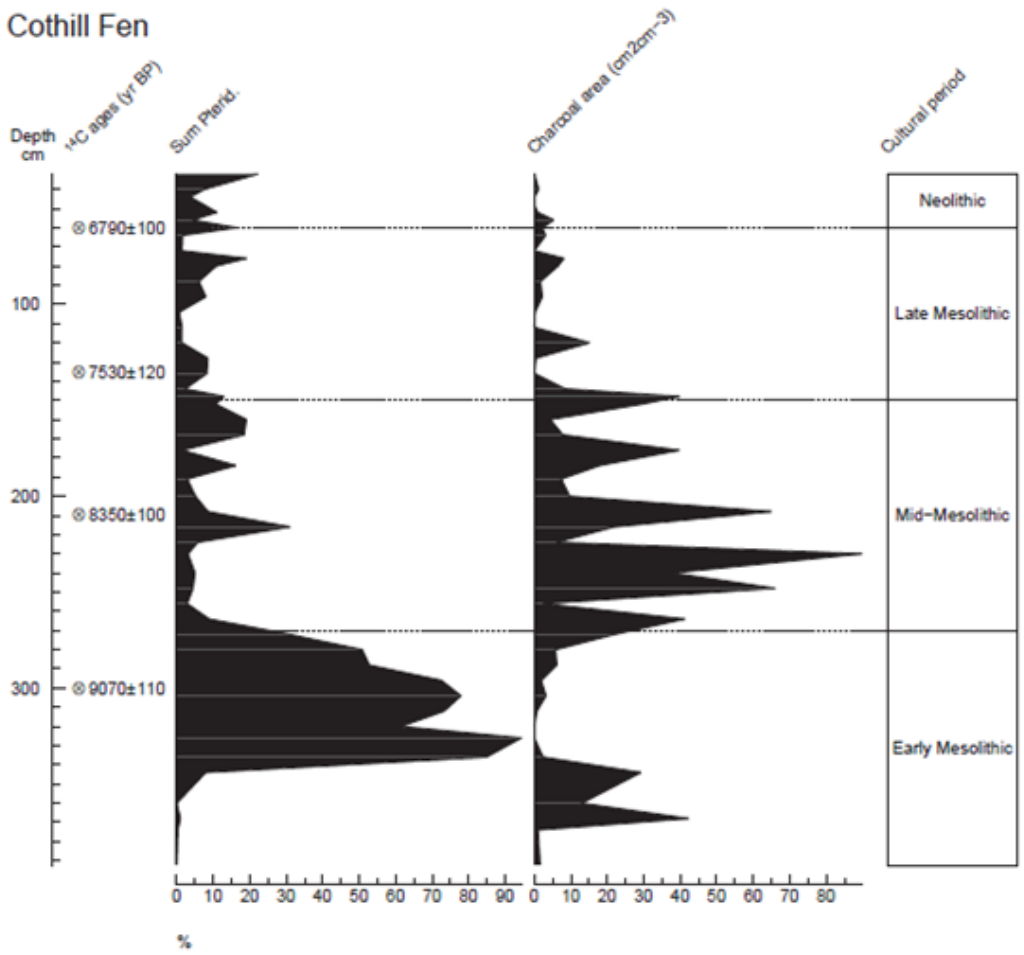
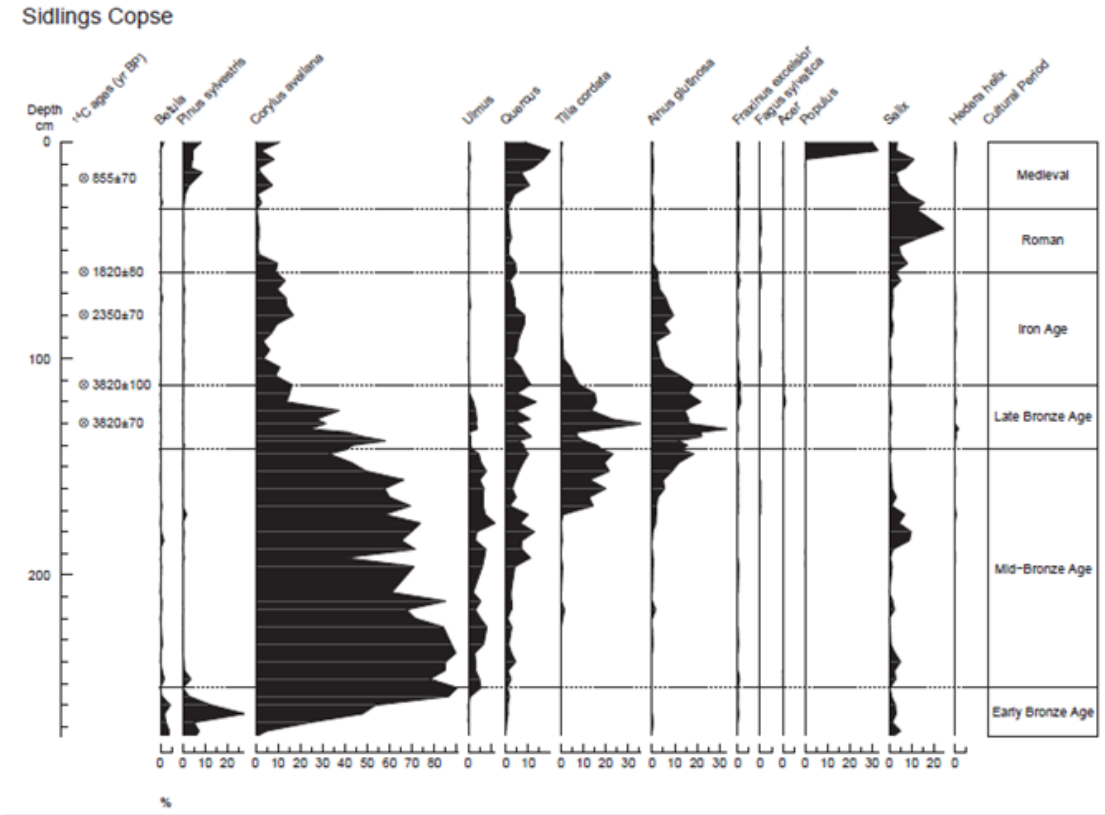
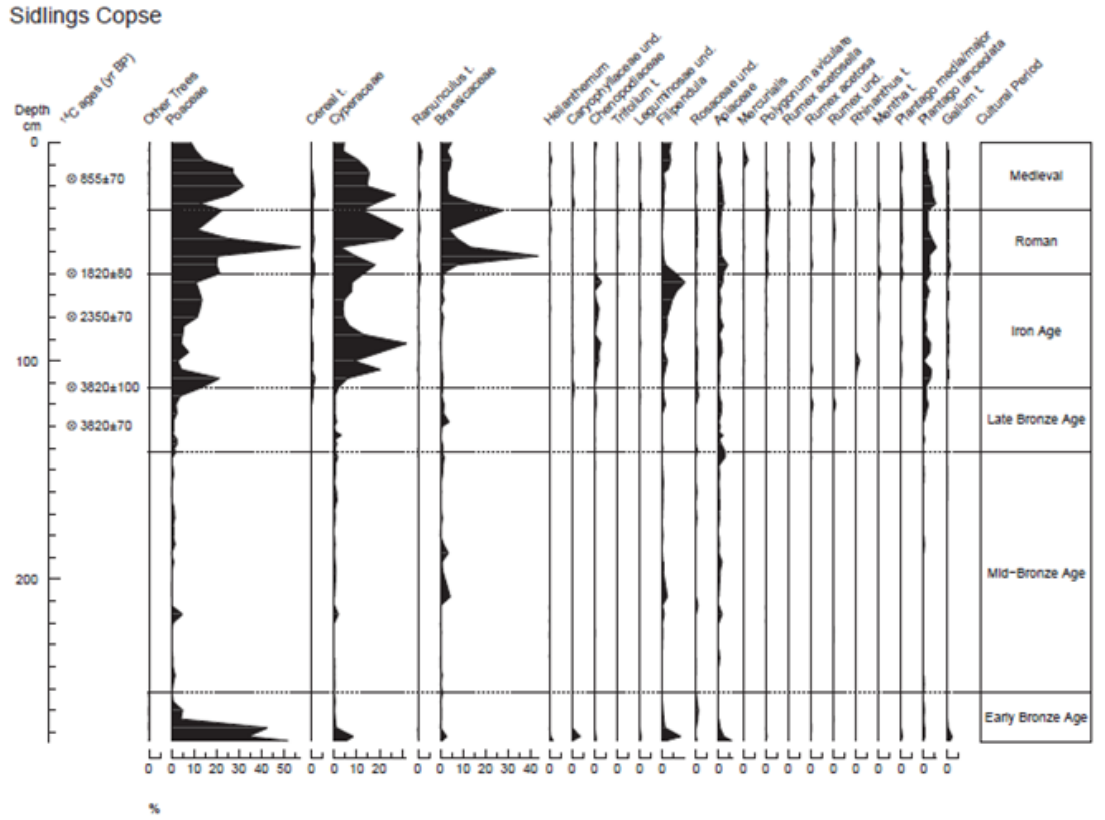


Figure 1 Pollen diagram of Cothill Fen. Pollen values are represented in abundance (%). The early Mesolithic was characterized by wet, open grassland which is replaced with woodland in the mid to late Mesolithic. Open wetland conditions return in the Neolithic.


Sidlings Copse
Depth cm
14C ages (yr BP)
Betula
Pinus sylvestris
Corylus avellana
Ulmus
Quercus
Tilia cordata
Alnus glutinosa
Fraxinus excelsior
Fagus sylvatica
Acer
Populus
Salix
Hedera helix
Cultural Period
0
100
200
855±70
1820±80
2350±70
3820±100
3820±70
Medieval
Roman
Iron Age
Late Bronze Age
Mid-Bronze Age
Early Bronze Age
%


Sidlings Copse
Depth cm
14C ages (yr BP)
Other Trees
Poaceae
Cereal t.
Cyperaceae
Ranunculus t.
Brassicaceae
Helianthemum
Caryophyllaceae und.
Chenopodiaceae
Trifolium t.
Leguminosae und.
Filipendula
Rosaceae und.
Apiaceae
Mercurialis
Polygonum aviculare
Rumex acetosella
Rumex acetosa
Rumex und.
Rhinanthus t.
Mentha t.
Plantago media/major
Plantago lanceolata
Galium t.
Cultural Period
0
100
200
855±70
1820±80
2350±70
3820±100
3820±70
Medieval
Roman
Iron Age
Late Bronze Age
Mid-Bronze Age
Early Bronze Age
%

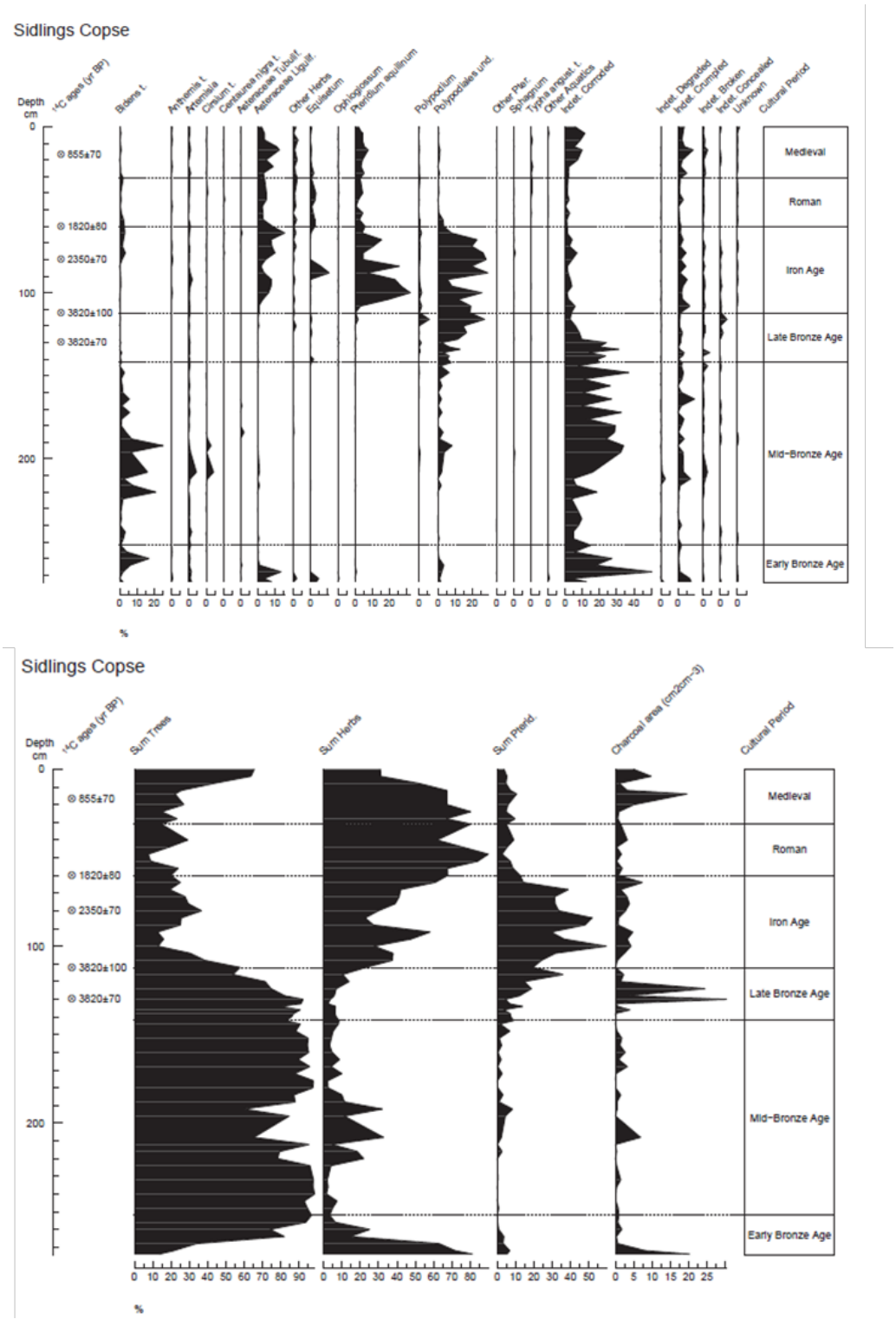


Figure 2 Pollen diagram of Sidlings Copse. Pollen values are represented in abundance (%). The early Bronze Age was initially open grassland, which was replaced by woodland cover in the mid to late Bronze Age. Open conditions return during the Iron Age. The Roman landscape was characterized by intensive arable cultivation and deforestation. Agricultural intensification decreased during the medieval period and the landscape became more 'pastoral,' characterized by species typical of meadow and wood pasture.

Table 2 Palaeoecological Evidence for Grassland Development and Arable Cultivation at Cothill Fen and Sidlings Copse. Indicators of grassland expansion are highest in the early Bronze Age, Iron Age, Roman and medieval periods. Indicators of arable cultivation are highest in the Roman period. Indicator species refer to taxa indicative of grasslands or arable fields. EM = early Mesolithic, MM = mid-Mesolithic, LM = late Mesolithic, Neo = Neolithic, EBA = early Bronze Age, MBA = mid-Bronze Age, LBA = late Bronze Age, IA = Iron Age, Rom = Roman period, Med = Medieval period. A * means that pollen from this taxa is not present

	Indicator Species	EM	MM	LM	Neo	EBA	MBA	LBA	IA	Rom	Med
Wetland	Cyperaceae	0.203 ± 0.04	0.045± 0.06	0.10± 0.005	0.29 ± 0.08	0.025 ± 0.022	0.003 ± 0.025	0.007± 0.028	0.105 ± 0.027	0.166 ± 0.031	0.134 ± 0.039
	Typha type	0.014 ± 0.009	*	*	0.001 ± 0.019	*	*	*	*	0.002 ±9.7^{-4}	0.004± 8.84^{-4}
Grassland	Poaceae	0.03 ± 0.004	0.017 ± 0.007	0.016 ± 0.006	0.014 ± 0.009	0.23 ± 0.035	0.007 ± 0.039	0.017 ± 0.043	0.10 ± 0.042	0.26 ± 0.049	0.20 ± 0.045
	Plantago lanceolata	*	*	*	*	0.001 ± 0.004	*	0.004 ± 0.004	0.016 ± 0.004	0.031 ± 0.005	0.024 ± 0.005
	Ranunculus type	*	*	*	*	0.001 ± 0.001	*	*	0.003 ± 0.001	0.006 ± 0.002	0.008 ± 0.001
	Filipendula type	*	*	*	*	0.019 ± 0.007	0.002 ± 0.008	0.001 ± 0.009	0.027 ± 0.009	0.013 ± 0.010	0.019 ± 0.01
	Trifolium type	*	*	*	*	*	*	*	1.538^{-4} ± 3.6^{-4}	0.001 ± 4.3^{-4}	0.001 ± 3.9^{-4}
	Rhinanthus type	*	*	*	*	*	*	*	0.002 ± 1.01^{-3}	*	0.0004 ± 1.07^{-3}
	Asteraceae	0.002 ± 0.001	*	*	0.003 ± 0.002	0.048 ± 0.012	0.003 ± 0.013	0.002 ± 0.014	0.061 ± 0.014	0.047 ± 0.016	0.062 ± 0.015
Arable	Ceralia	*	*	*	*	*	*	*	0.003 ± 0.001	0.008 ± 0.001	0.003 ± 0.001
	Brassicaceae	0.003 ± 0.003	0.004 ± 0.005	0.01 ± 0.005	0.004 ± 0.006	0.008 ± 0.02	0.008 ± 0.02	0.01 ± 0.03	0.008 ± 0.3	0.128 ± 0.3	0.077 ± 0.3

Table 3 Changes in vegetation species composition over time based on macrobotanical samples. Woodland species dominate Neolithic and Bronze Age samples while species indicative of arable cultivation, grassland and wetland increase after the Iron Age.

Cultural Period	Characteristic species	Interpretation of vegetation
Neolithic	*Alnus glutinosa* (L.) Gaertn. , *Arrhenatherum elatius* (L.) P.Beauv. ex J.Presl & C.Presl, *Carpinus betulus* L., *Corylus avellana* L., *Fagus sylvatica* L., *Fraxinus excelsior* L., *Malus* sp., *Prunus spinosa* L., *Quercus* sp., *Sorbus* sp.	Woodland
Bronze Age	*Alnus glutinosa, Arrhenatherum elatius, Carpinus betulus, Corylus avellana, Fagus sylvatica, Festuca gigantea/pratensis, Fraxinus excelsior, Malus* sp., *Prunus spinosa, Quercus* sp., *Sorbus* sp., *Trifolium* sp.	Wood pasture
Iron Age	*Aethusa cynapium* L., *Atriplex* spp., *Bromus hordeaceus* L., *Carduus* sp., *Cirsium* sp., *Carex* sp., *Chenopodium album* L., *Fraxinus excelsior, Galium aparine* L., *Juncus* sp., *Lathyrus* sp., *Lycopus europaeus* L., *Medicago lupulina, Bromus* sp., *Eleocharis palustris* (L.) Roemer & Schultes, *Plantago major* L., Poaceae, *Salix* sp., *Trifolium pratense* L., *Trifolium repens* L., *Rumex* sp., *Urtica dioica* L.	Arable fields and pastures
Roman	*Achillea* sp., *Aethusa cynapium, Alisma* sp., *Atriplex* spp., *Ballota nigra* L., *Bromus hordeaceus, Carduus* sp., *Carex* sp., *Chenopodium album* L., *Coriandrum sativum* L., *Daucus carrota* L., *Eleocharis palustris, Euphrasia* sp., *Hyoscyamus niger* L., *Juncus* sp., *Medicago lupalina, Plantago major,* Poaceae, *Prunella vulgaris* L., *Rumex* sp., *Sonchus asper* (L.) Hill, *Trifolium* sp., *Trifolium pratense, Urtica dioica, Urtica urens* L., *Valerianella dentata* (L.) Pollich, *Vicia* sp., *Vicia cracca* L.	Arable fields, pastures and meadows
Early medieval	*Alisma* sp., *Arrhenatherum elatius, Bromus* sp., *Carex* spp., *Centaurea* sp., *Chenopodium album, Corylus avellana, Eleocharis palustris, Juncus* spp., *Lathyrus* sp., *Lolium perenne* L., *Medicago lupalina, Mentha arvensis* L., *Phleum sp.,* Poaceae, *Prunella vulgaris, Rumex* sp., *Rumex crispus* L., *Salix* sp., *Trifolium arvense* L., *Trifolium* sp., *Trifolium pratense, Urtica dioica, Vicia* spp., *Vicia cracca, Zannichellia palustris* L.	Arable fields, meadows, wood pasture, rush-pasture
Late medieval	*Alisma, Arrhenatherum elatius, Carex* sp., *Chenopodium album, Corylus avellana, Cratageus* sp., *Fraxinus excelsior, Juncus* spp., *Lathyrus* sp., *Poaceae, Prunus spinosa, Rumex* sp., *Salix* sp., *Trifolium* sp., *Urtica dioica, Vicia spp.*	Arable fields, wood pasture, rush-pasture

Table 4 Crop Wild Relatives found in Oxfordshire. 36 crop wild relatives were found throughout the county.

CWR Type	Genus	Species	Number of Occurrences
Grass	Alopecurus	*Alopecurus geniculatus* L.	9
		Alopecurus myosuroides Huds.	3
	Festuca	*Festuca filiformis* Pourr.	1
		Festuca glauca Lam.	1
	Holcus	*Holcus mollis* L.	8
	Lolium	*Lolium multiflorum* Lam.	7
	Phleum	*Phleum bertolonii* DC.	29
		Phleum phleoides (L.) H. Karst.	2
		Phleum pratense L.	12
	Poa	*Poa angustifolia* L.	2
		Poa compressa L.	1
		Poa humilis Ehrh. ex Hoffm.	1
		Poa nemoralis L.	13
Legume	Hippocrepis	*Hippocrepis comosa* L.	4
	Lathyrus	*Lathyrus latifolius* L.	18
		Lathyrus nissolia L.	6
		Lathyrus odoratus L.	1
	Lotus	*Lotus pedunculatus* Cav.	13
		Lotus tenuis Willd.	7
	Medicago	*Medicago arabica* (L.) Huds.	26
		Medicago lupulina L.	13
		Medicago minima (L.) Bartal.	1
		Medicago polymorpha L.	1
	Melilotus	*Melilotus altissimus*Thuill.	4
	Trifolium	*Trifolium arvense* L.	1
		Trifolium campestre Schreb.	17
		Trifolium dubium Sibth.	51
		Trifolium fragiferum L.	1
		Trifolium hybridum L.	8
		Trifolium medium L.	1
		Trifolium micranthum Viv.	2
	Vicia	*Vicia cracca* L.	46
		Vicia hirsuta (L.) Gray	15
		Vicia sativa L.	5
		Vicia sepium L.	17
		Vicia tetrasperma L.	11

References

Adler, P.B., Raff, D.A. & Lauenroth, W.K., 2001. The effect of grazing on the spatial heterogeneity of vegetation. *Oecologia*, 128(4), pp.465–479.
Allen, T. 2006. "Oxfordshire: Later Bronze Age and Iron Age." *Historic Environment Resource Assessment.* Oxford: Oxford Archaeology.
Altieri, M.A., Anderson, M.K. & Merrick, L.C., 1987. Peasant Agriculture and the Conservation of Crop and Wild Plant Resources. *Conservation Biology*, 1(1), pp.49–58.
Arthur, J., 1962. Botanical material from the soil samples. In: Biddle M. The deserted medieval village of Seacourt, Berkshire. *Oxoniensia*, 26, pp.70–210.
Behre, K.E., 1981. The interpretation of anthropogenic indicators in pollen diagrams. Pollen Spores 23: 225-245. *Pollen et Spores*, 23, pp.225–245.
Bennett, K., 1994. *Annotated Catalogue of Pollen and Pteridophyte Spore Types of the British Isles*, Department of Plant Sciences, Cambridge.
Benton, T.G., Vickery, J.A. & Wilson, J.D., 2003. Farmland biodiversity: is habitat heterogeneity the key? *Trends in Ecology & Evolution*, 18(4), pp.182–188.
Berglund, B., M. Gaillard, L. Björkman, and T. Persson. 2008. "Long-term changes in floristic diversity in southern Sweden: palynological richness, vegetation dynamics and land-use." *Vegetation History and Archaeobotany* 17:573–583.
Birks, H. and J. Line. 1992. "The use of Rarefaction Analysis for estimating palynological richness from Quaternary pollen-analytical data." *The Holocene* 2: 1.
Birks, H. 2012. "Ecological palaeoecology and conservation biology: controversies, challenges, and compromises." *International Journal of Biodiversity Science, Ecosystem Services & Management* 8.4: 292-304.
Booth, P. 2009. "Roman Oxfordshire." Report for Oxford Archaeology. Oxford: Oxford Archaeology.
Breed, M.F. et al., 2012. Which provenance and where? Seed sourcing strategies for revegetation in a changing environment. *Conservation Genetics*, 14(1), pp.1–10.
Brown, A., 1971. Botanical material. 33. In: Hassall T.G. Excavations at 44-46 Cornmarket Street, Oxford, 1970. *Oxoniensia*, 36, pp.15–33.
Brown, A. 2010. "Pollen analysis and planted ancient woodland restoration strategies: a case study from the Wentwood, southeast Wales, UK." *Vegetation History and Archaeobotany* 19: 79-90.
Brown, A., 1968. Plant remains. *Oxoniensia*, 34, pp.5–20.
Brown, F., 1957. Charcoals N. Thomas, ed. *Oxoniensia*, 22, pp.11–53.
Brown, T.A. et al., 2009. The complex origins of domesticated crops in the Fertile Crescent. *Trends in Ecology & Evolution*, 24(2), pp.103–109.
Brush, G.S., 2001. Natural and anthropogenic changes in Chesapeake Bay during the last 1000 Years. *Human and Ecological Risk Assessment: An International Journal*, 7(5), pp.1283–1296.
Burger, J.C., Chapman, M.A. & Burke, J.M., 2008. Molecular insights into the evolution of crop plants. *American Journal of Botany*, 95(2), pp.113–122.
Caldararo, N., 2002. Human ecological intervention and the role of forest fires in human ecology. *Science of The Total Environment*, 292(3), pp.141–165.
Carruthers, W., 1990. The carbonised and mineralised plant remains, Halfpenny Lane. In: Ford S (et al.) Archaeology of the Cleeve-Didcot pipeline, South Oxfordshire. *Oxoniensia*, 60, pp.19–38.
Carruthers, W., 1990. The carbonised and waterlogged plant remains, Aston Tirrold Site 2 S. Ford, ed. *Oxoniensia*, pp.38–20.
Castañeda-Álvarez, N.P. et al., 2016. Global conservation priorities for crop wild relatives. *Nature Plants*, 2(4), p.16022.
Chalk, L., 1946. The species of charcoal R. J. C. Atkinson, ed. *Oxoniensia*, 25, pp.11–12.
Chambers, F.M., E.W. Cloutman, J.R.G. Danniell, D. Mauquoy, P.S. Jones. 2013. "Long-term

ecological study (palaeoecology) to chronicle habitat degradation and inform conservation ecology: an exemplar from the Brecon Beacons, South Wales." *Biodiversity Conservation* 22:719–736.
Cheffings, C. & Farrell, L., 2005. *The Vascular Plant Red Data List for Great Britain*, JNCC.
Chorlton, K.H. et al., 2000. A forage grass and legume plant collecting expedition in Portugal. *Genetic Resources and Crop Evolution*, 47(2), pp.157–162.
Colombaroli, D. & Tinner, W., 2013. Determining the long-term changes in biodiversity and provisioning services along a transect from Central Europe to the Mediterranean. *The Holocene*, 23(11), pp.1625–1634.
Court-Picon, M., A. Buttler, and J-L. de Beaulieu. 2006. "Modern pollen/vegetation/land-use relationships in mountain environments: an example from the Champsaur valley (French Alps)." *Vegetation History and Archaeobotany* 15: 151–168.
Crawley, M.J., 2011. *Statistics: an introduction using R.*, Hoboken: John Wiley & Sons.
Critchley, C.N.R., Burke, M.J.W. & Stevens, D.P., 2004. Conservation of lowland semi-natural grasslands in the UK: a review of botanical monitoring results from agri-environment schemes. *Biological Conservation*, 115(2), pp.263–278.
Dambrine, E. et al., 2007. Present forest biodiversity patterns in France related to former Roman agriculture. *Ecology*, 88(6), pp.1430–1439.
Day, P. 1991. "Post-glacial vegetational history of the Oxford region." *New Phytologist* 119: 445-470.
Day, P. 1992. "Woodland origin and 'ancient woodland indicators': a case-study from Sidlings Copse, Oxfordshire, UK." *The Holocene* 3: 45-53.
Dark, P. 2000. *The Environment of Britain in the First Millennium AD*. London: Duckworth.
Dark, P. 2006. "Climate deterioration and land-use change in the first millennium BC: perspectives from the British palynological record." *Journal of Archaeological Science* 33: 1381-1395.
Derreumaux, M., 2005. How to detect fodder and litter? A case study from the Roman site "Le Marais de Dourges," France. *Vegetation History and Archaeobotany*, 14(4), pp.373–385.
Dimbleby, G.W., 1962. Charcoal. In: Biddle M. The deserted medieval village of Seacourt, Berkshire. *Oxoniensia*, 26, pp.70–210.
Dimbleby, G.W., 1960. Charcoals. In: Fowler P J. Excavations at Madmarston Camp, Swalcliffe, 1957-8. *Oxoniensia*, 25, pp.46–48.
Dimbleby, G.W., 1957. Charcoals. In: Bayne N. Excavations at Lyneham Camp, Lyneham, Oxon. *Oxoniensia*, 22, pp.1–10.
Dimbleby, G., 1966. Charcoals. In: Case H, Bayne N, Steele S, Avery G and Sutermeister H. Excavations at City Farm, Hanborough, Oxon. *Oxoniensia*, 94(29), pp.1–98.
Dimbleby, G.W., 1956. Charcoal. In H. Case, ed. *The Neolithic causewayed camp at Abingdon*. Berkshire Antiquities Journal. pp. 12–30.
Duigan, S.L., 1955. *Plant remains from the gravels of the Summertown Radley Terrace near Dorchester, Oxfordshire*
Dupouey, J.L. et al., 2002. Irreversible impact of past land use on forest soils and biodiversity. *Ecology*, 83(11), pp.2978–2984.
Eriksson, O., S. Cousins and H. Bruun. 2002. "Land-use history and fragmentation of traditionally managed grasslands in Scandinavia." *Journal of Vegetation Science* 13.5:743-748.
Erskine, W., Smartt, J. & Muehlbauer, F.J., 1994. Mimicry of lentil and the domestication of common vetch and grass pea. *Economic Botany*, 48(3), pp.326–332.
Esquinas-Alcazar, J., 2005. Protecting crop genetic diversity for food security: political, ethical and technical challenges. *Nature Reviews Genetics*, 6(12), pp.946–953.
Fahrig, L., 2003. Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, 34(1), pp.487–515.
Fielder, H. et al., 2015a. Enhancing the conservation of Crop Wild Relatives in England. *PLoS ONE*,

10(6), p.e0130804.
Fielder, H. et al., 2015b. Enhancing the conservation of Crop Wild Relatives in Wales. *New Journal of Botany*, 5(3), pp.178–191.
Fischer, M. K. Rudmann-Maurer, A. Weyand, and J. Stöcklin. 2008. "Agricultural land use and biodiversity in the Alps: How cultural tradition and socioeconomically motivated changes are shaping grassland biodiversity in the Swiss Alps." *Mountain Research and Development* 28.2: 148–155.
Franklin, P.G.H., 1974. Excavations at Oxford 1972. 5th Interim report. *Oxoniensia*, 38, pp.268–98.
Froyd, C. A., and K. J. Willis. 2008. "Emerging issues in biodiversity & conservation management: The need for a palaeoecological perspective." *Quaternary Science Reviews* 27(17–18): 1723–32.
Fuller, D.Q., 2007. Contrasting patterns in crop domestication and domestication rates: Recent archaeobotanical insights from the Old World. *Annals of Botany*, 100(5), pp.903–924.
Gepts, P., 2006. Plant genetic resources conservation and utilization. *Crop Science*, 46(5), p.2278.
Giorgi, J. & Robinson, M., 1984. The environment. 38-45. In: Foreman M and Rahtz S. Excavations at Faccenda Chicken Farm, near Alchester, 1983. *Oxoniensia*, 49, pp.23–46.
Goman, M. & Byrne, R., 1998. A 5000-year record of agriculture and tropical forest clearance in the Tuxtlas, Veracruz, Mexico. *The Holocene*, 8(1), pp.83–89.
Gray, M., 1971. *Excavations at Northfield Farm, Long Wittenham*, Oxford: Oxford Architectectural & Historical Society.
Grime, J. P., J. Hodgson, and R. Hunt. 1990. *The Abridged Comparative Plant Ecology*. London: Unwin Hyman.
Groves, J.A. et al., 2012. Long-term development of a cultural landscape: the origins and dynamics of lowland heathland in southern England. *Vegetation History and Archaeobotany*, 21(6), pp.453–470.
Hardy, A., 2003. *Aelfric's abbey: excavations at Eynsham Abbey, Oxfordshire, 1989-1992*, Oxford: Oxford Archaeological Unit.
Harlan, J.R., 1992. *Crops & Man*, American Society of Agronomy.
Heckenberger, M.J. et al., 2007. The legacy of cultural landscapes in the Brazilian Amazon: implications for biodiversity. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 362(1478), pp.197–208.
Helgadóttir, Á. et al., 2000. Prebreeding of red clover (*Trifolium pratense* L.) for Northern areas. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, 50(3), pp.187–190.
Hey, G., 2004. *Yarnton: Saxon and medieval settlement and landscape : results of excavations 1990-96*, Oxford: Oxford University School of Archaeology, for Oxford Archaeology.
Hey, G. 2006. "Neolithic and Early Bronze Age Oxfordshire." *Solent Thames Research Framework*. Oxford: Oxford Archaeology.
Hjelle, K., S. Kaland , M. Kvamme , T. Klungseth Lødøen and B. Natlandsmyr. 2012. "Ecology and long-term land-use, palaeoecology and archaeology – the usefulness of interdisciplinary studies for knowledge-based conservation and management of cultural landscapes." *International Journal of Biodiversity Science, Ecosystem Services & Management* 8.4: 321-337.
Hodder, K., P. Buckland, K. Kirby and J. Bullock. 2009. 'Can the pre-Neolithic provide suitable models for rewilding the landscape in Britain?" *British Wildlife* 20.5: 4-15.
Hodgson, J.G. et al., 1999. 'Functional interpretation of archaeobotanical data: Making hay in the archaeological record.' *Vegetation History and Archaeobotany*, 8(4), pp.261–271.
Hoekstra, J.M. et al., 2005. Confronting a biome crisis: global disparities of habitat loss and protection. *Ecology Letters*, 8(1), pp.23–29.
Hunt, C.O. & Rabett, R.J., 2014. Holocene landscape intervention and plant food production strategies

in island and mainland Southeast Asia. *Journal of Archaeological Science*, 51, pp.22–33.
Jarvis, S. et al., 2015. Distribution of crop wild relatives of conservation priority in the UK landscape. *Biological Conservation*, 191, pp.444–451.
Jones, M., Plant remains from dry bulk samples. 103-4. In: Lambrick, G. and Robinson M. (eds). 1979. Iron Age and Roman riverside settlements in Farmoor, Oxfordshire. *CBA Research Report*, 32.
Jones, M., 1975. Seed report. In: Parrington M. Excavations at the Old Gaol, Abingdon. *Oxoniensia*, 40, pp.59–78.
Jones, M., 1979. The carbonised plant remains. In: Parrington M. 1979. Excavations at Stert Street, Abingdon, Oxon. *Oxoniensia*, 44, pp.191–205.
Jones, M., 1975. Seed report C. Balkwill & M. Parrington, eds. *Oxoniensia*, 40, pp.50–52.
Jones, M., 1978. *The plant remains*. CBA Research Report 28. The Excavation of an Iron Age Settlement at Ashville Trading Estate (Oxon) 1974-6.
Joshi, J. et al., 2001. Local adaptation enhances performance of common plant species. *Ecology Letters*, 4(6), pp.536–544.
Kleijn, D. et al., 2009. On the relationship between farmland biodiversity and land-use intensity in Europe. *Proceedings of the Royal Society B-Biological Sciences*, 276(1658), pp.903–909.
Klink, C.A. & Machado, R.B., 2005. Conservation of the Brazilian Cerrado. *Conservation Biology*, 19(3), pp.707–713.
Jeffers, E.S., Nogué, S. & Willis, K.J., 2015. The role of palaeoecological records in assessing ecosystem services. *Quaternary Science Reviews*, 112, pp.17–32.
Johansson, L., K. Hall, H. Prentice, M. Ihse, T. Reitalu, M. Sykes, and M. Kindström. 2008. "Semi-natural grassland continuity, long-term land-use change and plant species richness in an agricultural landscape on Öland, Sweden." *Landscape and Urban Planning* 84: 200–211.
Jutila, H.M., 2001. Effect of flooding and draw-down disturbance on germination from a seashore meadow seed bank. *Journal of Vegetation Science*, 12(5), pp.729–738.
Kershaw, A.P. et al., 1997. The contribution of humans to past biomass burning in the tropics. In J. S. Clark et al., eds. *Sediment Records of Biomass Burning and Global Change*. NATO ASI Series. Springer Berlin Heidelberg, pp. 413–442.
Kirby, K.J. et al., 1995. Pasture-woodland and its conservation in Britain. *Biological Journal of the Linnean Society*, 56, pp.135–153.
Lambrick, G. & Robinson, M., 1979. Iron Age and Roman riverside settlements in Farmoor, Oxfordshire. *CBA Research Report*, 32.
Leake, J. et al., 2004. Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning. *Canadian Journal of Botany*, 82(8), pp.1016–1045.
Lin, G., McCormack, M.L. & Guo, D., 2015. Arbuscular mycorrhizal fungal effects on plant competition and community structure. *Journal of Ecology*, 103(5), pp.1224–1232.
Lindbladh, M., 1999. The influence of former land-use on vegetation and biodiversity in the Boreo-Nemoral Zone of Sweden. *Ecography*, 22(5), pp.485–498.
Lindbladh, M., Niklasson, M. & Nilsson, S.G., 2003. Long-time record of fire and open canopy in a high biodiversity forest in southeast Sweden. *Biological Conservation*, 114(2), pp.231–243.
Lomas-Clarke, S.H. & Barber, K.E., 2004. Palaeoecology of human impact during the historic period: palynology and geochemistry of a peat deposit at Abbeyknockmoy, Co. Gaiway, Ireland. *The Holocene*, 14(5), pp.721–731.
Magurran, A.E. et al., 2010. Long-term datasets in biodiversity research and monitoring: assessing change in ecological communities through time. *Trends in Ecology & Evolution*, 25(10), pp.574–582.
Maloney, D.B.K., 1998. The long-term history of human activity and rainforest development. In

B. K. Maloney, ed. *Human Activities and the Tropical Rainforest*. The GeoJournal Library. Springer Netherlands, pp. 65–85.
Marchese, C., 2015. Biodiversity hotspots: A shortcut for a more complicated concept. *Global Ecology and Conservation*, 3, pp.297–309.
Marshall, A. et al., 2010. Introgression of novel traits into white clover (*Trifolium repens* L.) from related *Trifolium* species. In C. Huyghe, ed. *Sustainable use of Genetic Diversity in Forage and Turf Breeding*. Springer Netherlands, pp. 539–543.
Maxted, N. et al., 2006. Towards a definition of a crop wild relative. *Biodiversity & Conservation*, 15(8), pp.2673–2685.
Maxted, N. et al., 2007. Creation and use of a national inventory of crop wild relatives. *Biological Conservation*, 140(1-2), pp.142–159.
Maxted, N. et al., 2012. Toward the systematic conservation of global Crop Wild Relative diversity. *Crop Science*, 52(2), p.774.
Meltsov, V., A. Poska, T. Reitalu, M. Sammul, and T. Kull. 2013. The role of landscape structure in determining palynological and floristic richness. *Vegetation History and Archaeobotany* 22:39–49.
Metera, E. et al., 2010. Grazing as a tool to maintain biodiversity of grassland - a review. *Animal Science Papers and Reports*, 28(4), pp.315–334.
Mitchell, D., 2005. *The Esri Guide to GIS Analysis, Volume 2: Spatial Measurements and Statistics* 1 edition., Esri Press.
Moffett, L., 1991. Pignut tubers from a Bronze Age cremation at Barrow Hills, Oxfordshire, and the importance of vegetable tubers in the Prehistoric period. *Journal of Archaeological Science*, 18, pp.187–191.
Moffett, L., 1990. The charred plant remains. In T. Allen, ed. *An Iron Age and Romano-British enclosed settlement at Watkins Farm Northmoor, Oxon.* Oxford University Committee for Archaeology Monograph. pp. 60–64.
Moffett, L., Robinson, R. & Straker, S., 1989. Cereals, fruit and nuts: charred plant remains from Neolithic sites in England and Wales and the Neolithic economy. *British Archaeological R*
Moore, J., 2000. Forest fire and human interaction in the early Holocene woodlands of Britain. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 164(1–4), pp.125–137.
Morrison, M., 1960. Carbonised cereals from the Roman Villa of North Leigh, Oxon. *Oxoniensia*, 24, pp.13–21.
Murphy, D.J., 2007. *People, Plants and Genes*, Oxford University Press.
Needham, I. et al., 2015. Pollen of Malagasy grasses as a potential tool for interpreting grassland palaeohistory. *Grana*, 54(4), pp.247–262.
Odgaard, B.V., 1999. Fossil pollen as a record of past biodiversity. *Journal of Biogeography*, 26(1), pp.7–17.
Oxford Archaeological Unit, 2009. College Farm, College Farm Close, Oddington, Oxfordshire, Archaeological Watching Brief Report.
Patterson III, W.A., Edwards, K.J. & Maguire, D.J., 1987. Microscopic charcoal as a fossil indicator of fire. *Quaternary Science Reviews*, 6(1), pp.3–23.
Pearson, R.G. & Dawson, T.P., 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology and Biogeography*, 12(5), pp.361–371.
Peglar, S.M., 1993. The development of the cultural landscape around Diss Mere, Norfolk, UK, during the past 7000 years. *Review of Palaeobotany and Palynology*, 76(1), pp.1–47.
Pilcher, J., 1968. Charcoal. In: Hamlin A. Early Iron Age sites at Stanton Harcourt. *Oxoniensia*, 31, pp.1–27.
Plieninger, T. et al., 2015. Wood-pastures of Europe: Geographic coverage, social–ecological values,

conservation management, and policy implications. *Biological Conservation*, 190, pp.70–79.
Pollock, C.J., Abberton, M.T. & Humphreys, M.O., 2005. Grass and forage improvement: temperate forages. In D. A. McGilloway, ed. *Grassland: a Global Resource*. Netherlands: Wageningen Academic Publishers, pp. 57–68.
Poudevigne, I. & Baudry, J., 2003. The implication of past and present landscape patterns for biodiversity research: introduction and overview. *Landscape Ecology*, 18(3), pp.223–225.
Richardson, K.M. & Young, A., 1951. An Iron Age A site on the Chilterns. *Antiquities Journal*, pp.134–148.
Robinson, M. 1992. "Environmental Archaeology of the River Gravels: Past Achievements and Future Directions." *The Developing Landscapes of Lowland Britain: The Archaeology of the British Gravels: A Review*. Edited by M. Fulford and E. Nichols. London: The Society of Antiquaries of London.
Robinson, M. 2011. "The paleoecology of alluvial hay meadows in the Upper Thames valley." *Fritillary* 5: 47-57.
Robinson, M., 1984a. Agricultural debris against Norman bridge and early habitation. In: Durham B. 1984. The Thames crossing at Oxford: Archaeological Studies 1979-82. *Oxoniensia*, 49, pp.57–100.
Robinson, M., 1984b. Environmental evidence from late Saxon levels. In: Durham B. 1984. The Thames crossing at Oxford: Archaeological Studies 1979-82. *Oxoniensia*, 49, pp.57–100.
Robinson, M., 1976. Note on biological remains. In: Palmer N. Excavations on the outer city wall of Oxford in St. Helen's Passage and Hertford College. *Oxoniensia*, 41, pp.140–160.
Robinson, M., 1983a. Plant and invertebrate remains, 11-12 Queen St. In: Halpin C. Late Saxon evidence and excavation of Hinxey Hall, Queen Street, Oxford. *Oxoniensia*, 48, pp.1–69.
Robinson, M., 1983b. Plant and invertebrate remains, New Inn Court.In: Halpin C. Late Saxon evidence and excavation of Hinxey Hall, Queen Street, Oxford. *Oxoniensia*, 48, pp.1–69.
Robinson, M., 1985. Plant and invertebrate remains. 196-201, fiche D14, F1. In: Lambrick G. 1985. Further excavations on the second site of the Dominican priory, Oxford. *Oxoniensia*, 50, pp.130–208.
Robinson, M., 1979. Plant Identification. In: Blair J. Frewin Hall Oxford: a Norman mansion and a monastic college. *Oxoniensia*, 43, pp.48–99.
Robinson, M., 1988. The plant remains from Tiddington. In: Greig J. The interpretation of some Roman well fills from the midlands of England. In H. Kuster, ed. *Der prahistorische Mensch und seine Umwelt. Landesdenkmalamt Baden-Wurttemberg*. Stuttgart: Konrad Theiss, pp. 367–378.
Robinson, M., 1990. The waterlogged seeds. In T. Allen, ed. *An Iron Age and Romano-British enclosed settlement at Watkins Farm Northmoor, Oxon*. Oxford University Committee for Archaeology Monograph. pp. 64–72.
Robinson, M., 1980. Waterlogged plants and invertebrates. In: Palmer, N. Beaker burial and medieval tenements at The Hamel, Oxford. *Oxoniensia*, 45, pp.124–155.
Robinson, 19871, 1981. Roman waterlogged plant and invertebrate evidence. In J. Hinchcliffe & R. Thomas, eds. *Archaeological investigations at Appleford*. Oxoniensia. pp. 9–111.
Robinson, M., 1976. Appendix 2. The environment of the Roman defences at Alchester. In C. J. Young, ed. *The defences of Roman Alchester*. Oxoniensia. pp. 161–70.
Robinson, M., 1974. Carbonised seed. 109-11. In: Robinson M A. 1973. Excavations at Copt Hay, Tetsworth, Oxon. *Oxoniensia*, 38, pp.41–115.
Rosenthal, G., Schrautzer, J. & Eichberg, C., 2012. Low-intensity grazing with domestic herbivores: A tool for maintaining and restoring plant diversity in temperate Europe. *Tuexenia*, (32), pp.167–205.
Salzmann, U., 2000. Are modern savannas degraded forests?-A Holocene pollen record from the

Sudanian vegetation zone of NE Nigeria. *Vegetation History and Archaeobotany*, 9(1), pp.1–15.

Scotton, M., Sicher, L. & Kasal, A., 2014. Semi-natural grasslands of the Non Valley (Eastern Italian Alps): Agronomic and environmental value of traditional and new Alpine hay-meadow types. *Agriculture, Ecosystems & Environment*, 197, pp.243–254.

Simon, U., 1994. Collecting and evaluating genetic resources of fodder plants from subalpine and alpine permanent grassland. *Euphytica*, 77(3), pp.175–181.

Thuiller, W. et al., 2005. Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Sciences of the United States of America*, 102(23), pp.8245–8250.

Vitolo, M., 2009. *Cold Harbour Farm Crowmarsh, Wallingford: plant remains from the corn drying oven*, Portsmouth: English Heritage.

Walther, G.-R. et al., 2002. Ecological responses to recent climate change. *Nature*, 416(6879), pp.389–395.

Weng, C., Hooghiemstra, H. & Duivenvoorden, J.F., 2007. Response of pollen diversity to the climate-driven altitudinal shift of vegetation in the Colombian Andes. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 362(1478), pp.253–262.

Western, A., 1982. Charcoals. In H. J. Case & A. W. R. Whittle, eds. *Settlement patterns in the Oxford region: excavation at the Abingdon Causeway enclosure*. CBA Research Report. Oxford: Dept Antiquities, Ashmolean Museum.

Western, A.C., 1982. Charcoals from the Big enclosure ditch, 1951 cutting H. J. Case & A. W. R. Whittle, eds. *CBA Research Report*, 44, p.150.

Willis, K.J. et al., 2010. Biodiversity baselines, thresholds and resilience: testing predictions and assumptions using palaeoecological data. *Trends in Ecology & Evolution*, 25(10), pp.583–591.

Willis, K.J., Gillson, L. & Knapp, S., 2007. Biodiversity hotspots through time: an introduction. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 362(1478), pp.169–174.

van der Heijden, M.G.A. et al., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396(6706), pp.69–72.

Van der Heijden, M.G.A., Bardgett, R.D. & Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, 11(3), pp.296–310.

Vera, F. 2002. “The dynamic European forest.” *Arboricultural Journal: The International Journal of Urban Forestry* 26.3: 179-211.

CHAPTER 6

DISCUSSION

1. The Agricultural Value of Grassland Biodiversity

In this thesis, I show that species-rich grasslands have a strong, and often untapped, agricultural value. Despite the fact that biomass production is one of the most important grassland ecosystem services to those who manage-and rely on- these landscapes, few studies have looked at the agricultural value of grassland biodiversity. Previous ecological research has focused on the value of grassland biodiversity in the provision of ecosystem services and in mitigating the effects of climate change (Isbell et al. 2011; Reich et al. 2001), yet these results rarely inform grassland management practices. Throughout this thesis, I show how the unique diversity of plants found on these fields strongly contributes to forage quality, bioenergy potential, and microbial diversity, all aspects of agricultural productivity of central importance to grassland stakeholders, from farmers and graziers to conservation agents. Grassland biodiversity thus has an economic, as well as environmental, value. This chapter provides a brief overview of the key results of this thesis while highlighting the significance of these results for the agricultural use of grasslands and making recommendations to farmers, land managers, and conservation agents.

2. Forage Production from Species-Rich Grasslands

This study found that forage quality of fodder from species-rich grasslands was comparable to commercial feeds and may provide livestock with additional health benefits and may affect meat and

dairy quality. Forage samples from species-rich grasslands contained comparable amounts of dry matter and sugar to improved leys and were not significantly higher in lignin content (which can reduce digestibility). This forage also contained up to 27 % more protein, 56 % more Phosphorus (P), 106 % more Potassium (K), and 183 % more Calcium (Ca) than cereals and conventional hay. These results were unexpected based on previous research which reports the low nutritional value of forage from unimproved grassland (Tallowin and Jefferson 2008). Perhaps most importantly, this study found that farmers valued species-rich grasslands primarily as a source of low-input nutritious fodder. Through ecological surveys and ethnobotanical research, I found that species-rich grasslands contained medicinal plants not found on improved grasslands. These include wild mint (*Mentha longifolia*), meadowsweet (*Filipendula ulmaria*), betony (*Stachys officinalis*), and yarrow (*Achillea millefolium*) all of which contain plant secondary metabolites with antibiotic and anthelmintic (de-worming) properties. Although previous studies have highlighted the medicinal and/or antimicrobial properties of these plants (e.g. Waghorn and McNabb 2003), few have identified the extent to medicinal plants with potentially beneficial effects on livestock health are found on species-rich grasslands. My results suggest that forage production from species-rich grasslands could contribute to creating more sustainable livestock systems in the future.

These results could be used as a basis for further research on the agricultural value of species-rich grasslands. Specifically, future studies could determine what plants and metabolites from species-rich grasslands most affect the nutritional composition of meat and milk quality. Connections between specific metabolites and specific benefits (e.g. increased fatty acid content) should be sought. In addition, how to harness grassland metabolites to improve livestock health, e.g. by developing new supplements aimed at lowering antibiotic use etc., requires further research.

From a practical standpoint, these results should encourage the use of species-rich grasslands for forage production. Conservation agents responsible for protected species-rich grasslands (e.g. SSSIs) should communicate these results on forage quality to farmers, graziers and other stakeholders who manage species-rich grasslands (SSSIs and grasslands managed/created under agroenvironmental schemes) to encourage the agricultural use of these grasslands in livestock production systems. Based on these results, farmers could also look into specialty markets for hay produced from species-rich grasslands and meat and dairy products produce from livestock raised on these landscapes.

Finally, grassland conservation and agricultural production can go hand in hand, but this will require sharing knowledge and resources among stakeholders as well as willingness to find compromises. Conservation agencies should endeavor to work more closely with stakeholders (farmer, graziers, and landowners) to create more flexible guidelines around when grasslands can be grazed and/or cut for hay to encourage active management and decrease invasive plants. At the same time, they could facilitate knowledge sharing regarding grassland management through workshops or pairing experienced farmers with new farmers.

3. Turning Excess Grassland Biomass into Bioenergy

Analysis of grassland biomass samples from Oxfordshire suggests that the bioenergy yield from species-rich grasslands was competitive with other crops (e.g. wheat, Miscanthus). In contrast to previous studies which claim that biomass from grasslands contains too much lignin for bioenergy production to be feasible (Frigon and Giuiot 2010), I found that the lignin content of biomass from species-rich grasslands was similar to other current feedstocks like cereals and significantly lower than other feedstocks such as Miscanthus and rapeseed. My analysis showed that the average biogas yield of species-rich grasslands was comparable to, or surpassed, other current feedstocks. The average

biogas yield of Miscanthus was (811.1 ± 118.36 Nm^3 per ton), followed by biomass from species-rich grasslands (637.1 ± 54.79 Nm^3 per ton), switchgrass (455.5 ± 111.36 Nm^3 per ton), crop waste (498.8 ± 87.86 Nm^3 per ton), rapeseed (498.8 ± 85.32 Nm^3 per ton), monoculture grass leys (439.7 ± 98.01 Nm^3 per ton), legumes (285.2 ± 81.68 Nm^3 per ton), and cereals (222.9 ± 111.36 Nm^3 per ton). These results are significant given the lack of research which compares the bioenergy potential of grasslands to other bioenergy crops. This study also demonstrated that species-rich grasslands could potentially provide more bioenergy per area (ha^2) than other feedstocks because grasslands are (in general) larger than arable fields. The average size of SSSI grasslands in Oxfordshire was 20.57 ha while that of Miscanthus was 5.95 ha. Due to larger average field size, the average potential biogas output of SSSIs and grasslands managed under agroenvironmental schemes exceeds that of fields currently sown with Miscanthus by up to 117%. The schemes with the highest total biogas yield were permanent grasslands maintained under Article 13 (69, 879, 544 Nm^3), permanent grasslands with low inputs (outside SDA and ML) (24, 598, 946 Nm^3), permanent grasslands with very low inputs (outside SDA and ML) (20, 756, 514 Nm^3), SSSIs (11, 223, 459 Nm^3), and maintained species-rich, semi-natural grasslands (9, 490, 812 Nm^3) due to the large number of fields managed under these schemes (6, 396 fields in Oxfordshire).

To improve these projections, future research could focus on refining estimates of the potential biogas yield of grasslands. This could be achieved by involving farmers. Specifically, farmers could provide annual accounts of the biomass yields of their species-rich grasslands. At the same time, biomass from species-rich grasslands could be given a trial run at one or two active anaerobic digestion plants to see what the actual annual bioenergy output would be. This would also help to determine what technical, social, and economic problems might arise.

These initial results are encouraging and can be used to initiate discussions about using excess grassland biomass in the UK for bioenergy production. Many conservation groups own or actively manage species-rich grasslands across the country. Management of grasslands for optimal biogas production aligns closely with grassland management activities under conservation guidelines (e.g. late harvests) and could potentially coincide with conservation objectives. However, care should be taken to ensure that only grasslands not used for grazing and/or hay making are used for this purpose.

4. Managing and Exploiting Grassland Microbial Diversity

Metagenomic analysis of soil samples from 30 grasslands and six arable fields across Oxfordshire demonstrated that microbial diversity was highest on species-rich grasslands. Conversion of species-rich grasslands to arable production did not cause bacterial diversity to decline but fungal diversity decreased by up to 40%. Although beneficial microbiota (such as N-fixing bacteria) were found across the study sites, specific species were unique to species-rich grasslands, including several types of mycorrhizas. This agrees with previous research which has shown that agricultural intensification tends to affect fungal communities more than bacterial communities (Hansen et al. 2000). I also found that species-rich grasslands had the lowest level of potential soil pathogens and specific pathogens were ten to twenty times more abundant on agriculturally improved fields. *Fusarium sp., Olpidium brassicae, Pseudomonas viridiflava,* and *Verticillium dahliae* were the most abundant plant pathogens on improved arable fields. In addition, several potential human and livestock pathogens (e.g. *Chaetomium spp., Cryptococcus spp., Enterobacter amnigenus, Stenotrophomonas maltophilia and Yersinia spp.*) were only found on improved arable fields.

Further research could refine this picture of how agricultural intensification effects microbial diversity by using a multi-omics approach. Specifically, metagenomics investigation could be combined with

metaproteomics and metatranscriptomics. This could provide more accurate recommendations to farmers regarding the fertility and health of their grasslands. In addition, further microbial analysis of fields where digestate from anaerobic digesters are used should be prioritized, particularly given the increased promotion of using digestate from anaerobic digesters as a 'sustainable' fertilizer.

The metagenomic data from Oxfordshire indicates species-rich grasslands contain a high level of microbial diversity that could actively be managed-and exploited-by farmers to improve the health of their fields and by conservation groups to achieve biodiversity objectives. Farmers could consider inoculating fields with soil from species-rich grasslands to boost fungal diversity and decrease soil pathogens. This would help improve overall crop productivity. At the same time, conservation groups working on reverting intensively cultivated arable fields back into species-rich grasslands should promote the use of locally sourced seed and soil inoculum in grassland restoration activities to 'restore' microbial diversity.

5. The Dependence of Grassland Biodiversity on Human Management

Palaeoecological data from the region suggests species-rich grasslands developed ca. 2,000 years ago and without human management these landscapes would revert to oscillating between open grassland and woodland. This agrees with other palaeoecological studies in northern Europe which propose species-rich grasslands developed through human management (Lindbladh 1999; Berglund et al. 2008). Although experimental, GIS analysis of combined palaeoecological and modern vegetation survey data found that hotspots of historic biodiversity showed a great degree of persistence and were linked to specific environmental conditions (areas of low elevation and annual flooding) and continuity in pastoral use of the landscape (e.g. for grazing and hay-making). Finally, this analysis found that these historic landscapes contain 36 wild relatives of fodder crops. This agrees with a recent study by

Jarvis et al. (2015) which found that grasslands in the UK contained a high abundance of crop wild relatives compared to other habitats (e.g. field margins, woodland). It also lends some support to claims that hotspots of crop wild relatives may be linked to historic areas of human agricultural activity (Altieri et al. 1987).

Future research could focus more intensively on monitoring, collecting, and using the plant genetic resources from these hotspots of biodiversity. Species-rich grasslands contain locally adapted forage grasses and legumes which could serve as an additional source of breeding material for creating forage crops that are more resilient to climate change and that require lower inputs. Current conservation activities could incorporate monitoring and collection of crop wild relatives into their pre-existing activities and more research and development projects could make active use of this germplasm to ensure that plant genetic resource conservation and use are cyclical and re-inforce one another. Finally, future research could look more closely at the ecological and socio-economic impact of allowing species-rich grasslands to 'revert' by monitoring changes in vegetation and how these landscapes were utilized in a long-term study. This would help inform in greater detail the potential losses, and benefits, of losing species-rich grasslands.

6. The Future of Grasslands: Biodiversity and the Bio-economy

Grassland biodiversity is a natural resource that needs to be *monitored*, *sustainably managed*, and *actively used* in order to play an important role in the current and future UK bioeconomy. While this vision of grassland biodiversity as functional and as a cornerstone for economic development is optimistic, it is not unattainable. This perspective seeks to engage two communities with often conflicting motives: farmers and conservationists. Although we may have lost many of our species-

rich grasslands in the UK, we do have the tools, technology, and social capacity to help stem, and even reverse, these losses.

References

Altieri, M.A., Anderson, M.K. & Merrick, L.C., 1987. Peasant Agriculture and the Conservation of Crop and Wild Plant Resources. *Conservation Biology*, 1(1), pp.49–58.

Barry, T.N. & McNabb, W.C., 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *The British Journal of Nutrition*, 81(4), pp.263–272.

Berglund, B.E. et al., 2007. Long-term changes in floristic diversity in southern Sweden: palynological richness, vegetation dynamics and land-use. *Vegetation History and Archaeobotany*, 17(5), pp.573–583.

Frigon, J.-C. & Guiot, S.R., 2010. Biomethane production from starch and lignocellulosic crops: a comparative review. *Biofuels, Bioproducts and Biorefining*, 4(4), pp.447–458.

Hansen, B. et al., 2000. Nitrogen leaching from conventional versus organic farming systems — a systems modelling approach. *European Journal of Agronomy*, 13(1), pp.65–82.

Isbell, F. et al., 2011. High plant diversity is needed to maintain ecosystem services. *Nature*, 477(7363), pp.199–202.

Jarvis, S. et al., 2015. Distribution of crop wild relatives of conservation priority in the UK landscape. *Biological Conservation*, 191, pp.444–451.

Lindbladh, M., 1999. The Influence of Former Land-Use on Vegetation and Biodiversity in the Boreo-Nemoral Zone of Sweden. *Ecogeography*, 22(5), pp.485–498.

Reich, P.B. et al., 2001. Plant diversity enhances ecosystem responses to elevated CO2 and nitrogen deposition. *Nature*, 410(6830), pp.809–812.

Tallowin & Jefferson, 1999. Hay production from lowland semi-natural grasslands: a review of implications for ruminant livestock systems. *Grass and Forage Science*, 54(2), pp.99–115.