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PATTERNS AND PROCESS: BIODIVERSITY AND ECOSYSTEM FUNCTION RESPONSE TO CHANGES IN THE ARABLE LANDSCAPE

by

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Patterns and Process: Biodiversity and Ecosystem Function Response to Changes in the Arable Landscape

Abstract: Land use change is a major driver of species loss worldwide, the extent and intensity of agricultural land use poses particular pressures for biodiversity and the ecosystem services it provides. In recent years, agroecosystems have seen the introduction of 2nd generation bioenergy crops in order to tackle anthropogenic climate change, providing a renewable alternative to fossil fuels. In this thesis I study the impact of cultivating two commercial perennial energy crops (PECs), *Miscanthus x giganteus* and willow short-rotation coppice, when compared to the cereal crops they replace. I investigate processes relevant to the provisioning of pollination and decomposition services and explore patterns of soil element bioaccessibility alongside analyses of the similarity and diversity of soil bacterial communities. When compared to cereals, I find a consistent increase in pollinator (hoverfly, bumblebee and butterfly/moth) wildflower visitation in the margins of willow but not *Miscanthus*. In *Miscanthus,* opposing trends arose for different pollinator taxa: butterflies/moths were more frequent flower visitors in *Miscanthus* margins than cereal margins, while hoverfly flower visits were most frequent in cereal margins. Furthermore, the availability of margin wildflowers was enhanced in willow but not *Miscanthus* and the seed set of margin phytometers was similar between *Miscanthus* and cereals. Cultivation of willow, in particular, may therefore yield local conservation benefits for both wildflowers and pollinators. However, there was no evidence for enhancement of pollinator activity in cereals adjacent to either PEC, indicating that the strategic cultivation of these crops is unlikely to enhance pollinator service provision in the wider agri-environment. For investigated soil elements, bioaccessibility in PECs did not differ significantly to cereal controls, and denaturing gradient gel electrophoresis (DGGE) revealed no difference in the diversity of bacterial communities. Similarly, DGGE fingerprint patterns did not indicate the development of crop specific assemblages, demonstrating that the mobility of soil elements and structure of bacterial communities were principally determined by factors other than the identity of the crop cultivated. Investigation of meso-microfaunal decomposition rates in *Miscanthus* using litter bags demonstrated an impact on decomposition processes, with a significant increase in winter decomposition rates in the PEC when compared to cereals.

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Abbreviations and definitions

Agri-environment Schemes- AES

Ammonia-oxidising *Archaea*- AOA

Ammonia-oxidising *Bacteria*- AOB

Bioenergy Crop- BEC

Carbon- C

Chemometric Identification of Substrates and Elemental Distributions- CISED

Denaturing Gradient Gel Electrophoresis- DGGE

Ecosystem Process- EP

Ecosystem Service- ES

Indirect Land Use Change- ILUC

Mass-flowering crop- MFC

Miscanthus- *Miscanthus x giganteus*

Oven-dried Tonnes- ODT

Oilseed Rape/Canola- OSR

Perennial Energy Crop- PEC

Potentially Toxic Element- PTE

Short-rotation Coppice- SRC

Soil Organic Matter- SOM

Soil Organic Carbon- SOC

Authors Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee.

Work submitted for this research degree at University of Plymouth has not formed part of any other degree either at University of Plymouth or at another establishment.

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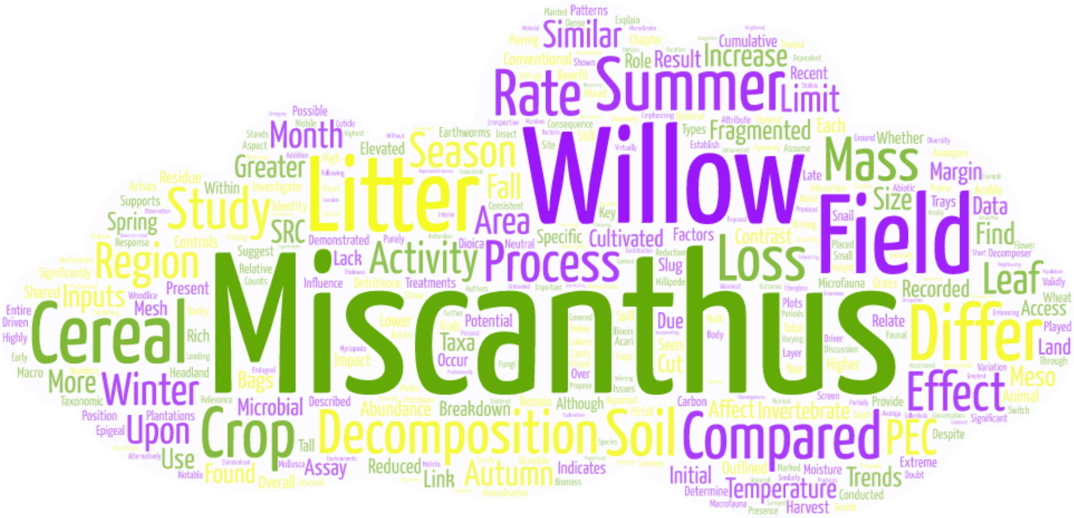
Chapter 1: General Introduction



1.1 Introduction

Recent figures derived from United Nations data suggest a global population size of 9.6 billion by 2050, with the potential to rise to 12.3 billion by 2100 (Gerland *et al*., 2014). As the human population continues to grow there is increased pressure to achieve higher crop yields from a finite amount of land (Foley *et al*., 2011). Tilman *et al*. (2011) forecast a 100-110% greater demand on crop production from 2005 to 2050. To date, the expansion and intensification of British agriculture, following the world wars and the green revolution of the mid-1900s, has allowed for rapid yield increases (Tilman *et al*., 2001). Developed nations have been able to support expanding populations and achieve improved food security. Foley *et al.* (2011) suggest that between the years of 1985 and 2005 global crop yield increased by 25% and by as much as 56% from 1965 to 1985. However, the reduction in the size of yield increases in recent decades suggests that, globally, yields are beginning to plateau (Foley *et al*., 2011). Global average yield increase fell from 3% in 1960 to just 1.5% in 2000 and pressure upon land resources is expected to increase in the coming decades (FAO, 2009).

Alongside limited improvements in yield there has been a growing awareness that current agricultural practices are unsustainable, with detrimental impacts on the land, water, biodiversity and climate (Tilman *et al*., 2001; Foley *et al*., 2011). The heavy reliance on pesticides has been implicated as a key factor in the decline of important pollinator species (IPBES, 2016). The detrimental impact of eutrophication as a result of the over-use and leaching of fertiliser is also a problem that has been recognised for many decades. A notable example being the ‘dead zones’ in the Gulf of Mexico, spanning an area of over 6400 square miles in 2015 due to excessive nutrients entering the Mississippi River (NOAA, 2015). Furthermore, use of nitrogen fertilisers has had concomitant negative effects on greenhouse gas (GHG) emissions, accounting for a major proportion of fossil fuel use in agroecosystems (Vitousek *et al*., 1997). Although intensive inputs and monoculture farming practices are recognised as problems requiring urgent attention, it is important to note that without intensification agriculture would dominate as much as 2.5 times more of the land area than it does today (FAO, 2009). In addition, Dr Norman Borlaug, seen by many as the founder of the 'Green Revolution', has been credited with saving an estimated 1 billion people from starvation through improved plant breeding, as well as the international dissemination and application of intensive agricultural practices. Developing nations are those most likely to have yields below their potential and small future increases in intensification, targeted to these areas, could still theoretically result in large yield increases (Twomlow *et al.*, 2010). Nonetheless, in the main, it is now widely understood that biodiversity has already paid significantly for human population growth and the agricultural practices that have made it possible (Krebs *et al.*, 1999).

1.2 Agriculture and Insect Pollinator Declines

In recent decades there have been concerns internationally about the plight of pollinators, with declines seen in the abundance and species richness of taxa worldwide (Gallai *et al.*, 2009; Potts *et al.*, 2010; Senapathi *et al.*, 2015). Given their widespread use as commercial pollinators, particular focus has been directed at the western honeybee (*Apis melifera* Linnaeus), the commercially reared bumblebee *Bombus terrestris* Linnaeus and the solitary bee *Megachile rotundata* Fabricius (Goulson *et al.*, 2008). However attention has also begun to shift to the ~20,000 wild pollinating species that include, amongst others, many bumblebees, solitary bees, flies, butterflies, moths and beetles (IPBES, 2016).

In the USA *Apis melifera* colony loss is recorded as 59% between 1947 and 2005 with a 25% loss of colonies in central Europe between 1985 and 2005 (Potts *et al.*, 2010). A wide range of drivers are thought to have played a role in the decline of pollinators, and of bees in particular. Colony collapse disorder (CCD), a generic term for the abrupt and disastrous population declines in *Apis melifera*, is thought to have >60 contributing factors (Wu *et al*., 2011). For example, there are a large number of parasites that affect bees, including mites, protozoans, fungi, bacteria, and viruses (Goulson and Hughes, 2015). *Apis mellifera* is thought to be under synergistic pressures from parasites such as the varroa mite (*Varroa destructor* Anderson and Trueman) (an ectoparasite and an invasive species native to Asia) and *Nosema ceranae* Fries *et al*., an endoparasite (Wagoner *et al*., 2013).

International trade of both honeybee and bumblebee colonies has potentially been a major factor in the spread of diseases worldwide (Goulson and Hughes, 2015). Commercially reared bumblebees are thought to have higher incidences of pathogen infections than wild bumblebees (Goulson, 2003). A study by Colla *et al*. (2006) in Ontario, Canada, implicates pathogen spill-over from commercial bees to wild bees as potential driver in their decline. The authors demonstrated that pathogens such as *Crithidia bombi* Gorbunov and *Nosema bombi* Fantham & Porter, which may be transmitted during flower visitation, were more prevalent in bees collected near to greenhouses than those at greater distance.

Over 1 million bumblebee colonies are produced and exported globally each year (Goulson and Hughes, 2015). Since 1961 the number of honey bee hives has increased by ~45% worldwide (Aizen *et al*., 2009). However this does not match the >300% increase in the proportion of agricultural crops dependent on pollination services, likely placing pressure on pollination capacity (Aizen *et al*., 2009). The need for these commercial colonies may also be, in part, the result of declines in wild species, potentially indicating that crops are already limited by availability of wild pollinator services (Gallai *et al*., 2009). Ellis *et al*. (2016) suggest that the commercial trade in bees may also mask the effect of declining pollination services from wild bees. The authors argue that if this was more apparent, farmers would be more incentivised to invest in wild pollinator conservation. In the main, the majority of insect pollinated crops are still largely reliant, to some extent, on wild pollination services (Breeze *et al*., 2011). Concern has been voiced regarding the over-dependence farmers and growers are currently placing on a small number of commercial species to provide their pollination services. Such dependence leaves crop yields and profits vulnerable to fluctuations in the wellbeing of commercial species. This is supported by one of the principle arguments for maintenance of biodiversity- the ecological redundancy of species is likely an important buffer to the provision of ecosystem services. If two species fulfil the same ecological niche then declines in one can be compensated by the other taking its place, acting as an 'insurance policy' for continued pollination service provision (Hallet *et al*., 2017).

Alongside the drivers of bee decline discussed above there is also widespread evidence for the detrimental impact of agricultural intensification and agricultural expansion on bees and other pollinating insects (Kremen *et al*., 2002; Bergmann *et al*., 2004). Given that reductions in habitat quality and quantity are believed to cause population declines this is perhaps unsurprising (Schultz and Dlugosch, 1999). Carvell *et al*. (2006) argue that agricultural land-use and management practices are likely to be the principle factor in the decline of social bumblebee species. Loss of flower rich hay meadows following a move to silage as a fodder crop, a fall in the use of clover leys and loss of semi-natural habitats, being key examples (Corbet *et al*., 1992). Senapathi *et al*. (2015) showed that sites surrounded by an expansion of arable land in the landscape had greater declines in bee and wasp populations than sites that did not, supporting the conclusion of other studies which demonstrate the ameliorating effect of heterogeneous semi-natural habitat on pollinating species (Bergmann *et al*., 2004; Klein *et al*., 2012).

Declines in wild pollinators not only have implications for agricultural crop pollination but also for the pollination of wild plants in field margins and semi-natural habitats

(Biesmeijer *et al*., 2006; Potts *et al*., 2010). It is thought that 80% of wild plants are directly dependent on insects for pollination with 62-73% of studied populations showing pollen limitation (Potts *et al*. 2010). Biesmeijer *et al*. (2006) has shown that in Britain, obligatory outcrossing plants reliant on insect pollinators have declined alongside declines in bee species diversity, whilst wind pollinated plants have increased. Similarly, in the Netherlands where species richness of bees has declined but hoverflies have increased it was shown that obligatory bee pollinated plants had decreased in abundance whilst plants pollinated by a variety of insect taxa had increased (Biesmeijer *et al*., 2006).

Pesticides are one of a suite of environmental stressors on pollinators and considerable research has been carried out to determine their effects on bees. Although exposure levels of bees to pesticides are generally not lethal, lethal effects have been shown (see below) and there is now considerable evidence for sub-lethal effects on managed (Stanley and Raine, 2016) and wild bees (Hanley & Wilkins, 2015; Woodcock *et al*., 2016). Wu *et al*. (2011) has shown that pesticide residues in honeybee brood comb (a total of 39 chemicals) resulted in delayed larval development and adult emergence, reduced adult longevity, and foraging activity with premature shifts in hive roles. Application of insecticide phosmet to tackle codling moth (*Cydia pomonella* Linnaeus) in orchards in the western United States also resulted in adult honeybee declines and a fall in progeny production (Alston *et al*., 2007).

Following their introduction in the early 1990s, neonicotinoids are a group of pesticides (five chemicals) that have received much scientific, media and policy attention (The Guardian, 2016; Stanley and Raine, 2016). Three neonicotinoid seed dressing treatments (thiamethoxam; clothianidin and imidacloprid) have been subject to a partial moratorium of use in Europe (Regulation (EU) No 485/2013) and a blanket ban on their outdoor use was announced in April 2018 (European Commission, 2018). A study in

Italy by Girolami *et al*. (2009) demonstrated lethal concentrations of these three insecticides in the guttation fluid of neonicotinoid seed coated maize plants. Using field realistic thiamethoxam concentrations (10 ppb), Stanley and Raine (2016) have also demonstrated sub-lethal effects on the foraging behaviour of exposed bumblebees. These bumblebees exhibited differing initial forage preferences, increased flower handling learning time, and greater frequency of pollen collection. Additive effects of the neonicotinoid thiacloprid have also been demonstrated on *Apis melifera* infected by the microbial pathogens *Nosema ceranae* and black queen cell virus (BQCV) (Doublet *et al*., 2015). Providing evidence for the role of synergistic effects in CCD and the additional pressures agricultural inputs place on bee populations.

The effect of pesticides on non-bee pollinators is still poorly understood. Even for neonicotinoids, only a single study appears to have been carried out on hoverflies for example, focusing on a single insecticide, thiamethoxam (Basley *et al.*, 2018). Exposure of *Eristalis tenax* (Linnaeus) to 500 ppb thiamethoxam concentration had a significant negative effect on survival but no effect was seen at field realistic concentration (Basley *et al.*, 2018). In contrast studies of imidacloprid on the aquatic (non-pollinating) insect *Ceriodaphnia dubia* Richard demonstrate significant LC_{50} mortality at only 2.1 ppb (Chen *et al*., 2010). It is clear more research is needed on the impact of widespread pesticides upon non-bee insects (Pisa *et al*., 2015). However given the purpose of insecticides, and their often limited specificity, future evidence of detrimental effects on non-target pollinating insects would not be unexpected. Pyrethroids (insecticides accounting for 23% of the global insecticide market, Piccolomini *et al*., 2018) are potential candidates to replace neonicotinoids and may, similarly, have adverse effects on non-target pollinators (Raimets *et al*., 2018), although few studies are currently available (Oliver *et al*., 2015).

Additionally, alongside losses in flower rich habitats, widespread herbicide use is also likely to be playing a role in pollinator declines (Kluser and Peduzzi, 2007). Just as reductions in the provision of pollinator services to wild plants may have contributed to plant declines, the converse is also likely, with falls in the availability of forage resources understood to have contributed to declines in pollinating insects such as bumblebees (Goulson *et al*., 2005; Carvell *et al*., 2006). Given that plant and pollinator species are functionally linked this is perhaps unsurprising. In both the UK and Netherlands, bee and hoverfly species which are more functionally specific, in terms of having narrow habitat requirements and/or dietary preferences, have shown greater declines in diversity than generalist species (Biesmeijer *et al*., 2006).

1.3 Mass Flowering Crops: A Means to Reverse Declines?

It has been proposed by Westphal *et al*. (2003) that mass-flowering crops (herein: MFCs) may be incorporated into agri-environment schemes (AES) in order to help tackle pollinator declines. Compared to wild plants present in the landscape, MFCs represent an energetically rewarding source of forage due to their high floral unit counts. However, studies to date have shown mixed effects of mass-flowering crops on pollinator taxa. The majority of MFC studies have focused on oilseed rape (*Brassica napus* Linnaeus; herein: OSR), likely due to the crop's widespread cultivation for both food and biofuel (Diekötter *et al*., 2010; Stanley *et al*., 2013). Several studies have shown positive impacts of mass-flowering crop cultivation on bumblebee densities, in both the crop and adjoining margins (Westphal *et al*., 2003; Westphal *et al*., 2009; Hanley *et al*., 2011). However this response has been shown to be short-lived, with densities falling rapidly post-flowering (Hanley *et al*., 2011). Westphal *et al*. (2009) showed that bumblebee reproductive success (queen and male production) did not increase with greater OSR cultivation in the landscape and suggest that this may be due to lack of continuity in resource provision throughout the season. Using molecular techniques however, Knight *et al*. (2009) demonstrated a positive correlation in the amount of OSR (in a 1000 m radius) and the density of *Bombus pascuorum* (Scopoli) colonies. Stanley (2013) also demonstrated high numbers of bumblebee colonies using OSR fields, with as many as 880 using a single field.

Although most studies have focused on bumblebees, studies on other pollinators, such as solitary bees have also drawn variable conclusions regarding the benefits of mass-flowering crop cultivation. Holzschuh *et al*. (2013) demonstrated positive effects of greater OSR cultivation on populations of the solitary bee *Osmia bicornis* Linnaeus*,* both in the OSR field and in adjacent grassland habitats. The number of brood cells in trap nests was also 55% higher in grassland adjacent to OSR compared to isolated grassland (Holzschuh *et al*., 2013). Conversely, Montero-Castano *et al*. (2016) showed that, at the landscape scale, French Honeysuckle (*Hedysarum coronarium* Linnaeus, a fodder crop) had negative effects on generalist pollinators present in nearby semi-natural habitats, competing with native plants for honeybee and wild bee pollination in the nearby native shrubland. This, again, is in contrast to a bumblebee study by Hanley *et al*. (2011) which demonstrated that when in flower, field bean increased bumblebee visits to wild margin plants compared to conventional wheat. It was also shown that relative bumblebee species abundances did not differ with proximity to a MFC. This in turn differs from the findings of Diekötter *et al*. (2010), who demonstrated apparent distortions of plant-pollinator interactions as increasing proportions of OSR at the landscape scale resulted in declines of long-tongued bumblebees likely due to indirect, disproportional, benefits to short-tongue bumblebees. Other studies looking at pollination outcomes, using single phytometers, have shown variable contrasting effects of MFCs; Cussans *et al*. (2010) for example compared the reproductive success of both birds-foot trefoil (*Lotus corniculatus* Linnaeus) and ground ivy (*Glechoma hederacea* Linnaeus) when planted next to MFCs and non-MFCs. Despite higher fruit set of *L.*

corniculatus in OSR margins compared to wheat, *G. hederaceae* seed set did not increase. Hanley *et al* (2011) state that the variability we see between studies may result from concentrating on only a single plant species rather than whole floral communities, as in their study. It is also worth considering that the effects of the MFCs may also have been influenced by management (Stanley and Stout, 2013), particularly given the evidence for negative effects of neonicotinoid treated OSR on wild bees, with the potential for future pesticide alternatives to be either more or less damaging (Woodcock *et al*., 2016). Nonetheless, regardless of the variable impacts of MFCs upon pollinators reported in the literature, their potential to play a strategic conservation role, and thus increase proportionally in the landscape, exemplifies the dynamic nature of agricultural land use, a landscape in flux due to policy, market demand and rotational cropping, with important implications for biodiversity.

1.4 Agricultural Land Use Change

As the land-share of agriculture increases, semi-natural habitats are being lost rapidly across the globe, with significant negative effects on biodiversity, ecosystems processes and services (Gonthier *et al*., 2014). In the United Kingdom, agriculture accounts for ~57% of the island's total surface area (Rae, 2017), where it has replaced species-rich semi-natural habitats such as peatlands, heathlands and unimproved grasslands (NEA, 2011; Kahn *et al*., 2013). The 'State of Nature' report published in 2016 revealed that 56% of the species studied (2137 of 3816) had declined in the UK between 1970 and 2013 (Hayhow *et al*., 2016). Following the first report in 2013, Burns *et al*. (2016) highlighted two important drivers of biodiversity change; 'agricultural management' and 'preceding climatic change'. Intensive 'agricultural management' was a key factor in biodiversity declines with primarily negative impacts for investigated taxa whilst 'preceding climatic change' had mixed effects but remained the factor with the second greatest negative impact. Butterfly Conservation's report, 'State of the UK's Butterflies

2015', emphasises the likely role of agricultural and forestry land use change in the decline of many habitat specialist butterflies, and suggests the latitudinal differences in the status of wider countryside species may result from more prevalent land use change in southern England (Fox *et al*., 2015). The 'Farmland Bird Index', which monitors the state of 19 species, has similarly seen a 43% fall between 1970 and 1988 (NEA, 2011). Such data sets are some of the best long-term records available due to a long-established tradition of natural history recording in the UK. The concomitant declines in populations of such diverse taxa have been ongoing for centuries due to anthropogenic activities, often associated with agricultural land-use change.

Increased demand for land to cultivate food crops is not a new phenomenon; between 1940 and 1980 the cropped area in the UK increased by 40% (NEA, 2011). Furthermore, in recent decades the development of first generation biofuels has created a novel demand on land resources and renewable energy is expected to be one of the key drivers of future land use change (Manning *et al*., 2015; Santangeli *et al*., 2016). Tilman *et al*. (2009) succinctly summarised this as the 'Food, Energy and Environment Trilema'.

1.5 Climate Change and Renewable Energy

In recent decades there have been sustained warnings from the scientific community of the urgent need to address anthropogenic climate change. Evidence set out in the fifth report of the Intergovernmental Panel on Climate Change (IPCC, 2014) shows that mean land and sea temperatures have increased by 0.85 °C between 1880 and 2012; there have been reductions in spring snow cover, glaciers have shrunk and ocean acidity has increased by 26%. The report's authors state that the concomitant increase in anthropogenic GHG emissions (i.e. CO_2, CH_4, N_2O and fluorinated gases) since pre-industrial times is "extremely likely to be the dominant cause of warming since the mid-twentieth century".

Projections of future climatic change vary in severity but it is expected that global temperatures will increase under all emission scenarios. Representative concentration pathway (RCP) 2.6 (the lowest emission scenario) is expected to result in a temperature increase of between 0.3 °C to 1.7 °C by 2100 relative to 1986-2005 levels, whilst RCP 8.6 (baseline/worst case scenario) is likely to see temperatures increase to between 2.6 °C and 4.8 °C (IPCC, 2014). Such temperature increases, alongside factors such as further sea level rise and ocean acidification, are predicted to have negative consequences for humanity, especially for the poorest and most vulnerable, where problems such as food security and disease are likely to be exacerbated by a changing climate (IPCC, 2014; Myers *et al*., 2017). Similarly, evidence is already widespread for negative impacts on biodiversity with range contractions reported for many taxa, often as a result of population extinctions at lower latitudes and elevations (Parmesan, 1996). Species extinctions have likewise been reported for range-restricted species such as cloud-forest-dependent amphibians in Costa Rica (Pounds *et al*., 2006; Parmesan, 2006). Differences in the rate of climate induced changes in phenology have also led to mismatches between predators and prey (Visser and Both, 2005), interacting plants and insect species (Hindle *et al*., 2015; Schenk *et al*., 2018) and even nesting bird phenology and farming practices (Santangeli *et al*., 2018). Likewise, taxonomic variability in climate induced range shifts (Parmesan and Yohe, 2003) has the potential to disrupt trophic interactions and biogeochemical cycling (Chivers *et al*., 2017). Such negative impacts on biodiversity, whilst important in their own right, will also have damaging effects on human health due to a loss or reduction in the ecosystem goods and services which it provides (Haines *et al*., 2006; Runting *et al*., 2017). Given that scenarios with higher atmospheric GHG concentration will result in greater negative effects for both humanity (Kovats *et al*., 2005) and biodiversity (Martay *et al*., 2017), it is imperative that action is taken to rapidly reduce emissions (Rockström *et al*., 2017).

In the UK and Europe, recent policy has aimed to stimulate a movement away from fossil fuels and towards more renewable forms of energy generation. Frameworks under the EU Renewable Energy Directive (RED, Council Directive 2009/28/EC) commit member states to collectively produce at least 20% of total energy consumption from renewables by 2020, expected to rise to 27% by 2030 (COM(2016)767/F2). The UK Climate Change Act 2008 (HM Government, 2008) concomitantly establishes a target of an 80% reduction in GHG emissions below 1990 levels by 2050 and 34% by 2020. This has since been followed by the release of 'The Carbon Plan' (HM Government, 2011) detailing potential pathways to 2050 emission reductions and by the UK government's 'Bioenergy Strategy' (DECC *et al.*, 2012), which highlights its approach and commitment to bioenergy to help fulfil these goals. The recent Conference of the Parties in Bonn reaffirmed the international commitment of the 174 Parties (nations and territories) whom have ratified the Paris Agreement (United Nations, 2016), and so committed to pursuing efforts to limit temperature increases to 1.5 °C above pre-industrial levels. Some governments have now begun to implement carbon tax or cap-and-trade schemes in order to incentivise businesses to reduce emissions through economic drivers. Examples of such schemes are present or planned in nearly 40 countries, including the nations of the European Union, China, India, Japan and regions of the US (World Bank, 2016). In order for the UK to meet its 2050 target, a carbon price trajectory of £33/tonne of CO_2 was expected in 2020 rising to £78/tonne in 2030, the 2017 Budget currently sees the carbon price frozen at £18/tCO_2 until April 2021 however (Committee on Climate Change, 2015; Parliament, House of Commons, 2018). Nevertheless, these carbon emission penalties are aimed at stimulating investment in cleaner energy technologies (Aldy and Stavins, 2012).

The Royal Commission on Environmental Pollution (2004) has suggested that by 2050 as much as 12% of the UK's energy (excluding transport) will be derived from biomass alone. Bioenergy crops (BECs) are therefore expected to make a large contribution to the British renewable energy portfolio alongside other forms of biomass, such as by-products of forestry and agriculture (Sims et al., 2006). The UK Biomass Strategy predicted that perennial energy crops will occupy some 350 000 ha by 2020 and provide 278 PJ of energy (DEFRA, 2007). Additional drivers for bioenergy production include a desire for greater energy security as well as the acknowledgement that alternatives must be found to replace the finite reserves of coal, oil and gas. Furthermore, food production can also be decoupled from fluctuating fossil fuel prices which influence oil and fertiliser costs and thus, indirectly, affect food prices (Valentine *et al*., 2012). It is also suggested that bioenergy crop cultivation should stimulate the rural economy, allowing farmers to diversify from arable, reducing labour requirements, benefiting older and part-time farmers (Edwards *et al*., 2008; Valentine *et al.*, 2012).

1.6 Bioenergy and Biomass

In order to meet future energy demand sustainably it has become evident that a diverse range of renewable energy technologies will need to be utilised and developed. Such a mix includes bioenergy as well as wind, solar, hydro-electric, tidal, and geothermal. The appropriate combination of fuels will vary between nations, dependant on factors such as climate and available land area (EASAC, 2012).

Bioenergy is the conversion of biomass into energy carriers such as heat, electricity and transport fuels. Bioenergy can therefore be seen as the energy derived from biomass. Field *et al.* (2008) define biomass energy as, ‘any source of heat energy produced from non-fossil biological material’. Biomass can be utilised from a range of sources including agricultural and forest residues, organic municipal waste and energy crops (European Commission, 2017). Data on global final energy consumption shows that in 2013 renewables accounted for 18% of energy demand compared to 38% from oil and only 2% from nuclear fuels (World Energy Council, 2016). Biomass is the predominant source of this renewable energy (~77%), principally woody biomass. Woody biomass accounts for ~87% of all utilised biomass, although the sustainability of sources is variable as data encompass 'traditional bioenergy' sources which, when used inefficiently can lead to deforestation (IEA, 2009; World Energy Council, 2016). However in the UK, BECs still only account for a relatively small percentage of arable land, just over 2% in 2016 (DEFRA, 2017) with 53% of this land being used for 1st generation biofuel production for the road transport market. First generation biofuels consist of oil and starch rich crops that are principally grown for food and used to produce bioethanol and biodiesel.

Bioethanol is produced from predominantly sugar and starch rich crops such as sugarcane (*Saccharum* spp. Linnaeus), maize (*Zea mays* Linnaeus) and wheat (*Triticum* spp. Linnaeus) using microbial fermentation (Rulli *et al.*, 2016). Biodiesel is largely produced from oil rich crops such as soybean (*Glycine max* Linnaeus), oil palm (*Elaeis guineensis* Jacquin) and OSR using the chemical process of trans-esterification (Rulli *et al.*, 2016). Global consumption of biofuels in 2013 was ~86 million tonnes with bioethanol accounting for 75.6% and biodiesel 24.4% (Rulli *et al.*, 2016). In many cases first generation biofuels have had devastating consequences for natural environments and biodiversity due to direct and indirect land use change (ILUC) (Fargione *et al.*, 2008). The European Union has been the biggest importer of palm oil derived biodiesel from Malaysia, Indonesia and Papua New Guinea (Rulli *et al.*, 2016). It has already made attempts in 2012 to reduce environmental damage from palm oil cultivation in these nations by refusing biofuels produced on land of high biodiversity value (European Union, 2012). Biodiesel has a land footprint 100% that of bioethanol and the direct and indirect effects of production remain hard to verify (Hermele, 2014 in Rulli *et al.*, 2016).

In the US, the Renewable Fuels Standard has encouraged the widespread cultivation of wheat and maize feedstock (EISA, 2007). Whilst the Renewable Energy Directive in Europe has similarly encouraged the cultivation of first generation crops alongside imports, with targets having been set for 10% of transport fuel to be renewable by 2020. In April 2015 a 7% cap was placed on food crop based biofuels for the transport sector, due to recognition of the damaging effects of ILUC, and followed by a European Parliament approved reform of the Renewable Energy Directive, recognising the need to reduce the proportion of food based biofuels in favour of advanced biofuels, principally waste & residues, and potentially second generation BECs (COM(2016)767/F2).

This study focuses on second generation lignocellulosic bioenergy crops, 'modern' biomass, primarily grown for energy production and not used as food. These have benefits both over 'traditional' biomass and also the more widespread, first generation biofuels. Lower water consumption, reduced land footprint and less competition with food crops are suggested benefits of 2nd generation BECs (IEA, 2008), although, as pointed out by Fargione *et al.* (2008), these crops may still act as a possible driver of land clearance in the future if they are not grown on abandoned agricultural lands. However, if 2nd generation BECs are indeed grown on marginal agricultural lands, potential may still remain for rapid increases in their proportional land area, both in the UK and abroad. Perhaps even stimulated by targets to phase out combustion engines in favour of electric vehicles by 2040, following pledges by the UK, China, India, France, Germany, Norway, The Netherlands and eight states in the US (World Economic Forum, 2017).

1.7 Lignocellulosic Bioenergy Crops: Cultivation and Management

A wide range of 2nd generation lignocellulosic BECs have been considered as future sources of biomass energy. In temperate regions these crops are expected to be

Miscanthus x giganteus Greef *et* Deuter (herein: *Miscanthus*) and short-rotation coppice (SRC) crops such as willow (*Salix* spp. Linnaeus) that will be the focus of this study (Bauen *et al*., 2010; Hastings *et al*., 2014). This investigation is undertaken in arable areas of central England (East Midlands) and southwest England as these areas have received significant plantings with grants principally awarded to growers in arable regions (Natural England, 2008 in Haughton *et al*., 2009).

1.7.1 *Miscanthus*

Miscanthus x giganteus is a fast growing perennial rhizomatous grass which originates from Asia; a sterile hybrid of the two grasses *M. sinensis* Anderson and *M. sacchariflorus* (Maximowicz) Hackel It is a C4 grass and therefore has high photosynthetic and water use efficiencies. Long roots reaching depths of 2 m assist in water acquisition, reducing the likelihood of yield losses from water stress. However its rapid growth means higher water use than conventional crops (Heaton *et al.*, 2010). Despite its C4 physiology, the grass is capable of growing at temperatures as low as 6°C and produces good yields in temperate climates such as the UK. This chilling tolerance means that unlike maize, a first generation biofuel crop and also a C4 grass, *Miscanthus* can take advantage of a longer growing season (Dohleman and Long, 2009). In England, yields average between 12-15 ODT (oven dried tonnes) ha^{-1} yr^{-1} once the crop is established (DEFRA, 2017). Although preferring soils with a pH of 5.5-7.5, *Miscanthus* is tolerant of a wide range of pH and soil types. Additional factors important to crop yield include sunlight, temperature, water availability and site aspect.

Miscanthus is a long-lived plant; the rhizome remains viable in the soil for 15-20 years before replanting becomes necessary. This in turn means tillage and soil disturbance are reduced. Once mature, the bamboo-like canes show impressive annual growth of between 2.5-3.5 m. In field trials in Illinois, biomass increase between June and August corresponded to 4.4% conversion of solar energy into biomass, one of the highest recorded (Dohleman *et al*., 2012). Productivity and yields are much reduced in Europe, due to climatic factors, but the efficiency of the crop is clear.

The canes are harvested annually and provide a regular source of income for the grower. In economic terms this is a key benefit over willow SRC, which tends to be harvested on a 3 year cycle (Rowe *et al.*, 2009). Unlike conventional arable crops, *Miscanthus* has a low requirement for agrochemical inputs. The grass exhibits a high nitrogen use efficiency and recycles many nutrients back to either its rhizome or to the soil (as leaf litter) prior to harvesting in the winter/spring. It may be necessary to use a conventional non-selective cereal herbicide as a weed control prior to crop establishment in some instances however. This can be applied in the second year preceding new growth where weeds remain competitive. There were ~7000 hectares of *Miscanthus* grown on agricultural land in the UK in 2016, mainly grown in Yorkshire, the East Midlands and southwest region, this is down from a peak of ~9213 ha in 2009 (DEFRA, 2015; DEFRA, 2017).

1.7.2 Willow Short-rotation Coppice (SRC)

As with other SRC crops trialled in the UK, such as poplar (*Populus* spp. Linnaeus), willow is a fast growing tree species that may re-sprout from stools after harvest. Following removal of low yielding biomass during the initial establishment year, willow is typically coppiced on a 3 year rotation (DEFRA, 2017). Willow plantations therefore have lower disturbance than the annually harvested *Miscanthus* (DEFRA, 2004). Cuttings are planted in spring and harvested in winter using conventional farm machinery. Harvested material is chipped and dried for use in dedicated biomass burners or for co-firing (Rowe *et al.*, 2009). The crop is densely planted with ~15,000 stools ha^{-1}, using varieties that have predominantly been developed from the osier shrub (*Salix viminalis* Linnaeus), with traits such as resistance to disease, erect growth habit and high yields selected for (DEFRA, 2004; Rowe *et al.*, 2009). The plantations can remain viable for up to 30 years before replanting becomes necessary. UK willow yields are expected to fall between 7 and 12 ODT ha^{-1} year^{-1} with annual rainfall requirements of 600-1000 mm per year being preferable (DEFRA, 2004).

In Europe in 2009 the largest amount of SRC was grown in Sweden, with 15,000 ha under production. Italy had 6000 ha of predominantly poplar, Poland and the UK each had 3000 ha of mostly willow and Germany 1500 ha of mainly poplar (Dimitriou *et al.*, 2009). Recent figures from DEFRA (2017) show the area of UK arable land cultivated with SRC remained at ~3000 ha in 2016.

Although numbers of studies are still low, some striking differences are appearing between these 2nd generation BECs and when related to either 1st generation BECs or traditional food crops. When compared to these altenative agricultural land uses, studies to date have documented predominantly positive or neutral effects of *Miscanthus* and willow SRC cultivation upon a variety of taxa. Densities of birds in hedgerows surrounding willow SRC are seen to be higher compared to arable and grassland for example, with higher numbers of winter migrants and more species using the fields (Sage *et al.*, 2006). Sage *et al.* (2010) describe *Miscanthus* as having largely neutral impacts on birds. When comparing *Miscanthus* to winter wheat Bellamy *et al.* (2009) show seasonal variation in the effects of each crop upon birds, with benefits from *Miscanthus* in summer and winter but greater insect prey in wheat fields during the breeding season. Some negative effects may arise for birds preferring open farmland, however, such as skylark (*Alauda arvensis* Linnaeus), yellow wagtail (*Motacilla flava* Linnaeus) and lapwing (*Vanellus vanellus* (Linnaeus)), although abundances can be high in SRC following harvest (Sage *et al.*, 2006).

In rural areas, SRC is shown to provide a novel habitat that increases phytodiversity (Baum *et al.*, 2012), with richer ‘weed’ vegetation reported in recently established

Miscanthus compared to reed canary-grass (*Phalaris arundinacea* Linnaeus) or arable fields (Semere and Slater, 2007b). *Miscanthus* also provides suitable margin habitats for mammals but numbers are seen to remain similar to both reed canary grass and conventional arable (Semere and Slater, 2007a). Studies to date have largely focused on birds or plants with limited research of other groups. However Rowe *et al*. (2013) have shown significantly more non-coleopteran arthropod predators in willow than arable whilst rove beetle (Staphylinidae) abundance was higher in willow but ground beetle (Carabidae) abundance unaffected by crop type.

Conversion of semi-natural habitats to BECs is expected to have negative consequences however, with BEC monocultures more closely approximating conventional arable than complex biodiversity rich habitat (Rowe *et al.,* 2009). Nonetheless, perennial energy crops (PECs) offer a potential opportunity to promote agricultural sustainability, enhancing regulation of key ecological processes (Rooney *et al*., 2009). Positive and benign effects of *Miscanthus* and largely positive effects of willow SRC, have led to the suggestion that PECs may be suitable for strategic planting in landscapes dominated by annual rotational cropping (Haughton *et al*., 2015), with potential inclusion in agri-environment schemes (Sage *et al*. 2006).

1.8 Bioenergy Crops: Biodiversity, Ecosystem Processes and Services

Ecosystem services are the collective benefits that society obtains from ecosystems and a key justification of the benefits of conservation (Liss *et al.,* 2013). The National Ecosystem Assessment (NEA, 2011) recognises four broad and inter-related categories of ecosystem services.

- Supporting services (including soil formation and nutrient cycling)
- Regulating services (including pollination, pest and disease regulation).

- Provisioning services (products obtained from ecosystems, including food, fibre, fuel and fresh water)
- Cultural Services (including cultural identity, aesthetic appreciation and inspiration)

The discussion here is directed towards those processes and services in *Miscanthus* and willow SRC that are the principle focus of this study: pollination, biogeochemical cycling (encompassing nutrient cycling and climate regulation) and decomposition. Despite the evidence for positive impacts on biodiversity, few studies have been carried out to investigate how ecosystem processes and services in 2nd generation lignocellulosic BECs differ from conventional agricultural land use (Rowe *et al*., 2013).

1.8.1 Pollination Processes

From an ecological standpoint insect pollination can be viewed as the biophysical transfer of pollen grains, a service which involves the processes of insect flower visitation, pollen collection and transport, pollen deposition onto conspecific stigmas and, if successful, pollen tube growth into the style (Willmer, 2011). Estimates of the success of this service can be inferred from measures of reproductive output (i.e. seed production and/or fruit set) which, at least in the context of crop yields, is a metric with a clear monetary value and, in the context of wild plants, vital to the development of viable progeny (Cussans *et al*., 2010).

The decline of pollinating insects has attracted increasing concern regarding the provision of pollination services, making potential negative or positive impacts from future land use change an important area of investigation. Insect pollinators contribute to the yield of ~75% of crop species with an estimated value to agriculture in 2005 of €153 billion globally (Gallai et al., 2009). Despite its magnitude this is a conservative estimate and does not account for biofuel, ornamental flower pollination or pollination of livestock crops (Gallai et al. 2009). Although commercial bee colonies are increasingly used as a management tool, pollination by wild insects provides a vitally important ecosystem service to a large number of crop and wild plants (Gallai et al., 2009).

To date there have been no direct attempts to specifically quantify pollination service provision in *Miscanthus* or willow SRC. Studies have instead focused on population parameters such as abundance, species richness and diversity, important components of pollination but not direct measures of pollination process or service provision. Studies in Ireland have compared pollinator populations in *Miscanthus* to those in conventional arable and a 1st generation BEC, OSR (Stanley and Stout, 2013). Responses of pollinator assemblages differed between the two BECs. Solitary bees benefitted from the cultivation of both BECs with higher abundances and species richness compared to wheat, whilst community composition differed between the two BECs. The authors suggest that elevated wildflower abundance may explain these trends. Investigation of trap nesting bees revealed higher numbers in *Miscanthus* than OSR whilst bumblebee abundance, species richness and nest searching activity was no greater in *Miscanthus* than any of the other crop types. Similarly, Lepidoptera and hoverfly abundance and species richness in *Miscanthus* did not differ to wheat and the authors posit that this may arise from the differing larval food preferences of these taxa. Lepidoptera were more abundant in *Miscanthus* fields than grass silage however, with differences in wild plant communities also documented between land uses. Overall, these studies demonstrate fairly neutral effects on pollinator taxa when changing from conventional arable to *Miscanthus* but highlight variability in the direction of response between different taxa, there is consequently a need to avoid using coarse taxonomic resolution when investigator pollinator assemblages.

Comparison of recently established *Miscanthus* (≤3 years) to an alternative 2nd generation BEC, reed canary-grass, suggests some benefits of *Miscanthus* upon invertebrate populations over alternative biomass BECs (Semere and Slater, 2007b). Although relevance to pollination is constrained due to a limited focus on ground beetles, butterflies and arboreal invertebrates (the latter principally at family level), it is shown that all three invertebrate groups were more abundant and diverse in *Micanthus* with greater diversity of wild plant species. Lepidoptera in *Miscanthus* were more abundant in both the cropped region (likely due to within-crop 'weeds') and margin region of the fields compared to reed canary grass. Similar to *Miscanthus*, a study of commercial willow SRC in Yorkshire showed increased butterfly abundance and species richness in the field margins and headlands compared to conventional arable (Cunningham *et al*., 2004) and grassland (Cunningham *et al*., 2006). The less intensive, more sheltered, wider headlands are suggested to drive these trends, providing good butterfly habitat, with numbers seen to increase in more established crops (Cunningham *et al*., 2004).

In willow SRC, floral resources are seen to change throughout the harvest cycle, recently harvested crops providing disturbed sites, largely for annuals, with crop establishment resulting in a proportional increase perennial and invasive species (Cunningham *et al*., 2004). Although I concentrate on pollination services during the summer months this study, the willow crop itself is in flower during the spring, providing an important source of forage for pollinators at this time. In Germany Haß *et al*., (2012) showed that male willow flowers appear to be an important source of forage for early emerging bumblebees and oligolectic spring solitary bees such as *Andrena clarkella* (Kirby), *Andrena vaga* Panzer and *Colletes cunicularius* (Linnaeus), leading the author to recommend planting a high proportion of male plants, however given seed and nectar production by female flowers, entirely single sexed stands are unlikely to be desirable for biodiversity generally. The benefit of multiple cultivars, due to variation in the time of anthesis, is also emphasised, and the authors suggest that willows should be favoured over alternative poplar SRC due to the predominantly wind pollinated nature of latter crop. Across the whole study (April 1st to 24th June 2010) Haß *et al*., (2012) record a respectable 29 bee species on the SRC, indicating the value of incorporating this crop into farmland landscapes. In another European study, in The Netherlands, Redderson (2001) demonstrate that catkin number increases with year since harvest whilst harvesting reduced catkin numbers to nil in the subsequent year, demonstrating the value of varying the harvest cycle for different regions of a given plantation, preventing a discontinuity in resources for spring pollinators. Redderson (2001) report few bees in their study but suggest that this largely results from an otherwise impoverished landscape and small plantation sizes (mean= 1.1ha). Across investigations, studies in both bioenergy crops suggest little benefit to particularly rare pollinators; for example butterflies in SRC tend to consist of common ‘browns’ and ‘skippers’ such as meadow brown (*Maniola jurtina* Linnaues) and large skipper (*Ochlodes sylvanus* (Esper)) (Cunningham *et al*., 2004).

1.8.2 Biogeochemical Cycling and Decomposition Processes

Biogeochemical cycling is the process by which elements move through biotic (biosphere) and abiotic (lithosphere, hydrosphere, atmosphere) compartments of the Earth. Biogeochemical cycling has particular importance for ecosystem services such as climate regulation (Dennan *et al*., 2007) and soil nutrient status (Jobbágy and Jackson, 2004), highly relevant in the context of anthropogenic climate change and farmland soil fertility. In this study, attention is given to patterns of soil elements and *Bacteria* under BECs with consideration of decomposition processes at the soil surface. Soil is a major reservoir of nutrients and yet many gaps still exist in our knowledge regarding the cycling of even abundant elements such as carbon, nitrogen and phosphorus (Quinton *et* *al.*, 2010). Land use change can have consequences for biogeochemical cycling due to its effect on both biotic and abiotic processes (Guo and Gifford, 2002). To date most studies in *Miscanthus* and willow SRC have focused on processes directly relevant to C and N cycling.

Although the global warming potential of N_2O (a by-product of incomplete nitrification and denitrification) is 298 times that of CO_2, disparities in their atmospheric concentration result in a far greater contribution of CO_2 to radiative forcing (+1.68 [1.33 to 2.03 W m^{-2}), greater in fact than all other GHGs combined (total forcing = + 2.29 [1.13 to 3.33] W m^{-2}; IPCC, 2014). Understanding how changing land use might alter carbon cycle processes relevant to atmospheric CO_2 concentrations is clearly necessary. In terrestrial systems, soils are the most important pool of stored carbon, storing more globally than both terrestrial vegetation and the atmosphere combined (Averill *et al.*, 2014). The global soil organic carbon (SOC) pool is estimated to be ~1550 PgC, which is approximately double that of the atmospheric pool (~770 PgC) and 2.5 times the biotic pool (~610 PgC) (King *et al.*, 2004).There is now a growing body of research seeking to understand the potential of soils to act as carbon 'sinks' (i.e. sites of net carbon storage as opposed to net loss, Smith, 2014) under differing land uses.

In the UK >95% of land carbon stocks are in soils (Ostle *et al.*, 2009). The 57% land share of agriculture in Britain (~74% in England; Rae, 2017) highlights the important contribution that agricultural land use may have in controlling national GHG emissions, through effects on processes that control the accumulation, stability and composition of soil organic matter (SOM) (Batjes, 1996, Ostle *et al.*, 2009, Averill *et al.*, 2014). Although smaller in magnitude than the oceanic carbon pool, the short term labile nature of soil carbon adds to its importance (Batjes, 1996). Based on Countryside Survey (2007) data, arable and horticultural below-ground carbon stocks are the third highest (198 ± 19 million tonnes) of any broad habitat in the UK; only bog (>550 million tonnes) and improved grassland (274 ± 25 million tonnes) habitats have greater stores of soil carbon (Ostle *et al*., 2009). In addition, the low baseline carbon stock, per unit area, of arable and horticultural croplands in the UK (43 ± 0.5 tonnes ha^{-1} to 15 cm depth; Countryside Survey, 2007) presents a major opportunity for future improvements through changes in land use and management practices (Davidson and Janssens, 2006; Dondini *et al*., 2009).

On average, British croplands are estimated to lose ~140 ± 100 kg C ha^{-1} yr^{-1} (Dawson and Smith, 2007) with analyses revealing arable soils as net carbon sources in 33 additional European countries (Janssens *et al*., 2006). Conservation agricultural practices such as crop residue retention and no-till farming have been shown to increase soil carbon sequestration in Europe (Smith *et al*., 1998; Blanco-Canqui and Lal, 2007) and elsewhere (e.g. North America, Hollinger *et al*., 2005; South America, Bayer *et al*., 2006 and Africa, Saber and Mrabet, 2002). Increasing the amount and quality of non-farmed habitats such as hedgerows, woodland and field margins, is also a means of increasing C (as SOM) in farmed landscapes (Falloon *et al*., 2004; van Vooren *et al*., 2018). Likewise, research by Guo and Gifford (2002) has demonstrated significant increases in soil carbon stocks with wholescale land use change from cropland to lower intensity pasture (+19%), plantation (+18) and forestry (+53%).

CO_2 release through soil respiration plays a major role in CO_2 efflux and highlights the importance of decomposition processes for land use carbon balance. Decomposition is the breakdown of non-living organic material (detritus) into simpler compounds; this may result in immobilisation of nutrients (i.e. the accumulation of elements in biomass for biosynthesis or storage) or complete oxidation/mineralisation, whereby inorganic substances are released into the environment, where they are potentially bioavailable to plants and microbes. Although influenced by abiotic processes such as physical (i.e. leaching) and chemical (i.e. hydrolysis, photolysis) degradation, biodegradation is often a major driver in the breakdown of organic material in many environments (Jenny, 1961), with the biota responsible providing an important ecosystem service that can result in SOC accumulation.

Soil invertebrates can have a considerable effect on decomposition rate (Chapin *et al*., 2002). In the soil and litter layer soil animals are important for the fragmentation of litter and microbes (*Bacteria* and fungi) have a major role in the transformation of molecules and elements into different forms, different strains having differing capabilities, with considerable redundancy likely contributing to the resilience of this ecosystem process (Kertesz and Frossard, 2015). Interactions between soil organisms (e.g. between consortia within microbial biofilms, between earthworms and microbes etc.) can also have implications for soil processes. As such, changes in land use are likely to pose differing pressures that affect survival, growth and reproduction of soil organisms and in turn the decomposition process (Chapin *et al*., 2002). Microbial predation by protozoa may be greater under certain land uses and for certain microbial species for example, influencing microbial community composition and even soil C and N turnover, due to high microbial concentration of these elements (Chapin *et al*., 2002). Furthermore, changes in soil disturbance through altered cultivation activity can influence oxidation of slowly decomposing SOM by microbes. Farming practices that reduce the intensity, frequency and depth of soil cultivation have therefore been proposed as one means to reverse carbon efflux (Ogle *et al*., 2005; UNEP, 2013).

In some regions, such as the Po Valley in northern Italy, PECs have been grown with the duel aim of reducing carbon emissions and improving soil quality, tackling SOC losses resulting from intensive agriculture; sequestering carbon from litter, roots and harvest residues into SOC (Agostini *et al*., 2015). Cattaneo *et al*. (2014) used denaturing gradient gel electrophoresis (DGGE) to investigate the microbial communities present in 9-year-old *Miscanthus* and giant reed (*Arundo donax* Linnaeus) whilst comparing them to two 40 year old arable cropping systems, continuous wheat and a wheat-maize rotation. The activities of three enzymes involved in soil carbon, nitrogen and phosphorus cycles (β-glucosidase, urease and alkaline phosphatase) were also investigated. Both bioenergy crops increased the activity of the enzymes, particularly in the topsoil (0-0.15 m) where carbon and nitrogen were most prevalent. The authors attribute this activity to reduced soil disturbance (i.e. tillage) in the perennial crops, highlighting the findings of King *et al*. (2004) who showed that under lower aeration (i.e. reduced cultivation) soil carbon storage increased due to lower microbial oxidation. Numerous studies have also shown that afforestation and no-tillage provide microbes with a large supply of nutrients in the form of root and leaf litter to the top soil layer, leading to greater microbial biomass (Mao and Zeng, 2010; Toenshoff *et al*., 2013). Due to fine roots primarily colonising the upper 10 cm of soil under fast growing tree-plantations, microbes in top soil are also thought to benefit from root exudates (Heinsoo *et al*., 2009). Cattaneo *et al*. (2014) showed that organic carbon was positively correlated with enzyme activities, although surprisingly not β-glucosidase, and a significant but weaker correlation existed for total nitrogen. DGGE fingerprinting demonstrated that community richness was higher in perennial crops than annual, but no crop-linked variation in species diversity was recorded and no depth effect observed. The authors conclude that perennial grasses may represent a sustainable choice for soils depleted by intensive agriculture (Cattaneo *et al*., 2014).

Furthermore, Schmitt *et al*. (2010) have also reported higher microbial biomass C under willow than popular clones. The authors suggest that lower lignin content and C/N ratio of willow leaves made them more degradable. Additionally, a study of willow SRC by Rowe *et al*. (2013) has demonstrated elevated soil surface processes with higher litter fragmentation rates in the PEC compared to cereals, an important aspect of SOC turnover rates. This is, again, attributed to the lower levels of tillage as well as reduced pesticide use; lower pesticide is understood to benefit soil biota, increasing both soil faunal abundance and microbial activity (Curry *et al*., 2002; Minor and Cianciolo, 2007). The authors also highlight a study by Makeschin (1994) who report higher earthworm and woodlice numbers in willow SRC plantations than in arable systems (but see Coates and Say, 1999), with willow SRC also having higher microbial biomass and soil faunal diversity compared to arable controls (Rowe *et al*., 2013). However, in contrast to willow, a study comparing *Miscanthus* and two other PECs (switchgrass and planted prairie) by Zangerl *et al*. (2013) found no difference in litter decomposition rates or arthropod abundance between PECs. Furthermore, the application of insecticide, which reduced arthropod abundance, did not result in differences in litter decomposition rates, inorganic nitrogen leaching or carbon- nitrogen ratios for any crop. Comparison of soil and epigeal invertebrate abundance by Bellamy *et al*. (2009) failed to find significant differences between *Miscanthus* and wheat for many taxa, although some were more abundant, for example, flies (Diptera), millipedes (Diploda), slugs and snails (Mollusca) were twice as abundant in *Miscanthus* and earthworm abundance significantly higher.

In depth investigation into the effects of PECs on microbial nitrogen cycling have been carried out by Mao *et al*. (2011) in Illinois, focusing on the impact of *Miscanthus* and switchgrass (*Panicum virgatum* Linnaeus) in comparison to first generation maize (*Triticum* spp.) and tallgrass prairie. During the first two years of crop establishment differing microbial communities developed in the crops with each functional group responding differently to land use change. The authors investigated the quantity and diversity of key functional genes involved in nitrogen cycling, namely *nifH*, bacterial/archaeal *amoA* and *nosZ*. 16S rRNA genes were also studied. The quantities of the nitrogen cycling genes remained stable in all crops except fertilised maize. Cultivation of N-fertilised maize had the greatest effect on nitrogen cycling communities with increases in the abundance of ammonia-oxidising bacteria (AOB; doubling in <3 months) and denitrifying bacteria, whilst the diversity of ammonia-oxidising Archaea (AOA), archaeal denitrifiers and diazotrophs decreased. Significant changes also occurred in the structure of the denitrification community. The fact that maize was the only crop fertilised implicates nitrogen fertilisation as having a role in these changes and the authors point to a previous study by Okano *et al*. (2004) that showed the rapid and long term effects of nitrogen fertilisation on AOB. AOA are suggested to be responsible for the nitrification rate in this crop due to the significant correlation with the quantity of archaeal *amoA* (Mao *et al*., 2011). Under *Miscanthus*, the abundance of the genus *Herbaspirillum* Baldani *et al*. increased. These species are known to be endophytic diazotrophs which benefit from cultivation of C4 grasses (Mao *et al*. 2011). The authors suggest that root exudates released by *Miscanthus* may be the reason for populations of this genus being favoured. It is also suggested that only specific diazotrophs are enhanced by *Miscanthus*, rather than the whole community.

A follow up study, Mao *et al*. (2013), investigated the effect of mature (established for 6 years) bioenergy crops on soil microbial communities. Although differences existed between crops in terms of the compositions and quantities of the microbial communities and functional N-cycling genes present, site-to-site variation surpassed this and no single crop type converged on a typified species assemblage. However, similar to the previous study, the largest differences at each site were found between maize and the two perennial grasses (Mao *et al*., 2013). Positive effects of *Miscanthus* and switch grass cultivation were shown, with significantly higher abundance of the *nifH* gene and total soil N compared to maize, the authors concluding that cultivation of perennial grasses as a bioenergy feedstock improve soil nitrogen sustainability over maize, increasing N-fixing bacteria.

Previous models of the growth dynamics of the four crops investigated by Mao *et al.* (2011, 2013) also showed that cultivation of *Miscanthus* would result in greatest GHG reductions, with biological nitrogen fixation contributing to the crops N demand (Davis *et al*., 2010). Unsurprisingly, first generation N-fertilised maize was also shown to be the worst GHG emitter, with 956-1899 g CO_{2eq} m^{-2} y^{-1} more emissions than the other bioenergy crops studied (Davis *et al*., 2010). Keymer and Kent (2014) have shown that in relation to recently established *Miscanthus* plants, ~16% of new plant nitrogen is likely to be derived from nitrogen fixation, even in conditions with non-limiting soil nitrogen. The authors suggest that breeding lines that maximise this trait could improve the nitrogen sustainability of this crop. Agricultural land is the dominant source of anthropogenic N_2O emissions (van Groenigen *et al*., 2011); the nitrogen sustainability of *Miscanthus* feedstock is therefore an evident benefit over 1st generation BEC maize. The ability of *Miscanthus* to associatively obtain nitrogen will better enable it to tolerate low nutrient soils, perhaps unsuitable for other crops, and will reduce fertiliser inputs.

Bioenergy crop cultivation along watercourses has further consequences for N cycling, reducing nutrient run-off from agricultural land (Börjesson, 1999). In a recent study, Ferrarini *et al*. (2017), investigated N-removal processes in willow and *Miscanthus* buffer strips of two different widths (5 and 10 m) along a soil-groundwater continuum; NO_3-N was effectively removed from groundwater by the buffer strips (62%- 5m and 80%-10m) and removal rate increased with elevated nitrate inputs from fertilised fields. Rhizosphere priming by the two crops stimulated microbial functioning by providing a bioaccessible source of dissolved organic carbon, leading to greater carbon in microbial biomass and microbial immobilisation of nitrogen (Ferrarini *et al*., 2017). Willow outperformed *Miscanthus* in terms of total and root biomass production, and willow harvesting resulted in greater nitrogen removal (the negative or positive connotations dependant on where and why the crops are grown). This may be useful when buffering watercourse from groundwater nitrogen pollution, but not beneficial in terms of productivity on low fertility land.

Although the nutrient requirements of willow are low compared to conventional arable, a study in Canada by Hangs *et al*. (2014) demonstrates that some inputs are likely to be necessary during the crops life cycle. Following a single coppice cycle of 4 years, deficits of 17, 39, 112, 271 and 74 kg ha^{-1} of N, P, K, Ca and Mg are reported for recently established plantations. Such studies investigating the response of a diverse range of soil nutrients to land use change are limited and there is a need for better understanding of the response of other elements, particularly in terms of bioaccessible concentrations relevant to soil biota.

1.9 Project Aims

Despite the growing influence of ecosystem services upon policy and decision making (Liss *et al*., 2013), there remain significant gaps in the understanding of how land use change from conventional arable to 2nd generation BECs may affect ecosystem processes and services (Manning *et al*., 2015). This study aims to begin to address gaps in the literature by investigating ecosystem processes in two commercial lignocellulosic BECs, *Miscanthus* and willow SRC, compared to rotational cereal crops, including patterns relevant to soil chemistry and bacterial communities.

Outline of chapter content:

Chapter 2: A study of the frequency of insect flower visitation (principally hoverflies, bumblebees and Lepidoptera) to wild plants in the margins of *Miscanthus* and willow fields. BECs are compared to adjacent and distant cereal controls, making it possible to investigate whether insect visitation in surrounding arable fields is locally enhanced or reduced by the cultivation of the two BECs.

Chapter 3: Following documentation of insect visitation patterns in chapter 2, seed-set is investigated as an indication of pollination success in *Miscanthus* and cereal control fields. Two morphologically distinct phytometers, Californian poppy (*Eschscholzia californica* Chamisso) and red clover (*Trifolium pratense* Linnaeus) are used alongside a common hedgerow plant, red campion (*Silene dioica* (Linnaeus) Clairville). Visitation rates to margin wildflower patches in *Miscanthus* are described for focal insect taxa. Due to the potential conservation value of MFCs, and following evidence of increased bumblebee visitation to margin wildflowers by Hanley *et al*. (2011), comparisons of seed-set are also presented for paired wheat and field bean (*Vicia faba* Linnaeus) crops in southwest England.

Chapter 4: Soil chemistry is investigated in *Miscanthus* and willow and compared to cereal controls, focusing specifically on the extractability of 28 different elements that might indicate biologically relevant effects of PEC land use change on biogeochemical processes and soil fertility. Bacterial communities are investigated to establish whether broad differences occur between BECs and conventional cereal crops.

Chapter 5: Following investigation of soil element and microbial communities, and building upon previous investigations in willow SRC by Rowe *et al*. (2013), this chapter looks to discern the effect of *Miscanthus* cultivation on the decomposition process of litter fragmentation. Litter degradation rates in BECs are compared to conventional cereals in each season.

Chapter 6: General discussion, bringing together findings across all chapters in the context of past research, outlining implications for ecosystem service provision, conservation, management and policy.

Chapter 2: Influence of bioenergy crops on pollinator activity varies with crop type and distance

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2.1 Abstract

Second-generation perennial energy crops (PECs) are generally correlated with increased biodiversity, compared to traditional annual crops, but experimental studies with adequate replication and controls are few. I expect pollinator visitation to wildflowers in crop margins to be augmented by PECs, but this process has not been tested. I tested this hypothesis in a 4 year study across multiple sites using two PECs which did not themselves constitute pollinator resources: *Miscanthus x giganteus* and willow short-rotation coppice. I recorded flower visits to PEC and cereal crop margins by three pollinator groups: hoverflies, bumblebees and butterflies/moths. Data on other flower visiting insects are also presented. Each PEC field was paired with two cereal fields, one adjacent to the PEC and one distant. I also measured floral resources, since crop-specific management seemed a likely means of influencing margin wildflowers and thus pollinator activity. My results add quantitative support to the suggestion that PECs should enhance ecosystem processes in agri-landscapes. However, benefits were highly context-dependent. Consistent enhancement of pollinator activity in margins of PEC fields was found for one species (willow) and not the other (*Miscanthus*). This arose from opposing trends for different pollinator taxa: Lepidoptera were more frequent flower visitors around *Miscanthus* than at cereal margins, while hoverfly flower visits were most frequent along margins of distant cereals. Results for willow were more consistent: flower visits were more frequent at willow than at cereal margins across all three focal pollinator taxa. Further, bumblebee and Lepidoptera species richness, Lepidoptera diversity, and preferred flowers were all higher in willow margins than cereal margins. Of the most frequent non-focal flower visiting taxa, *Scathophaga stercoraria* visits were significantly higher along distant cereal margins than the margins of *Miscanthus* or adjacent cereals, whilst *Rhagonycha fulva* visitation was significantly lower in distant cereals compared to willow and adjacent cereals. Network

parameters associated with plant-pollinator interaction indicated that willow networks were slightly more robust than cereals, whilst *Miscanthus* networks differed little to cereal controls. Future land-use practices should consider how PEC identity affects both target species and ecosystem processes. Tackling anthropogenic climate change through cultivation of willow, in particular, may yield local benefits for both wildflowers and pollinators, even if evidence for increased pollinator activity in the wider agri-environment is limited.

2.2 Introduction

A global decline in insect pollinator abundance and diversity is well established and associated ramifications for crop and wildflower pollination widely debated (Potts *et al.*, 2010; Vanbergen, 2013; De Palma *et al.*, 2016; Hallmann *et al*. 2017; but see Ghazoul 2005). The impact of agricultural intensification on insect pollinators like bees, has received particular attention, driving major declines in both wild and managed species (Goulson *et al.*, 2005; Biesmeijer *et al.*, 2006; Vanbergen, 2013). Other pollinators are also faring poorly; European hoverfly communities are represented by fewer species (Biesmeijer *et al.*, 2006) while European Lepidoptera have declined in abundance and distribution (van Swaay *et al.*, 2008; van Dyck *et al.*, 2009; Fox *et al.*, 2013, 2015). Conservation actions to halt and reverse these declines are necessary, both to retain biodiversity and the key ecosystem service (ES) pollinators provide (Gallai *et al.*, 2009; Goulson *et al.*, 2011).

Counter to historic trends, future land use changes could help reverse pollinator decline. Although the success of ‘pollinator-friendly’ agri-environment schemes (e.g. wildflower strips) are debated (Batáry *et al.*, 2015), and further research necessary (Wood *et al.*, 2016), effects on pollinator communities and associated ES provision appear largely positive (McCracken *et al.*, 2015). Organic farming has similarly been associated with benefits to pollinator communities (Holzschuh *et al.*, 2007; Tuck *et al.*, 2014), but the likely contribution of organic farms to the global food supply remains doubtful (Seufert *et al.*, 2012). Changes in conventional cropping systems may also contribute to pollinator conservation. The cultivation of mass-flowering crops (MFCs) such as oilseed rape (*Brassica napus*) and field bean (*Vicia faba*) has, for example, been linked to increased bumblebee abundance at the landscape-scale and more visits to wild flowers in the margins adjacent to the crop (Holzschuh *et al.*, 2007; Hanley *et al.*, 2011).

As part of a global commitment to reduce greenhouse gas emissions, the cultivation of bioenergy crops (BECs) is now widely practised. In particular, so-called ‘second generation’ lignocellulosic BECs such as the fast-growing perennial grass, *Miscanthus*, and short-rotation coppice (SRC) species (principally willow and poplar) are widely grown throughout Europe and North America (Somerville *et al.*, 2010). Although there is concern that perennial energy crops (PECs) may displace traditional food crops (Gelfand et al., 2013), it is widely held that PECs are locally beneficial for farmland biodiversity, due to the relatively low chemical inputs and disturbance regimes they require (Dauber et al., 2010; Rowe et al., 2011; Wiens et al., 2011; Bourke et al., 2014). In addition, key ecosystem processes such as decomposition and predation, are enhanced within the PEC compared to adjacent cereal crops (Rowe *et al.*, 2013). Taken together, these findings have led to the suggestion that the strategic location of PECs could boost local pollinator abundances and thus benefit ecosystem service provision, enhancing ecosystem processes (EPs) such as flower visitation to margin wildflowers, both within the field and in the local arable landscape (Manning *et al.*, 2015; see also Holland *et al.*, 2015; Milner *et al.*, 2016).

As an important ES in the agri-environment, any benefits accruing to pollinators and pollination from the strategic cultivation of PECs are of considerable importance, not least because of widespread concerns about global pollinator declines. Consequently, there is a pressing need to understand how pollinator communities respond to PEC cultivation (Rowe *et al.*, 2013; Manning *et al.*, 2015). Local pollinator spill-over from MFCs to adjacent field margin wildflowers (Hanley *et al.* 2011) offers a precedent to investigate whether PECs may similarly enhance pollination processes to margin flowers, both within the field and surrounding landscape. This hypothesis remains largely untested however. In this study I investigated the influence that *Miscanthus* and willow SRC cultivation had upon guild-specific pollinator visits to native plants in margins adjacent to the crop, and compared this to margins of traditional annual cereals in a replicated, paired design. Visitation within the PEC field is principally management driven rather than 'spill-over' of pollinators from the crops themselves, as the crops are either sterile (*Miscanthus*) or do not provide floral resources during the study period (willow). In addition, I investigated the potential for locally enhanced flower visitation in the surrounding landscape by comparing frequency of pollinator visits along the margins of adjacent and distant conventional cereal fields. It is understood that habitat modification (Tylianakis *et al*., 2007) and land use change (Weiner *et al*., 2014) can alter properties of insect interaction networks with potential effects on ecological functioning; (Valiente-Banuet *et al.*, 2014) consequently I also compare PEC to cereals using a selection of indices that describe the architecture of insect plant visitation networks. All surveys were undertaken in England, United Kingdom.

2.3 Materials and methods

2.3.1 Study crop and sites

Miscanthus x giganteus is a perennial grass of Asian origin; a sterile hybrid of *M. sinensis* and *M. sacchariflorus*. Despite its C4 physiology, *Miscanthus* produces good yields in temperate climates averaging between 12-16 ODT (oven dried tonnes) ha^{-1} in England and growth rates in excess of 2.5 m per year (DEFRA, 2007). The rhizome remains viable in the soil for 15-20 years, reducing tillage and soil disturbance. Moreover, unlike conventional arable crops, *Miscanthus* has a low requirement for agrochemical inputs; high nitrogen-use efficiency enables nutrient recycling back to the rhizome or soil (as leaf-litter) prior to winter/spring harvest. Willow (typically *Salix viminalis*) SRC re-grows rapidly from stools over a typical 3-4year coppice cycle (DEFRA, 2004). Willow is densely planted with ~15,000 trees ha^{-1}; an erect growth habit produces high yields (up to 12 ODT ha^{-1} year^{-1}) for up to 30 years (DEFRA, 2004).

Surveys of crop margin visits by pollinators for *Miscanthus* centred on six locations in southwest England, and for willow SRC, five locations in central England; i.e. Nottinghamshire and Lincolnshire (Table 2.1; Plate 2.1). Each site comprised three fields (Fig. 2.1), a focal PEC, an adjacent cereal and a distant cereal situated a minimum of 920 m from the focal PEC or any other BEC or MFC (based on field centres); this distance is thought to minimise non-independence of mobile bumblebee pollinators (Knight et al., 2005). As far as possible, cereal fields in each 'triplicate' were matched with the focal PEC for margin characteristics, field area, slope, altitude and aspect and for the most part were represented by wheat (*Triticum* spp.) with barley (*Hordeum vulgare* Linnaeus) as the alternative where wheat was unavailable. Both crops are principally wind-pollinated, hence not themselves key floral resources, and represent the two most commonly cultivated crops in the UK (DEFRA 2015b). Investigation of *Miscanthus* and cereal controls involved fieldwork in 24 fields across 3 years (2012, 2014 and 2015), whilst 22 fields were studied across 2 years (2013 and 2015) for comparison of willow and controls. During data collection it was established that treatments did not differ significantly in terms of relative land cover classes in the landscape (1 km radius), wind speed, cloud cover or temperature (Appendix 1- 10).

Table 2.1: Locations (approximate field centres) and characteristics of study sites in southwest and central England. Coordinates: WGS 1984. Information obtained using Google Earth (Google Earth, 2017). N, S, E and W are representative of cardinal directions.

Year	Location	Crop	Lat. Lon.	Field Area (ha)	Altitude (m)	Aspect
2012	Buckfastleigh, Devon	Miscanthus	50° 28.330'N, 3° 47.621'W	12.6	95	SE
		Adjacent Cereal	50° 28.532'N, 3° 47.487'W	20	101	SE
		Distant Cereal	50° 26.144'N, 3° 47.016'W	5	133	SE
	Pelynt, Cornwall	Miscanthus	50° 21.886'N, 4° 31.222'W	4.8	142	S
		Adjacent Cereal	50° 21.916'N, 4° 31.118'W	4.4	145	S
		Distant Cereal	50° 22.925'N, 4° 31.239'W	4.2	121	NW
	St Minver, Cornwall	Miscanthus	50° 33.124'N, 4° 51.373'W	3.8	44	SW
		Adjacent Cereal	50° 33.240'N, 4° 51.334'W	5.6	53	SW
		Distant Cereal	50° 32.434'N, 4° 51.050'W	5.5	17	NW
	Wadebridge, Cornwall	Miscanthus	50° 31.518'N, 4° 46.450'W	2.2	106	NW
		Adjacent Cereal	50° 31.551'N, 4° 46.288'W	4.3	103	N
		Distant Cereal	50° 31.529'N, 4° 44.517'W	6.1	100	SW
2014	Egloshayle, Cornwall	Miscanthus	50° 31.560'N, 4° 48.191'W	7.1	76	W
		Adjacent Cereal	50° 31.560'N, 4° 48.024'W	7.9	75	SE
		Distant Cereal	50° 31.021'N, 4° 49.055'W	7.8	42	SW
	Pelynt, Cornwall	Miscanthus	50° 21.886'N, 4° 31.222'W	4.8	142	S
		Adjacent Cereal	50° 21.916'N, 4° 31.118'W	4.4	145	S
		Distant Cereal	50° 21.831'N, 4° 29.760'W	4.7	102	SE
	St Minver, Cornwall	Miscanthus	50° 33.124'N, 4° 51.373'W	3.8	44	SW
		Adjacent Cereal	50° 33.240'N, 4° 51.334'W	5.6	53	SW
		Distant Cereal	50° 33.226'N, 4° 50.177'W	2.9	18	NE
	Wadebridge, Cornwall	Miscanthus	50° 31.518'N, 4° 46.450'W	2.2	106	NW
		Adjacent Cereal	50° 31.551'N, 4° 46.288'W	4.3	103	N
		Distant Cereal	50° 31.380'N, 4° 44.462'W	4.3	100	W
2015	Lostwithiel, Cornwall	Miscanthus	50° 25.704'N, 4° 37.887'W	5.6	113	SW
		Adjacent Cereal	50° 25.857'N, 4° 37.483'W	7.1	134	NE
		Distant Cereal	50° 25.811'N, 4° 35.418'W	2.6	163	NW
	Pelynt, Cornwall	Miscanthus	50° 21.886'N, 4° 31.222'W	4.8	142	S
		Adjacent Cereal	50° 21.916'N, 4° 31.118'W	4.4	145	S
		Distant Cereal	50° 21.901'N, 4° 29.433'W	5.9	84	NE
	St Minver, Cornwall	Miscanthus	50° 33.076'N, 4° 51.233'W	5.4	47	SW
		Adjacent Cereal	50° 33.100'N, 4° 50.914'W	9.3	55	SE
		Distant Cereal	50° 33.226'N, 4° 50.177'W	2.9	18	NE
	Wadebridge, Cornwall	Miscanthus	50° 31.518'N, 4° 46.450'W	2.2	106	NW
		Adjacent Cereal	50° 31.551'N, 4° 46.288'W	4.3	103	N
		Distant Cereal	50° 31.380'N, 4° 44.462'W	4.3	100	W

Table 2.1 continued: Locations (approximate field centres) and characteristics of study sites in southwest and central England. Coordinates: WGS 1984. Information obtained using Google Earth (Google Earth, 2017). N, S, E and W are representative of cardinal directions.

Year	Location	Crop	Lat. Lon.	Field Area (ha)	Altitude (m)	Aspect
2013	Newark, Nottinghamshire	Willow	53° 15.122'N, 0° 49.430'W	8.18	17	SW
		Adjacent Cereal	53° 15.089'N, 0° 49.257'W	7.35	21	S
		Distant Cereal	53° 14.835'N, 0° 48.686'W	10.96	10	SE
	Gainsborough, Lincolnshire	Willow	53° 26.036'N, 0° 48.150'W	5.49	2	E
		Adjacent Cereal	53° 25.898'N, 0° 48.098'W	8.93	2	E
		Distant Cereal	53° 27.172'N, 0° 47.815'W	7.8	5	SE
	Stapleford, Nottinghamshire	Willow	53° 6.590'N, 0° 43.029'W	11.01	17	E
		Adjacent Cereal	53° 6.557'N, 0° 42.898'W	7.82	18	S
		Distant Cereal	53° 6.703'N, 0° 42.037'W	9.36	16	SE
	Retford, Nottinghamshire	Willow	53° 16.490'N, 0° 59.394'W	7.49	40	SW
		Adjacent Cereal	53° 16.490'N, 0° 59.669'W	6.19	36	SW
		Distant Cereal	53° 16.951'N, 0° 59.071'W	8.84	37	NE
2015	Newark, Nottinghamshire	Willow	53° 14.758'N, 0° 49.295'W	13.1	15	SW
		Adjacent Cereal	53° 14.820'N, 0° 49.130'W	11.8	15	SE
		Distant Cereal	53° 12.814'N, 0° 49.838'W	5.1	27	E
	Gainsborough, Lincolnshire	Willow	53° 26.036'N, 0° 48.076'W	8.8	2	E
		Adjacent Cereal	53° 26.044'N, 0° 48.252'W	2.1	2	W
		Distant Cereal	53° 27.172'N, 0° 47.815'W	2	5	SE
	Stapleford, Nottinghamshire	Willow	53° 6.590'N, 0° 43.029'W	11.1	17	E
		Adjacent Cereal	53° 6.557'N, 0° 42.898'W	7.7	18	S
		Distant Cereal	53° 6.015'N, 0° 42.597'W	13	14	SW
	Whatton, Nottinghamshire	Willow	52° 55.840'N, 0° 54.436'W	4.6	30	NW
		Adjacent Cereal	52° 55.946'N, 0° 54.436'W	6.2	30	NW
		Distant Cereal	52° 56.489'N, 0° 54.342'W	14.2	26	NW

Plate 2.1: Perennial energy crops (top) and cereal control crops (bottom). From top left to bottom right: willow SRC (*Salix* spp.); *Miscanthus x giganteus*, wheat (*Triticum* spp.) and barley (*Hordeum vulgare*) (Photographs: N. Berkley).

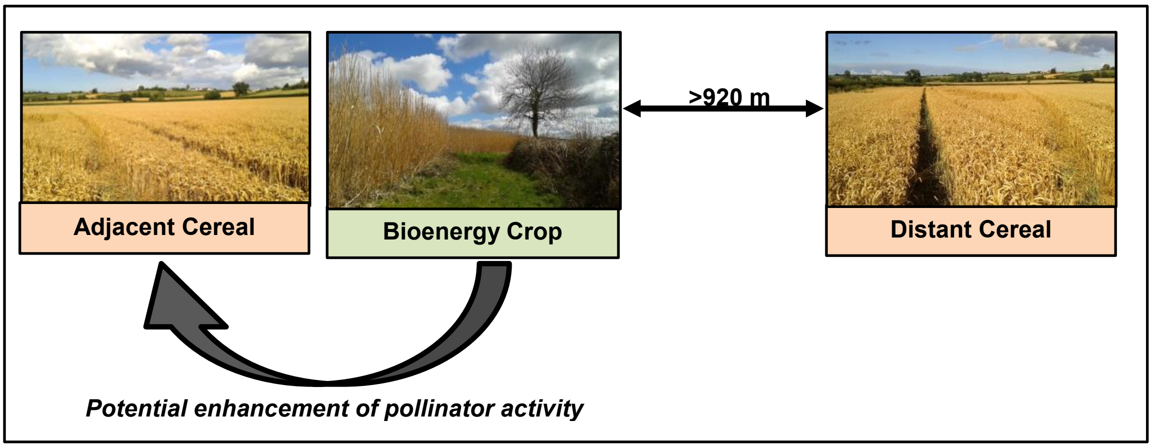

Figure 2.1: Triplicate experimental design. Figure shows field arrangement used at each site in both southwest and central England in order to determine potential local enhancement of flower visitor activity in the margins of cereal fields neighbouring bioenergy crops.

2.3.2 Pollinator surveys

Investigation of pollinator activity along crop margins followed the methods outlined by Hanley *et al*. (2011) and Hanley & Wilkins (2015). For *Miscanthus*, 50 m × 2 m transects were set out along the centre part of opposing margins in each field, with equivalent margin aspect between the three crop fields comprising each site; 100 m × 2 m transects were walked in SRC fields. Differences in transect length were purely for logistical reasons and consistent across control fields for each PEC. In southwest England (*Miscanthus*) field boundaries were comprised of established hedgerows dominated by native woody plants e.g., hawthorn (*Crataegus monogyna* Jacquin), English oak (*Quercus robur* Linnaeus), blackthorn (*Prunus spinosa* Linnaeus), dog rose (*Rosa canina* Linnaeus), blackberry(*Rubus fruticosus* Linnaeus agg.), and gorse (*Ulex europaeus* Linnaeus) with a naturally colonising, diverse basal flora including cock's-foot (*Dactylis glomerata* Linnaeus), foxglove (*Digitalis purpurea* Linnaeus), cleavers (*Galium aparine* Linnaeus), herb Robert (*Geranium robertianum* Linnaeus), hogweed (*Heracleum sphondylium* Linnaeus), creeping buttercup (*Ranunculus repens* Linnaeus), red campion (*Silene dioica*) hedge woundwort (*Stachys sylvatica* Linnaeus), and common nettle (*Urtica dioca* Linnaeus). Current UK agricultural policy requires a 1m border between the field boundary and crop edge; for our fields this border was comprised of perennial herb and grass species typical of hedgerow margins. This border, plus an additional 1m extending into a point about half way into the hedgerow, formed the 2m width of our transects in SW England. In Nottinghamshire and Lincolnshire (SRC) field margins were dominated by hawthorn (*C. monogyna*). Species composition was similar to southwest England although thistles were a dominant component (e.g. spear thistle (*Cirsium vulgare* (Savi) Tenore) & meadow thistle (*C. dissectum* (Linnaeus) Hill)). Fields at Gainsborough did not contain hedgerows;

uncultivated field margins were therefore compared using 2m wide transects from the crop edge, delimited by ditches.

Miscanthus transects encompassed walks in both the morning and afternoon between the hours of 09:00 and 16:00 on each of 3 or 4 separate occasions beginning late June, through July and August, on days favourable to pollinator activity (Goulson and Darvill 2004). The longer willow transects were walked once per day between 10:00 and 18:00. Due to separation between sites, it was impossible to survey all fields in a single day, but it was ensured that fields within each triplicate were surveyed during the same day, with the relative order randomly assigned.

We identified and recorded actively foraging insects (i.e. observed visiting an inflorescence) belonging to three major pollinators groups, hoverflies, bumblebees and Lepidoptera (Plates: 2.2-2.4), together with the plants upon which they foraged. Generally, individuals were identified to species-level. However, due to the difficulty of separating workers of the subgenus *Bombus* s. str. (i.e. buff-tailed bumblebee (*Bombus terrestris* (Linnaeus)), white-tailed bumblebee (*B. lucorum* (Linneaus)), northern white-tailed bumblebee (*B. magnus* Vogt) and the cryptic white-tailed bumblebee (*B. cryptarum* (Fabricius)) in the field (Williams *et al.* 2012), no attempt was made to distinguish between these species and throughout this group is collectively referred to as *B. terrestris* agg. Although not the study's focus, insects outside the focal guilds were also recorded, except in central England in 2013. The limited taxonomic resolution of these 'other taxa' (Plate: 2.5) precluded the analysis of richness and diversity but patterns of flower visitation frequency are presented for highly recorded taxa. Small black flower beetles (Meligethinae Thomson) were excluded from analysis as they were localised to single plants but exhibited high abundance, likely to skew overall trends. Foraging insects were not captured, but because transects were linear and completed relatively rapidly, it is extremely unlikely that the same individual was recorded more than once during each transect walk. A total of 564 insect visitation transects were undertaken.

Immediately after completing insect surveys, I estimated the number of flowers of each plant species likely to be visited by pollinators along each transect to determine variation in floral resource availability. Estimates for total flower number were achieved by counting the number of flowers on 10 separate inflorescences of a given plant species and then to multiply this mean value by the estimated total number of inflorescences observed along the transect. For Apiaceae and Asteraceae, an umbel and a capitulum were each considered to be a single ‘flowering unit’.

Plate 2.2: Common butterflies found in English arable field margins. From top left to bottom right: comma (*Polygonia c-album* (Linnaeus)); small skipper (*Thymelicus sylvestris* (Poda)); speckled wood (*Pararge aegeria* (Linnaeus); small tortoiseshell (*Aglais urticae* (Linnaeus)); common blue (*Polyommatus icarus* (Rottemburg)) and brimstone (*Gonepteryx rhamni* (Linnaeus)) (Photographs: N. Berkley).

Plate 2.3: Common bumblebee species found in English arable field margins. From top left to bottom right: common carder-bee (*Bombus pascuorum* (Scopoli)); buff-tailed bumblebee (*Bombus terrestris*); tree bumblebees (*Bombus hypnorum* (Linnaeus)); red-tailed bumblebee (*Bombus lapidarius* (Linnaeus)); vestal cuckoo bumblebee (*Bombus* (*Psithyrus*) *vestalis* (Geoffroy in Fourcroy)) and garden bumblebee (*Bombus hortorum* (Linnaeus)) (Photographs: N. Berkley).

Plate 2.4: Common hoverfly species found in English arable field margins. From top left to bottom right: *Merodon equestris* (Fabricius); marmalade hoverfly (*Episyrphus balteatus* (De Geer)); *Sphaerophoria* sp. Le Peletier & Serville; pellucid hoverfly (*Volucella pellucens* (Linnaeus)); *Rhingia campestris* Meigen and *Syritta pipiens* (Linnaeus) (Photographs: N. Berkley).

Plate 2.5: Examples of additional flower visitors to flowers in English arable field margins. From top left to bottom right: solitary bees such as *Colletes* sp. Latreille; *Empis tessellata* Fabricius; broad centurion (*Chloromyia formosa* (Scopoli)); flesh fly (*Sarcophaga* sp. Meigen); honey bee (*Apis mellifera*) and yellow dung fly (*Scathophaga stercoraria* (Linnaeus)) (Photographs: N. Berkley).

2.3.3 Statistical analysis

Following graphical and statistical (Shapiro-Wilk) consideration of normality and homogeneity of variance (Levene's test), one-way ANOVA or Kruskal-Wallis tests were conducted to investigate 'crop type' effects. Where appropriate, non-normal data were $\mathrm{Log}_{10}+1$ transformed in preference to non-parametric analyses; Welch's ANOVAs were run for normal but heteroskedastic data. It was not possible to use repeated measures analysis s because the crops present at some field sites differed between years (i.e. PECs were removed at certain sites or became unusable when cereal controls were rotated to alternative crops); sites with data across multiple years were therefore averaged across years for each site-crop combination, with either the mean proportion of flower visits, species richness, diversity or flower number acting as the replicate in the analyses. Tukey's HSD and pairwise comparisons were used respectively for parametric and non-parametric post-hoc analyses. Shannon-Wiener Diversity indices were used as a measure of diversity for species level identifications only. Species richness data encompassed all unique taxa including those at higher resolution than species, when no overlap occurred with species level identifications. Analyses were carried out using SPSS (version 22, IBM Inc.). Generation and analysis of quantitative insect-plant interaction networks was carried out using the bipartite package (Dormann *et al.*, 2009) in R (R Core Team, 2017). The following network indices were generated for PEC and distant cereal controls, connectance, generality, H2, interaction evenness, links per species, nestedness, specialisation asymmetry, vulnerability and web asymmetry (Dormann *et al.*, 2009). For southwest data, *t*-tests were used to compare site indices for *Miscanthus* and distant cereal treatments with means used for sites visited in multiple years. Due to an absence of species level floral data in 2013, *t*-tests in central England only incorporated network data from sites visited in 2015.

2.4 Results

In total, 7747 insects belonging to the three target taxa (hoverflies, bumblebees, Lepidoptera) were observed visiting flowers in the margins of the three crop types studied. Across treatments there were 52 and 55 insect ‘species’ (unique taxa) described for focal pollinator taxa in central and southwest England, respectively. For bumblebee and Lepidoptera pollinator guilds, species richness was significantly higher next to willow than along distant cereal controls. Shannon-Wiener indices also revealed that Lepidoptera diversity was significantly higher in the margins of willow than of adjacent and distant cereals respectively (Table 2.2). *Miscanthus* exhibited significantly higher Lepidoptera richness than distant cereals. However, no differences in species richness or diversity were seen between *Miscanthus* and cereal controls for any other taxa.

Table 2.2: Species richness and Shannon-Wiener diversity across triplicate fields for each PEC. Mean of site counts, standard error of the mean (S.E.) and output of statistical analysis are given for each focal pollinator guild that was recorded visiting flowers, as well as the respective floral make-up, for each of the triplicate fields - margins, adjacent cereal and distant cereal - for both *Miscanthus x giganteus* and willow short-rotation coppice bioenergy crops.

		Hoverfly				Bumblebee				Lepidoptera				Margin Wildflowers			
		Mean	**S.E.**	**Test Output**	***P***	**Mean**	**S.E.**	**Test Output**	***P***	**Mean**	**S.E.**	**Test Output**	***P***	**Mean**	**S.E.**	**Test Output**	***P***
Species Richness	Willow	8	0.88	$F_{2,12}$= 3.053	0.085	5.1^{a}	0.29	$F_{2,12}$= 4.657	0.032	9.4^{a}	0.98	$H_{2,12}$= 10.231	0.006	18.8	3.35	$F_{2,9}$= 0.661	0.54
	Adjacent Cereal	5.3	0.97			3.7^{b}	0.3			$4.9^{a,b}$	0.87			15.5	2.96		
	Distant Cereal	5.9	0.51			$4.1^{a,b}$	0.4			3.8^{b}	0.46			14.5	1.55		
	Miscanthus	6.6	1.13	$F_{2,15}$= 0.544	0.591	3.6	0.6	$F_{2,15}$= 0.147	0.865	3.7^{a}	0.92	$F_{2,15}$= 5.857	0.013	18.1	1.8	$F_{2,15}$= 0.612	0.555
	Adjacent Cereal	6.2	0.84			3.5	0.59			$2.3^{a,b}$	0.38			16.8	1.05		
	Distant Cereal	7.8	1.38			3.2	0.34			1.1^{b}	0.48			19.2	1.59		
Species Diversity	Willow	0.92	0.08	$F_{2,12}$= 0.486	0.627	1.11	0.05	$H_{2,12}$= 4.16	0.125	1.73^{a}	0.17	$F_{2,12}$= 5.288	0.023	1.68	0.18	$F_{2,9}$= 0.712	0.516
	Adjacent Cereal	0.78	0.1			0.86	0.11			$1.22^{a,b}$	0.16			1.75	0.18		
	Distant Cereal	0.85	0.12			0.83	0.21			1.07^{b}	0.12			1.95	0.14		
	Miscanthus	1.33	0.2	$F_{2,15}$= 0.728	0.499	1	0.17	$F_{2,15}$= 0.105	0.901	0.87	0.22	$F_{2,15}$= 2.228	0.142	1.05	0.19	$F_{2,15}$= 0.851	0.447
	Adjacent Cereal	1.11	0.11			0.92	0.2			0.57	0.16			1.24	0.23		
	Distant Cereal	1.11	0.11			0.91	0.1			0.31	0.18			1.46	0.25		

α=0.05, different letters indicate significant difference between treatment means derived from Tukey's post-hoc test or pairwise comparison.

Of the three target insect taxa, the majority of individuals observed in southwest England (*n*= 2531 total flower visiting insects) were hoverflies (76%), followed by bumblebees (17%) and Lepidoptera (7%). In central England (*n*= 5,216 total flower visiting insects), hoverflies (49%) and bumblebees (35%) dominated, with Lepidoptera much less common (16%). Bumblebees represented the vast majority (88.2%) of bees for both regions and PECs, and all analyses of bees focus on this group only. *Episyrphus balteatus* was by far the most common of the hoverflies (62% of all observations), but the relative abundance of hoverflies in general (and this species in particular) varied considerably with respect to PEC type.

The top five most visited plant species accounted for at least 50% of visits; often exceeding 80% of flower visits for some taxa in certain treatments (Appendix 11-16). In southwest England most visits were to hogweed (*Heracleum sphondylium*) (27.4%), whilst in central England most visits were to spear thistle (*Cirsium vulgare*) (20.3%). The most visited plants were not the most regionally abundant however; hedge bedstraw (*Galium mollugo* L.) dominated in southwest England with 64.5% of flowers and *H. sphondylium* had greatest floral abundance in central England, accounting for 15.3% of flowers.

When all three pollinator taxa were considered together, cumulative margin flower visits were not seen to differ between *Miscanthus* and cereal controls, ($F_{2,15}$= 3.262; P= 0.067; Appendix 17). This was not the case between willow and cereals however, ($F_{2,12}$= 20.256; P= < 0.001), with multiple comparisons demonstrating significantly higher pollinator flower visitation in willow when compared to both adjacent (P= 0.001) and distant (P= < 0.001) cereals, with no difference between controls (P= 0.903; Appendix 17). In order to determine if all three taxa exhibited a similar lack of response to *Miscanthus*, and to establish which taxa were driving the elevated flower visits in willow, further taxonomic breakdown of flower visitation was conducted.

2.4.1 Hoverflies

A significant ‘crop type’ effect was found for flower-visiting hoverflies in *Miscanthus* field triplicates, ($F_{2,15}$= 9.2; P= 0.002). Multiple comparisons revealed significantly greater flower visits in the field margins of distant cereals than those of *Miscanthus* (Fig. 2.2a), there were no significant differences between *Miscanthus* and adjacent cereal margins or between cereal controls. For willow, multiple comparisons revealed that a significant ‘crop type’ effect, ($F_{2,12}$= 7.216; P= 0.009), arose due to greater hoverfly visitation in the PEC margins compared to both distant cereal controls and adjacent cereal controls with no difference between the two controls (Fig. 2.3a).

2.4.2 Bumblebees

Abundances of bumblebees actively visiting flowers was significantly different among willow and cereal controls (Fig. 2.3b, $F_{2,12}$= 27.653; P= <0.001), but varied remarkably little between *Miscanthus* and cereal crops, (Fig. 2.2b, $F_{2,\ 9.507=}$ 0.832; P= 0.465). Pairwise testing across willow triplicates highlighted a more than three-fold significant increase in bumblebee visitation along SRC margins, compared to both adjacent- and distant cereals (Fig. 2.3b). *B. terrestris* agg. dominated bumblebee visits in all treatments (with 54.2% of visits). Red tailed bumblebees (*B. lapidarius*, with 14.9% of visits) and common carder bees (*B. pascuorum*, with 12.2% of visits) were relatively common, but patchily distributed.

2.4.3 Lepidoptera

Lepidoptera were more responsive to PEC cultivation than the other two guilds. A significant ‘crop type’ effect was found in *Miscanthus* (Fig. 2.2c, $F_{2,15=}$ 10.508; P= 0.001) and in willow (Fig.2.3c, $F_{2,12}$= 108.387; P= < 0.001). For both willow and *Miscanthus*, Lepidoptera were significantly more abundant floral visitors along PEC margins than along margins of distant cereals. In willow, a significant difference additionally emerged between margins of willow and adjacent cereal fields. In central

England, three species represented >45% of visits with meadow brown (*Maniola jurtina* (Linnaeus)) comprising 16.6%, small white (*Pieris rapae* (Linnaeus)) 15% and *Aglais urticae* 13.5% of Lepidoptera floral visits. Gatekeeper (*Pyronia tithonus* (Linnaeus)) had highest overall abundance in the southwest, accounting for 20% of flower visits.

2.4.4 Other taxa

A total of 8283 non-focal insect taxa were recorded visiting flowers, 5451 in *Miscanthus* triplicates across the years of 2012, 2014, 2015 and 2832 in willow field triplicates in 2015. Across all crop types and in all regions, most insects belonged to the orders Diptera, Coleoptera and Hemiptera. The most frequent visitor in willow triplicates were unidentified Diptera followed by *Empis tessellata*, the latter accounting for 50% of all non-syrphid dipterans and cumulatively both groups account for 69% of all non-focal taxa. Of the most frequent insect taxa accounting for 80% of visits, margin flower visitation frequency of common red soldier beetle (*Rhagonycha fulva* (Scopoli)) was significantly lower in distant cereals than willow and adjacent cereal (Table 2.3), visitation by other taxa did not differ significantly. In the southwest, unidentified Diptera were again most frequent but *E. tessellata* visitation is surpassed by *R. fulva* (18% of non-focal visits) and *Scathophaga stercoraria* (12% of non-focal visits). Flower visits by *S. stercoraria* were significantly higher in distant cereal controls compared to *Miscanthus* and adjacent cereals (Table 2.4), visitation did not differ significantly for other taxa contributing to the top 80% of visits.

2.4.5 Floral resources

There were a total of 93 flowering plant species recorded along transects in southwest England and 59 in central England. Floral availability did not vary between *Miscanthus* and cereal controls, ($H_{2,15}$= 0.082; *P*= 0.96), but did differ significantly between willow and cereal controls, ($F_{2,9}$= 9.001; *P*= 0.007). Post-hoc comparisons showed significant differences between both willow and distant cereal (*P*= 0.01) and willow and adjacent cereal (*P*= 0.016) with greater floral abundance in willow. Flowers of species important to focal pollinator guilds (i.e. the 2-8 plant species accounting for 80% of visits; Appendix 11-16) were more abundant in willow margins for flowers visited by bumblebees ($F_{2,9}$= 11.259; *P*= 0.004) and Lepidoptera ($F_{2,9}$= 11.401; *P*= 0.003), compared to adjacent (*P*= 0.025) and distant controls (*P*= 0.003) (Appendix 20; 26;27). Although non-significant, flower counts of plant species receiving 80% of hoverfly visits were 3 and 4 fold higher in willow than adjacent and distant cereals respectively (Appendix 25). Trends are similar for each pollinator group as similar subsets of plants were preferred across pollinator guilds. The dominant individual pollinator species for bumblebees (*B. terrestris* agg.) and for butterflies (*M. jurtina*) similarly had access to significantly higher abundances of their preferred flowers (*C. vulgare*; *R. fruticosus*; *H. sphondylium*; *C. dissectum*; *Trifolium repens* Linnaeus) in willow compared to adjacent and distant cereals (P= < 0.05; Appendix 23). Flowers highly visited by non-focal taxa did not differ in availability between crop types.

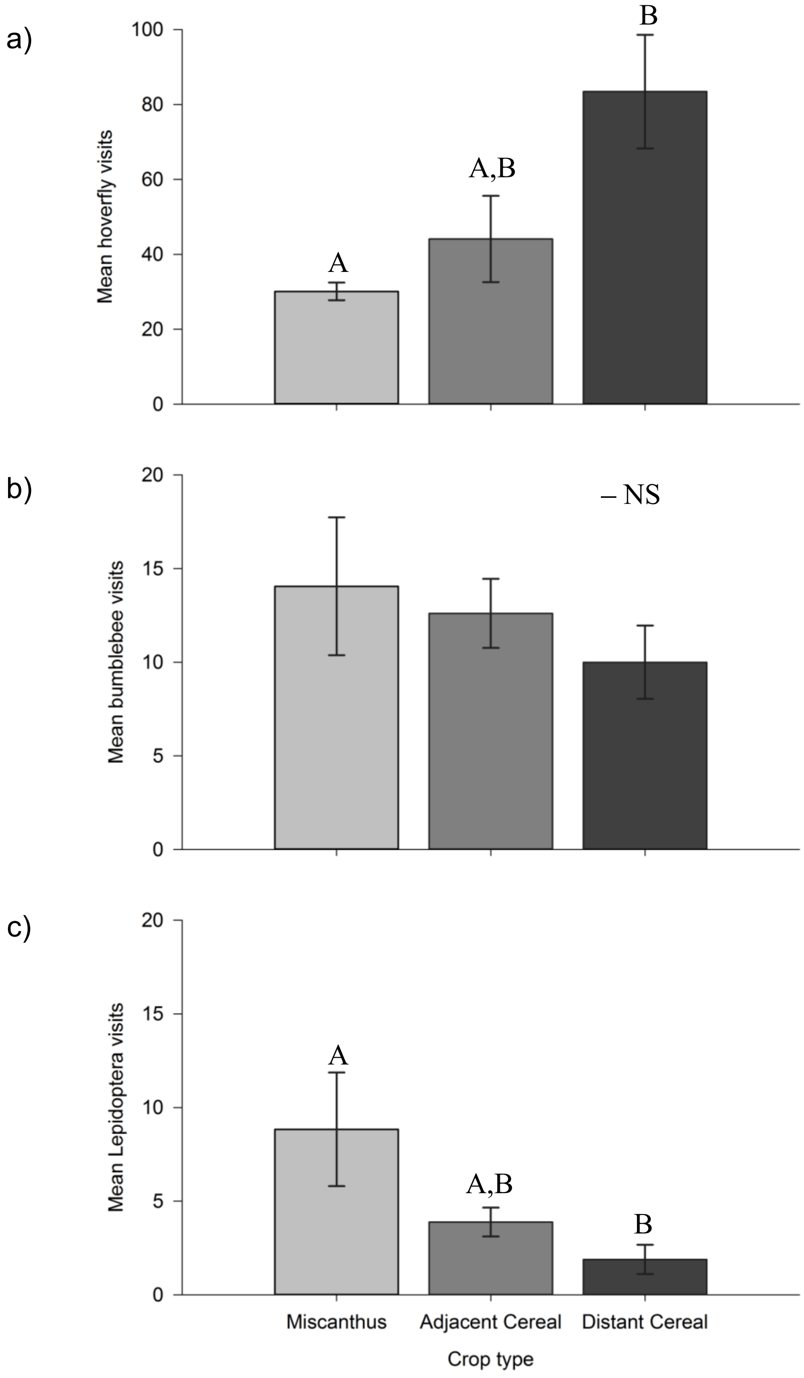

Figure 2.2: Mean (± standard error, S.E.) margin wildflower visits by target insect guilds across triplicate fields, *Miscanthus x giganteus*, adjacent- and distant-cereals, at sites in southwest England. Different letters indicate significant differences between treatment means ($P < 0.05$). NS= non-significant.

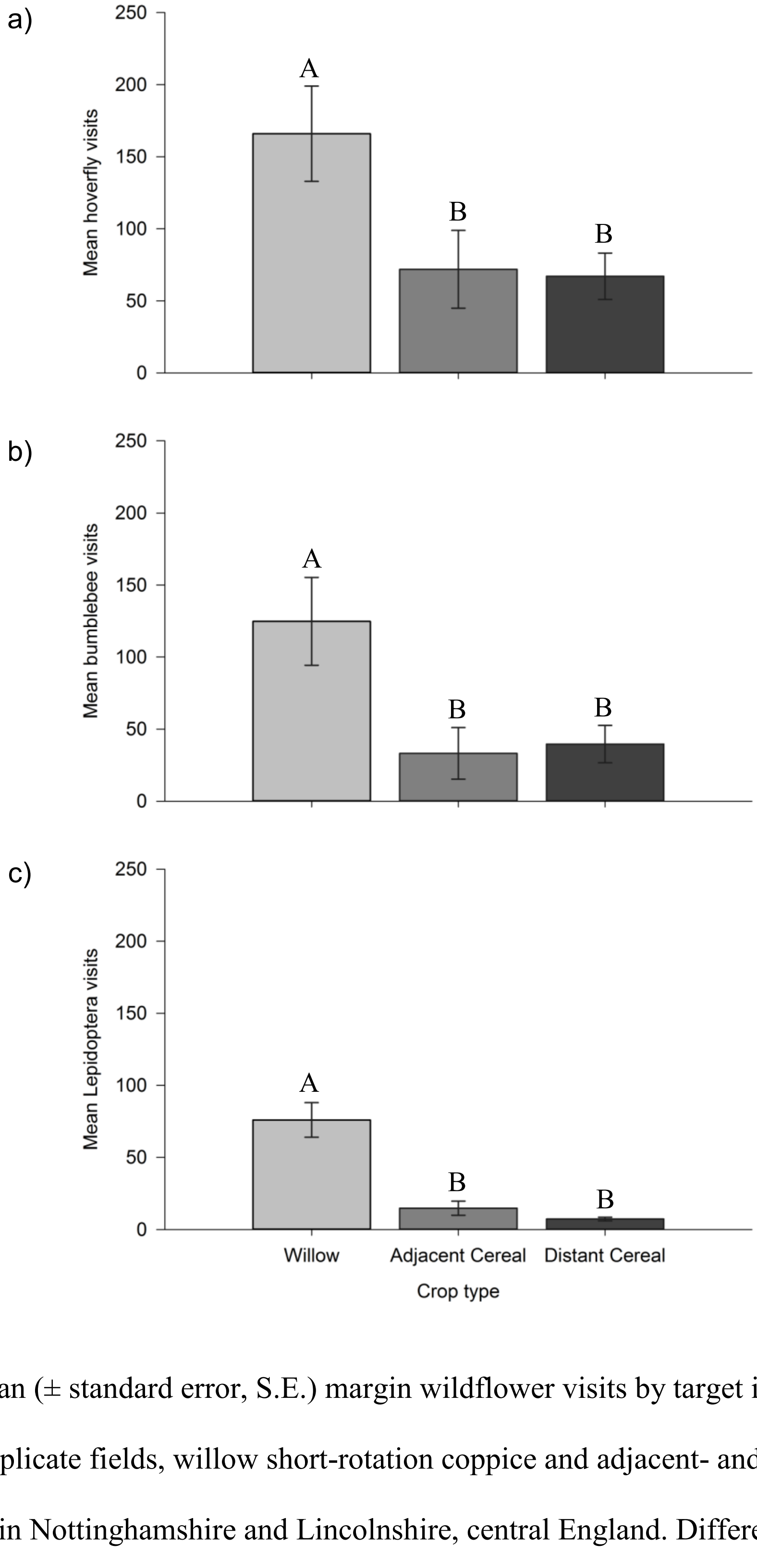

Figure 2.3: Mean (± standard error, S.E.) margin wildflower visits by target insect guilds across triplicate fields, willow short-rotation coppice and adjacent- and distant-cereals, at sites in Nottinghamshire and Lincolnshire, central England. Different letters indicate significant differences between treatment means ($P < 0.05$).

Table 2.3: Mean site flower visitation counts of non-focal pollinator taxa recorded visiting wildflowers in the margins of *Miscanthus x giganteus* and cereal controls in southwest England during the summers of 2012, 2014 and 2015. α= 0.05.

	Other Insects (Non-focal Taxa)				***Rhagonycha fulva***			
	Mean	**S.E.**	**Test Output**	***P***	**Mean**	**S.E.**	**Test Output**	***P***
Miscanthus	98.06	13.08			18.39	5.16		
Adjacent Cereal	143.00	51.83	$F_{2,15}$= 1.608	P = 0.233	15.78	10.74	$F_{2,7.991}$= 3.654	P = 0.075
Distant Cereal	186.67	36.55			30.67	15.78		
	Scathophaga stercoraria				***Empis tessellata***			
Miscanthus	8.89^{a}	3.61			13.33	2.60		
Adjacent Cereal	13.06^{a}	6.23	$F_{2,15}$= 9.807	P = 0.002	9.06	2.05	$F_{2,15}$= 0.905	P = 0.425
Distant Cereal	23.94^{b}	7.49			14.11	3.35		
	Coleoptera				***Oedemera nobilis***			
Miscanthus	37.06	8.73			11.28	3.64		
Adjacent Cereal	27.28	13.53	$F_{2,15}$= 3.26	P = 0.067	3.56	1.32	$F_{2,15}$= 2.575	P = 0.109
Distant Cereal	58.61	21.66			4.94	1.62		
	Diptera (Non-syrphid)							
Miscanthus	53.94	10.12						
Adjacent Cereal	102.39	45.34	$F_{2,15}$= 1.317	P = 0.297				
Distant Cereal	113.61	24.83						

Table 2.4: Mean site flower visitation counts of non-focal pollinator taxa recorded visiting wildflowers in the margins of willow short-rotation coppice and cereal controls in central England during the summers of 2013 and 2015. α= 0.05.

	Other Insects (Non-focal taxa)				**Diptera (Non-syrphid)**			
	Mean	**S.E.**	**Test Output**	***P***	**Mean**	**S.E.**	**Test Output**	***P***
Willow	275	31.44			187.75	28.77		
Adjacent Cereal	250.5	81.40	$F_{2,9}= 1.289$	$P = 0.322$	170.75	99.37	$F_{2,9}= 0.485$	$P = 0.631$
Distant Cereal	182.5	27.21			146.75	20.02		
	Empis tessellata				***Rhagonycha fulva***			
Willow	67.5	24.67			51^{a}	15.06		
Adjacent Cereal	55.75	23.54	$F_{2,9}= 2.765$	$P = 0.116$	53.5^{a}	38.52	$F_{2,4.09}= 14.495$	$P = 0.014$
Distant Cereal	119.75	25.28			4^{b}	3.37		

2.4.6 Visitation networks

Visualisation of visitation networks (Fig. 2.4-2.5) conveys the high frequency of visits by a subset of insect taxa to a subset of plant taxa; a pattern that is consistent across both PECs and their respective controls. Many of these taxa contribute a high proportion of interactions in all treatments, irrespective of region. *E. balteatus* (A24) and *B. terrestris* agg. (A16) dominate network activity whilst *R. fruticosus* agg. (B45) and *Heracleum sphondylium* Linnaeus (B32) receive universally high flower visits. The frequent insect visitors also tend to be highly generalist, visiting a large number of different plants, but nonetheless directing a high proportion of visits to only a small number of highly abundant taxa (i.e. B45 and B32). However, visual inspection of networks indicates that cereal controls are particularly dominated by visits from *E. balteatus*, whilst relative visitation in both the PECs is contributed by a larger number of insect species.

Statistical analyses of network indices (Table 2.5) indicate a high similarity for most of the investigated measures of network structure. However, willow networks have significantly greater 'links per species' and are significantly more 'nested' (i.e. value closer to zero; Rodriguez-Girones and Santamaria, 2006) than cereal controls. Difference in 'vulnerability' (mean number of pollinators per plants) was marginally non-significant in the southwest (*P*= 0.056), being higher in *Miscanthus* compared to cereals. Furthermore, although non-significant, inverse trends in the number of plants species visited are seen between PECs, with more plants visited in willow than cereals but fewer plants visited in *Miscanthus* than cereals.

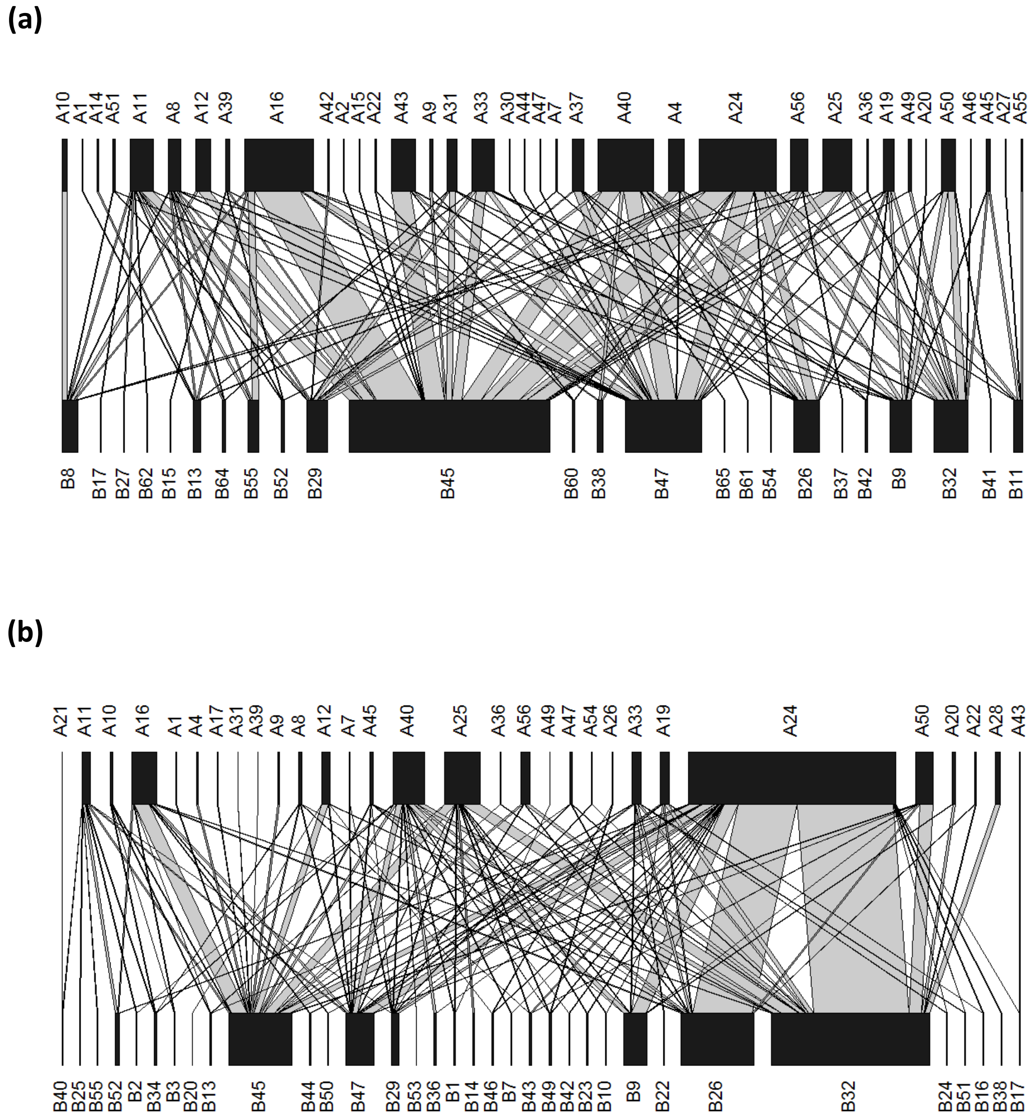

Figure 2.4: Flower-visitor (top level) and plant (lower level) visitation networks in *Miscanthus x giganteus* (a) and distant cereal (b) treatments. Networks incorporate focal taxa only (taxa identified above genus level also excluded). Data pooled from all transects and sites. Width of boxes indicates treatment specific relative visits per species, width of lines indicates interaction frequency. For legend see Appendix 31.

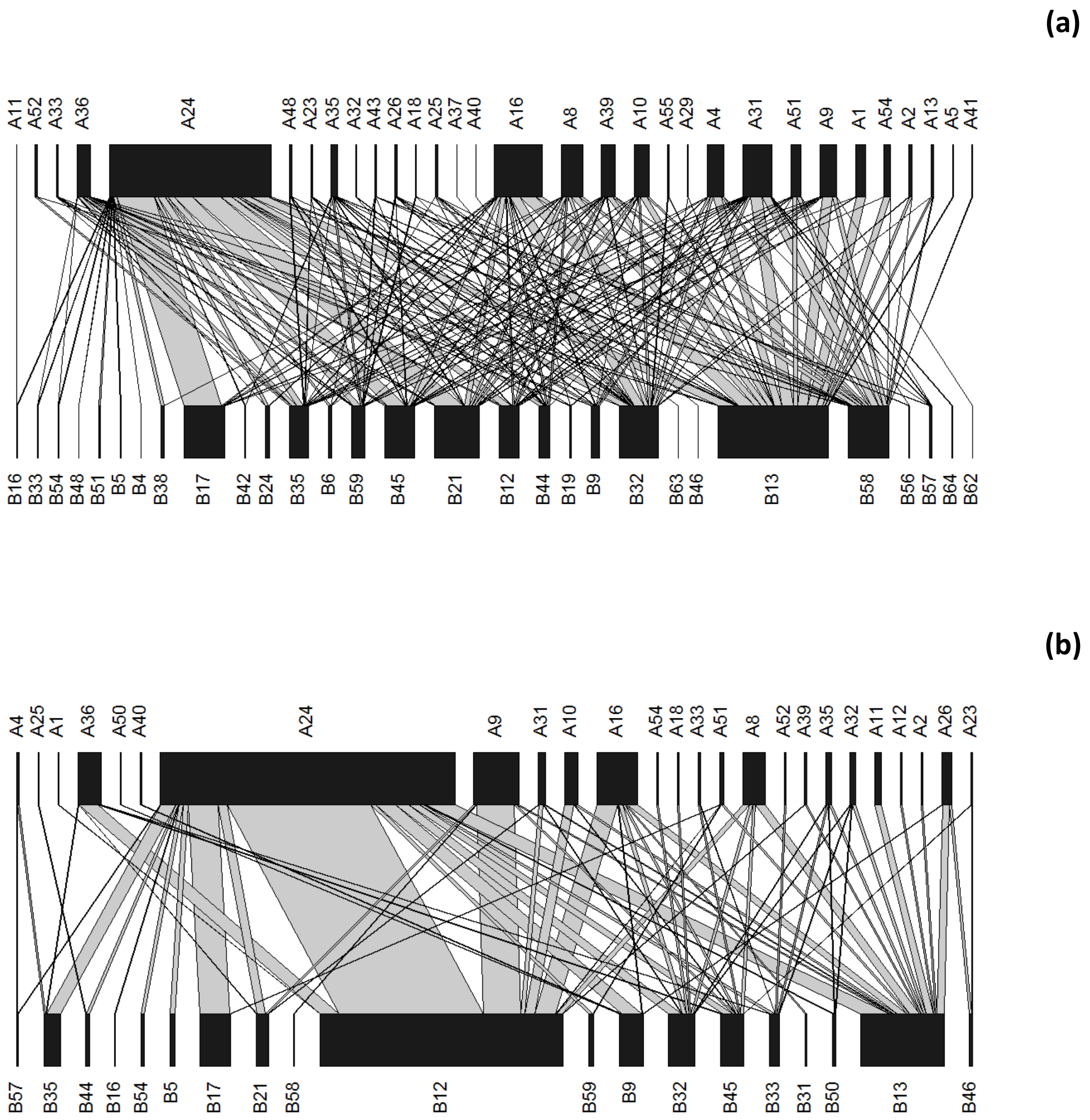

Figure 2.5: Flower-visitor (top level) and plant (lower level) visitation networks in willow short-rotation coppice (a) and distant cereal (b) treatments. Networks incorporate focal taxa only (taxa identified above genus level also excluded). Data pooled from all transects and sites. Width of boxes indicates treatment specific relative of visits per species, width of lines indicates interaction frequency. For legend see Appendix 31.

Table 2.5: Mean, standard error and output of *t*-test analyses of network parameters in perennial energy crops *Miscanthus x giganteus* and willow short-rotation coppice and their corresponding cereal controls. NS= non-significant; *= significant. α= 0.05.

Indice	Miscanthus		Cereal		Sig.	Willow		Cereal		Sig.
	Mean	± S.E.	Mean	± S.E.		Mean	± S.E.	Mean	± S.E.	
Connectance	0.23	0.02	0.24	0.02	NS	0.24	0.04	0.27	0.03	NS
Generality	2.86	0.46	2.62	0.48	NS	3.88	0.58	3.24	0.63	NS
H2	0.40	0.02	0.36	0.04	NS	0.30	0.04	0.30	0.04	NS
Interaction Evenness	0.59	0.03	0.54	0.03	NS	0.59	0.02	0.56	0.03	NS
Links per species	1.31	0.11	1.32	0.11	NS	1.70	0.14	1.21	0.08	*
Nestedness	19.95	1.93	19.41	2.32	NS	11.33	1.36	19.34	0.35	*
Specialisation Asymmetry	-0.20	0.10	-0.08	0.04	NS	-0.09	0.07	-0.12	0.15	NS
Vulnerability	5.46	0.75	3.63	0.38	NS	6.17	1.49	3.47	0.53	NS
Web Asymmetry	0.24	0.10	0.08	0.06	NS	0.29	0.12	0.26	0.10	NS
Number of plant species	9.83	1.22	11.67	1.12	NS	12.25	2.10	7.75	1.38	NS

2.5 Discussion

When taken together, I found evidence that willow cultivation significantly increased pollinator flower visitation all focal guilds (hoverflies bumblebees and Lepidoptera), while *Miscanthus* had a positive effect only for Lepidoptera. However, these effects attenuated quickly with distance from the PEC. For focal pollinator taxa, I found no evidence to support the hypothesis that PECs facilitate greater pollinator visitation in the local landscape; i.e. there was no instance where focal pollinator numbers along adjacent cereal margins were significantly higher than distant cereals.

Cultivation of second generation BECs has been widely observed to have positive implications for biodiversity compared to conventional arable cropping systems (Rowe *et al*. 2011, 2013, Milner *et al*., 2016); my results generally corroborate this pattern. However, for focal taxa, I found no evidence to support the (largely untested) hypothesis that the increases in biodiversity associated with PEC cultivation would have concomitant benefits to EP provision in the surrounding landscape (Manning *et al.*, 2015). The nearly three-fold decline in the numbers of flower visiting hoverflies when going from the edge of the distant cereals to *Miscanthus* margins was particularly unexpected. My results contrast markedly with the neutral effect observed by Bourke *et al*. (2014) working on *Miscanthus* in Ireland. While regional differences in community composition may be important, i.e. tiger hoverfly (*Helophilus pendulus* (Linnaeus)) rather than *E.balteatus* dominated in Bourke *et al.'s* (2014) study, methodology also differed. Bourke *et al*. (2014) used pan traps (likely attractive to *H. pendulus* given its aquatic larvae), whilst I quantify abundance of insects specifically visiting flowers (an EP rather than a biodiversity metric).

As floral resource availability varied little between *Miscanthus* and cereal margins in my study, other factors likely explain the differences in Lepidoptera and hoverfly abundance I observed in southwest England. One possibility for hoverflies is that there are few major *Miscanthus* pests in Britain (DEFRA, 2007); aphid abundance has been shown to be particularly low (Semere and Slater, 2007b). It is possible that female hoverflies in particular, foraged in areas (i.e. cereal crops and margins) where aphid populations and thus larval brood sites were more abundant (Almohamad *et al*., 2007). Regardless of the underlying mechanism, lower hoverfly activity in *Miscanthus* implies not only a reduction in pollination services to margin plants, but perhaps diminished aphid biocontrol by aphidophagous hoverfly larvae (Tenhumberg and Poehling, 1995).

Lepidoptera visitation, in contrast, was enhanced along *Miscanthus* margins, although the relatively low overall frequency of visits, even within the PEC margins, might suggest limited ES benefit, particularly as Lepidoptera often visit only a small number of preferred nectar plants (Jennersten, 1984). Since floral resource availability was unlikely to account for crop-specific variation in imago numbers, the variation in larval food plant abundance (i.e. uncut *D. glomerata* used by large skipper *Ochlodes sylvanus*) may explain these observations. Nonetheless, for a group of species suffering widespread decline in the agri-environment (Fox et al 2013, 2015); my findings corroborate other studies showing benefits of *Miscanthus* to Lepidoptera (Semere and Slater, 2007b).

Generally thought to be widely involved in pollinator service provision (Garratt *et al*., 2014), the limited response of bumblebees to *Miscanthus* cultivation observed here suggests that local enhancement of pollinator visitation in the agri-environment (Manning *et al.* 2015), or indeed wider conservation value of this crop to bumblebees, is unlikely (see also Stanley & Stout 2013). In contrast to *Miscanthus*, bumblebee abundance (in addition to Lepidoptera) was higher along willow SRC margins than either adjacent or distant cereals; although again, this did not enhance visitation in adjacent cereal fields. Given that I observed consistently more preferred (and total) flowers in willow SRC margins than cereal margins, the most parsimonious explanation for increased pollinator visitation (and diversity) in willow is that insects were responding to higher floral resource availability (Sutherland *et al.* 2001; Pywell *et al.*, 2011; Hanley & Wilkins 2015). Several authors (Cunningham *et al.* 2004; Rowe *et al.* 2011) have noted how the low chemical input and low disturbance regimes applied to woody PECs have a positive effect on associated plant communities. Remarkably however, aside from butterflies, to my knowledge, no previous study has attempted to examine how cultivation of woody SRC crops (e.g. willow or poplar) affects the flower visitation of other pollinator taxa compared with conventional arable crops. My data are therefore the first to underscore the importance of willow cultivation for hoverflies and bumblebees which likely benefit from elevated floral resource provision.

Given the varied and distinct crop type differences seen for focal pollinator taxa, it is surprising that only a few of the frequently recorded non-focal insect taxa showed any significant difference in flower visitation between crops, though species specific variation may have been obscured by the coarse nature of some of the taxonomic groupings. Although less morphologically adapted for pollination compared to conventional pollinators such as bumblebees, many of the non-focal taxa remain likely to be fairly effective pollinators due to their high visitation frequency. Elevated *R. fulva* visitation in willow and adjacent cereals, when compared to distant cereals, therefore emphasises the potential local benefits of willow and provides the only evidence of a positive effect of PEC cultivation upon pollination process in the surrounding landscape. However few studies exist on the contribution of *R. fulva* to wildflower pollination, the species is thought to be an important pollinator of *H. sphondylium* (carrying significant amounts of pollen, Grace and Nelson, 1981) , and visitation data from this study certainly support this notion, with *H. sphondylium* accounting for ~70% of visits across willow triplicates in 2015. Nonetheless, given the predominance of *H. sphondylium* visits, increased *R. fulva* visitation frequency recorded in and around willow might therefore have a limited benefit for the wildflower community more generally, although *C. vulgare* accounted for approximately 12% of visits and *Conium maculatum* Linnaeus*, Jacobaea vulgaris* Gaertner*, Chaerophyllum temulum* Linnaeus each accounted for 4% of visits, and thus may also benefit. *R. fulva* is known to feed upon pollen and nectar as an adult but also eats aphids and the larvae feed on epigeal invertebrates such as slugs and snails (van Emden, 2013), potentially providing an important contribution to biocontrol services. Conversely, reduced *S. stercoraria* visits in *Miscanthus* and surrounding cereals may have a negative effect on crop pests, although local livestock densities will likely be a far more important determinant of abundance/activity of this dipteran given its association with fresh cow dung (a single cow pat can produce several hundred flies, Blanckenhorn *et al*., 2010). Nevertheless, the consequences of reduced *S. stercoraria* flower activity in *Miscanthus* might be expected to reduce pollination as both sexes take nectar, and the males are both large and hairy, and thus probable pollinators; however lower *S. stercoraria* activity may increase the number of flower visiting Dipteran prey, potentially countering or reversing decreases in *S. stercoraria*.

Similar to findings by Stanley (2013), I show that indices describing flower-visitation networks in *Miscanthus* are generally similar to those of cereals, suggesting that network properties are fairly robust to land use change. However, for willow, some differences were discernible. For example, I find significantly higher 'nestedness' in willow than cereal networks, which may prove an added benefit of this particular PEC. 'Nestedness' is thought to indicate more stable networks (Bascompte *et al*., 2003) and is generally understood to increase with greater complexity (i.e. more unique interactions). As such, it is not unexpected that 'links per species' is also significantly higher in willow, visually evident in the bipartite graphs and indicative of a more complex network. Although, Stanley (2013) did not find differences in vulnerability when comparing *Miscanthus* to either winter wheat or grassland, they do find significantly greater numbers of flowering plants in *Miscanthus* networks which contrasts to the, marginally non-significant, but higher ‘vulnerability’ of *Miscanthus* networks and lower numbers of interacting plant species recorded in this study, although again, the latter is not in-itself significant. It is possible that these opposing trends may arise as a consequence of the notably species rich fields margins of Irish agroecosystems (Stanley, 2013). The authors also find lower connectance in *Miscanthus* compared to wheat, a measure near identical in my analyses and, again, a possible outcome of the greater floral richness of *Miscanthus* networks not found in this study.

Consequently, although I found limited evidence for the enhancement of pollination process over long-distances as a result of *Miscanthus* or willow PEC cultivation (Manning *et al.* 2015), willow offered considerable local benefits to the pollinator community. However I acknowledge that this study focuses on a single EP (flower visitation) and the consequences for plant reproduction (i.e. fruit/seed set) require further attention. Although the dominant flower visitors in this study are understood to be effective pollinators in many systems, future investigation of pollinator effectiveness (King *et al.*, 2013) would also expand on my findings. In addition, to determine whether elevated bumblebee visitation seen in willow is a consequence of increased colony size or increased nest number, genetic analyses are necessary. Furthermore, while I explicitly set out to investigate whether PECs enhance pollinator flower visitation in adjacent cereal crops, I did not consider how the presence of the PEC at the landscape-scale influenced overall pollinator numbers and thus pollinator service provision. In MFCs for example, a putative "dilution" effect has been evidenced at the landscape scale, as wild bee pollinators are widely dispersed over the large quantity of MFC floral resources (Holzschuh et al., 2011; 2016). Should PECs differ to MFCs in this regard, they may play a particularly important role in insect conservation when grown as an alternative to mass-flowering biofuel crops, even when cultivated at high densities in the landscape. Nonetheless, from a conservation perspective, my findings suggest that willow crops, in particular, offer the potential to enhance farm-scale biodiversity, with positive effects evident for at least three threatened pollinator groups. Incorporation of willow SRC into conventional mixed farming systems may yet help to support pollination services to wild margin plants.

Chapter 3: Effects of a bioenergy crop and a mass-flowering crop on the seed set of field margin phytometers

3.1 Abstract

Cultivation of perennial energy crops (PEC) and mass-flowering crops (MFC) has been shown to affect pollinator numbers. However research is necessary to determine the reproductive implications for field margin plants. Here I investigate seed set for patches of two planted phytometers California poppy (*Eschscholzia californica*) and red clover (*Trifolium pratense*) as well as a hedgerow wildflower, red campion (*Silene dioica*), in the PEC *Miscanthus x giganteus* and the MFC field bean (*Vicia faba*). Comparison of seed counts in *Miscanthus* to adjacent and distant cereal controls revealed no significant difference in seed set for any phytometer. Similarly, seed set of *E. californica* and *S. dioica* did not differ significantly between *V. faba* and wheat (*Triticum* spp.) controls. However seed set of *T. pratense* was significantly lower in *V. faba* fields. There was no evidence that flower visitation was driving trends, with no significant difference in visitation between crops for any planted phytometer species, and total recorded flower visits in fact higher in *V. faba*. In *Miscanthus*, cumulative Lepidoptera visitation rates to naturalised flower patches of *S. dioica* and *Rubus fruticosus* agg. were significantly higher than cereals. However visitation to individual plant species was not significantly different, indicating that increased flower visits may be too sparsely distributed across species to affect reproductive outcomes for individual species. Similarly, investigation of total pollinator (i.e. hoverfly, bumblebee, Lepidoptera) visitation rates revealed no significant difference compared to cereals. Despite the need for further replication, these findings suggest neutral effects of *Miscanthus* cultivation for pollination process and potentially negative effects of field bean upon the reproduction of a low density bumblebee pollinated plant.

3.2 Introduction

Pollination by wild pollinators is of direct value to humanity, improving the fruit-set of crop plants. Increased wild pollinator flower visits enhances fruit-set by twice the magnitude seen with equivalent increases in managed honeybees (Garibaldi *et al.*, 2013). Furthermore, many of these crop pollinators also pollinate wild plants, with as many as 87.5% of plant species dependent on animal pollination (Ollerton *et al.*, 2011). Pollinators thus indirectly support the provision of ecosystem services provided by plants, including habitat for pest predators, reduced soil erosion and flood prevention (Kremen *et al.*, 2007). Land use and management practices are understood to affect pollinator numbers (Herrmann *et al.*, 2007); with potential consequences for pollinator service provision and wildflower reproduction in arable margins (Westphal *et al.*, 2003).

Following variable trends in the frequency of flower visitation between different insect taxa in *Miscanthus* and cereal crops (chapter 2), and given the evidence of increases in the abundance of specific pollinating insect taxa in previous studies (Semere and Slater, 2007b; Stanley and Stout, 2013), I hereby investigate implications for seed set of non-crop margin plants in the perennial energy crop (PEC) *Miscanthus* (*Miscanthus* x *giganteus*). The widespread cultivation of *Miscanthus* in temperate climates confirms the potential for this PEC to become an increasingly abundant land use in the UK and abroad, including Europe, North America and its native Asia. To date, this is the first study to investigate the consequences of *Miscanthus* cultivation upon the reproductive success of margin plants.

Miscanthus is a sterile wind-pollinated crop and so any influence upon pollinator abundance or activity principally arises from its morphology and unconventional, low intensity management. In contrast to PECs such as *Miscanthus*, mass-flowering crops (MFCs) provide a brief and highly concentrated abundance of flowers in the agri-environment and therefore, despite their conventional, intensive management, MFCs also have the potential to influence pollination service provision to margin plants. Understanding the impact of MFCs has particular contemporary relevance; globally, the area of crops dependent upon insect pollination has increased rapidly in recent years with consequences for pollination of crops and native plants (Aizen and Harder, 2009).

MFC and co-flowering wild plants can display either facilitative or competitive effects on plant-pollinator interactions. This can occur through effects on either visitation rates or pollen transfer (Stanley and Stout, 2014), for example pollinators may spill-over from crop to margin flowers, or *vice versa*, potentially enhancing or decreasing overall visitation and pollination service provision. Studies of OSR (Stanley and Stout, 2014), sunflower (*Helianthus* sp. Linnaeus; Chamberlain *et al*., 2013) and mango (*Mangifera* sp. Linnaeus; Carvalheiro *et al*., 2012), for example, have shown shared pollinators between MFCs and co-flowering wildflowers. Facilitation of pollination occurs when increases in shared pollinators concomitantly result in increases in the transfer of conspecific pollen between stigmas. Conversely, deposition of heterospecific pollen may inhibit pollination, obstructing access of conspecific pollen to stigmas. Furthermore, interactions between pollinators might reduce overall visitation, for example aggressive interactions (i.e. contact, pollen theft) have been recorded between *Apis mellifera* and native pollinators, although there is currently little evidence for such aggression in MFCs (Geslin *et al*., 2017).

Due to their high resource abundance, strategic planting of MFCs has been suggested as a potential means to benefit pollinators and the ecosystem services they provide (Westphal *et al*., 2003). Evidence of increased bumblebee densities around mass-flowering oilseed rape (OSR) provides support for this hypothesis (Westphal *et al*., 2009; Knight *et al*., 2009). Nonetheless there still remains a need to better understand the implications of MFC floral resources upon the seed set of field margin plants

(Cussans *et al*., 2010). As such, in this study, I additionally investigate phytometer seed set in field bean (*Vicia faba*) crops with comparison to wheat controls. Hanley *et al.* (2011) has shown double the number of bumblebee visits to wildflowers in the margins of field bean when compared to wheat, this potential facilitation of pollination service provision provides the basis to investigate seed set in this particular MFC.

To date, most MFC studies have focused on spring flowering OSR; field bean, in contrast has received limited attention, likely due to the considerably lower area under cultivation, at ~4% of UK farmed land (Hanley *et al*., 2011). Nonetheless, despite a 41% decline in global field bean production, from 5.4 million tons in 1961-1962 to 3.2 million tons in 1991-1993, production has since improved, increasing by 33% to 4.52 million tons in 2008-2010 (Fouad *et al.*, 2013) with a global land area of 2 million hectares in 2014 (Bailes *et al*., 2018). The UK remains in the top three global exporters of bean and a recent Anderson Report (Redman *et al*., 2015) for the John Innes Centre has suggested that pea and bean markets could potentially double in size in the near future. In addition, in some regions, where OSR is less frequent, such as southwest England, field bean can be a locally important MFC. The growing recognition of the need for more sustainable soil management practices is a further incentive for greater incorporation of field bean into crop rotations, as a representative of the Fabaceae this crop enriches the soil with slowly released N, mitigating the need for inorganic inputs.

As field bean flowers later than spring-sown OSR, i.e. in mid-summer (June-August), semi-natural habitats contain more floral resources at this time and pollinator responses may therefore differ between these two seasonally distinct MFCs. Furthermore, in contrast to OSR, field bean also provides a concentrated abundance of flowers with long-tubed corollas that are principally only accessible to long-tongued bumblebees, but also attractive to nectar-robbing short-tongued bumblebees (Cussans *et al*., 2010; Garratt *et al*., 2014).

To investigate seed set in *Miscanthus* and field bean, I focus on the planted phytometers red clover (*Trifolium pratense*) and California poppy (*Eschscholzia californica*) as proxies for wildflower pollination. Each phytometer has morphologically distinct flowers and thus appeals to broadly different functional groups of pollinators. Both phytometers are self-sterile, the deep florets of red clover are understood to require long-tongued pollinators, principally bumblebees but also Lepidoptera and to a limited extent honey bees (Bohart, 1957; Peterson *et al*., 1960), whilst the large open flowers of California poppy are likely to be more dependent on smaller insect pollinators, capable of alighting upon them, such as Diptera (i.e. Syrphidae) and solitary bees. Given the lack of variation in bumblebee visits outlined in chapter 2, I hypothesise that seed-set of red clover will differ little between *Miscanthus* and cereal controls, whilst increased cumulative bumblebee visitation recorded by Hanley *et al*. (2011) will lead to increased seed set in bean compared to wheat fields. Furthermore, I hypothesise that the low hoverfly flower visitation recorded in *Miscanthus* fields in chapter 2 will result in reduced seed set of California poppy in relation to cereals, whilst seed set in bean fields will be similar to that of wheat, assuming little effect of bean upon non-bumblebee pollinators. I also investigate seed-set in the margin wildflower red campion (*Silene dioica*) which is pollinated by a vast array of insects and robbed by others (i.e. short-tongued bumblebees). The overall similarity in cumulative pollinator visitation seen in *Miscanthus*-cereal comparisons (chapter 2) suggests that seed set may differ little between these crops, although minor increases in *Miscanthus* may occur due elevated Lepidoptera activity; whilst in bean greater bumblebee visitation may increase seed set.

3.3 Methods

3.3.1 Phytometer Seed Set

Two 0.5 m^2 phytometer plots were set out in a single field margin of four *Miscanthus* and four spring field bean crops as well as their respective cereal control fields (*Triticum* spp. regards field bean and a mix of *Triticum* spp., *Hordeum vulgare* and oat (*Avena sativa* Linnaeus) regards *Miscanthus*) during June 2016. *Miscanthus* controls consisted of adjacent and distant cereals, following the experimental design used in Chapter 2, the study encompassed a total of 20 fields (Appendix 32). One phytometer plot consisted of red clover (*Trifolium pratense*) and the other California poppy (*Eschscholzia californica*). Plots at triplicate/paired sites were situated in margins with a similar aspect and spaced a distance of two metres apart. Ground layer vegetation was removed from an area ~ 1 m^2 around plots and flowers from an additional 1m^2 in order to limit the confounding influence of variation in local wild flower density upon pollinator visits (Sih and Baltus, 1987; Kunin, 1993). All patches were surrounded by chicken wire attached to bamboo canes in order to deter damage from mammalian herbivores. However this met with variable success and clover at Yarford adjacent cereal site required replanting as it was completely grazed by deer within 7 days. Clover plants also exhibited some mollusc damage at many sites despite use of slug pellets within the fenced area (Neudorff, Sluggo), mollusc damage was not excessive however and none was seen on California poppy. All phytometers used in the study were grown from seed sown 31st January (red clover) and 10th March (California poppy) in greenhouses. 'John Innes Seed Sowing Compost' was initially used before repotting in John Innes No.2 Potting on Compost on 19th March (Clover) and 19th - 26th April (California poppy). All developed plants were placed outside greenhouses in a sunny sheltered position one week before planting in the field to aid acclimatisation. All 200 plants were planted out in the field between the 3rd and 7th of June, with the addition of fresh ‘John Innes No.2 Potting on Compost’. Roots were not disturbed because of the sensitivity of California poppy to such disturbance. Due to a limited number of plants being ready to flower each patch consisted of five plants at different growth stages; one plant with well-developed flower buds, and so likely to flower within a few days, two plants had developed flower buds, likely to flower within a week and two had no flower buds. Fortunately this meant that there would be an ongoing floral display. Plants were arranged in a die like pattern of five with the most developed plant in the centre. All patches were watered upon planting and repeatedly on each visit over the following weeks, with addition of further slug pellets. Ideally potted plants would have been used, but this was initially seen as unfeasible due to the distance between sites, making such regular watering labour intensive. Nonetheless many clover plants struggled to establish and flowering was delayed and poor for some plants. California poppy, although more sensitive to physical disturbance and being very delicate to handle, faired far better due to its drought tolerance and apparent unpalatability to herbivores. Sites were regularly revisited and patches watered and weeded. As poppy seed pods developed and clover flowers senesced they were covered in breathable organza mesh bags (CraftPlanet) with elastic bands used to help secure them, this was intended to prevent seed loss but allow the seed to develop and ripen. Unfortunately farmers began to cut margins in late July and early August, with patches at Yarford *Miscanthus* and Lostwithiel *Miscanthus* being destroyed, fortunately some bagged seed pods could still be collected however. In order to prevent complete loss of samples, all remaining seed pods and senescing clover flowers were collected from all sites from the 3rd to the 6th of August, all bioenergy and MFC samples were collected on the same day as control fields. No seed had yet been released by any plants but most were well developed and assumed to contain the maximum number of seeds that would be produced. Seed pods were frozen at -20 °C upon returning to the laboratory, preceding determination of seed number.

Plate 3.1: Red clover (*Trifolium pratense*) and California poppy (*Eschscholzia californica*) phytometers used for seed-set determination in the bioenergy crop *Miscanthus x giganteus*, the mass-flowering crop field bean (*Vicia faba*) and their corresponding cereal controls.

Where present, seed pods were also collected from red campion (*Silene dioica*) growing in field margins. A minimum of 5 developing seed pods were bagged in each field using organza mesh bags and collected and frozen when ripe. Efforts were made to select flowers from different margin aspects at each site but this was not always possible because female plants were not always present in each margin and some were infected by the smut fungus (*Microbotryum lychnidis-dioicae* (DeCandolle) G. Deml & Oberwinkler). Investigation of collected red campion seed pods revealed that many had granivore damage from Lepidoptera larvae (e.g. *Sideridis rivularis* Fabricius) and red clover florets also regularly supported phytophagous larvae (likely weevil larvae, possibly *Apion* spp. Herbst). Two bean fields (Merton and Sowton) did not contain any viable red campion pods which prevented statistical analyses of differences in bean and cereal seed set for this species.

3.3.2 Insect visitation rates and margin floral transects

Plots of each phytometer were surveyed for 20 minutes on four occasions between 12/07/16 and 25/07/16 with the abundance of wild flowers estimated using two 50 m floral transects, one either side of the phytometer plots. Flower counts were estimated based on floral unit availability (see *Methods* chapter 2). All hoverfly and bumblebee flower visitors were recorded to at least genus level in the field with other taxa recorded to varying taxonomic resolution.

Hoverfly, bumblebee and Lepidoptera visitation rates to red campion and bramble (*R. fruticosus* agg.) were also recorded in the hedgerows at *Miscanthus* and cereal sites for 20 mins between 07/06/16 and 27/06/16. Where flower density differed between patches, large patches were cut, achieving approximately similar flower numbers between sites and crop types, preventing treatment differences in patch size from biasing flower visitation rates between treatments.

3.3.3 Statistical analyses

T-tests were used to discern differences in seed counts between crop types for MFC bean and wheat fields. One-way ANOVA was run to compare clover seed-set in *Miscanthus* and cereal controls, however due to low replication of seed counts for poppy and red campion phytometers it was not possible to incorporate distant cereal treatments into these analyses and therefore *Miscanthus* and nearby cereals were compared using *t*-tests. Due to low visitation rates to phytometer patches, permutation tests were run to test for crop differences, using the ‘coin’ package in R (R Core Team, 2017). ANOVA and Kruskal-Wallis tests were run on visitation rates to hedgerow species. Prior to analyses, normality (based on visual inspection and Shapiro-Wilk tests) and homogeneity of variance (based on sample variance and Levene’s test) were investigated. Where treatment variances were unequal, tests assumed non-equal variance. Differences in margin wildflower counts excluded *Galium mollugo* as this species was only present in a few fields but disproportionally skewed trends due to high floral unit abundance. The small flowers likely provided limited resources to pollinators individually and therefore give an unrealistic impression in the availability of floral resources. This does not alter conclusions however and uncorrected data are presented in Appendix 33.

3.4 Results

3.4.1 Phytometer seed set

Phytometer seed counts in *Miscanthus* fields (Fig. 3.1) were not significantly different from those in cereal fields for poppy (t_5= 0.431; P= 0.685), red cover ($F_{2,9}$= 0.62; P= 0.941) or red campion (t_4= 1.473; P= 0.215). However, mean red campion seed counts were 34.5% greater in *Miscanthus* fields than in adjacent cereal controls, the fact that this was non-significant emphasises the high variability of seed counts within treatments.

Red clover flowers had significantly lower seed set in bean than wheat fields (Fig. 3.2; t_6= 3.672; P= 0.01). However, there was no significant difference in poppy seed set (t_6= 0.403; P= 0.711) in bean fields compared to wheat. Low replication precluded statistical analysis of red campion seed set but graphical comparison (Fig. 3.2) indicates similar seed counts.

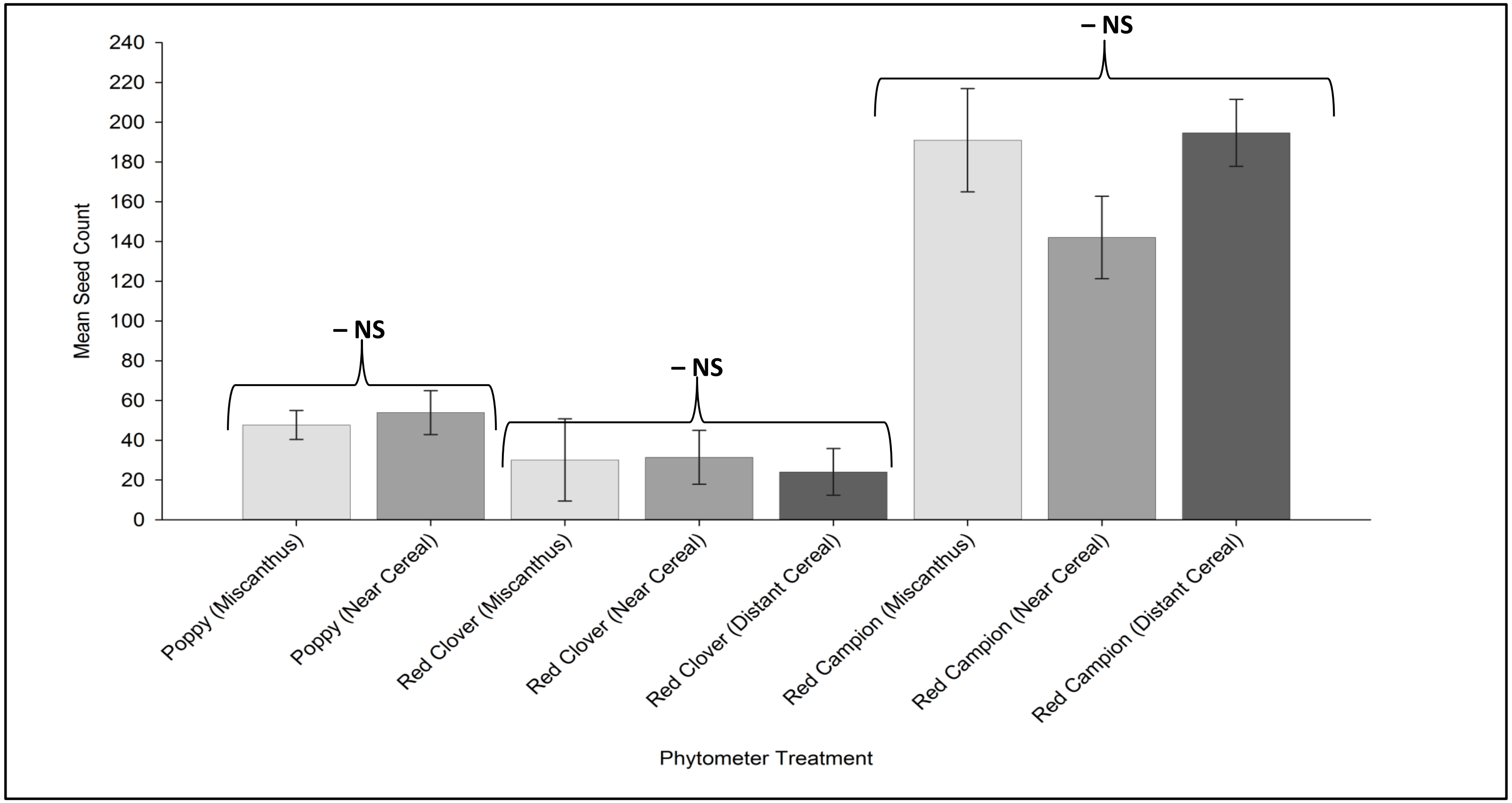

Figure 3.1 Mean seed counts of planted phytometers (California poppy (*Eschscholzia californica*), red clover (*Trifolium pratense*)) and hedgerow red campion (*Silene dioica*) in *Miscanthus x giganteus* and cereal control fields in southwest England, 2016. Error bars indicate ±1 S.E. α= 0.05. NS= Non-significant.

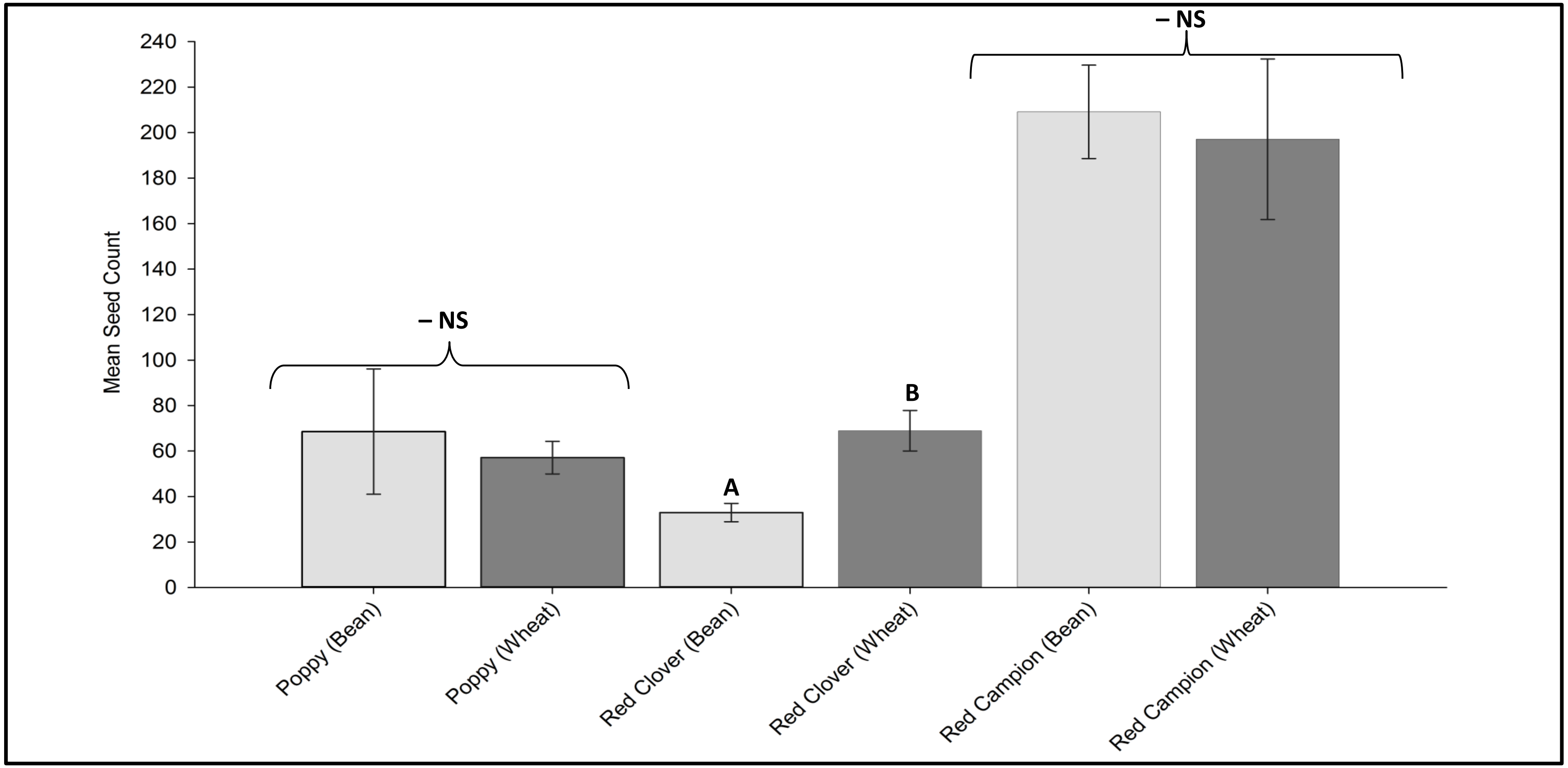

Figure 3.2: Mean seed counts of planted phytometers (California poppy (*Eschscholzia californica*), red clover (*Trifolium pratense*)) and hedgerow red campion (*Silene dioica*) in field bean (*Vicia faba*) and wheat (*Triticum* spp.) control fields in southwest England, 2016. Different letters indicate significant difference. Error bars indicate ±1 S.E. α= 0.05. NS= Non-significant.

3.4.2 Insect flower visitation rates

Both planted phytometers received low rates of insect visitation, a total of 57 visits were recorded across *Miscanthus* (16), adjacent (31) and distant (10) cereal controls and 32 across bean (19) and wheat (13) controls. Visits to red clover were particularly low, with only 9 recorded across *Miscanthus* (1), adjacent (4) and distant cereals (4) and, similarly, only 11 across bean (7) and wheat (4) controls. No significant difference was seen in visitation between *Miscanthus* and cereal controls (P= 0.342) or between bean and wheat (P= 0.612). Poppy visits totalled 48 across *Miscanthus* (15), adjacent (27) and distant controls (6) with 21 visits across bean (12) and wheat (9). Again, no significant difference was seen in visitation between *Miscanthus* and cereal controls (P= 0.808) or bean and wheat (P= 0.603).

For naturalised red campion and bramble patches, visitation rates were greatest for bumblebees (accounting for 66%, 68% and 72% of cumulative visits in *Miscanthus*, adjacent and distant cereal respectively) followed by hoverflies (respectively 17%, 24% and 25%) and Lepidoptera (17%, 9% and 3%). When considering patches of both species, cumulative Lepidoptera visitation rates were significantly higher in *Miscanthus* compared to distant cereal fields (Table 3.1). However, visits from other focal taxa did not differ significantly between crops, nor when considering all three groups collectively.

3.4.3 Margin wildflower abundance and species richness

The availability of margin wildflowers was not significantly different between bean and wheat (t_6= 0.849; P= 0.429, Appendix 36A) or between *Miscanthus* & cereal controls ($F_{2,4.6}$= 0.812; P= 0.498, Appendix 36B). Furthermore, there was not seen to be any difference in species richness between bean and wheat (t_6= 1.351; P= 0.225; Appendix 37A) or *Miscanthus* and cereal controls ($F_{2,9}$= 0.141; P= 0.871; Appendix 37B).

Similarly, floral diversity did not differ for *Miscanthus* ($H(2,9)= 0.615$; $P= 0.735$) or bean ($t(6)= 1.167$; $P= 0.288$) when compared to controls.

Table 3.1: Mean, S.E. and statistical output of tests for difference in insect visitation rate to bramble (*Rubus fruticosus* agg.) and red campion (*Silene dioica*) patches at *Miscanthus x giganteus* and cereal control sites in southwest England, 2016.

Test	Crop	Mean Visits (20 min^{-1})	S.E.	Output
Total Insect Visitation (Red Campion & Bramble)	Micanthus	38.25	6.52	$F_{2,9}= 0.848$; $P = 0.46$
	Near Cereal	26	3.54	
	Distant Cereal	32.25	8.82	
Total Insect Visitation (Bramble ONLY)	Micanthus	25.5	5.98	$F_{2,9}= 0.767$; $P = 0.492$
	Near Cereal	16	3.24	
	Distant Cereal	22.25	6.70	
Total Insect Visitation (Red Campion ONLY)	Micanthus	12.75	3.68	$F_{2,9}= 0.335$; $P = 0.724$
	Near Cereal	10	1.78	
	Distant Cereal	10	2.42	
Hoverfly Visitation (Red Campion & Bramble)	Micanthus	5.75	2.72	$F_{2,9}= 0.246$; $P = 0.787$
	Near Cereal	6	1.83	
	Distant Cereal	7.75	1.93	
Hoverfly Visitation (Bramble ONLY)	Micanthus	2.75	1.38	$F_{2,9}= 1.407$; $P = 0.294$
	Near Cereal	4.25	1.11	
	Distant Cereal	5.5	0.96	
Hoverfly Visitation (Red Campion ONLY)	Micanthus	3	1.78	$F_{2,9}= 0.218$; $P = 0.809$
	Near Cereal	1.75	1.11	
	Distant Cereal	2.25	1.03	
Bumblebee Visitation (Red Campion & Bramble)	Micanthus	22.5	4.99	$F_{2,9}= 0.357$; $P = 0.709$
	Near Cereal	17.25	4.01	
	Distant Cereal	23	6.65	
Bumblebee Visitation (Bramble ONLY)	Micanthus	16.25	4.33	$F_{2,9}= 0.427$; $P = 0.665$
	Near Cereal	11.25	2.95	
	Distant Cereal	16.25	5.57	
Bumblebee Visitation (Red Campion ONLY)	Micanthus	6.25	1.65	$H_{2,9}= 0.088$; $P = 0.957$
	Near Cereal	6	1.83	
	Distant Cereal	6.75	1.65	
Lepidoptera Visitation (Red Campion & Bramble)	Micanthus^{a}	5.75	2.10	$H_{2,9}= 6.667$; $P = 0.036$
	Near Cereal^{a,b}	2.25	0.75	
	Distant Cereal^{b}	1	0.41	
Lepidoptera Visitation (Bramble ONLY)	Micanthus	0.75	0.48	$H_{2,9}= 0.588$; $P = 0.745$
	Near Cereal	1.75	1.75	
	Distant Cereal	0.25	0.25	
Lepidoptera Visitation (Red Campion ONLY)	Micanthus	2.75	1.03	$F_{2,4.592}= 1.926$; $P = 0.201$
	Near Cereal	2.25	0.75	
	Distant Cereal	0.75	0.25	

3.5 Discussion

Similarity in seed set counts between *Miscanthus* and cereal fields suggests that cultivation of this PEC has little effect upon the outcomes of pollination service provision for margin plants. This finding complements trends in insect flower visitation presented in chapter 2, where no overall differences were found in the cumulative visitation frequency of hoverflies, bumblebees and Lepidoptera. Therefore, despite the differential effects this crop has on the abundances of specific flower visiting insect taxa (see chapter 2), I find only neutral effects for processes relevant to overall service provision.

Visitation rates to red campion growing wild in the field margins did not differ significantly between *Miscanthus* and cereals for any of the three focal taxa. The only positive effect of *Miscanthus* upon a pollinator taxon occurs when Lepidoptera visitation rates to red campion are combined with bramble, another widely available margin plant. This supports the significantly higher Lepidoptera flower visitation recorded upon *Miscanthus* transects in chapter 2. However, the lack of significance when investigating visitation rates to each plant separately implies that elevated pollination service provision may have little consequence for individual species, with overall increases in visitation appearing sparsely spread across multiple plant species. Insect visitation rates to planted phytometers are difficult to interpret given the low absolute numbers of insect visits recorded in this study. However there is no evidence of elevated visitation in *Miscanthus* for red clover or Californian poppy and, although non-significant, hoverfly visitation is in fact lower in *Miscanthus*, supporting transect trends reported for hoverflies in chapter 2. Likewise, data on floral resource availability did not differ between *Miscanthus* and cereals, supporting previous transect data (chapter 2), despite the inclusion of two unique fields sites in this study. Furthermore, there is no significant difference in floral richness and diversity between crop types.

Similar to *Miscanthus*, seed set of California poppy and red campion in bean fields did not differ significantly in comparison to wheat controls. This is perhaps predictable for California poppy as, in general, there was no *a priori* expectation for non-bumblebee flower visitors to benefit from field bean cultivation, given their inability to access its flowers (Garratt *et al*., 2014; although it was seen that bean plants often supported high aphid numbers, attractive to many hoverflies, *personal observation*). Conversely, red campion is likely to share some legitimate and illegitimate pollinators with field bean but I find no evidence of pollinator facilitation for this wild flower. However, in contrast to *Miscanthus*, seed set trends differed between field bean and cereal controls for red clover flowers, with reduced seed counts in field bean, suggesting an antagonistic effect upon this principally bumblebee pollinated wildflower. This trend does not conform to expectations of greater pollinator service provision that one might infer from the elevation in cumulative margin flower visits recorded by Hanley *et al*. (2011). Furthermore, these local findings also contrast with neutral effects on seed set documented for another MFC at the landscape scale; despite a decline in long-tongued bumblebees, Diekotter *et al*. (2010) found no effect of an increased proportion of OSR in the landscape upon the seed set of red clover. Findings are closer to those of Holzschuh *et al*. (2011) who showed that increase in the proportion of OSR in the landscape, from 5% to 15%, resulted in a 20% decrease in the seed set of co-flowering cowslip (*Primula veris* Linnaeus). However, comparison of local and landscape scale effects is likely to be of little value, for example Westphal *et al*. (2003) found elevated bumblebee density with extensive landscape scale OSR cultivation whilst failing to find field scale effects. Furthermore, due to differences in floral morphology, and thus pollinator assemblages (Garratt *et al*., 2014), any comparison of field bean and OSR also has limited relevance (Cussans *et al*., 2010). Field bean provides far fewer floral resources than OSR, the latter having >100 flowers per plant over a four week period, with as many as 350,000-700,000 plants per hectare, and thus, most likely, a greater impact on plant-pollinator interactions (Geslin *et al*., 2017). In addition, the position of MFCs in pollination networks is seen to differ depending on the crop studied (Geslin *et al*., 2017), alfalfa (*Medicago sativa* Linnaeus) for example has a low degree whilst OSR is highly generalist (Pocock *et al*., 2012; Stanley, 2013).

Although treatment differences were non-significant, total insect visitation was seen to be higher in bean than wheat for both planted phytometers, suggesting that low seed set is unlikely to have arisen from reduced insect visitation. However, visitation counts are so low in this study that interpretation of trends is of limited value, particularly given that many insect visitors may have been ineffective pollinators (i.e. there were few recorded bumblebees or Lepidoptera visits, *personal observation*). Based on these data, it is not possible to give a robust explanation for lower red clover seed set in bean fields. However, if it is assumed that effective pollinators are in approximately equal abundance in bean and cereal fields, and that as crypsis of wild flowers declines flower constancy declines (Goulson, 2010), one hypothetical explanation for lower seed set in bean could be that flower constancy is lower in cereal fields than bean. Lower crop height and the absence of crop flowers may result in effective pollinators being better able to locate margin flowers in cereal fields, resulting in greater seed set. Alternatively, reductions in red clover seed-set in bean fields may result from the clogging of clover stigmas due to increased heterospecific pollen deposition, a consequence of mutual pollinators being shared between both the crop and the phytometer (Morales and Traveset, 2008). This explanation must be somewhat more nuanced however, given that red clover patches in cereal fields will also receive heterospecific pollen, the phytometer patches being too small to support any individual pollinator in isolation, in both bean and cereal fields. Although long-tongued bumblebees are legitimate pollinators of field bean it is likely that illegitimate short-tongued bumblebees also benefited from field bean cultivation, perhaps disproportionately so, leading to greater nectar robbing of clover phytometers in bean fields and thus resource depleted florets, less attractive to legitimate pollinators.

There is no evidence that variation in the abundance of margin wildflowers influenced pollinator behaviour/activity between crop treatments. Bean and wheat floral abundance, species richness and diversity trends do not differ significantly, supporting floral transect trends reported by Hanley *et al*. (2011). The lack of difference seen in margin flowers between bean and cereal is not unexpected; to prevent pests and disease field bean is generally grown on a 5 year rotation (Finch *et al*., 2014) and therefore, at the field scale, for a given rotation, cereal management is likely to exert a greater selective pressure on margin flora than field bean.

Across treatments, low insect visitation was seen for all phytometers, possibly the result of surveying in a largely biologically impoverished agricultural landscape; additional factors may also have contributed however. For example, red clover only appeals to a limited guild of pollinators, i.e. those adapted to accessing or robbing the deep florets. Additionally, although native, red clover was not naturally present in the field margins at any of the investigated field sites and the presence of only a single patch of this phytometer may have had low appeal to bumblebees, particularly given their large foraging ranges (Knight *et al*., 2009) and flower constancy behaviour (Goulson, 1994; also documented for Lepidoptera, see Goulson and Cory, 1993). It is probable that many bumblebees will have moved away from the dense field bean resource to high density wildflower patches for instance, overlooking or avoiding (i.e. neophobia, Rands and Whitney, 2010) the single, novel, patch of red clover (or sparsely distributed red campion). If red clover flowers (or those of any other phytometer) had exceeded a certain threshold number, and/or density, findings may have differed.

Flower constancy has also been shown in hoverfly species (Goulson and Wright, 1998) which prefer more open flowers, it is therefore possible that small poppy patch sizes similarly failed to attract pollinators away from more abundant resources (i.e. Apiaceae) and so possibly explain the low visitation rates for this phytometer. For example, Sutherland *et al*. (2001) have shown that higher flower density results in greater hoverfly visitation in arable fields. Nonetheless, flower constancy to poppy patches was recorded for individual representatives of the genus *Eristalis* Latrielle who, likely attracted by the orange-yellow flowers (Sutherland *et al*., 1999), adopted highly territorial behaviour, often chasing away other prospective flower visitors (*personal observation*). These points emphasise the potential consequences that the choice of phytometer species (Hanley *et al*., 2011), the number of phytometer plants and the floral density of phytometer patches may have had upon experimental findings, both here and in other phytometer investigations. Larger, denser flower patches would likely have strengthened analyses of insect visitation rate data by attracting more pollinators for a given time period. Clearly this has logistical constraints and therefore it would also be sensible to increase duration of patch surveys in future studies. Alternatively, given the diel variation in activity of flower visiting species documented by others (Inouye *et al*., 2015), a better means of understanding the peaks and troughs in foraging activity may be to cluster visitation rate surveys into distinct temporal periods, with a concomitant increase in survey frequency.

Alongside pollinator visitation, seed set of entomophilous plants can be affected by numerous post-pollination processes (e.g. disease, climate factors and pre-dispersal granivory), as such, the crop specific seed set trends described here cannot be assuredly disentangled from the influence of other, confounding, factors. Data collection using a greater number of sites, and/or a greater number of years, would limit the statistical noise of such factors, strengthening confidence in discernible trends, time constraints therefore place limitations on the robustness of statistical analyses. In addition, I do not distinguish between insect abundance/activity and population trends in this study, for either crop. However, unlike *Miscanthus* which is a longstanding feature of the landscape, field bean is an annual crop and therefore a single year of survey data is likely to purely reflect foraging activity and worker abundance, rather than population level trends. To detect population level effects in field bean, genetic analyses would be necessary in the year post flowering, with effects only likely to persist in the long-term if field bean is grown year on year in the local landscape.

Nevertheless, as far as I am aware, this study is the first attempt to investigate seed set of wild margin plants in *Miscanthus*. Findings support those of chapter 2, with no clear benefit of *Miscanthus* for pollination processes. Apparent negative effects of field bean on clover seed set do not support the hypothesis of improved pollinator service provision to wild margin plants from the cultivation of this MFC. However, in order to ensure conclusions are robust, there remains a need for further study, with the inclusion of a more diverse array of phytometer species, a greater number of field sites and annual replication.

Chapter 4: The influence of two perennial energy crops upon soil element bioaccessibility and bacterial communities when compared to cereals

4.1 Abstract

The effect of cultivating the perennial energy crops (PECs) *Miscanthus* x *giganteus* and willow short rotation coppice (SRC) upon the bioaccessibility of 28 elements, and the structure and composition of bacterial communities, was investigated in the topsoil (0-15 cm depth) of four plantations and compared to cereal controls for both cropped and margin regions. Element bioaccessibility was investigated using a sequential extraction procedure (CISED). Community bacterial nucleic acids were extracted from the soil surrounding crop plants and from the centre of field margins. PCR was used to amplify 16S ribosomal RNA and the amplicons subjected to denaturing gradient gel electrophoresis (DGGE), followed by fingerprint analyses. Prominent DGGE bands were excised and sequenced to determine the taxonomic identities of dominant bacterial populations. For all elements, extractable concentrations did not differ significantly compared to cereal controls for either PEC. Similarly, no significant difference in the diversity of bacterial communities arose between treatments. Sequenced DGGE bands showed relatedness to genera of common soil bacteria, belonging to 21 families and three phyla (Actinobacteria; Firmicutes; Proteobacteria), including *Actinomadura*, *Bacillus, Microvirga* and *Streptomyces*. The DGGE fingerprint patterns were similar between PECs and cereal controls in both crop and margin regions, with no consistent treatment-specific clustering. Findings indicate that the cultivation of *Miscanthus* and willow SRC has little effect upon the bioaccessibility of elements or the structure and composition of bacterial communities in topsoil when compared to cereals.

4.2 Introduction

Land use change has the potential to affect microbial communities and the ecological processes they regulate (Lauber *et al*., 2008), this can have important consequences for biogeochemical cycling and soil fertility (Post and Kwon, 2000). In agroecosystems, variation in soil disturbance (Lupwayia *et al*., 1998), chemical inputs (Bulluck III *et al*., 2002) and crop identity (Grayston *et al.* 1998; Houlden *et al*., 2008) are some of the many factors that can impact microbial communities, with implications for the complex plant-microbe-mineral interactions taking place in soil. For example, differing management practices (i.e. soil cultivation) and differences in crop nutrient uptake may impact bioaccessible element concentrations, whilst the volume and composition of root exudates may differ between crop species, leading to plant specific microbial communities in the surrounding soil (Bais *et al*., 2006). Furthermore, this interaction is reciprocal, soil microorganisms play an important role in the cycling of all major plant nutrients (Yao *et al*., 2000). *Bacteria* can modify soil chemistry for example, releasing extracellular peptides such as siderophores which bind elements and alter their bioaccessibility (Radzki *et al*., 2013), active microbial phosphate solubilisation can assist metal uptake (Richardson and Simpson, 2011), biosurfactants can increase the bioavailability of metals (Primo *et al*., 2015), and oxidation-reduction reactions can influence element mobility and bioaccessibility (Burgin *et al*., 2011). Some *Bacteria*, deemed growth-promoting-*Bacteria*, are even important in bioremediation due to their ability to increase element bioavailability to plants, and thus enhance uptake of toxic elements by hyperaccumulator plants (Glick, 2010).

Given their high annual biomass production and fundamental differences in management when compared to rotational crops, such as cereals; cultivation of the perennial energy crops (PECs) *Miscanthus* x *giganteus* (herein: *Miscanthus*) and willow short rotation coppice (SRC; *Salix* spp.) may be expected to alter microbial community structure and composition. Evidence for this comes from Cattaneo *et al*. (2014) who show greater community richness in *Miscanthus* compared to wheat, and by Truu *et al*. (2009) who show effects of willow SRC upon soil community structure and soil biological activity, when compared to land previously under agriculture.

However, there are currently few studies investigating microbial communities or element cycling under BECs, and where available, most investigations are limited to the consequences of wastewater irrigation and remediation (Truu *et al*., 2009; Dimitriou *et al*., 2011) or restricted to carbon and nitrogen cycling (Davis *et al.,* 2010; Mao *et al*., 2013). Although topics of evident importance, there remains a need to investigate effects of PEC cultivation upon the geochemistry of a broader range of elements, and to consider possible implications for soil microbes. Understanding the effects of elements, such as metals, on the microbial metagenome at low, environmentally relevant, concentrations is poor even under conventional land uses (Sobolev and Begonia, 2008).

Many of the elements yet to be investigated in PEC are vital for life whilst others are highly toxic. Moreover, whilst important in their own right, element availability can also have important consequences for the cycling other elements; for example, molybdenum or vanadium can act as a key component of the nitrogenase enzyme, and therefore play an important role in the process of nitrogen fixation, modifying the demand for fertiliser inputs. Increased bioaccessibility of these elements may therefore be expected to shift bacterial communities in favour of nitrogen fixers for example. Similarly, increases in the mobility/bioaccessibility of heavy metals (HMs), such as toxic chromium (i.e. chromate, Cr^{VI}), may be expected to increase the dominance of bacterial genetic variants that are tolerant, or resistant, to this heavy metal. Although the presence of heavy metals (HMs) within soils is principally related the natural weathering of parent material and anthropogenic activities (i.e. inputs of sewage sludge & fertiliser), plants and bacteria have the potential to alter the bioavailability of HMs through biosorption, sequestration, and changes to soil chemistry (Abdu *et al*., 2017). Elevated HM concentrations are also understood to reduce microbial diversity, activity and population size (Abdu *et al*., 2017). In addition, increased HM bioaccessibility has the potential to affect plant mineral nutrition, influencing nutrient uptake (Marschner *et al*. 1986), with element solubility often explaining toxicity in soils (Rieuwerts *et al*., 1998). Furthermore, as potentially toxic elements (PTE), any increases in the bioaccessibility of HMs to plants may also have important consequences for human health, potentially increasing uptake by food crops grown on the same land in the future.

The aim of this study was therefore to improve understanding of element bioaccessibility in the soils of two novel, commercial, PECs when compared to annual cereals, and to establish the diversity, distribution and composition of microbial communities under these differing land uses. As fungi are understood to be less sensitive than *Bacteria* to HMs I focus on the latter group in this study. Element mobility and bacterial communities were investigated in soil samples collected from established crops in order to provide a more accurate reflection of long-term, field realistic, patterns that reflect bacterial communities which have had time to adapt to prevailing environmental conditions, whilst developing a soil chemistry more broadly reflective of the prevailing land use. The topsoil or ‘A horizon’ is the region of greatest biological relevance, as roots, animals and microbes are at greatest density in this layer (Alexander, 1961). Although both *Miscanthus* and willow SRC have deep roots the majority of root biomass is found in the top 30 cm of soil, as such, this section of the soil profile was the focus of this study. Furthermore, analysis of total soil element composition is likely to principally reflect the soils geologic origins (Rao *et al*., 2007); I therefore use a sequential extraction methodology in order to determine the concentrations of a series of elements when subject to extractants of increasing acidity.

Elements readily extracted at low acidity (i.e. the elements in the chemically active soils fractions, such as colloidal clays and organic matter) are likely to be both mobile and bioaccessible, and thus potentially bioavailable to soil organisms.

4.3 Methods

4.3.1 Soil collection

Soil samples were collected between 22nd June – 3rd July 2015 from PECs, *Miscanthus x giganteus* and willow SRC, and nearby wheat (*Triticum* spp.) or barley fields (*Hordeum vulgare*). *Miscanthus* samples were collected from four sites in Cornwall and willow SRC samples collected from four sites in central England (Nottinghamshire and Lincolnshire) using the same fields and nearby cereal controls described for pollinator transect work in 2015 (Chapter 2, Table 2.1). Soil was collected from around the base of crop plants to a depth of 15 cm and placed into sterile, air-tight plastic containers (Wilko, Plymouth, United Kingdom). In Cornwall soil samples were collected from the central point of a north-south gradient running approximately central through each field. Margin samples were obtained 1 m into the southern margin. In central England samples were collected 5 m into the crop at central points along the west margin and from 1 m into the southern margin. The samples were collected at a shorter distance into the crop compared than Cornwall samples in order to limit damage to the cereal crops. DNA samples were stored at -20 °C using portable freezers following collection in the field, with permanent storage at -20 °C upon reaching the laboratory.

4.3.2 Sequential element extraction

The soils for metal analysis were subject to a sequential extraction procedure derived from the Chemometric Identification of Substrates and Elemental Distributions or CISED methodology (Cave and Wragg, 1997; Cave et al., 2004). Margin and crop soils were processed for each bioenergy crop field and nearby cereal. This resulted in 32 samples, 16 from Cornwall (8 miscanthus & 8 cereals) and 16 from central England (8 willows & 8 cereals). Each sample was well mixed and dried to constant weight in acid washed glass beakers using an electric oven before being subsampled to produce triplicates for analysis. Using an acid rinsed mortar and pestle, soil aggregates were broken and stones removed. All soils samples were then sieved, using an acid rinsed sieve, to give 2g of soil with a particle size range of 125 to <149 µm and placed into sterile 15 ml polypropylene falcon tubes (Fisher Scientific, Loughborough, UK). Each replicate soil was then processed in the same manner. This involved sequentially washing the soil in extractant solutions of increasing acidity, followed by mixing using a vertical rotator (Grant-bio, PTR-60 Multi-Rotator, Grant Instruments, Cambridgeshire, UK) and finally centrifugation for 10 min at 2000 RCF (Harrier 18/80, MSE (UK) Ltd, London, UK) to elute the supernatant. Each soil replicate was subject to a total of fourteen extractant washes (Table 4.1) with soil material resuspended using a vortex (Vortex-Genie 2, Scientific Industries, Inc., Bohemia, NY) in-between washes. Mixed acids were prepared using concentrated HNO_3 acid (S.G. 1.42 (>62%), Fisher Scientific, Loughborough, UK) and HCl acid (S.G. 1.18 (~37%), Fisher Scientific, Loughborough, UK) and diluted to concentration using ddH_2O. All pipettes and volumetrics used in the preparation of acid reagents were dedicated CISED Class A glassware. Where applicable H_2O_2 (>30% w/v, Fisher Scientific, Loughborough, UK) was added to washes prior to rotation (Table 4.1). A total of 1428 elutions were collected (including tube controls) (Plate 4.1) with eluent volumes determined using dedicated Class A measuring cylinders. Eluents were subsequently processed using ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy, iCAP 7000 series, Thermo Fisher Scientific, Wilmington, DE). The 28 elements investigated, their limits of detection and their biological relevance are outlined in Table 4.2. Element concentrations were low, parts-per million (ppm) concentrations were therefore converted to nanomole per gram equivalent (nmol g^{-1} eq.) soil concentrations, facilitating comparison of PEC and cereal treatments.

4.3.3 Soil pH and soil characteristics

Soil pH was determined using a 1:2.5 soil:solution mixture with 1 M KCl and measured using a pH meter with a glass electrode (FiveEasy™ FE20, Mettler-Toledo AG, Schwerzenbach, Switzerland). The pH meter was calibrated and samples centrifuged in order to settle sediment that might interfere with the pH reading of the supernatant. Although understood to be an average of 0.85 pH units lower than water pH (Kabala *et al.*, 2016), KCl pH is thought to be a better measure of total available H^+ concentration as it includes both active and reserve/exchangeable acidity. For broader comparability, 1:5 H_2O pH was calculated using the model presented in Kabala *et al.*, (2016): $pH_{H2O\ 1:5} = -1.95 + 11.58*\log10(pH_{KCl\ 1:2.5})$. Similarity in soil types and parent bedrock was established between PEC and cereal control fields using British Geological Survey data through the *my*Soil (British Geological Survey, 2018a) and iGeology (British Geological Survey, 2018b) applications.

Table 4.1: List of extractants as designated by their molar (M) acid concentrations and in the order sequentially applied to soil samples. ‘A’ refers to first wash and ‘B’ to the second wash using the same extractant concentration, i.e. ‘B’ concentration is equivalent to ‘A’.

Extractant	Extractant volume (mL)	Volume H_2O_2 (mL)
Water A	10	N/A
Water B	10	N/A
0.01 M A	10	N/A
0.01 M B	10	N/A
0.05 M A	10	N/A
0.05 M B	10	N/A
0.10 M A	9.75	0.25
0.10 M B	9.75	0.25
0.50 M A	9.5	0.5
0.50 M B	9.5	0.5
1.00 M A	9.25	0.75
1.00 M B	9.25	0.75
5.00 M A	9	1
5.00 M B	9	1

Plate 4.1: Falcon tubes (15 mL) containing mixed acid extractants following processing of soil samples collected in the crop and margin regions of *Miscanthus x giganteus*, willow short-rotation coppice and their respective cereal controls. Extractant solutions subsequently processed using ICP-OES for element analysis.

Table 4.2: Elements analysed, their ICP-OES limits of detection (LOD) and their biological relevance. LGV= Lower Guideline Value (source: MEF, 2007).

Element	LOD (ppm)	Soil & Biological Relevance
Aluminium (Al)	0.02	A heavy metal/metalloid. A highly abundant soil element. Non-essential. Directly toxic (as soluble Al^{3+}) to plants & most *Bacteria* in acid soils (< pH 5.5), due to competition with Fe & Mg & binding to DNA, membranes & cell walls. Increases soil acidity by removing hydroxide ions, acids produced by microbes can mobilise Al from minerals. Reduces availability of cations, particularly those of P & S.
Antimony (Sb)	0.03	Non-essential. Toxic at high concentration (rarely are soil concentrations high enough to effect plants or animals), potentially carcinogenic. Environmental Sb concentrations are low, soils average 0.3-8.6 mg/kg. Uptake by plants is proportional to soluble soil concentration, little is known about uptake mechanisms. LGV= 10 mg/kg.
Arsenic (As)	0.03	Occurs in soils as a trace element due to weathering of parent rock, mining & arsenical pesticides use. Arsenic is found in most plants, excessive levels disrupt enzyme function & phosphate flow. Microbes can increase As mobility in soils. Some microbes can tolerate & even respire As compounds. Microbial cycling can occur in both aerobic & anaerobic environments. LGV= 50 mg/kg.
Barium (Ba)	0.003	Non-essential to terrestrial organisms. At fairly high abundance in soils but low mobility. Ba is toxic to plants at elevated levels but uptake is generally low. Ba compounds bind strongly to clay and readily precipitate with P & Mn compounds. Microbial action can release Ba^{2+}.
Beryllium (Be)	0.01	Non-essential. Toxic metal that may interact with Ca and Mg and influence P uptake in plants, possibly replacing each other. Potential carcinogen. Plant uptake from soils interferes with uptake of similar minerals. Appears to accumulate in plant roots. Impairs plant growth and yield. High Be concentrations can reduce soil microbial biomass & nitrogen mineralisation & inhibit enzymes.
Boron (B)	0.01	Essential plant micronutrient. Mobile in soil, available to plants from soluble borate minerals and organic compounds. Leachable but fixed to minerals at high pH. Widespread deficiencies but toxic at elevated concentration. Many biological functions, reduces polarity of sugars, aiding movement across membranes. Contributes structural integrity to plant cell walls, facilitates synthesis of plant hormones & nucleic acids,

		maintains the plasma membrane and metabolic processes. Boron concentrations in soil water > 1 ppm are toxic to plants. Soil microbes influence B availability through organic matter breakdown and B content of soil affects their activity. Most *Bacteria* and fungi do not require B but it is necessary for N_2 fixation in cyanobacteria.
Cadmium (Cd)	0.01	Non-essential, no biological function. Heavy metal. Toxic. Natural sources (i.e. bedrock) and anthropogenic sources (e.g. sewage sludge, manure and phosphate fertiliser). Generally poorly mobile in soils, mobility increased with acidity. Many negative effects on microbes reported, < N-fixation, < biomass, <cellulose decomp., < urease, < phosphatase, < CO_2 etc. LGV= 10 mg/kg.
Calcium (Ca)	0.01	Essential secondary plant nutrient, required in high amounts. Activates plant enzymes. Regulates plant nutrient transport. Calcium is important for *Bacteria*, including maintenance of cell structure, motility, transport and sporulation. Combats soil salinity.
Chromium (Cr)	0.02	A heavy metal. Present as chromite (Cr^{III} low solubility, less toxic) and chromate (Cr^{VI}, soluble, highly toxic, mutagenic, teratogenic, carcinogenic) in the environment. Some microbes show resistance to Cr & ability to detoxify. LGV= 200 mg/kg.
Cobalt (Co)	0.02	Essential micronutrient but toxic at relatively high concentrations. A heavy metal. Not essential for all plants. Needed by nitrogen fixing plants. LGV = 100 mg/kg.
Copper (Cu)	0.01	Essential micronutrient but toxic at relatively high concentrations. A heavy metal. LGV = 150 mg/kg. Deficiencies lead to blind grain sites infected with ergot.
Iron (Fe)	0.03	Essential plant micronutrient. A heavy metal. Generally abundant in soils but poorly available. Important cofactor in plant enzymes & for chlorophyll formation. The interaction of plant-microbe-organic substance increase availability of Fe^{III} for plants. *Bacteria* and plants bind iron extracellularly using siderophores and phytosiderophores respectively. Low Cu can lead to Fe deficiency.
Lead (Pb)	0.1	Naturally present in soil. No biological function. A heavy metal. LGV= 200 mg/kg. Little evidence of concentrations above LGV in European soils. Less toxic to *Bacteria* than other HM in culture media but concentrations as low as 1 ppm alter community diversity. Low mobility & long soil retention time. At high concentrations Pb sensitive strains are lost. Pb is rapidly bound by soil components.
Magnesium (Mg)	0.003	Essential plant macronutrient. Important constituent of chlorophyll and necessary for photosynthesis. High importance in cellular metabolism, activates plant

		enzymes including those in glycolysis, stabilises genetic material and activates ATP.
Manganese (Mn)	0.003	Essential plant micronutrient. A heavy metal. Necessary for chloroplast development. Can replace Mg in various metabolic processes. May reduce disease susceptibility, including take-all in wheat. Bioaccessibility affected by bacterial oxidation.
Molybdenum (Mo)	0.02	Essential plant micronutrient. A heavy metal. Component of the microbial enzymes used in the nitrogen cycle. Acid soils (< pH 6.3) can have low Mo. Mo can be applied as fertiliser. Detrimental at high concentrations.
Nickel (Ni)	0.02	Essential plant micronutrient. A heavy metal. Needed in nitrogen metabolism, activating urease in plants. LGV= 100 mg/kg.
Phosphorus (P)	0.03	Essential plant macronutrient. Under high demand by plants and microbes, vital for energy transfer (as a component of ATP), structurally integral to DNA, RNA and cell membrane phospholipids. Important in modifying enzyme activity by phosphorylation.
Potassium (K)	0.05	Essential plant macronutrient. Important in controlling stomata aperture, balancing anions, modifying enzyme activity involved in photosynthesis & respiration. High soil concentrations result in luxury consumption. Deficient plants are poor at synthesising starch, proteins, cellulose and susceptible to disease.
Selenium (Se)	0.1	Toxic metalloid but required by animals and some microbes at low concentration, accumulated by crops. Non-essential in plants. Chemical behaviour similar to S, concentrated in the major sulphide minerals. Little free selenide exists in aerobic soils below pH 9, elemental Se is non-bioavailable. Average soil concentrations are between 0.01 & 2 mg/kg. Oxidation state of Se is primarily controlled by microbes.
Silicon (Si)	0.01	Non-essential. Abundant in soil. Total Si typically ranges from 1- 30 mg/kg. Beneficial to plants, precipitates toxic metals, increases yields, strengthens cell walls & improves tolerance to frost & drought. Accumulates in plants, such as wheat, at levels comparable to essential macronutrients. Solubilised from silicate minerals by *Bacteria* and plant roots, releasing K & Si, reducing the need for K fertiliser.
Sodium (Na)	0.01	Necessary for regeneration of phosphophenol pyruvate in C4 plants such as miscanthus. Can substitute for K in some plants & performs similar functions.
Sulfur (S)	0.03	Essential plant macronutrient. Limited SO_2 input with rain & organic S in manure. Important structurally in amino acids (cysteine & methionine), in enzymes involved in N uptake & production of proteins. Important for chloroplast formation and function. Microbes such as *Thiobacillus* Beijerinck transform sulfides to root available sulfates. Gypsum is also a

		source of sulfates.
Tellurium (Te)	0.03	No known biological function. Toxic. Present at low concentrations (ppb) in environmnetal samples. Average soil content is 0.5-37.5 mg/kg, topsoil median is 0.03 mg/kg.. Low mobility. Oxidation state modified by *Bacteria* can affect toxicity.
Thallium (Tl)	0.1	Not found free in nature. Mostly associated with potassium or metal-sulfide ores. Highly toxic at trace levels. It interferes with K^+ transport & proteins. Affects ATPase activity & transmembrane potential. Solubility increases with acidity. Plant uptake lower than soil concentrations, anthropogenic Tl shows greater uptake than geogenic Tl. Wheat accumulates. Barley accumulation highest in roots. Impacts on soil microbes little understood.
Titanium (Ti)	0.01	Beneficial to plants, aids in Fe acquisition, stimulates enzyme activity, increases chlorophyll content & photosynthesis, improves nutrient uptake. Greater plant uptake with lower pH.
Vanadium (V)	0.01	Required by some plants at low concentrations. A heavy metal. Can substitute for molybdenum, important in the nitrogen cycle. Mainly accumulates in plant roots. At high concentrations may be toxic. EC50 of microbial assays reported as 28-690 mg/kg & 18-510 mg/kg in plants. May inhibit phosphate metabolising enzymes and nitrification at high concentrations. LGV= 150 mg/kg.
Zinc (Zn)	0.01	Essential micronutrient but toxic at relatively high concentrations; may cause gastrointestinal and immunological problems. Deficiencies may cause *Rhizoctonia cerealis* van der Hoeven infection in wheat. A heavy metal. Vital in many enzymes & DNA transcription. LGV= 250 mg/kg. Very little risk to food safety in Europe.

4.3.4 DNA extraction and PCR amplification

Soils subsampled from the locations investigated for metal analysis were subject to Community DNA extraction using the FastDNA® Spin Kit for Soil (MP Biomedicals, Santa Ana, CA), producing 32 samples which were stored at -20 °C. A 177 bp DNA fragment was isolated from the V2 region of the small subunit rRNA gene of eubacteria using the primer set 341f and 518r. For each PCR reaction, 0.5ul of each primer (25 pmol), 12.5 uL of DreamTaq® master mix and 9.5 uL of sterile nuclease free water (Thermo Fisher Scientific, Wilmington, DE) and 2 uL of template to make up a total volume of 25 mL. Prior to PCR, a Nano-Drop® Spectrophotometer ND-1000 (Thermo Fisher Scientific, Wilmington, DE) was used to confirm successful DNA extraction, furthermore, samples were diluted 10-fold with addition of 0.5 mg/mL bovine serum albumin (BSA; Sigma-Aldrich, St Louis, MO) due to initial issues with amplification. A GeneAmp® PCR System 9700 thermocycler (Applied Biosystems, Waltham, MA; Formerly: PE Applied Biosystems) performed PCR amplification using the following touchdown conditions: 95°C for 5 min; 20 cycles of 94°C for 30 sec, 65 °C for 30 sec, 72 °C for 30 sec; 12 cycles of 94 °C for 30 sec, 55 °C for 30 sec, 72 °C for 30 sec with a final extension of 72 °C for 5 min. PCR products were then visualised on a 1% agarose gel and processed using Nano-Drop (as above) in order to confirm amplification, determine concentrations and to check for contaminants.

4.3.5 Denaturing gradient gel electrophoresis (DGGE)

DGGE was performed using a Dcode™ Universal Detection System (Bio-Rad, Hercules, CA). A standardised concentration of the PCR product (120 ng) was loaded onto a 10% (wt/vol) acrylamide gel (acrylamide/bis solution, 29:1) containing a linear gradient ranging from 40% to 55% denaturant [17 M urea and 32% formamide], produced using a Model 475 Gradient Delivery System (Bio-Rad, Hercules, CA). The gels were run for 16hrs at 70 V and 60°C in 1 X TAE electrophoresis buffer (0.04 M

Tris-acetate 1mM EDTA, pH 8.5). The gels were stained in SYBR® Gold (Thermo Fisher Scientific, Wilmington, DE) solution for 25 min and rinsed in water for 5 min. Gels were imaged in a UV Transilluminator using ImageQuant D210 (Molecular Dynamics, Sunnyvale, CA). DNA bands were excised from the gel using a sterile pipette tip and placed into a sterile microcentrifuge tube containing 20 uL of sterile, nuclease-free water. The microcentrifuge tubes were kept at 4°C overnight to allow passive diffusion of DNA into the water and 2 uL of the eluted rRNAs were further amplified using the bacterial universal primers described above. Diffinity RapidTip® tips (Sigma-Aldrich, St Louis, MO) were then used to purify the PCR products which were subsequently diluted to 20- 80 ng/uL, a volume of 5 uL mixed with 5 uL of forward primer (5 pmol) before being sent overnight to GATC Biotech AG (Konstanz, Germany) for sequencing.

4.3.6 Statistical analysis

4.3.6.1 Element bioaccessibility

For each element, mean site concentrations were compared between the PECs (*Miscanthus* and Willow SRC) and their respective cereal controls using *t*-tests and, where applicable, Mann-Whitney U-tests. Separate investigation was made for 'most bioaccessible' concentrations (washes 1-4) and total CISED extractable (washes 1-14) concentrations.

4.3.6.2 Bacterial Communities

DNA fingerprints of the 16S rRNA banding patterns on the DGGE gels were normalised and subject to background subtraction, the gel images were then straightened, aligned, and then analysed to give a densitometric curve for each gel. Profile similarity was calculated by determining Dice's coefficient for the total number of lane patterns and cluster analyses of the lane patterns were constructed. Lane diversity was calculated using Shannon-Wiener diversity indices. All processing and analyses were conducted using BioNumerics software (version 7.6, Applied Maths, Sint-Martens-Latem, Belgium).

4.3.6.3 Phylogenetic analysis

Sequences were compared to GenBank using the BLASTn algorithm on the NCBI website (National Centre for Biotechnology: https://blast.ncbi.nlm.nih.gov/Blast.cgi). Percent identity of sequences to their nearest species match was carried out using MEGA software (Molecular Evolutionary Genetics Analysis version 7, Kumar *et al*., 2015). Sample sequences and nearest matches were aligned using the Kimura 2-parameter model with Gamma distribution and complete gap deletion.

4.4 Results

4.4.1 Element bioaccessibility

Comparison of mean CISED extracted element concentrations to published mean x-ray fluorescence (XRF) values, for English and Welsh soils, indicated that CISED extractable elements likely accounted for very low percentages of the total concentration of elements present in soil samples with heavy metal concentrations well below the lower guideline values presented in Table 4.2. Of the 28 elements, 7 (Be, Cd, Mo, Sb, Se, Te, Tl) had total CISED concentrations below their limit of detection. Where analysable, element concentrations differed little between fields under PEC cultivation or cereal cultivation, both in terms of bioaccessible concentrations (Table 4.4 - 4.5) and total CISED concentrations (Table 4.6 - 4.7). In fact, for 58% of comparable PEC-cereal contrasts, extractable element concentrations were more divergent in non-cropped margin regions compared to the cropped area (Table 4.7; margin data- Appendix 38-41). Near significant differences were found for iron, magnesium and sodium; when compared to cereals, iron concentrations were lower in *Miscanthus* but concentrations of magnesium and sodium were higher, both in terms of the 'most bioaccessible' (pH 3 to ~ pH 7) and total CISED concentrations. Element mobility in willow SRC did not differ significantly to cereals for any element. Patterns of element extraction (i.e. the magnitude of element concentrations extracted for a given acidity/wash) were also highly similar between PECs and cereals, in both the cropped (Appendix 42-43) and margin regions. This is exemplified for manganese in Fig. 4.1; additionally, despite differences in the magnitude of extracted concentrations between regions, extraction patterns are remarkably similar between the two PECs and their paired controls.

Table 4.3: Mean ‘most bioaccessible’ concentrations (nmol g^{-1}) of each element (washes 1-4) and standard error (S.E.) of the mean within the cropped region of *Miscanthus x giganteus* and cereal controls. LOD = Limit Of Detection.

Element	**Al**		**As**		**B**		**Ba**		**Ca**		**Co**		**Cr**	
Land use	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**
Mean	340.50	389.47	0.22	0.13	12.04	12.39	1.25	1.10	6179.81	6094.85	0.07	< LOD	< LOD	< LOD
S.E.	55.21	21.78	0.15	0.08	2.64	4.01	0.36	0.30	1000.07	285.45	0.07	<LOD	< LOD	< LOD
Analysis	t_6= 0.825; *P*= 0.441		t_6= 0.547; *P*= 0.604		t_6= 0.074; *P*= 0.943		t_6= 0.318; *P*= 0.761		t_6= 0.082; *P*= 0.938		N/A		N/A	
Element	**Cu**		**Fe**		**K**		**Mg**		**Mn**		**Na**		**Ni**	
Land use	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**
Mean	0.22	0.38	84.83	123.10	875.73	652.60	1532.87	848.91	563.10	275.24	649.64	418.11	< LOD	0.13
S.E.	0.13	0.21	13.12	11.38	260.73	151.63	325.51	96.41	204.11	158.17	82.72	36.24	< LOD	0.13
Analysis	t_6= 0.664; *P*= 0.531		t_6= 2.204; *P*= 0.07		U_6= 4; *P*= 0.343		t_6= 2.015; *P*= 0.091		t_6= 1.115; *P*= 0.308		t_6= 2.564; *P*= 0.061		N/A	
Element	**P**		**Pb**		**S**		**Si**		**Ti**		**V**		**Zn**	
Land use	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**
Mean	1.25	2.17	< LOD	< LOD	47.92	35.11	463	352.73	1.38	1.58	< LOD	< LOD	2.01	2.23
S.E.	1.05	0.82	< LOD	< LOD	7.88	5.03	91.44	191.81	0.56	0.51	< LOD	< LOD	0.68	0.84
Analysis	t_6= 0.692; *P*= 0.515		N/A		t_6= 1.37; *P*= 0.22		$t_{4.297}$= 0.519; *P*= 0.629		t_6= 0.264; *P*= 0.8		N/A		t_6=0.205; *P*= 0.844	

Table 4.4: Mean ‘most bioaccessible’ concentration (nmol g^{-1}) of each element (washes 1-4) and standard error (S.E.) of the mean within the cropped region of willow short-rotation coppice and cereal controls. LOD = Limit Of Detection.

Element	**Al**		**As**		**B**		**Ba**		**Ca**		**Co**		**Cr**	
Land use	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**
Mean	1567.39	1401.16	0.51	0.37	56.84	24.58	5.09	9.52	6889.32	9488.56	< LOD	< LOD	0.68	0.59
S.E.	610.54	699.11	0.42	0.15	21.24	6.50	0.36	2.65	1205.53	2863.21	< LOD	< LOD	0.68	0.42
Analysis	$t_{(6)}$= 0.179; P= 0.864		$t_{(6)}$= 0.092; P= 0.93		$t_{(6)}$= 1.452; P= 0.197		$t_{(3.108)}$= 1.654; P= 0.194		$t_{(6)}$= 0.781; P= 0.464		N/A		$U_{(6)}$= 6; P= 0.686	
Element	**Cu**		**Fe**		**K**		**Mg**		**Mn**		**Na**		**Ni**	
Land use	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**
Mean	1.50	0.86	551.12	366.97	1237.47	1324.10	2922.95	1623.40	132.04	122.00	421.99	161.48	0.74	0.51
S.E.	0.57	0.30	260.94	172.36	265.89	303.72	871.34	632.68	44.80	53.96	228.73	33.18	0.27	0.36
Analysis	$t_{(6)}$= 0.985; P= 0.362		$t_{(6)}$= 0.589; P= 0.577		$t_{(6)}$= 2.15; P= 0.837		$t_{(6)}$= 1.233; P= 0.264		$t_{(6)}$= 0.454; P= 0.669		$t_{(3.126)}$= 1.127; P= 0.339		$t_{(6)}$= 0.672; P= 0.527	
Element	**P**		**Pb**		**S**		**Si**		**Ti**		**V**		**Zn**	
Land use	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**
Mean	21.72	25.30	< LOD	< LOD	72.11	32.86	3088.04	2628.52	13.04	11.78	1.29	1.13	3.83	4.03
S.E.	6.95	7.79	< LOD	< LOD	41.07	9.69	1387.91	1189.09	5.58	5.76	0.75	0.66	1.10	1.68
Analysis	$t_{(6)}$= 0.343; P= 0.743		N/A		$t_{(6)}$= 0.909; P= 0.398		$t_{(6)}$= 0.481; P= 0.648		$t_{(6)}$= 0.158; P= 0.88		$t_{(6)}$= 0.146; P= 0.888		$t_{(6)}$= 0.097; P= 0.926	

Table 4.5: Mean total CISED concentration (nmol g^{-1}) of each element (washes 1-14) and standard error (S.E.) of the mean within the cropped region of *Miscanthus x giganteus* and cereal controls.

Element	**Al**		**As**		**B**		**Ba**		**Ca**		**Co**		**Cr**	
Land use	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**
Mean	59714.98	40801.74	41.95	41.97	463.83	390.15	77.2	74.2	31608.01	20464.48	42.74	29.78	43.23	35.48
S.E.	10359.8	7464.94	5.72	8.07	36.16	18.73	13.16	18.23	16600.45	2159.12	12.01	7.01	4.96	4.86
Analysis	t_6= 1.481; *P*= 0.189		t_6= 0.002; *P*= 0.999		t_6= 1.809; *P*= 0.12		t_6= 0.133; *P*= 0.898		t_6= 0.246; *P*= 0.814		t_6= 0.933; *P*= 0.387		t_6= 1.115; *P*= 0.308	
Element	**Cu**		**Fe**		**K**		**Mg**		**Mn**		**Na**		**Ni**	
Land use	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**
Mean	189.86	112.83	52669.08	46580.40	3623.29	2922.3	6325.86	3783.13	12056.57	9135.97	866.48	614.70	30.41	26.06
S.E.	113.65	28.35	2388.81	455.54	969.35	790.28	2324	1416.6	3326.51	2966.09	76.87	70.40	5.73	6.51
Analysis	t_6= 0.046; *P*= 0.965		t_6= 2.504; *P*= 0.082		t_6= 0.676; *P*= 0.524		t_6= 0.934; *P*= 0.386		U_6= 5; *P*= 0.486		t_6= 2.415; *P*= 0.052		t_6= 0.501; *P*= 0.634	
Element	**P**		**Pb**		**S**		**Si**		**Ti**		**V**		**Zn**	
Land use	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**
Mean	794.21	736.38	57.68	44.43	343.43	262.01	9932.68	6684.02	205.11	193.98	205.11	193.98	177.16	100.12
S.E.	127.25	88.15	15.89	9.71	53.09	37.49	2413.48	2276.20	24.10	26.19	24.10	26.19	79.66	16.14
Analysis	t_6= 0.289; *P*= 0.783		t_6= 0.712; *P*= 0.503		t_6= 1.211; *P*= 0.271		t_6= 0.979; *P*= 0.365		t_6= 0.313; *P*= 0.765		t_6= 1.356; *P*= 0.224		t_6= 0.948; *P*= 0.38	

Table 4.6: Mean total CISED concentration (nmol g^{-1}) of each element (washes 1-14) and standard error (S.E.) of the mean within the cropped region of willow short-rotation coppice and cereal controls.

Element	**Al**		**As**		**B**		**Ba**		**Ca**		**Co**		**Cr**	
Land use	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**
Mean	26041.45	19950.2	16.05	17.36	231.94	146.50	305.31	221.53	54489.44	91085.61	8.48	11.04	25.90	21.26
S.E.	6460.49	3536.82	6.12	8.40	114.82	80.59	122.62	97.43	27156.44	65059.58	7.09	10.39	8.87	5.89
Analysis	t_6= 0.827; P= 0.44		t_6= 0.103; P= 0.922		t_6= 0.815; P= 0.446		t_6= 0.61; P= 0.564		t_6= 0.175; P= 0.867		U_6= 10; P= 0.686		t_6= 0.436; P= 0.678	
Element	**Cu**		**Fe**		**K**		**Mg**		**Mn**		**Na**		**Ni**	
Land use	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**
Mean	104.27	75.33	22143.31	22276.05	4113.96	4114.06	17541.76	26775.85	3767.23	2978.76	1252.34	1218.06	43.84	27.94
S.E.	57.68	25.36	8938.54	10788.64	452.92	995.27	9013.56	20079.18	1128.68	1008.49	961.24	990.84	18.69	12.32
Analysis	t_6= 0.278; P= 0.791		t_6= 0.225; P= 0.83		t_6= 0; P= 1		t_6= 0.187; P= 0.858		t_6= 0.521; P= 0.621		U_6= 6; P= 0.686		t_6= 0.71; P= 0.504	
Element	**P**		**Pb**		**S**		**Si**		**Ti**		**V**		**Zn**	
Land use	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**
Mean	616.05	624.17	70.94	58.39	438.54	292.21	12265.28	12113.45	214.61	178.29	74.41	62.99	185.74	137.41
S.E.	217.98	209.74	33.78	32.85	259.75	151.24	2729.82	3624.06	93.27	39.03	27.38	18.81	87.26	56.05
Analysis	t_6= 0.027; P= 0.979		U_6= 6; P= 0.686		U_6= 6; P= 0.686		t_6= 0.033; P= 0.974		t_6= 0.359; P= 0.732		t_6= 0.344; P= 0.743		t_6= 0.487; P= 0.644	

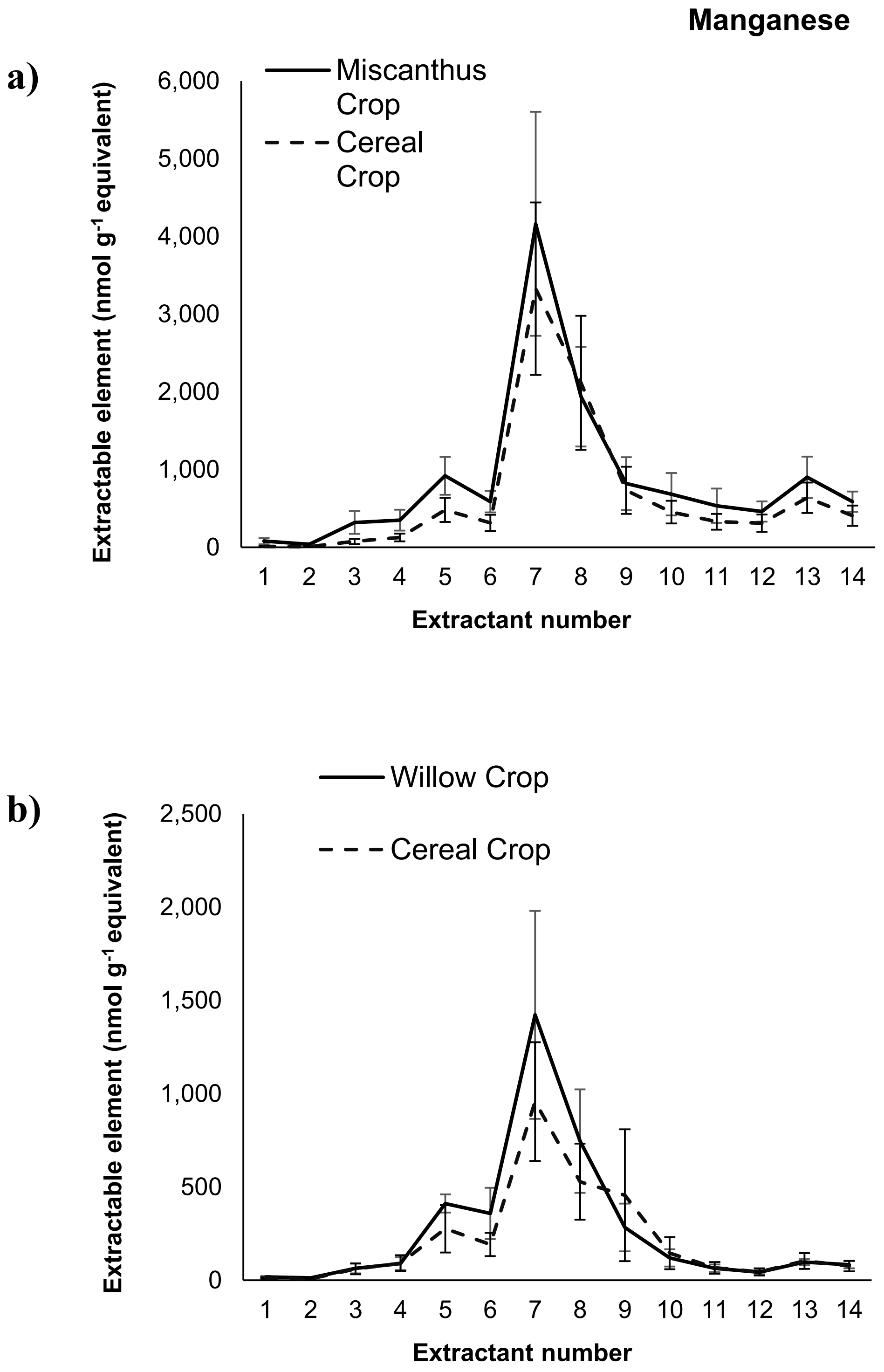

Figure 4.1: Mean manganese concentration extracted in *Miscanthus x giganteus* (a) and willow short-rotation coppice (b) perennial energy crops and their respective cereal controls with each one of the 14 CISED washes. Error bars show standard error of the mean.

Table 4.7: Mean percentage difference in soil element concentrations between two perennial energy crops (*Miscanthus x giganteus* and willow short-rotation coppice) and their respective cereal controls, split by field region (i.e. crop or margin). Grey highlights indicate elements where differences between crop types were greater in non-cropped (i.e. margin) areas. < LOD = below Limit Of Detection.

Element	Miscanthus vs. Cereal		Willow vs. Cereal	
	Crop vs. Crop	Margin vs. Margin	Crop vs. Crop	Margin vs. Margin
Al	17.67	50.97	41.76	61.69
As	125.48	< LOD	167.38	< LOD
B	65.15	78.37	84.61	49.44
Ba	61.76	46.91	54.38	46.89
Ca	25.68	15.34	24.73	27.33
Co	< LOD	< LOD	< LOD	< LOD
Cr	< LOD	< LOD	68.83	67.82
Cu	98.74	61.97	78.88	111.49
Fe	38.87	61.65	66.71	40.46
K	29.60	43.99	65.42	55.52
Mg	54.45	39.77	70.99	69.94
Mn	137.98	64.69	72.23	82.27
Na	41.76	29.62	67.28	42.68
Ni	< LOD	118.07	110.10	55.27
P	94.20	112.00	42.56	33.94
Pb	< LOD	< LOD	< LOD	< LOD
S	41.91	41.31	65.56	59.22
Si	88.73	38.45	63.82	68.58
Ti	71.15	80.82	70.95	62.00
V	< LOD	< LOD	115.06	44.10
Zn	81.47	86.56	44.13	38.20

4.4.2 Soil pH

Across soil samples, 1:5 H_2O pH measures showed a slightly acidic pH between 5-7. However there was some variation between fields with pH values slightly alkaline for 1-2 samples in specific regions at certain sites (e.g. Gainsborough, Appendix 44). Nonetheless, mean pH was similar between crop treatments, KCl pH averaging ~ pH 6 (Appendix 45-46).

4.4.3 Bacterial diversity

Shannon-Wiener diversity did not differ significantly between *Miscanthus* and cereals for either the cropped or non-cropped region, furthermore bacterial diversity did not differ between different areas of the field when considering each crop in isolation, $F_{3,12}$= 0.407; P= 0.751. Similarly, diversity did not differ significantly between willow and cereal controls for either the cropped or non-cropped region, or between different areas of the field when considering each crop in isolation, $H_{3,12}$= 2.404; P= 0.493.

4.4.4 Bacterial structure and composition

To investigate changes in bacterial communities, cluster analysis of the DGGE fingerprint banding patterns were compared between treatments using UPGMA and Dice's indices. No treatment specific clusters arose for any of the crop and field position combinations.

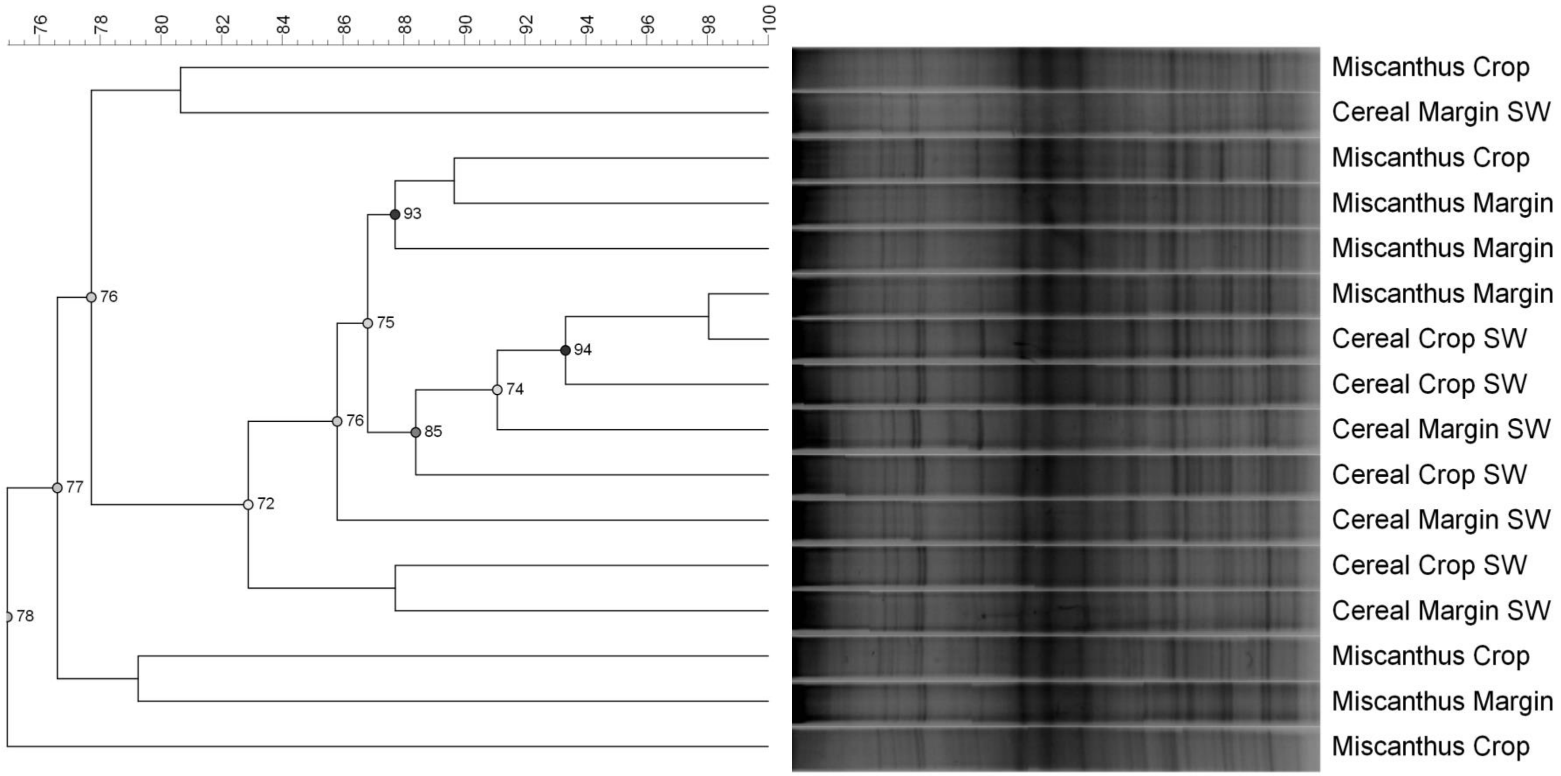

Figure 4.2: DGGE analysis of 16S rRNA fragments of the total bacterial population present in bulk soil around the base of established crop plants in *Miscanthus x giganteus* and cereal fields in 2015 and fragments of DGGE gel corresponding to each sample. SW= southwest.

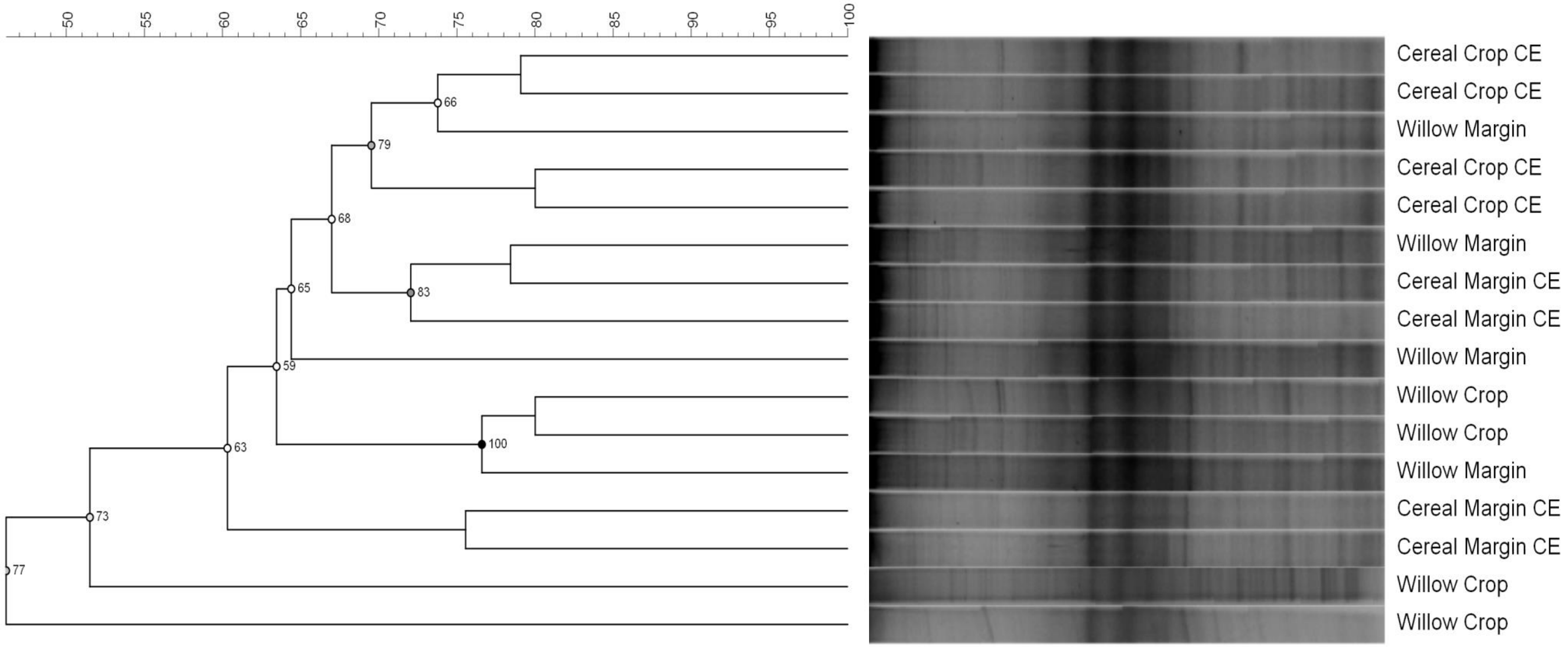

Figure 4.3: DGGE analysis of 16S rRNA fragments from the total bacterial population present in bulk soil around the base of established crop plants in willow short-rotation coppice and cereal fields in 2015, with fragments of DGGE gel corresponding to each sample. CE= central England.

BLASTn comparisons for the 57 sequenced DGGE bands yielded 42 unique species level matches, 64.3% of which were affiliated with the Actinobacteria, 21.4 % the Proteobacteria and 14.3% the Firmicutes and 88.1% of sequences allied with four Orders, Actinomycetales (47.6%), Rhizobiales (16.7%), Bacillales (11.9%) and Micrococcales (11.9%).

Table 4.8: Percent identity of *Miscanthus x giganteus* and cereal control DGGE band sequences in relation to the type strain of the closest species level sequence affiliation on the BLASTn database.

Rf	Related bacterial species	Identity (%)	GenBank accession no.
0.996	*Microvirga aerilata*	79.3	GQ421849
0.996	*Microvirga arabica*	78.3	JN989301
0.991	*Actinomadura flavalba*	81.2	FJ157185
0.946	*Pseudolabrys taiwanensis*	83.3	DQ062742
0.937	*Rhodococcus zopfii*	79.7	AF191343
0.937	*Geodermatophilus tzadiensis*	89	HE654545
0.933	*Rhodococcus fascians*	87.1	X79186
0.865	*Gordonia malaquae*	76.3	AM406674
0.857	*Microvirga aerilata*	83.9	GQ421849
0.798	*Streptomyces nodosus*	78.7	AF114033
0.776	*Actinomadura namibiensis*	80.8	AJ420134
0.749	*Actinomadura sediminis*	73.4	JF272484
0.731	*Chelatococcus reniformis*	75	KJ469373
0.695	*Lacibacterium aquatile*	84.1	HE795994
0.664	*Streptomyces griseorubens*	97.4	AB184139
0.588	*Microvirga ossetica*	90.7	KX576552
0.507	*Mycobacterium smegmatis*	75.4	AJ131761
0.457	*Bacillus drentensis*	83.6	AJ542506
0.372	*Arthrobacter globiformis*	75.8	X80736
0.368	*Bacillus korlensis*	76.9	EU603328
0.318	*Corynebacterium renale*	83.1	X81909
0.318	*Bacillus megaterium*	78.2	D16273
0.269	*Mycobacterium smegmatis*	75	AJ131761
0.251	*Bacillus subtilis*	77.9	AJ276351
0.206	*Mycobacterium rutilum*	80.7	DQ370011
0.184	*Lacibacterium aquatile*	88.2	HE795994
0.108	*Gordonia polyisoprenivorans*	69.4	Y18310

Table 4.9: Percent Identity of willow short-rotation coppice and cereal control DGGE band sequences in relation to the type strain of the closest species level sequence affiliation on the BLASTn database.

Rf	Related bacterial species	Identity (%)	GenBank accession no.
0.991	*Microvirga aerilata*	90.2	GQ421849
0.915	*Microlunatus phosphovorus*	90	Z78207
0.891	*Spirillospora albida*	81.2	D85498
0.864	*Microlunatus phosphovorus*	84.3	Z78207
0.824	*Microvirga aerilata*	83.2	GQ421849
0.816	*Spirillospora albida*	78.1	D85498
0.801	*Methyloceanibacter caenitepidi*	89.1	AB794104
0.756	*Friedmanniella sagamiharensis*	80.5	AB445456
0.737	*Dermacoccus nishinomiyaensis*	79.4	X87757
0.71	*Glutamicibacter nicotianae*	93.4	X80739
0.661	*Sphaerimonospora mesophila com*	82.5	AF002266
0.62	*Chelatococcus daeguensis*	93.2	EF584507
0.611	*Microvirga aerilata*	88.2	GQ421849
0.575	*Clostridium algidicarnis*	93.3	AF127023
0.489	*Jatrophihabitans huperziae*	89.2	KR184574
0.434	*Allochromatium vinosum*	70.5	AJ563291
0.389	*Microbacterium testaceum*	81.6	X77445
0.335	*Gordonia malaquae*	77.9	AM406674
0.33	*Actinomadura namibiensis*	80.4	AJ420134
0.267	*Terribacillus saccharophilus*	83.5	AB243845
0.262	*Mycobacterium smegmatis*	74.8	AJ131761
0.222	*Chelatococcus reniformis*	80.3	KJ469373
0.195	*Jatrophihabitans huperziae*	77.6	KR184574
0.181	*Microbacterium esteraromaticum*	76.1	Y17231
0.176	*Thermomonospora chromogena*	86.9	AF116558
0.131	*Jatrophihabitans huperziae*	90.1	KR184574
0.109	*Actinomadura sediminis*	76.3	JF272484
0.104	*Streptomyces erumpens*	71.3	AY999825
0.09	*Micromonospora wenchangensis*	75.1	JQ768361
0.072	*Nocardia brasiliensis*	78.3	AF430038

4.5 Discussion

Because PEC and cereal treatments had similar pH, and soil types and bedrock were comparable between paired sites, it is reasonable to assume that had any changes in element bioaccessiblity or bacterial community parameters been recorded, they would have been principally driven by crop differences. However, when compared to cereal controls, the results presented here do not indicate any crop specific effect of cultivating either *Miscanthus* or willow SRC upon element bioaccessibility or bacterial communities.

Broad patterns of element bioaccessibility conformed to expectations derived from the literature, with low mobility elements such as barium showing low CISED extractable concentrations, whilst others, such as aluminium, exhibited relatively higher concentrations. The similarity in element concentrations and patterns of extraction across treatments provides a clear indication that extractable elements have similar mobility, likely associated with similar soil phases (i.e. specific minerals, organic matter or pore water) irrespective of the crop grown.

Given that the PECS were cultivated on agricultural land in this study, the low absolute concentrations of heavy metals (HM) found in all treatments is not surprising, and many of the most toxic elements were undetectable using ICP-OES. Although there is a limited understanding of how most microorganisms respond to heavy metals (HM), it is understood that many are highly sensitive to their presence in soils (Giller *et al*., 2009). The universally low HM concentrations found in this study, and, importantly, the similarity between land uses, suggests that the HMs studied here are unlikely to pose differential stresses on microbial communities in the two investigated PECs, when compared to their cereal controls. If concentrations of HMs varied significantly between land uses it is possible, had toxicity thresholds been exceeded, that specific enzyme inhibition would have selected for declines in certain taxa, i.e. those with susceptible enzymes, and dominance of less sensitive taxa, with a probable reduction in diversity (Sobolev and Begonia, 2008). DGGE analyses confirm that this was not the case, with similar community diversity, and cluster analysis revealing overlapping, non-discrete, bacterial communities under the different land uses (i.e. soils under PECs were often more similar to non-cropped regions, or even cereal controls, than conspecific PECs). As such, potentially toxic elements (PTE), in the form of the HMs studied here, do not appear to be differentially affecting the diversity or structure of bacterial communities under the different crop types. Furthermore, Actinomycetes have been shown to be particularly sensitive to HMs (Hayat *et al*., 2002), their dominant affiliation to sequenced DGGE bands therefore further emphasise that cultivating these two PECs has little affect upon HM bioaccessibility.

Despite similar extractable element concentrations, and similar soil types and soil bedrock (for paired sites), variability in bacterial community structure within each of the crop treatments (i.e. PEC or cereal) supports the idea that site-specific edaphic factors played a greater role in determining bacterial community structure than the identity of the crop grown. This contrasts to the apparent PEC-specific effects on nitrogen cycling activity when compared to 1st generation BEC maize, reported by Mao *et al*. (2013); although the authors found no difference between the three 2nd generation PECs studied. In addition, tillage of rotational wheat has been shown to reduce bacterial diversity in comparison to no-till (Lupwayia *et al*., 1998), given the lack of soil disturbance under the established PECs, the similar diversity measures presented here are therefore unexpected. However, Cattaneo *et al.* (2014) also failed to find differences in Shannon-Wiener diversity for *Miscanthus* or giant reed (*Arundo donax*) when compared to rotational crops in Italy, although the authors report that community richness was higher in PECs. Nevertheless, results do correspond to the findings of studies more broadly relevant to land use effects on bacterial communities. For example, in Colarado, Lauber *et al*. (2008) demonstrated that different land use types did not harbour distinct fungal or bacterial communities; soil pH was in fact the best predictor of bacterial community composition, and fungal communities were determined by nutrient status. Similarly, Fierer and Jackson (2006) also found pH to be the main driver of bacterial richness and composition using 98 samples from across the Americas. Furthermore, in a nation-wide survey of UK soils (> 1000 samples) pH has been shown to influence both bacterial alpha- and beta-diversity (Griffiths *et al*., 2011). In this study pH was similar between paired PEC-cereal fields, and mean site pH near identical across treatments, however, not unexpectedly, variation was recorded between sites. Effects of PEC upon bacterial communities, and extractable element concentrations, may therefore be masked by more influential site-specific factors such as pH.

Although the structure of bacterial assemblages were inconsistent between crop types, investigation of sequences derived from dominant DGGE bands, did not indicate any particularly novel taxa across treatments, with sequences affiliated to common soil bacteria. For example, members of the Actinobacteria were well represented and included 8 genera understood to be widespread in soil. Many Actinobacteria are saprophytes important in the decomposition of organic material including recalcitrant plant residues such as lignocellulose as well as the decomposition of stover. Similarly, *Bacillus* spp. are ubiquitous, both in bulk soil as well as the rhizosphere of many plants, endospore formation facilitating their survival under a wide range of climatic conditions.

The value of DGGE is that it provides a cost-effective method to investigate broad patterns associated with microbial communities, however in comparison to next generation sequencing techniques (NGS) it provides limited coverage. Future study using NGS could provide a precise inventory of species assemblages, perhaps revealing more subtle shifts in bacterial communities not evident using fingerprint analyses. Furthermore this investigation was limited to *Bacteria*; it is possible that fungi may show a greater response to PEC cultivation, as such, there remains a need to better understand the consequence of PEC cultivation for fungal communities and the processes they control. For example, fungi are considered important in lignocellulose degradation, the high lignocellulosic residue inputs under these PECs may therefore have important consequences for the structure and composition of fungal communities. In order to better relate data collected on both soil *Bacteria* and soil element mobility in this study, bacterial communities were only investigated in samples corresponding to those obtained for processing by CISED methodology. Due to the time intensive nature of running the sequential extraction procedure on sample triplicates there were limitations on the number of samples it was feasible to process, however it is recognised that in future studies increased sample sizes are preferable particularly given the high variability in extractable element concentrations between fields under the same crop treatments (i.e. PEC or cereal).

This study was an exploratory investigation, facilitating the determination of generalisable, field relevant patterns of element mobility, and bacterial community structure and composition, collection of soil samples in this study was fairly coarse, with samples in the cropped region encompassing both bulk and rhizosphere soil. If soil samples had been constrained to the rhizosphere, i.e. the 2 - 80 mm zone directly surrounding the roots (Koo *et al*., 2005), it is possible that element mobility and bacterial community structure and composition would have differed more notably between the PEC and cereal treatments. However, if changes to element mobility are rapidly reversed outside of the rhizosphere, focusing on such a limited portion of the soil would have conveyed little information on consequences of land use change for biodiversity and soil biogeochemistry at the field scale, which I show here to be minimal in regards to the topsoil.

Chapter 5: Seasonal leaf litter decomposition rates in the perennial energy crop *Miscanthus x giganteus* with comparison to cereals

5.1 Abstract

Leaf litter decomposition processes have importance for soil fertility and climate change mitigation. However, there is currently limited understanding of how land use change to perennial energy crops may influence decomposition processes when compared to conventional arable. In this study I investigate the effect of *Miscanthus x giganteus* cultivation upon the breakdown of *Urtica dioica* leaf litter in the crop and margin regions of *Miscanthus* and cereal controls. Litter bags were placed upon the soil surface to specifically investigate the effects of land use change upon meso-microfauna fragmentation activity, with assays carried out for a two week period in each season. When compared to cereals, *Miscanthus* significantly increased winter decomposition rates, with mass loss comparable to that of the non-cropped field margins. During spring assays, rates of decomposition were reduced under both crops in comparison to field margins whilst, across treatments, decomposition rates were significantly greater during autumn assays when compared to other seasons. However, no significant difference in decomposition was found between *Miscanthus* and cereals during spring, summer, autumn or cumulatively across assays. Nonetheless, when compared to cereals, this is the first study to report elevated rates of meso-microfaunal leaf litter breakdown under *Miscanthus* during the winter months, such crop specific variation suggests that *Miscanthus* cultivation may have important consequences for decomposition processes over the crop's lifespan, when compared to annually rotated cereals.

5.2 Introduction

Decomposition is essential for the biogeochemical cycling of elements and for the maintenance of soil fertility. In terrestrial systems, the decomposition of litter from plants (i.e. leaves, twigs and bark) is an important component of global carbon and nitrogen budgets (Aerts, 1997; Yang *et al*., 2004) and described by Kuzyakov (2006) as one of the five main biogenic sources of soil CO_2 efflux. The decomposition of abscised leaves is also particularly important in crop systems, recycling nutrients back to the soil and so reducing nutrient offtake at harvest. In addition, whilst a source of soil organic carbon (SOC), the litter layer is itself an important reservoir/sink of carbon rich organic material. Therefore factors that influence litter decomposition processes have important implications for carbon sequestration and climate change mitigation.

Land use change from conventional cereals to perennial energy crops (PECs) has the potential to alter decomposition processes due to dissimilarities in crop morphology and management practices, including differences in harvest cycles, microclimate conditions and potentially the volume and composition of root exudates entering the soil (Técher *et al*., 2011; Hromádko *et al*., 2014). In particular, *Miscanthus x giganteus* and willow short-rotation coppice (SRC; *Salix* spp.) crops exhibit regrowth from rhizomes or stools respectively, and thus there is a consequential reduction in soil disturbance throughout their lifecycle. Evidence from studies on no-tillage systems (Ogle *et al*., 2005) has led to the suggestion that PECs may reduce aerobic microbial mineralisation and so slow decomposition processes occurring within the soil, leading PEC soils to act as net carbon sinks, with greater SOC stocks than conventional systems (Hansen *et al*., 2004).

Prior to incorporation into the topsoil, plant litter is subject to breakdown by physical and chemical processes and through biotic degradation by soil animals. When investigating the breakdown of common nettle (*Urtica dioca*) litter at the soil surface, Rowe *et al*. (2013) reported elevated decomposition in willow short-rotation coppice (SRC) when compared to wheat, providing support for differential effects of PEC plantations upon soil surface decomposition, and thus underscoring the need for further studies in additional PEC systems. To date, there has been no comparative investigation of litter decomposition processes in *Miscanthus* in relation to conventional arable crops such as cereals.

Soil microbes and invertebrate communities are understood to play an important role in decomposition with the meso-microfauna in particular contributing to the process of litter fragmentation (Wang *et al*., 2009). As such, following the work of Rowe *et al*. (2013), I hereby investigate whether the rate of *U. dioica* litter decomposition differs between *Miscanthus* and cereal crops using a litter bag methodology, specifically investigating the role of the meso-microfauna by imposing body size constraints on litter accessibility. Due to seasonal differences in crop morphology and management, I undertake assays in each season, in both cropped and non-cropped field regions, over the course of a year.

5.3 Methods

5.3.1 Field assays

To facilitate comparison between studies, methods largely follow those of Rowe *et al.* (2013). Leaf litter bags (170 mm x 100 mm) were placed in both *Miscanthus* and nearby cereal fields. Paired field site locations are described in Table 5.1 and soil properties in Appendix 47. Bags had a 1 mm mesh size and were made of nylon (Plymouth City Market, Colleens City Fabrics), sown on 3 sides using cotton fabric and the opening rolled closed and securely cable tied, allowing only meso- and microfauna access to the litter. Common nettle (*Urtica dioica* Linnaeus 1753) leaves were used to provide a single standardised tissue that would realistically occur within the field margins at all sites (*personal observation*, Rowe *et al.*, 2013). Leaves were collected from the field margins of an arable site in Cornwall (50°25.7800'N, 4°35.530'W). Following overnight wilting (to reduce sting cell activity), leaves were cut into 2 cm^2 sections to standardise size. Diseased, senescing and damaged leaves were discarded. Cut leaf sections were dried for 24 h at 60 °C and 2 g added to each litter bag. Litter bags were placed on the soil surface at ten locations within each of 8 fields using 2-3 metal tent pegs, 5 within the cropped area and five within the margins. Four bags were placed 20 m into the cropped region at points directly North, East and South of the field centre with four bags placed in the margins (~ equidistant from the crop and hedgerow) at corresponding positions and one in the southwest corner of each field. Litter bags were left *in situ* for two weeks before being collected. This time period allowed for an appreciable fall in mass without complete breakdown of material from all bags. For each *Miscanthus* and cereal field pair, bags were deployed and collected on the same day, and all deployments were completed within two days. Upon collection, bags were removed from the field and placed into paper bags to prevent litter loss during transport. Despite placement of 80 bags during each trial, there were variable bag losses in each season (likely due to mammal or bird damage). In the laboratory, litter was separated from any extraneous material and dried for 24 h at 60 °C to establish mass loss over the two week trial. The experiment was repeated four times to test for seasonal differences in decomposition, autumn (1st-16th November), winter (28th December- 12th January), spring (12th - 27th May) and summer (4th - 19th July).

Table 5.1: Location, grid references (approximate field centres) and characteristics of leaf-litter decomposition study sites in southwest England, 2016-2017. Coordinates: WGS 1984. Information obtained using Google Earth (Google Earth, 2017). N, S, E and W are representative of cardinal directions.

Location	Crop	Lat. Lon.	Field Area (ha)	Altitude (m)	Aspect
Coldrenick, Cornwall	Miscanthus	50° 30.290'N, 4°41.1850'W	1.9	210	NW
	Adjacent Cereal	50° 30.365'N, 4°41.1180'W	6.2	216	N
Lostwithiel, Cornwall	Miscanthus	50° 25.704'N, 4° 37.887'W	5.6	113	SW
	Adjacent Cereal	50° 25.857'N, 4° 37.483'W	7.1	134	NE
Pelynt, Cornwall	Miscanthus	50° 21.886'N, 4° 31.222'W	4.8	142	S
	Adjacent Cereal	50° 21.620'N, 4°31.110'W	4.5	131	NE
St Minver, Cornwall	Miscanthus	50° 33.076'N, 4° 51.233'W	5.4	47	SW
	Adjacent Cereal	50° 33.240'N, 4° 51.334'W	5.6	53	SW

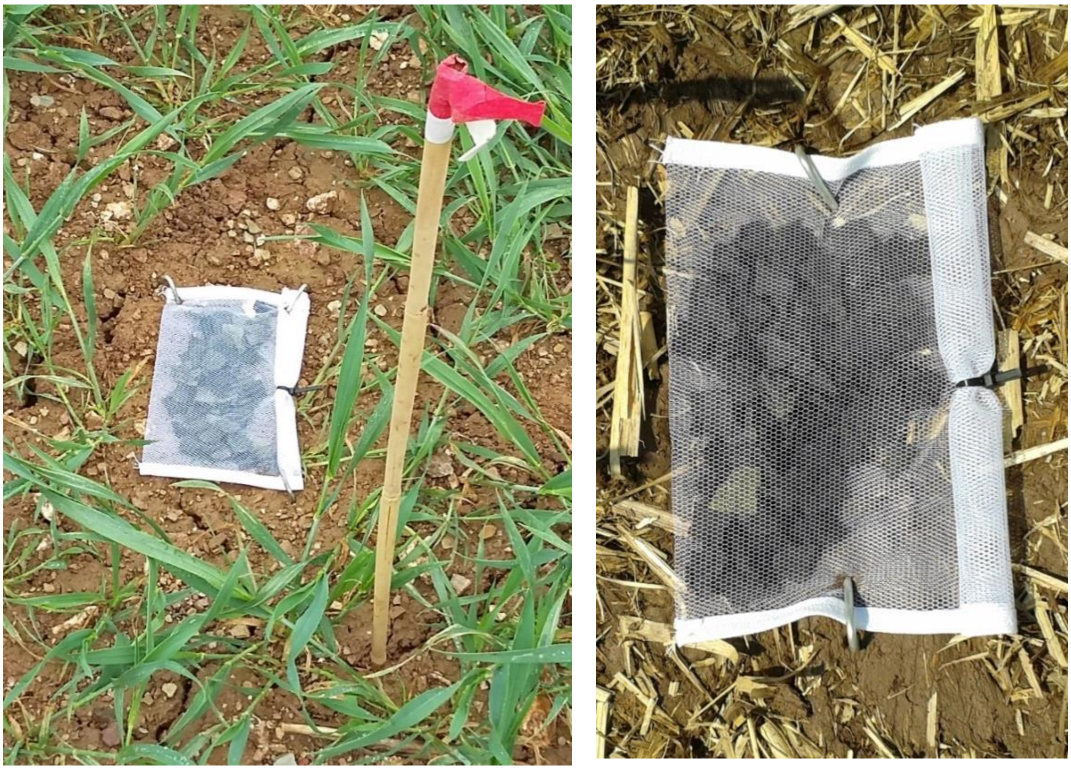

Plate 5.1: Leaf-litter decomposition bags situated within the cropped region of wheat (*Triticum* spp.) (May 2016).

Prior to all analyses, investigation of normality (graphical, Shapiro-Wilk) and homogeneity of variance (graphical, Levene's test, comparison of minimum and maximum variance) of residuals was undertaken, with $Log_{10}+1$ transformations used for non-normal data and Welch's ANOVA run for normal but heteroscedastic data. Mean percentage remaining mass, at the level of 'site-season-crop-field position', comprised the response variable in the analysis. For each season, it was necessary to separately investigate effects of crop type (i.e. *Miscanthus* or cereal), field position (i.e. cropped region or non-cropped region) and crop*field position interaction upon percentage remaining litter mass using 2-way ANOVAs. This arose as a result of heteroscedasticity preventing all main effects and interaction effects being testable using a single model and, similarly, led to two-way ANOVA's being implemented for overall analysis of the main effects of crop type and field position as well as the crop*field position interaction upon percentage remaining litter mass across seasons, with one-way ANOVAs and Welch's ANOVA necessary to investigate season and site effects when combining crop and field position treatments. Where interactions were significant, simple main effects tests were undertaken. Analyses were carried out using SPSS (version 22, IBM Inc.).

5.4 Results

During winter assays there was a significant interaction of crop type (i.e. *Miscanthus* or cereal) and field position (i.e. cropped region or margin region), $F_{1,12}$= 4.924; *P*= 0.047. This arose as fragmentation of litter in *Miscanthus* differed little between crop and margin regions, $F_{1,12}$= 0.002; *P*= 0.97, whilst in cereals winter decomposition was notably reduced in the cropped area of the field (Fig. 5.1) leading to differences in rates of mass loss between crop types in the cropped region of the field, $F_{1,12}$= 9.605; *P*= 0.009.

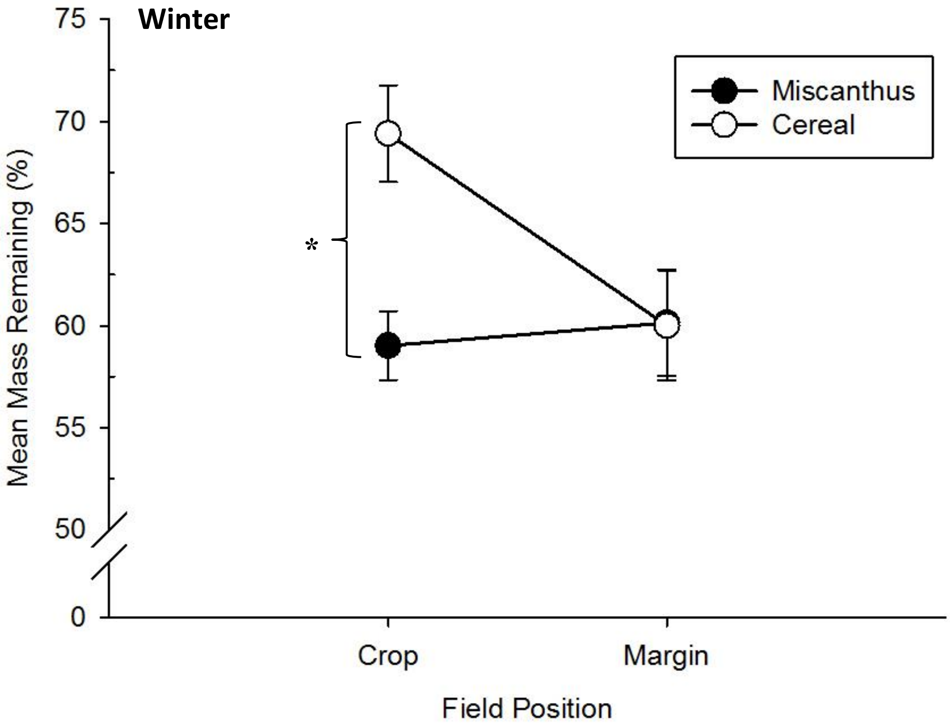

Figure 5.1: Interaction between crop type (*Miscanthus x giganteus* or cereal) and field position (crop or margin regions) upon the mean winter percentage remaining common nettle (*Urtica dioica*) leaf litter at sites in southwest England, 2016. Error bars show ±1 S.E. * = significant simple effect, α= 0.05.

In addition, during spring, there was seen to be a significant effect of field position, with greater decomposition in field margins, $F_{1,12}$= 5.309; P= 0.04 (Fig. 5.2), and across treatments a significant difference occurred between seasons, $F_{3,30.751}$= 15.645; P= <0.001, with autumn decomposition significantly higher than all other seasons (Fig. 5.3i). No difference in mass loss arose between sites, $F_{1,62}$= 0.288; P= 0.594, and cumulatively (i.e. across seasonal assays), no difference in mass loss was seen with no significant interaction of crop and field position across seasons, $F_{1,62}$= 0.288; P= 0.594. Similarly, for all seasons decomposition in cropped and margin regions did not differ when considering both crops collectively, $F_{1,62}$= 1.094; P= 0.3 (Fig. 5.3ii), and litter breakdown rates were non-significant between crops when considering both field regions and all seasons, $F_{1,62}$= 1.458; P= 0.232 (Fig. 5.3iii).

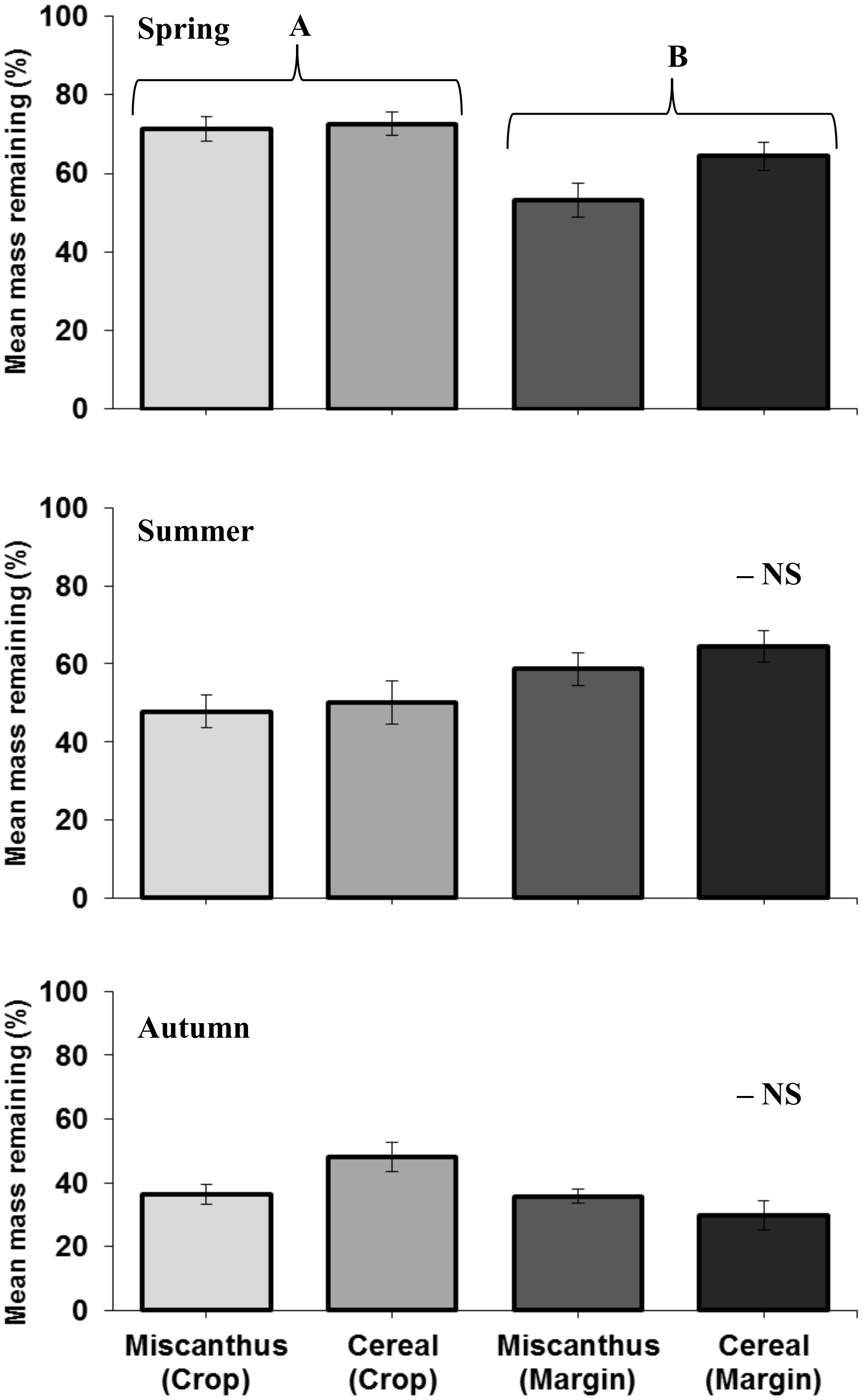

Figure 5.2: Spring-autumn specific percentage dry weight of common nettle (*Urtica dioica*) leaf-litter after two weeks of field exposure, split by crop (*Miscanthus x giganteus* or cereal) and field position (crop or margin). Error bars show ±1 S.E. Different letters denote significant difference between treatments following Tukey post-hoc tests, α= 0.05. NS= Non-significant.

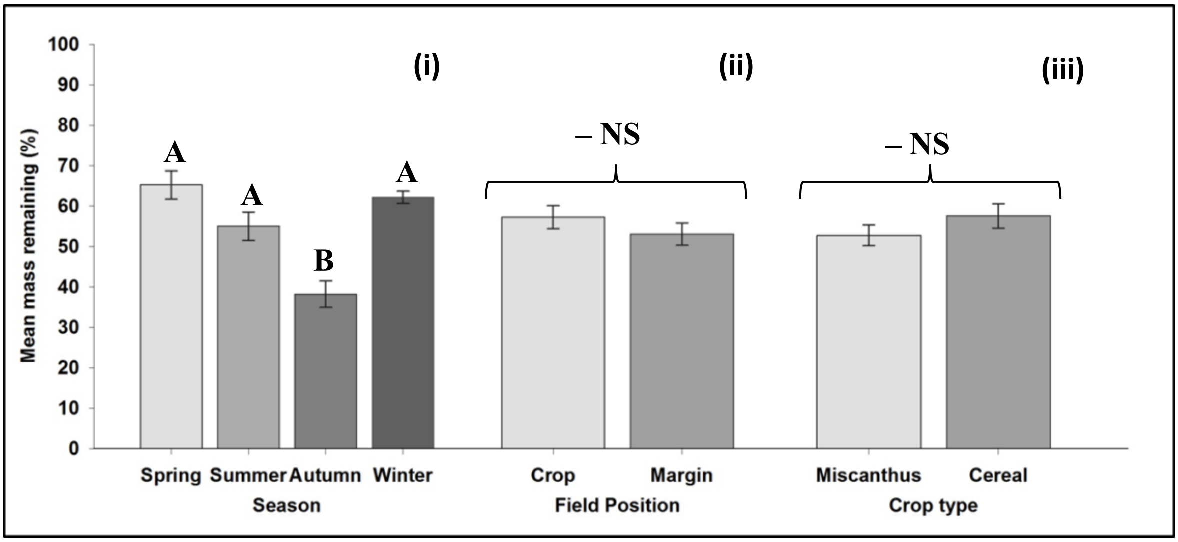

Figure 5.3: Comparison of mean percentage dry weight of common nettle (*Urtica dioica*) leaf-litter between (i) seasons, (ii) field positions and (iii) crop types, following two weeks of exposure in the field. Error bars show ±1 S.E. Different letters denote significant difference between treatments following Games-Howell post-hoc tests, α= 0.05. NS = Non-significant.

5.5 Discussion

Rates of leaf litter breakdown showed significant variation between PEC *Miscanthus* and cereal controls during the winter, with reduced litter breakdown under cereals. Differences in decomposition rates were specific to the cropped region with mass loss in margin regions near identical between crop types. This crop specific effect suggests that *Miscanthus* cultivation is likely to alter decomposition processes when replacing cereal crops, particularly given the PECs long, 15-20 year, lifespan. Despite leaf abscission in late summer, *Miscanthus* remains unharvested until spring whereas cereal crops are harvested between mid-July and mid-September. Given the height of *Miscanthus* stands, the visual contrast to the stubble of cereal fields during the winter is starkly apparent, potentially affecting microclimate conditions, thus providing a possible explanation for the differences in rates of decomposition recorded here, i.e. the soil of cereal fields are likely less buffered to climate and weather variables throughout the winter months.

The presence of *Miscanthus* leaf-litter and cereal residues help to retain moisture in each crop respectively, however the tall stands of *Miscanthus* likely provide a more sheltered microclimate, potentially favouring decomposition processes by increasing decomposer and detritivore activity. The extremely dense litter layer is also likely to have resulted in elevated temperatures when compared to more exposed cereal soils, where comparatively little organic material is left in the field. This is supported by the findings of Kucharik *et al*., (2013) in the US, who demonstrated that varying thickness of *Miscanthus* straw by 1-5 cm increased temperatures by 2.5°C to 6°C at 10 cm soil depth. When compared to cereals, inputs of *Miscanthus* residues are consistently higher throughout the year; however they are likely to be most important during the winter due to recent inputs from leaf-fall and as a consequence of lower average and minimum temperatures. The greater exposure of cereal soils may therefore explain the reduced winter decomposition when compared to *Miscanthus*.

The process of spring harvesting is an unusual aspect of *Miscanthus* management but despite potentially having an indirect effect upon winter decomposition, there was no evidence for a direct effect upon rates of litter breakdown in spring. Although spring specific differences in litter decomposition were documented between litter bags situated in different field positions/regions (i.e. cropped and non-cropped areas), these differences occurred across both crops, with greater litter breakdown in field margins. This may have arisen due to reduced vegetation cover in cropped areas, with cereals yet to fully develop and *Miscanthus* stems having been recently harvested. Nevertheless, the absence of crop specific differences in mass loss therefore suggests that *Miscanthus* harvesting has little effect on initial spring decomposition processes when compared to cereals.

Similarly, summer assays also failed to indicate any variation in decomposition between crops. These results contrast to increases in the decomposition of *U. dioica* litter described by Rowe *et al.* (2013) in another PEC, willow SRC, when compared to barley, and assayed for the influence of both macro- and meso-microfuana activity. Rowe *et al.* (2013) report elevated decomposition rates in willow when compared to controls, although, similar to summer *Miscanthus*, decomposition rates do not differ across field regions (i.e. headland or cultivated area), and no interaction between field position and land use is recorded. However, as stated, trials conducted by Rowe *et al.* (2013) relate purely to the summer months, so it is not possible to determine whether such trends would be found for willow in other seasons, or across seasons, when compared to the *Miscanthus* data presented here. Nonetheless, for summer data, trends in litter decomposition between the two PECs and their cereal controls may be validly compared.

Considering that decomposition rates in the winter '*Miscanthus*-cereal' comparison are specific to the crop region, whilst differences between the summer 'willow-cereal' comparison are reported in both cropped and non-cropped regions, it therefore appears likely that different mechanisms are driving decomposition processes in each of these season-specific comparisons. In willow, the attribute(s) of cultivation which increases the rate of decomposition might therefore be shared between both headland and cultivated regions of the fields or decomposition rates may be driven by different factors within each region, leading to similar outcomes upon decomposition processes. Alternatively, highly mobile decomposers, which benefit in one region of the field, may spill-over to neighbouring regions, enhancing decomposition processes in both areas. Regardless, it appears likely that PEC specific factors affecting summer decomposition rates in willow SRC are different to those occurring in winter *Miscanthus*.

Rowe *et al*. (2013) suggest that elevated litter decomposition in summer willow fields is likely to have resulted from elevated detritivore and decomposer activity, with Makeschin (1994) having reported that willow SRC supports higher numbers of earthworms and woodlice than arable, with generally higher microbial biomass and soil faunal diversity. By contrast, in *Miscanthus*, Zangerl *et al.* (2013) directly investigated the decomposition process for three grass PECs (*Miscanthus*, switch grass and planted prairie) in Illinois and found no effect of invertebrates on rates of decomposition. The authors compared the decomposition of insecticide treated BEC litter to untreated litter and found no effect upon remaining litter mass, inferring little impact of invertebrates on decomposition process in *Miscanthus* plantations. This casts doubt on the relative contribution of invertebrates and abiotic factors upon litter breakdown in *Miscanthus* in this study when compared to cereals. However, methodological limitations constrain the interpretation and representativeness of Zangerl *et al.*'s findings in relation to the data presented here. For instance, assays were conducted in small plots (0.7 ha) of recently established *Miscanthus* with litter placed in bottomless trays, and hence only likely to be accessible to below ground invertebrates (trays were 5 cm in height and covered with a fiberglass mesh screen of an unspecified mesh size). As far as I am aware, no other studies have investigated decomposition processes in *Miscanthus*. However, in a study investigating invertebrates in *Miscanthus* by Bellamy *et al.* (2009), significantly lower winter abundances of earthworms were recorded in pitfall traps compared to wheat, with no difference in the abundance of Collembola and Acari, two taxa clearly capable of accessing the litter bags used in this study. However, although non-significant, Myriapoda and Mollusca (slug and snail) abundances in *Miscanthus* were nearly double those in wheat, indicating a rather mixed taxonomic response of epigeal and endogeal inverterbrates to *Miscanthus*, despite broadly similar cumulative counts (i.e. total invertebrate abundance).

Despite the short duration of the field experiments in this investigation, fungi and bacteria likely played at least some role in litter decomposition because leaf-litter inputs were partially fragmented upon placement in the field (i.e. cut to a standardised size, comparable to Rowe et al., 2013). This cutting allows microbes to by-pass the cuticle and access the leaf interior without having to first wait for fragmentation activity. It is therefore possible that enhanced litter breakdown in winter *Miscanthus* arises from increased microbial activity. Nonetheless, the proportional influence of microbial activity on decomposition cannot be ascertained and invertebrate fragmentation is presumed to be the predominant driver of mass loss. Although invertebrate populations were not specifically surveyed in this study, it was seen that millipede and slugs were regularly present within litter bags (*personal observation*), organisms understood to be effective at litter fragmentation and the taxa reported to show elevated abundance by Bellamy *et al*. (2009). The presence of millipedes (predominantly classed as macrofauna) demonstrates that it is erroneous to assume that constraining mesh size produces consistent and universal limitations upon the taxonomic identity of invertebrates accessing the litter-bags, both here and in other studies. Variation in body size that occurs during the ontogeny of many species (i.e. earthworms) also makes clear that it would be unsubstantiated to assume complete exclusion of many other taxa typically classified as macrofuana.

As temperature is understood to be one of the key factors determining decomposition rates (Anderson et al., 1991; Hobbie, 1996), variation in the mass loss of litter between seasons is not unexpected. Previous studies have shown that decomposition processes (i.e. microbial respiration/organic carbon mineralisation) are greatest in the summer months when temperatures are highest (Chapin et al., 2002). In this study, decomposition is also seen to increase in the summer months when compared to spring and winter, however autumn is the only season to show significantly greater mass loss, exceeding that recorded in all other seasons. The greater decomposition in autumn is difficult to explain through temperature (i.e. autumn is neither the warmest or coldest month) and explanations are purely speculative. Nonetheless it is possible to propose hypotheses. For example, rather than temperature dependence, greater autumnal mass loss may instead result from stimulation of detritivore and decomposer activity, due to increased litter inputs during late summer and early autumn, following leaf-fall in *Miscanthus* and harvest residue inputs in cereal controls. In addition, this study focuses on litter fragmentation rates which are likely to be principally governed by animal activity (i.e. meso- and microfauna), rather than microorganisms. As many epigeal and soil animals are dependent upon moist environments, periods of reduced soil moisture during the summer months may have limited the effects of heightened temperatures that are understood to stimulate microbial activity, with increased moisture potentially benefiting litter fragmenting animals in the autumn, when temperatures are still relatively high.

I do not investigate post-fragmentation processes in this study, and it would be interesting to establish whether microbial mineralisation activity is enhanced under winter *Miscanthus*. The focus on the combined effects meso-microfauna activity and abiotic factors is a limitation of the study. As such, further studies incorporating macrofauna are necessary for a more holistic understanding, with the interactions between soil organisms likely playing an important role in ecosystem functioning. Nonetheless, I show that during the winter, fragmentation of nutrient rich, readily degradable, O horizon, leaf-litter differs between *Miscanthus* and conventional cereal crops, indicating that initial abiotic and meso-microfauna decomposition processes are altered by cultivation of PEC *Miscanthus*.

Chapter 6: General Discussion

6.1 Introduction

Agriculture accounts for ~40% of land area globally (Foley *et al.*, 2005); the scale and intensity of production are substantial contributors to the ecological footprint of humanity, placing a major strain on the Earth's biosphere. Over the next century, the pressures placed on agriculture systems will increase further, with human population growth set to generate greater demand for food and energy, placing further stress on the Earth's biocapacity. In addition, as a result of anthropogenic activity, this will occur against the backdrop of a rapidly changing climate. Agriculture is currently a net contributor to greenhouse gas (GHG) emissions, future changes to agroecosystems must therefore limit the carbon footprint of the industry and, where possible, counter emissions, through use of land as a carbon sink and/or by providing sources of renewable, low carbon, energy. Furthermore, current agricultural practices are widely recognised to have had detrimental impacts on biodiversity, the retention of which is vital to the functioning of healthy ecosystems, with rapid declines having been reported in diverse taxa. As such, it is imperative that changes in land use and management practices that take place in the forthcoming decades not only reduce negative impacts on biodiversity but, moreover, improve conditions, both for biodiversity and the services it provides.

In this thesis I aimed to advance understanding of how current and future changes in the agri-environment might affect the provisioning of ecosystem services (ES), studying not only patterns of biodiversity but, importantly, some of the key processes involved in service provision, in particular, those relevant to pollination and decomposition. I principally focus on the effects of cultivating two lignocellulosic bioenergy crops which present an emergent, novel land use, both in England and in temperate regions globally (Haughton *et al.*, 2009). I accomplished this through a combination of field observations, field experiments, soil chemical analysis and microbial fingerprint analysis. In chapter 2 I explore trends in the frequency of insect visits to wildflowers in the margins of two perennial energy crops (PECs), *Miscanthus x gigenteus* and willow short-rotation coppice (SRC, *Salix* spp.), when compared to the conventional cereals that they might replace. I also investigate whether pollinator visitation to margin wildflowers in cereals surrounding PECs differs to those at greater distance, examining effects on species richness and diversity as well as the availability of floral resources. Additionally, for PECs and isolated cereals, I compare the structure of plant-pollinator networks. In chapter 3 I use a selection of phytometers to examine whether seed-set differs in the field margins of *Miscanthus* when compared to cereals, exploring the impact of *Miscanthus* cultivation upon plant reproduction. I also compare phytometer seed-set in field bean to wheat fields, the former a mass-flowering crop (MFC); farm scale cultivation of field bean is understood to increase bumblebee visitation to margin wildflowers (Hanley *et al*., 2011), as such, the crop was investigated for its potential conservation benefits for wild plants. I also compare visitation rates of insects visiting phytometers and native wildflower patches in *Miscanthus* and cereal controls. In Chapter 4 I study patterns of element bioaccessibility for a broad range of soil elements that present important metabolic or toxicological implications for plants and soil *Bacteria*; I compare extractable element concentrations as well as patterns of element extractability in the soils of *Miscanthus* and willow SRC to adjacent cereals. Furthermore, I compare the structure and diversity of bacterial communities under cereal controls to those of the PEC soils. Lastly, in Chapter 5, I investigate soil surface litter decomposition, specifically seasonal litter fragmentation rates using a litter-bag approach. This final chapter is a synthesis of my key findings in the context of existing research; I also highlight the relevance of findings for ecosystem service provision, conservation, and management and policy, and present a discussion of methodological limitations and potential areas for future research.

6.2 Summary and Synthesis

6.2.1 Insect wildflower visitation, species richness and diversity

Differences in crop morphology and management practices between PECs and conventional cereals have the potential to differentially affect plant and pollinator populations and therefore plant-pollinator interactions. Past studies focusing on biodiversity metrics (i.e. abundance, species richness and diversity) have shown either positive or neutral effects for investigated insect taxa when compared to annual rotation crops (Dauber *et al*., 2010). However, aside from a *Miscanthus* study in Ireland (i.e. Stanley and Stout, 2013; Bourke *et al.* , 2014), most studies have either focused on butterflies (Haughton *et al.*, 2009), invertebrates in the PECs themselves (Sage and Tucker, 1997; Redderson, 2001) and/or reported general patterns for coarse taxonomic groups of insects (Cunnigham *et al*., 2004; Semere and Slater, 2007b); to my knowledge, no study in the primary literature has directly investigated effects on the processes involved in pollination service provision to wild margin plants, neither in *Miscanthus* or willow SRC. In chapter 2 I demonstrate that effects of PEC cultivation on visitation frequency were highly context dependent, with only willow exhibiting consistent enhancement of flower visitation by all three key pollinating taxa (i.e. hoverflies, bumblebees and butterflies/moths). For bumblebees and Lepidoptera, taxa specific trends in pollinator flower visitation found in *Miscanthus* correlate with patterns of abundance reported in the literature, with Semere and Slater (2007b) and Haughton *et al.* (2009) having reported elevated butterfly abundance and Bourke *et al*., (2014) having described neutral effects upon the abundance of bumblebees. Conversely, the negative effect of *Miscanthus* on hoverfly flower visitation was unexpected and in contrast to neutral effects on abundance reported by Bourke *et al*. (2014) who found little difference in pan-trap counts of hoverflies between *Miscanthus* and cereals. However, pan traps may have attracted hoverfly species not reflective of the flower visiting assemblages, including non-aphidophagous hoverflies, such as those seeking aquatic oviposition sites or territories. In addition, Bourke *et al.* (2014) show that hoverflies are particularly affected by landscape context, which may have been regionally distinct (i.e. differing between the agri-environments investigated in Ireland and England), and thus leading to the dissimilar land use trends for this insect taxa between studies. However, the authors do record negative effects on a non-pollinating taxa, carabids (but see Semere and Slater, 2007b), and suggest that lower aphid abundance may have driven this trend, a suggestion equally applicable to aphidophagous hoverflies that are seen to dominate transect counts in this study. Hoverflies, bumblebees and Lepidoptera are recognised as some of the most important pollinating insect taxa and variation in flower visitation trends between the two PECs, with regards to cereals, could have particularly important implications for ecosystem service provision. The visitation rate data to red campion and bramble patches presented in chapter 3 also adds support to the transect data. Lepidoptera visitation rate to flower patches is seen to be significantly higher when considering both wildflowers whilst, although non-significant, hoverfly visitation rates were lower in *Miscanthus*. However, despite a slight indication of increased Lepidoptera visitation in cereals surrounding *Miscanthus* I find no statistical evidence that either PEC positively affects focal pollinator visitation in adjacent cereal fields. Nevertheless, at the field scale, increased bumblebee and Lepidoptera species richness and Lepidoptera diversity in willow, as well as enhanced Lepidoptera species richness in *Miscanthus*, suggest benefits of PEC cultivation, for these taxa, beyond that of increased pollinator activity. In addition, despite the neutral effects of both PECs upon broad taxonomic groups of non-focal taxa, this study is the first to report the distinctive responses of the frequent flower visitors *Scathophaga stercoraria* and *Rhagonycha fulva. S. stercoraria* visitation was reduced in *Miscanthus* and *R. fulva* visitation enhanced in willow, with the increased *R. fulva* visitation in cereals adjacent to willow being particularly noteworthy, indicating benefits beyond the PEC field not seen for other taxa.

6.2.2 Floral resource

Past investigations have indicated positive effects of cultivating both willow and *Miscanthus* upon wild plants, both within the crop as well as in the non-cropped headlands and margins (e.g. greater plant species richness in *Miscanthus* (Bourke *et al.*, 2014) and both increased species richness and unique species assemblages in willow (Baum *et al.*, 2012)). However, additional studies in *Miscanthus* (Dauber *et al.*, 2015) and willow (Fry and Slater, 2008) have also shown that more mature plantations exhibit a decrease in floral biodiversity compared to patchier, recently established plantations. For example, for *Miscanthus* fields yielding 9.8 *t* d.m. ha^{-1} yr^{-1}, Dauber *et al.*, (2015) found no difference in plant species richness compared to data on wheat fields collected in the same region. Although I do not investigate within-crop flora in this study, the dense height of the well-established *Miscanthus* sites may provide a related explanation (i.e. light penetration) for the neutral effects of the PEC upon margin wildflower availability, species richness and diversity; dense vegetation may have shaded out many of the hedgerow plants and also visually obscured them from foraging pollinators, with margin encroachment of the crop discernible at many of the sites, but not seen in the wider willow margins. In addition, similar to patterns of visitation by focal pollinator groups, distinct differences are seen for trends in flower availability between

Miscanthus and willow. Willow cultivation increased flower abundance whereas *Miscanthus* had no effect on flower number in comparison to cereals. Furthermore, when compared to an alternative PEC, reed canary-grass (*Phalaris arundinacea*), Clapham and Slater (2008) report lower weed community species richness in *Miscanthus*, providing further balance to the argument that the impacts of *Miscanthus* on native wild plants may be limited and inconsistent. The neutrality of *Miscanthus* cultivation upon flowering plants is further substantiated by the similar phytometer seed counts when compared to cereals. Data presented on seed set in chapter 3 suggest that neither *Miscanthus* nor mass-flowering field bean improve seed-set of field margin phytometers; floral counts and species richness and diversity of margin wildflowers was also comparable between bean and wheat controls, with red clover seed-set reduced in bean fields.

6.2.3 Plant-pollinator networks

An ever increasing variety of indices are now available to describe parameters associated with mutualistic plant-pollinator networks. However, where parameters differ, tangible implications for pollinator and plant communities remain little understood. Nevertheless certain measures are thought to indicate greater network stability and robustness to anthropogenic or environmental perturbation. In the first study to attempt to compare network properties between willow SRC and cereals I found notable differences in network parameters, willow networks exhibited significantly greater ‘nestedness’ and ‘links per species’ when compared to cereals, indicating more complex and therefore more stable networks. In contrast, and similar to Stanley (2013), indices were similar between *Miscanthus* and cereal controls indicating neutral effects upon network parameters when replacing cereals with this PEC. Network vulnerability was only marginally non-significant however and higher in *Miscanthus*, a possible indication of a slight reduction in the stability of *Miscanthus* networks, although inclusion of additional field sites would be necessary to clarify this.

6.2.4 Soil elements and bacterial communities

Despite numerous studies on soil carbon processes there has been limited investigation of how PECs influence trace element availability or the structure and composition of soil microbial communities. In chapter 4 I find no evidence that cultivation of *Miscanthus* or willow leads to differences in the bioaccessibility of an array of elements that have the potential to have important consequences for plant health and bacterial communities. Bacterial populations also failed to converge on typical crop-specific assemblages with no discernible impact on diversity.

6.2.5 Decomposition

Investigation of above ground ecosystem processes in PECs are limited, however research by Rowe *et al.* (2013) demonstrated higher summer decomposition rates in willow SRC compared to cereals. In this study, using a similar methodology, I also report marked differences in the decomposition rates between the cropped areas of the PEC *Miscanthus* when compared to cereals, with reduced litter breakdown in the harvested cereal fields during the winter but decomposition rates in *Miscanthus* remaining comparable to non-cropped regions. However, in contrast to the findings of Rowe *et al.* (2013) in summer willow SRC, I find no evidence of enhanced decomposition during the spring, summer or autumn (at least in terms of meso-microfaunal activity), with rates of litter breakdown similar between land uses.

6.3 Ecosystem service and conservation relevance

There is a growing recognition of the value of nature, both intrinsically (Batavia and Nelson, 2017) and in terms of the benefits derived by humanity (Guerry *et al.*, 2015), this is coupled with an improved understanding of the role that land use change may have upon biodiversity and the services it provides (Foley *et al*., 2005). This has particular relevance for pollinators. Where adequate data are available (i.e. north-western Europe and North America) pollinators are seen to be faring poorly with similar trends having also been shown for insects more generally (Hallman *et al*., 2017), and regional declines expected in additional areas due to widespread evidence of local declines (IPBES, 2016). The low intensity management of PECs has been suggested to benefit biodiversity generally and, where investigated, evidence suggests that effects are principally neutral or positive, benefiting pollinating insects such as butterflies (Semere and Slater, 2007b), solitary bees, and trap nesting bees and wasps (Stanley and Stout, 2013). Here I find evidence that pollinator populations in willow SRC are likely to benefit from its cultivation given their enhanced activity in field margins, as well as greater species richness and diversity. Insect flower visitation is a key process involved in pollination, increased flower visits by all three dominant pollinator taxa therefore suggests reproductive benefits to margin wildflowers through enhanced service provision. The higher flower counts recorded in willow also adds support, potentially arising from increased pollination and/or a reduction in management intensity. However, because flowering plant communities did not become more complex, with similar species richness and diversity between willow and cereals, it appears unlikely that willow margins provide novel plant niches. Landscape context could hinder colonisation by plant propagules however, willow PEC plantations in less biologically impoverished landscapes might therefore support greater plant diversity; and active introductions might also assist.

As outlined above, the relationship connecting structural attributes of pollination network to ES provision is poorly understood. The conservation value of differences in network structure between PECs and cereals is thus difficult to ascertain without further development of this field (Tylianakis *et al*., 2008). Nevertheless, indication of increased robustness of willow networks would appear to suggest that pollinator visitation processes in this PEC are less susceptible to perturbation, with presumed conservation benefits for both flowering plants and flower visiting insects, as well as enhancement of the process of flower visitation itself, potentially increasing ES provision.

The divergent trends outlined in *Miscanthus* compared to willow suggest that the conservation value of the former PEC is, at best, mixed for pollinating insects. Significant increases recorded in Lepidoptera flower visitation are not expected to have major implications for the provision of pollination service, given the relatively low number of visits recorded, and thus the crop's value for diurnal Lepidoptera conservation, although positive, also remains limited. Reduced hoverfly activity suggests pollination by hoverflies is likely adversely affected by *Miscanthus* and raises concerns that pest control may also be reduced, although potentially ameliorated by, and a response to, a reduction in pest pressure. Negligible effects on bumblebees supports past studies, which report no impacts on overall abundance and no increases in nest searching activity when compared to cereals, indicating that *Miscanthus* has little relevance to bumblebee conservation and is unlikely to enhance bumblebee pollination service provision to wild plants (Stanley and Stout, 2013). In contrast to willow, the increased vulnerability of *Miscanthus* networks suggest that plant-pollinator interactions are less robust when compared to cereals and therefore consequences for pollination service provision, and conservation, may prove negligible or even negative.

Neither PEC positively affected flower visitation by focal pollinator taxa in surrounding cereal fields, indicating that, at the farm scale, both crops have limited implications for the enhancement of plant/pollinator conservation or ES provision beyond the field. Though, for willow, cereals margins may be less likely to attract pollinators away from the PEC due to their comparatively intensive management; therefore if insects do indeed spread out from the plantations, they may simply overlook cereals in favour of more rewarding habitats (i.e. semi-natural habitats and, potentially, MFCs). Investigation of less intensive and/or flower rich habitats surrounding PECs may therefore indicate localised enhancement of pollinator activity, as pollinators may be shared between land uses offering complementary or alternative resources. Nonetheless, the neutral effects on flower visitation in adjacent cereals surrounding both PECs directly opposes suggestions posited by Manning *et al*. (2015) that PECs may enhance service provision in the surrounding agricultural landscapes, at least in terms of pollination by the three focal pollinator taxa. However, enhanced *R. fulva* flower visits in cereals adjacent to willow does offer some support to the suggestions of Manning *et al.* (2015) with potential enhancement of both pollination and biocontrol services, indicating the value of investigating a wider range of species.

Despite the benefits willow provides for plants and focal pollinator taxa, and which *Miscanthus* provides for diurnal Lepidoptera, there is little evidence that either crop supports rare or endangered species. This is not unexpected as the plantations studied are located in a mixed farming landscape that tends to support a reduced flora and fauna compared to other land uses (Altieri, 1999). Furthermore, data do not encompass all species, rarer species are less likely to have been observed on transects and it is certain that flowering willow will itself attract a different assemblage of pollinators in the spring, whilst potentially supporting the larvae of rarer non-flower visiting insects that were not recorded in this study. Nevertheless, for summer flower visiting insects the conservation value of willow arises as a consequence of the crops ability to benefit common species when compared to conventional cereals; given widespread declines in insect biomass, keeping common species common remains an important objective (Gaston, 2010). Furthermore, in terms of ES provision, it is likely to be the most frequently recorded (and efficient) species that make the greatest contribution to service provision (Bommarco *et al.* 2013).

Alongside differences in pollinator flower visitation, I find evidence that *Miscanthus* is likely to have consequences for the provisioning of another ES when compared to cereals. Elevated rates of litter mass loss in *Miscanthus* during the winter may have implications for decomposition processes, particularly given the crop's long lifespan. However across the large number of soil elements investigated, I find no indication that either of the two PECs will affect element bioaccessibility, with indirect effects upon bacterial communities likely to be minimal given the similarity in bacterial community diversity and the absence of unique assemblages between crop treatments. Site variation in edaphic soil properties is therefore likely to be a greater determinant of bacterial community parameters under both PECs and cereals. Soil data therefore indicate that nutrient cycling and biogeochemical processes will be little altered by either PEC when compared to cereals. Such patterns are in line with recent investigation of soil carbon processes undertaken in willow and *Miscanthus* which, using an improved methodology to previous studies, casts doubt on expectation of increased carbon sequestration under the two PECs, with no significant difference in soil C when compared to either grassland or arable (Rowe *et al.* 2016).

In summary, I show that willow cultivation, in particular, is likely to lead to farm scale benefits for pollinator conservation and provision of pollination service, but mixed effects may arise for *Miscanthus*. Largely neutral effects are found for MFC field bean, but with the possibility of negative effects on reproduction of some margin plants. Meso-microfauna decomposition processes are likely to be affected by *Miscanthus* cultivation during the winter but there is no indication of differing patterns in soil element accessibility or bacterial communities between land uses.

6.4 Methodological Considerations

This study encompassed a range of scientific fields, including pollination, soil chemistry, soil microbiology, and decomposition. This interdisciplinary investigation therefore required diverse methodologies. Here I discuss the methods used to investigate the effect of PEC and MFC land use change upon patterns and processes relevant to provision ecosystem services, with a focus on methodological limitations where they exist.

When surveying pollinating insects it is generally accepted that application of multiple methods is preferable, as I focus specifically on insects visiting flowers in this study methodology was constrained to direct observations. Passive sampling techniques (i.e. pan traps or trap nests) were unsuitable as they would have conveyed the abundances of all trapped insects, many of which may have little relevance to pollination service provision, with species perhaps favouring either PEC plantations or cereals due to features such as desirable nesting habitat, suitable oviposition sites, or favourable microclimate conditions, factors unrelated to wildflower pollination.

The observational nature of transects places limitations on the representativeness of species richness and diversity measures, with species level identifications of briefly encountered, morphologically similar species (i.e. *Syrphus* sp. Fabricius), difficult to distinguish in the field. However, in most circumstances active sampling on transects using a net was considered undesirable due to the likelihood of disturbing as yet unrecorded insect visitors on surrounding plants (consider hoverfly activity on Apiaceae for example). Related to this, without actively capturing flower visiting bumblebees of the *Bombus sensu stricto* subgenus it was not possible to determine the species identity as identification is reliant on genetic analyses. However, such bumblebee species may have different ecological preferences and respond differently to PEC cultivation not discernible in this study (Stanley, 2013). In addition, as mentioned above, transects were only undertaken in the summer months when wildflowers in field margins are most abundant, however flower visitation to willow crops in spring remained unconsidered.

Hedge cutting, granivory and disease pressures, adversely affected the phytometer seed set experiment. Loss of samples could have been better tolerated had the experimental design included a greater number of sites, a greater number of planted phytometers per site, multiple years of data collection, and a larger number of phytometer species. Nonetheless, for a single researcher, this was not feasible within the time constraints of the project.

Use of microbial fingerprint techniques such as DGGE is a cost effective means of investigating microbial communities. DGGE rapidly provides an overview of microbial biodiversity. Nonetheless this technique has its limitations, (i.e. it is largely qualitative technique and can suffer from issues with reproducibility) and more advanced high throughput techniques would have provided greater sequence information, potentially revealing more subtle changes in community structure.

6.5 Further Research

Given reported variation in the effectiveness of different pollinator taxa when depositing pollen upon plant stigmas (King *et al.*, 2013), measures of pollinator effectiveness, particularly for moderate-highly frequent flower visitors, would strengthen understanding of the contribution made by specific taxa to ES provision. Such data would bridge the gap between flower visitation and reproductive outcomes (i.e. seed/fruit set). The focal taxa studied in this investigation are already understood to be important pollinators but effectiveness for a given plant and in a given context (i.e. network) requires further research, with even greater relevance for non-focal taxa whose contribution to pollination is less understood. Furthermore, there also remains a need to establish whether measures of plant reproductive success differ between margin wildflowers in willow SRC when compared to cereal controls, and future investigation of seed set should seek to address the issues outlined in chapter 3, incorporating a greater number of flower patches and plant species for example.

In addition, the lower hoverfly visitation in *Miscanthus* fields and the lower carabid abundance reported by Bourke *et al.* (2014) suggest that investigation of aphid abundances in the margins of both PECs and cereals could help to explain trends. Although non-significant, the decline in the absolute abundance of hoverflies in cereals adjacent to *Miscanthus* may indicate lower aphid numbers on the cereal crops themselves and surveys comparing aphid pressure upon cereals that are adjacent and distant from PECs is also worth studying.

Although Bourke *et al.* (2014) have investigated impacts of landscape context on local *Miscanthus* biodiversity (demonstrating both independent field scale effects and, for some taxa, additive effects on species richness, diversity and abundance), inclusion of PECs into agricultural landscapes may be expected to have variable effects at the landscape scale, dependent on their relative proportion in the landscape; likely increasing landscape heterogeneity at low density but rendering landscapes homogenous at high density. This considered, there remains a need for landscape scale studies, both on biodiversity and ES. Limited cultivation in the UK means that these studies must necessarily occur in European nations with greater areas under cultivation.

Increased landscape homogeneity may occur in the future if PEC cultivation becomes not only widespread, but due to economic drivers, a concentrated locally dominant land use. Such developments emphasise the need to consider PEC impacts at far larger scales. For instance, competition with food crops currently remains virtually as relevant for PECs as it does for 1st generation biofuel crops. Imported biomass is currently the dominant source of biomass combusted in the UK (Haughton *et al*., 2009; Dauber *et al*., 2010). In the United States there is evidence of increased pressures on important biodiversity habitats due to wood harvesting from forests, whilst forests on marginal soils may be lost in order to produce purpose grown feedstocks (Tarr *et al*., 2017) as producers seek to meet biomass demands from the UK and Europe (~ 4.6 million tonnes, Dale *et al*., 2017); an undesirable outcome for conservation and ecosystem services at an international level. The reason for UK dependency on imports arises, in part, from the limited and disparate uptake of PECs nationally, hindering the ability of energy providers to cost effectively acquire biomass from national sources. An in-depth discussion of the reasons for limited PEC cultivation are beyond the scope of this investigation but include factors such as farmer uncertainty (on a range of issues, cost, yields, prices, biodiversity impacts; Sherrington, 2008) and, in many regions, limited demand for biomass by energy providers (Devon grower, *personal communication*). The latter is a possible outcome of frequent changes in policy stance (and thus market uncertainty) and exemplified by variable contribution of co-fired biomass to meeting renewable obligation requirements and, recently, the cessation of subsidy incentives. Acknowledgement by the EU of potentially negative effects of indirect land use change, including subsequent amendments to policy, is certainly unlikely to have encouraged uptake. As such, the landscape, national and international scale implications of PEC cultivation upon ecosystem services and conservation may well prove the dominant factor constraining the proportion of land under PEC production over the coming decades. However, it is worth considering that the demands currently placed by food crops on land resources are greatly exacerbated by market demand, and policy incentives, that encourage inefficient methods of food production, particularly in developed nations (Clark and Tilman, 2017; Shepon *et al*., 2018); in the long-term, efforts to improve the efficiency and sustainability of food production could therefore help to alleviate the conflict between land use for energy, food and biodiversity.

6.6 Conclusions, management and policy relevance

To date, limited attention has been given to the potential effect of PEC cultivation upon the ecosystem processes necessary for the maintenance of ecosystem service provision. This thesis presents evidence for differential effects of PEC cultivation upon ecosystem processes relevant to pollination and decomposition services. Here I outline some of the implications for management and policy.

In terms of pollination process, the consequence of replacing common rotational crops such as cereals with PECs is highly specific to the PEC cultivated (chapter 2). Willow SRC has almost exclusively positive impacts upon both pollinators and plants taxa. However impacts of *Miscanthus* were variable, encompassing positive, neutral and negative effects on pollinators and no clear benefit to plant communities. Furthermore, there is no suggestion that *Miscanthus* benefits the reproduction of margin plants (chapter 3), suggesting, that, for margin wildflowers, inclusion of this crop under any future agri-environment scheme would be ineffectual. Similarly, the apparent negative effects of MFC field bean on red clover flower patches suggests that some margin plants may in fact be adversely affected by incorporation of this crop as a conservation measure. These findings have significance for land managers and spatial planners, especially where there is an ambition to improve the conservation value of agricultural landscapes; in particular, willow SRC may prove an important refuge for pollinators and plants. The comparison here is limited to cereals however, and transitioning from other land uses may produce dissimilar trends, with detrimental impacts almost certain if semi-natural habitats are replaced (Rowe *et al.*, 2009). Low disturbance semi-natural habitats often support scarce and rare species; I find no evidence that either PEC benefits rare flower-visiting insects or margin plants species in this study (chapter 2).

Beyond the PEC field there is little evidence of enhanced insect flower-visitation for either willow SRC or *Miscanthus* (chapter2), at least at the field scales investigated here. Despite its local value, willow SRC may therefore be unlikely to benefit wildflowers in surrounding cereal fields. PECs would thus prove unsuitable as a management prescription where elevated pollination service provision in wider landscape is desirable (Manning *et al.*, 2015) and where cereals are prevalent. The increase in meso-microfauna decomposition rates under *Miscanthus* (chapter 5) has the potential to affect biogeochemical and nutrient cycles, although, without further investigation, the consequences for management and policy are unclear. Given that *Miscanthus* cultivation had no evident influence upon bacterial populations or element bioaccesibility (chapter 4), between-site variation in edaphic factors are likely to prove of greater relevance to farmers and land managers in this regard.

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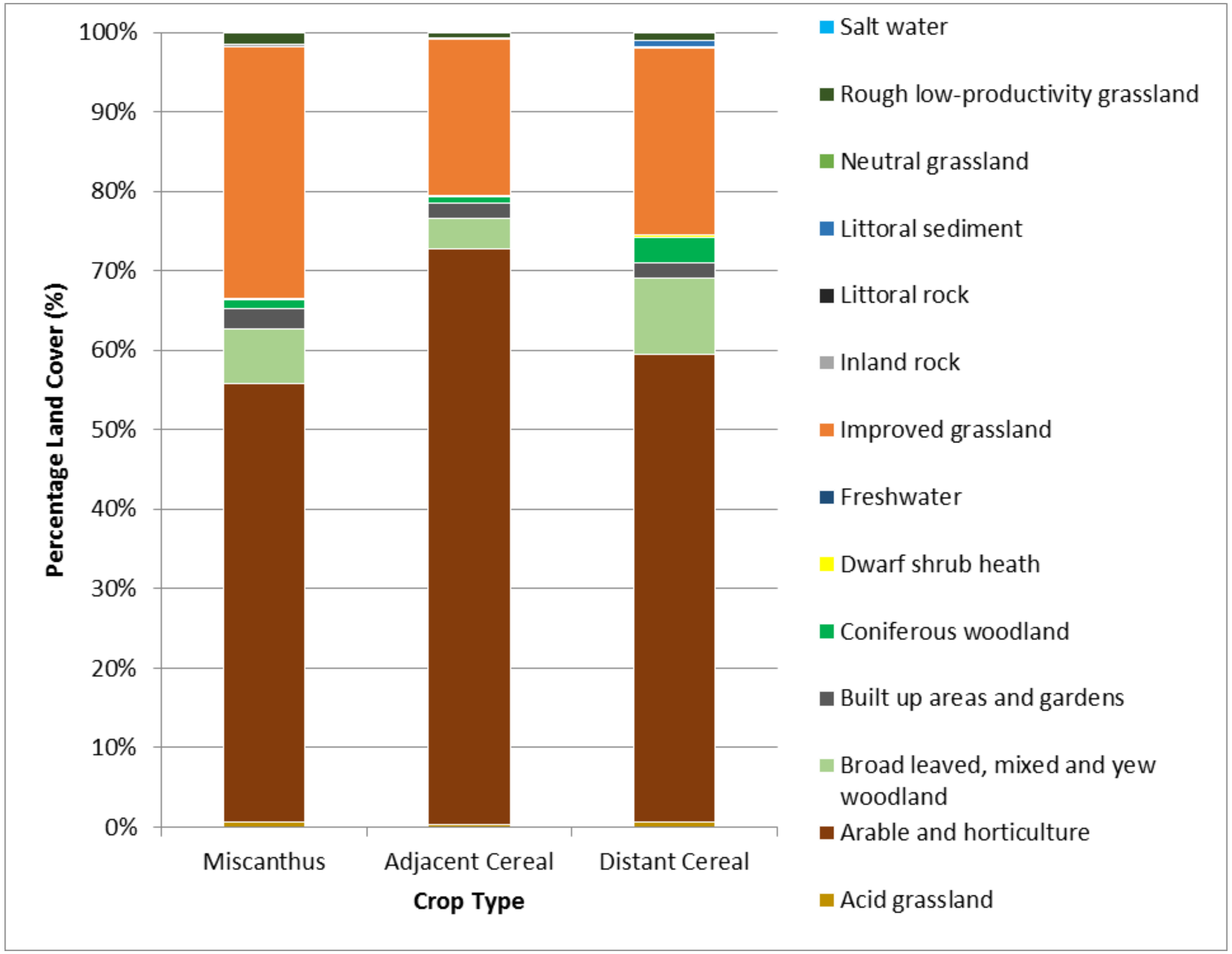
Appendices

Appendix 1: Mean land cover data within a 1 km radius, standard error of the mean (S.E.) and analyses of statistical differences around field sites present in each crop type in southwest England, α= 0.05.

Land Cover Type	Mean (±S.E.) Percentage Cover (%)			Statistical Output
	Miscanthus	Adjacent Cereal	Distant Cereal	
Acid Grassland	0.961 (0.361)	0.957 (0.369)	1.608 (0.717)	$H_{2,16}$= 1.435; p= 0.488
Arable & Horticulture	55.18 (4.632)	56.04 (5.908)	58.847 (5.955)	$H_{2,33}$= 0.074; p= 0.964
Broad-leaved, mixed & yew woodland	6.94 (1.91)	6.337 (1.443)	9.598 (2.233)	$H_{2,33}$= 0.956; p= 0.620
Built-up areas and gardens	2.977 (0.885)	3.119 (1.159)	1.899 (1.328)	$H_{2,30}$= 4.553; p= 0.103
Coniferous woodland	2.527 (0.283)	2.93 (0.525)	7.806 (3.38)	$H_{2,14}$= 1.060; p= 0.589
Dwarf shrub heath	0.76 (0.347)	0.412 (-)	1.108 (0.404)	N/A
Freshwater	-	-	0.197 (-)	N/A
Improved grassland	31.578 (3.112)	31.235 (4.483)	23.52 (3.192)	$F_{2,32}$= 1.558; p= 0.226
Inland rock	0.414 (0.082)	0.368 (0.071)	0.573 (-)	$H_{2,18}$= 1.057; p= 0.589
Littoral rock	-	-	0.621 (0.359)	N/A
Littoral sediment	-	-	2.361 (1.255)	N/A
Neutral Grassland	-	-	0.62 (-)	N/A
Rough low productivity grassland	1.525 (0.461)	1.16 (0.335)	1.54 (0.324)	$F_{2, 27}$= 0.419; P= 0.662
Salt water	-	-	0.432 (0.193)	N/A

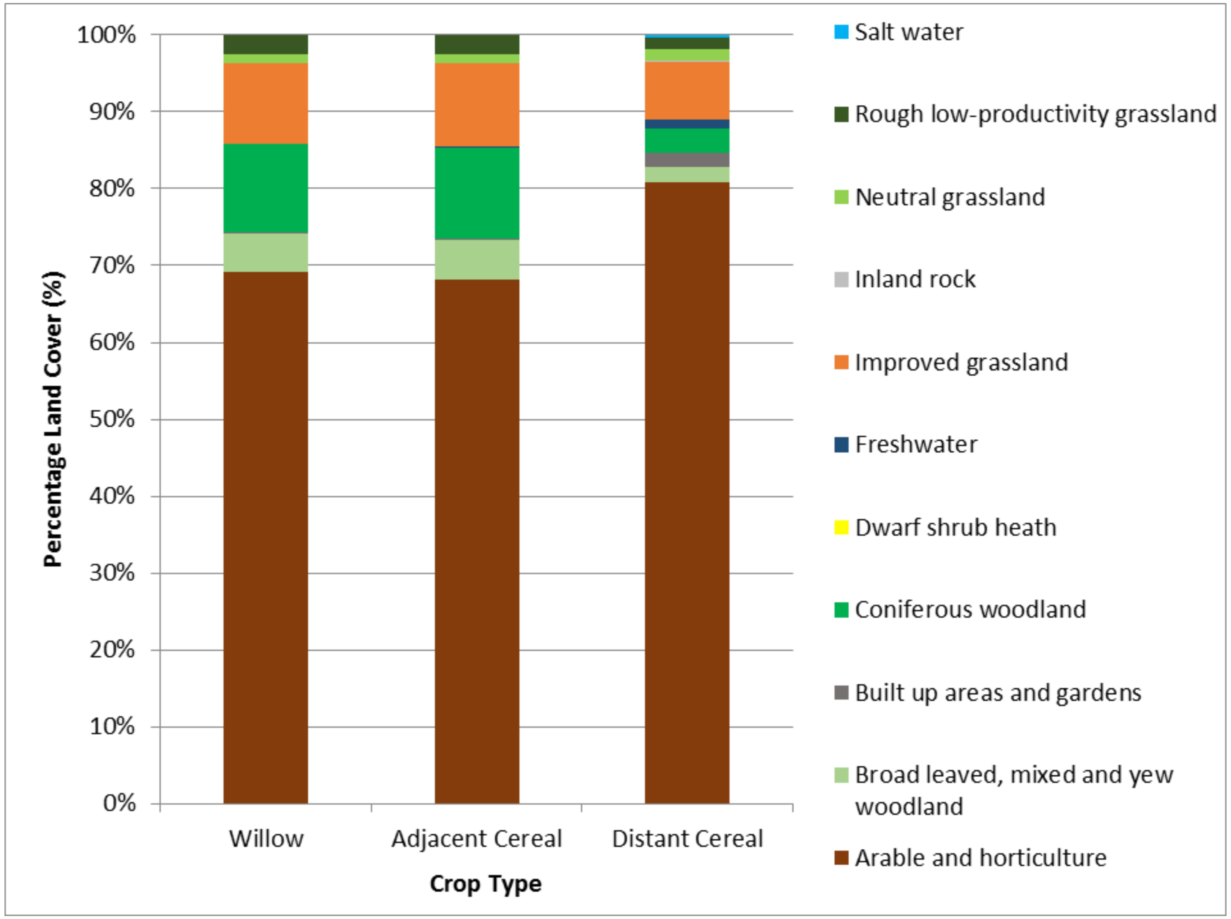
Appendix 2: Mean percentage area of land cover types for each crop type within a 1 km radius around field sites in southwest England.



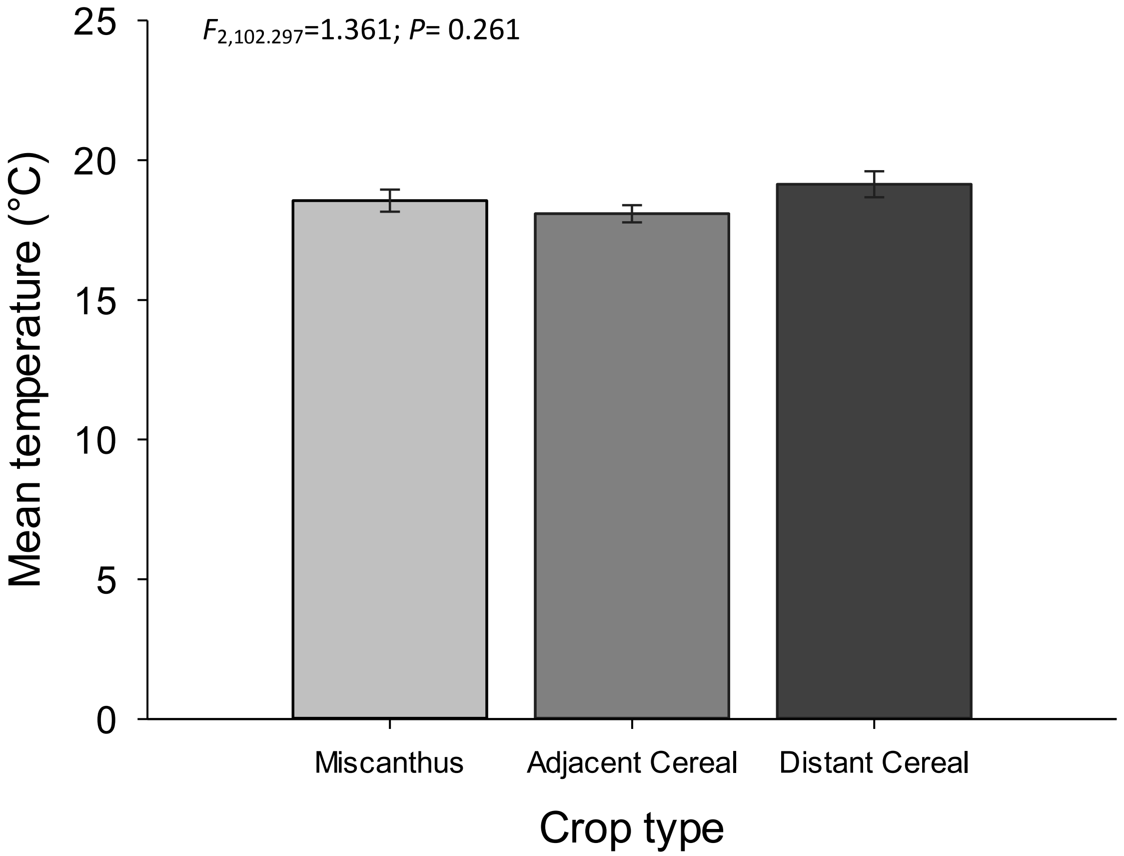
Appendix 3: Mean land cover data within a 1 km radius, standard error of the mean (S.E.) and analyses of statistical differences around field sites present in each crop type in Nottinghamshire and Lincolnshire, England. α= 0.05.

Land Cover	**Mean (±S.E.) Percentage Cover (%)**			**Statistical Output**
	Willow	**Adjacent Cereal**	**Distant Cereal**	
Arable & Horticulture	69.181 (7.316)	68.089 (6.957)	80.777 (5.283)	$H_{2,21}$= 2.075; p= 0.345
Broad-leaved, mixed & yew woodland	7.872 (2.138)	6.892 (1.978)	4.207 (2.127)	$F_{2,11}$= 0.715; p= 0.509
Built-up areas and gardens	0.7669 (0.268)	0.639 (0.205)	2.345 (1.123)	$H_{2,9}$= 4.847; p= 0.089
Coniferous woodland	22.919 (9.112)	31.296 (8.613)	6.378 (6.092)	$H_{2,8}$= 4.060; p= 0.131
Dwarf shrub heath	-	0.42 (-)	-	N/A
Freshwater	0.0493 (-)	1.222 (-)	2.17 (1.111)	N/A
Improved grassland	10.392 (2.535)	10.824 (3.248)	7.51 (2.586)	$F_{2,20}$= 0.412; p= 0.668
Inland rock	0.261 (-)	0.261 (-)	1.608 (-)	N/A
Neutral Grassland	1.676 (0.586)	1.9 (0.602)	2.396 (1.428)	$F_{2,12}$= 0.058; p= 0.944
Rough low productivity grassland	2.463 (1.033)	3.352 (1.156)	1.819 (0.61)	$H_{2,18}$= 0.495; p= 0.781
Salt water	-	-	2.291 (-)	N/A

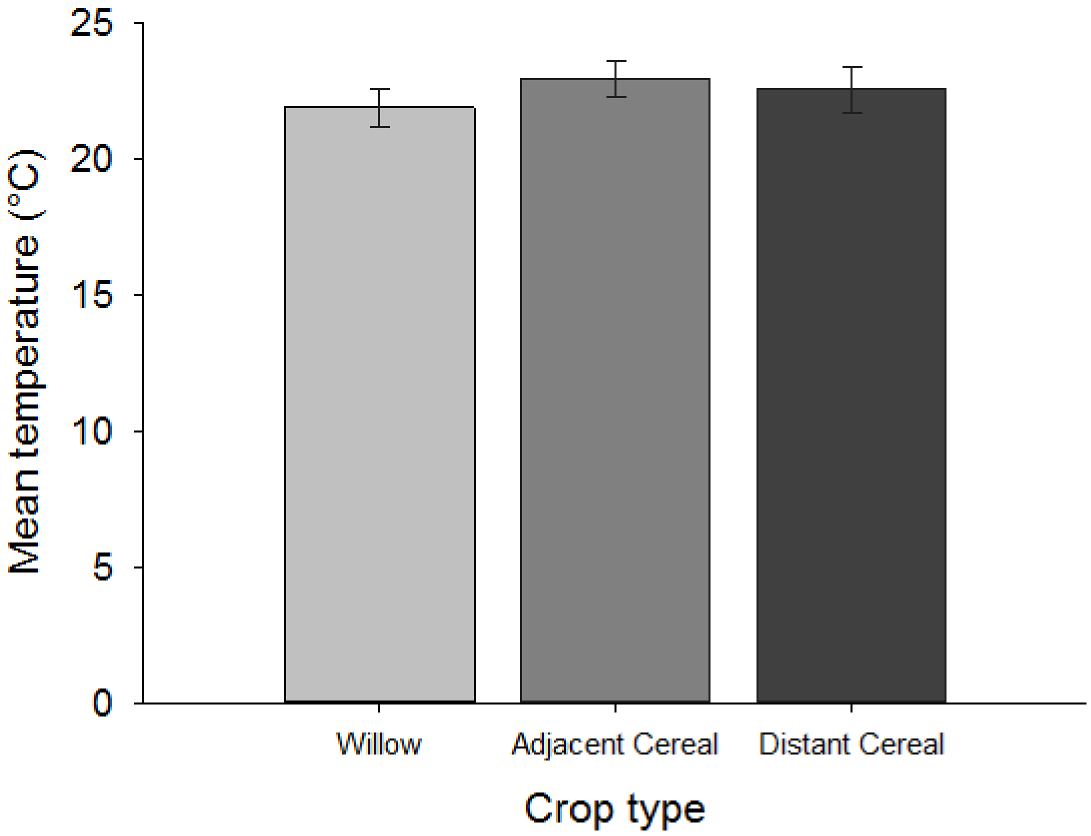
Appendix 4: Mean land cover data within a 1 km radius, standard error of the mean (S.E.) and analyses of statistical differences around field sites present in each crop type in Nottinghamshire and Lincolnshire, England. α= 0.05.



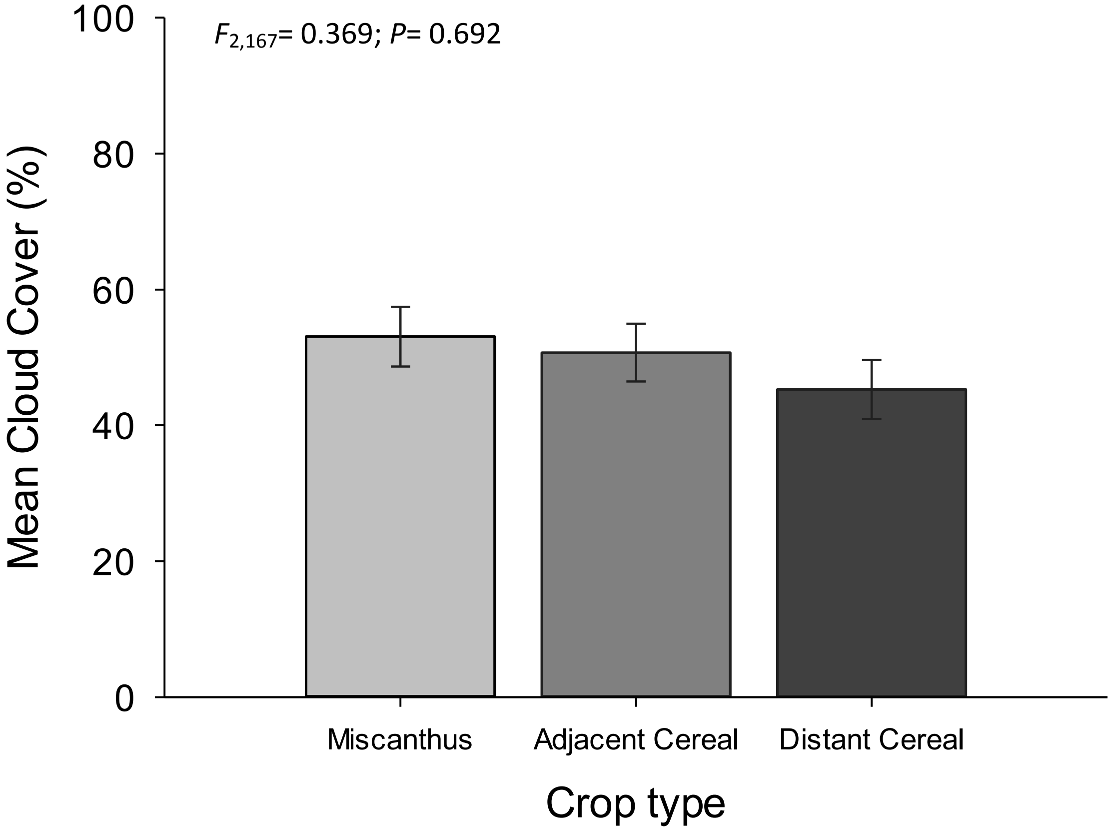
Appendix 5: Mean transect temperature (°C) for each crop treatment recorded in southwest in 2015. Error bars show standard error. α= 0.05.



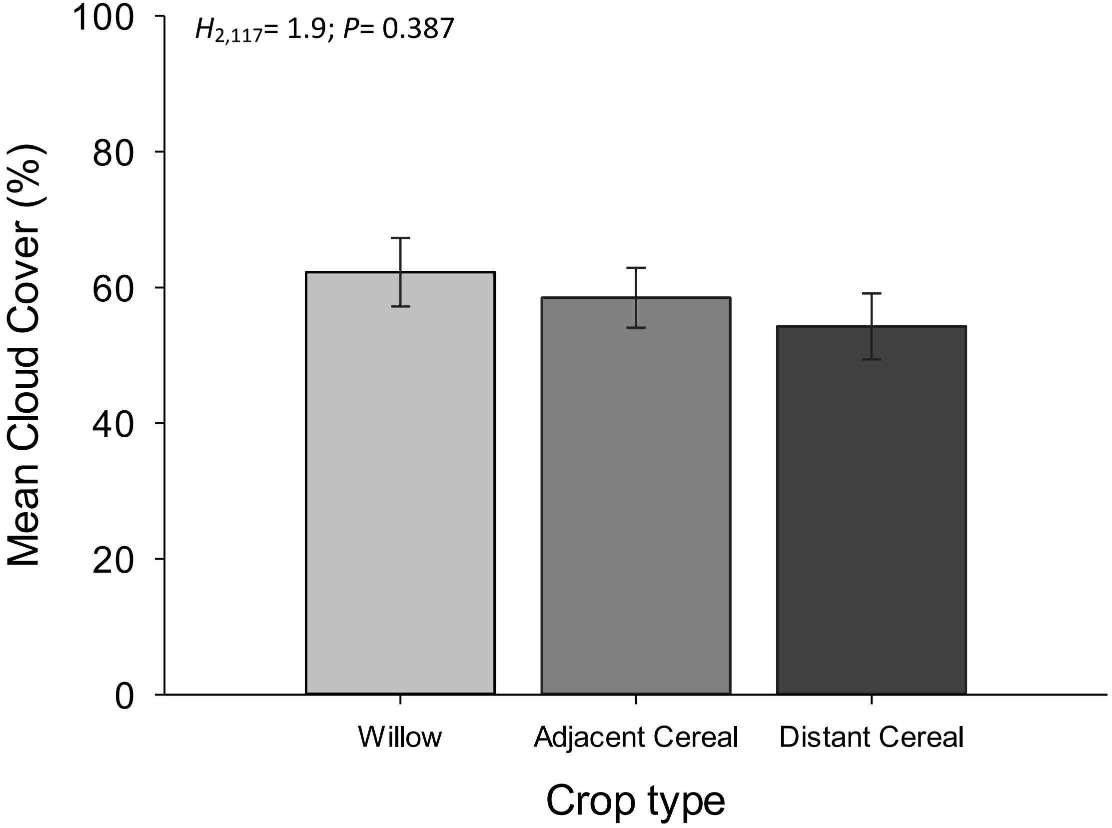
Appendix 6: Mean transect temperature (°C) for each crop treatment recorded in Nottinghamshire and Lincolnshire in 2015. Error bars show standard error. α= 0.05.



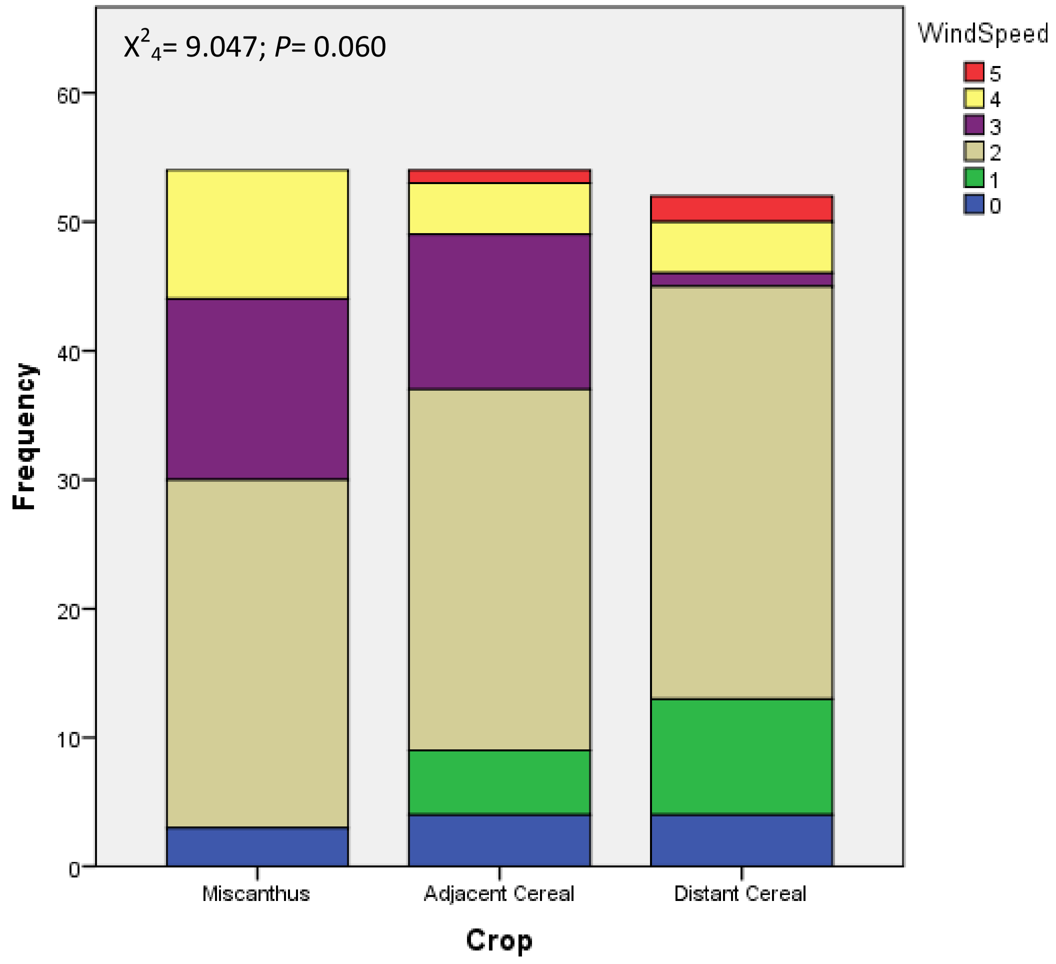
Appendix 7: Mean estimates of cloud cover upon commencing pollinator visitation transects for each crop type in southwest England, 2015. Error bars show standard error of the mean. α= 0.05.



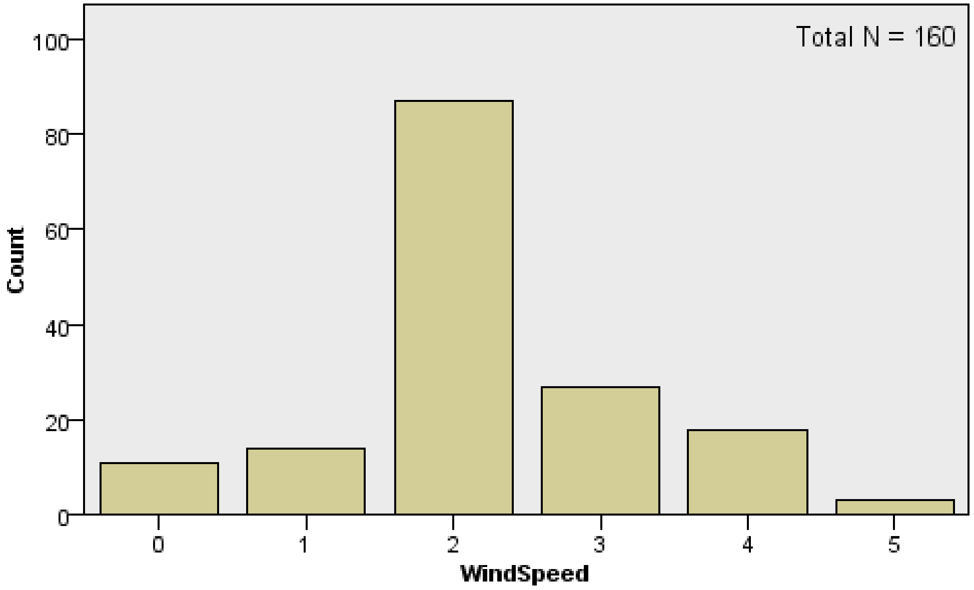
Appendix 8: Mean estimates of cloud cover upon commencing pollinator visitation transects for each crop type in Nottinghamshire and Lincolnshire, central England, 2015. Error bars show standard error of the mean. α= 0.05.



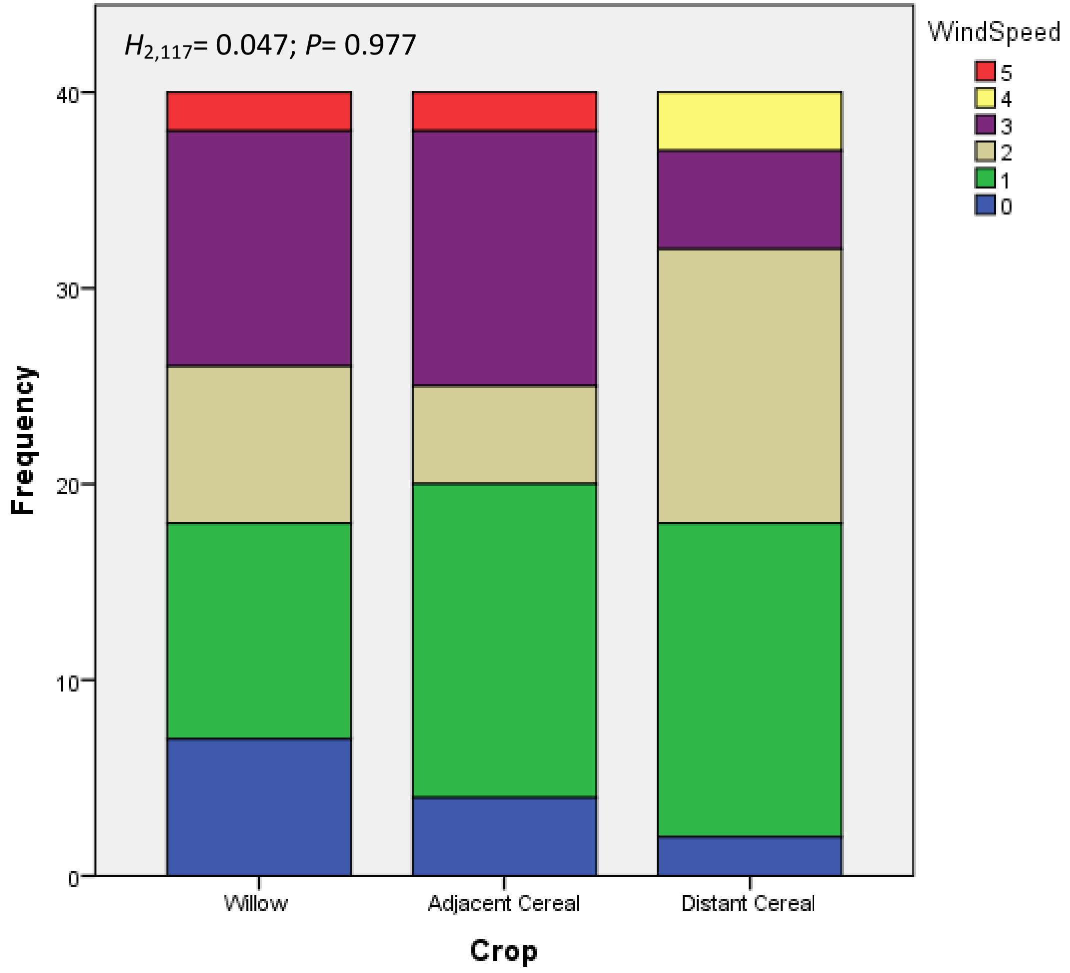
Appendix 9: Mean estimates of wind speed upon commencing pollinator visitation transects for each crop type in southwest England, 2015. Error bars show standard error of the mean. α= 0.05.



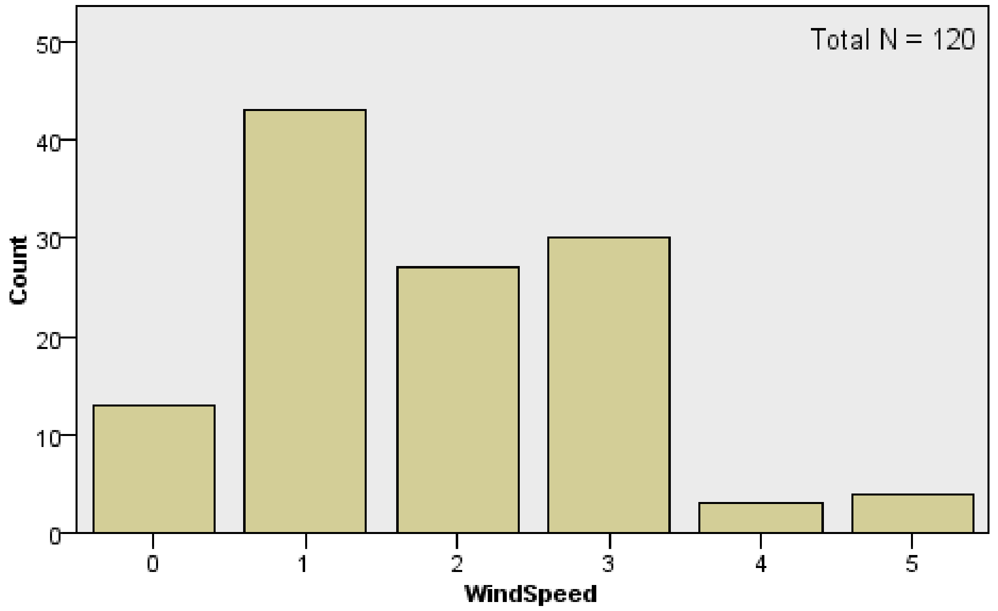
Statistical analysis was carried out on three broad wind speed categories. This approach was taken due to the subjective nature of drawing conclusions from the full six categories, some of which exhibit close similarity (i.e. ‘2’ & ‘3’; ‘4’ & ‘5’), the presence of zero counts also render X^2 analysis invalid for non-binned data. New categories involved binning data as follows (based on the Beaufort Scale); “None/Very Low”= “0” & “1”, “Low”= “2” & “3”, “Moderate”= “4” & “5”.



Appendix 10: Mean estimates of wind speed upon commencing pollinator visitation transects for each crop type in Nottinghamshire and Lincolnshire, England, 2015. Error bars show standard error of the mean. α= 0.05.



Statistical analysis was carried out using a Kruskal-Wallis test for midlands data as binning the data (described above for *Miscanthus* analysis) still resulted in >20% of midlands categories having < 5 counts, violating assumptions of X^2 analysis. Regardless, output is very similar between tests, X^2_4= 0.534; *P*= 0.970.



Conclusion: No significant difference between crop treatments (bioenergy crop, adjacent cereal, distant cereal) is seen for any recorded environmental variable, it is therefore assumed that variation in transect temperature, cloud cover and wind speed did not bias results for data collected in either southwest England or central England in 2015 or for land cover classes in any year (southwest: 2012, 2014, 2015; central England: 2013, 2015).

Notes on statistical analyses of environmental variables:

The individual transect data for the environmental variables described above (i.e. temperature, cloud cover, wind speed) are considered to be unique replicates in the analyses because they are not specifically dependent on crop treatment. Environmental conditions recorded upon any one transect cannot be considered to be influenced by the crop for the data shown here, environmental measures were simply taken in order to clarify that they were broadly similar across treatments when pollinator surveys were conducted. If any association had been found between crop type and environmental variables then this would imply a potential source of bias that may have confounded crop treatment effects on pollinator visitation. I do not find any difference in environmental variables (including variation in percentage land cover) between crop treatments, indicating that my conclusions are robust. One might argue that variation in shelter provided by the different crop treatments may vary between crop types and so influence these environmental measures, evidently this cannot be the case for cloud cover but may be true of wind speed or temperature. In the context of this study however, wind speed was a general indicator of wind speed/intensity in the local environment (i.e. field) and influence of the crop at small scales cannot be ascertained from this data, it may be that for at heights < 2m for example, wind speed may be lower in *Miscanthus* fields, this cannot be established from our data however given its resolution. Similarly it is not possible to establish whether crop shelter influenced temperature because temperature was only recorded at one location on the transect and would have had to have been recorded, simultaneously, away from the influence of the crop (or any landscape features) if a shelter effect was to be investigated, I do not have this data and so cannot draw any conclusions on this point. An additional justification for use of individual transect data as replicates arises from the fact that site averages would be unsuitable for wind speed measures because these data are ordinal and averages would therefore offer limited interpretation.

Appendix 11: The top five most highly visited plants for each of the three focal pollinator taxa, in each region, alongside the relative percentage and cumulative relative percentage of visits to each plant species (southwest: 2012, 2014 & 2015; central England: 2015).

Southwest: *Miscanthus* & Controls									
Rank	Hoverfly			Bumblebee			Lepidoptera		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Heracleum sphondylium*	35.33		*Rubus fruticosus* agg.	58.87		*Rubus fruticosus* agg.	46.86	
2	*Rubus fruticosus* agg.	18.72	54.05	*Silene dioica*	9.93	68.79	*Silene dioica*	10.29	57.14
3	*Galium mollugo*	16.76	70.81	*Stachys sylvatica*	5.91	74.70	*Galium mollugo*	7.43	64.57
4	*Silene dioica*	9.59	80.40	*Teucrium scorodonia*	3.78	78.49	*Chaerophyllum temulum*	5.71	70.29
5	*Chaerophyllum temulum*	7.68	88.09	*Centaurea nigra*	3.31	81.80	*Geranium robertianum*	5.71	76.00

Midlands: Willow SRC & Controls									
Rank	Hoverfly			Bumblebee			Lepidoptera		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Cirsium dissectum*	17.89		*Cirsium vulgare*	31.25		*Cirsium vulgare*	40.36	
2	*Rubus fruticosus* agg.	16.16	34.04	*Cirsium dissectum*	17.47	48.72	*Trifolium repens*	15.71	56.06
3	*Convolvulus arvensis*	14.83	48.87	*Rubus fruticosus* agg.	13.62	62.34	*Rubus fruticosus* agg.	10.14	66.20
4	*Cirsium vulgare*	8.98	57.85	*Heracleum sphondylium*	12.02	74.36	*Heracleum sphondylium*	7.75	73.96
5	*Heracleum sphondylium*	8.58	66.42	*Trifolium repens*	10.58	84.94	*Cirsium dissectum*	4.57	78.53

Appendix 12: Treatment specific breakdown (i.e. *Miscanthus x giganteus*, adjacent cereal, distant cereal) of the relative percentage and cumulative relative percentage of flower visits by the three focal pollinator taxa to the top 5 most visited plants in southwest England (2012, 2014, 2015).

Miscanthus									
Rank	Hoverfly			Bumblebee			Lepidoptera		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Heracleum sphondylium*	12.50		*Rubus fruticosus* agg.	56.55		*Rubus fruticosus* agg.	46.85	
2	*Rubus fruticosus* agg.	40.70	53.20	*Silene dioica*	11.03	67.59	*Silene dioica*	13.51	60.36
3	*Galium mollugo*	7.27	60.47	*Stachys sylvatica*	1.38	68.97	*Galium mollugo*	8.11	68.47
4	*Silene dioica*	18.90	79.36	*Teucrium scorodonia*	8.28	77.24	*Chaerophyllum temulum*	5.41	73.87
5	*Chaerophyllum temulum*	7.27	86.63	*Centaurea nigra*	9.66	86.90	*Geranium robertianum*	4.50	78.38

Adjacent Cereal									
Rank	Hoverfly			Bumblebee			Lepidoptera		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Heracleum sphondylium*	28.57		*Rubus fruticosus* agg.	62.26		*Rubus fruticosus* agg.	47.83	
2	*Rubus fruticosus* agg.	19.16	47.74	*Silene dioica*	8.18	70.44	*Silene dioica*	6.52	54.35
3	*Galium mollugo*	18.82	66.55	*Stachys sylvatica*	8.81	79.25	*Galium mollugo*	8.70	63.04
4	*Silene dioica*	10.80	77.35	*Teucrium scorodonia*	1.89	81.13	*Chaerophyllum temulum*	6.52	69.57
5	*Chaerophyllum temulum*	10.80	88.15	*Centaurea nigra*	-	-	*Geranium robertianum*	8.70	78.26

Distant Cereal									
Rank	Hoverfly			Bumblebee			Lepidoptera		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Heracleum sphondylium*	46.45		*Rubus fruticosus* agg.	56.67		*Rubus fruticosus* agg.	44.44	
2	*Rubus fruticosus* agg.	11.14	57.59	*Silene dioica*	10.83	67.50	*Silene dioica*	-	-
3	*Galium mollugo*	18.93	76.53	*Stachys sylvatica*	7.50	75.00	*Galium mollugo*	-	-
4	*Silene dioica*	5.82	82.35	*Teucrium scorodonia*	0.83	75.83	*Chaerophyllum temulum*	5.56	50.00
5	*Chaerophyllum temulum*	6.11	88.46	*Centaurea nigra*	-	-	*Geranium robertianum*	5.56	55.56

Appendix 13: Treatment specific breakdown (i.e. willow short-rotation coppice, adjacent cereal, distant cereal) of the relative percentage, and cumulative relative percentage, of flower visits by the three focal pollinator taxa in Nottinghamshire and Lincolnshire, central England, 2015.

Willow									
Rank	Hoverfly			Bumblebee			Lepidoptera		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Cirsium dissectum*	6.89		*Cirsium vulgare*	38.87		*Cirsium vulgare*	46.09	
2	*Rubus fruticosus* agg.	11.48	18.36	*Cirsium dissectum*	3.22	42.09	*Trifolium repens*	2.60	48.70
3	*Convolvulus arvensis*	19.08	37.45	*Rubus fruticosus* agg.	4.56	46.65	*Rubus fruticosus* agg.	20.57	69.27
4	*Cirsium vulgare*	10.33	47.78	*Heracleum sphondylium*	15.28	61.93	*Heracleum sphondylium*	7.29	76.56
5	*Heracleum sphondylium*	8.46	56.24	*Trifolium repens*	17.43	79.36	*Cirsium dissectum*	3.13	79.69

Adjacent Cereal									
Rank	Hoverfly			Bumblebee			Lepidoptera		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Cirsium dissectum*	4.48		*Cirsium vulgare*	8.24		*Cirsium vulgare*	20.73	
2	*Rubus fruticosus* agg.	40.34	44.82	*Cirsium dissectum*	4.71	12.94	*Trifolium repens*	-	-
3	*Convolvulus arvensis*	13.45	58.26	*Rubus fruticosus* agg.	67.06	80.00	*Rubus fruticosus* agg.	48.78	69.51
4	*Cirsium vulgare*	2.52	60.78	*Heracleum sphondylium*	10.59	90.59	*Heracleum sphondylium*	7.32	76.83
5	*Heracleum sphondylium*	13.17	73.95	*Trifolium repens*	-	-	*Cirsium dissectum*	2.44	79.27

Distant Cereal									
Rank	Hoverfly			Bumblebee			Lepidoptera		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Cirsium dissectum*	45.56		*Cirsium vulgare*	26.22		*Cirsium vulgare*	24.32	
2	*Rubus fruticosus* agg.	4.22	49.78	*Cirsium dissectum*	56.71	82.93	*Trifolium repens*	-	-
3	*Convolvulus arvensis*	9.33	59.11	*Rubus fruticosus* agg.	6.71	89.63	*Rubus fruticosus* agg.	2.70	27.03
4	*Cirsium vulgare*	12.00	71.11	*Heracleum sphondylium*	5.49	95.12	*Heracleum sphondylium*	13.51	40.54
5	*Heracleum sphondylium*	5.11	76.22	*Trifolium repens*	0.61	95.73	*Cirsium dissectum*	24.32	64.86

Appendix 14: Regional breakdown of the top five most highly visited plants for each of the three most frequent flower visiting species which represent each of the three focal pollinator taxa (hoverflies, bumblebees, Lepidoptera) alongside the relative percentage and cumulative relative percentage of visits to each plant.

Southwest: *Miscanthus* & Controls									
Rank	*Episyrphus balteatus*			*Bombus terrestris* agg.			*Pyronia tithonus*		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Heracleum sphondylium*	37.80		*Rubus fruticosus* agg.	63.80		*Rubus fruticosus* agg.	77.14	
2	*Galium mollugo*	27.37	65.17	*Silene dioica*	9.95	73.76	*Galium mollugo*	8.57	85.71
3	*Rubus fruticosus agg.*	11.14	76.30	*Stachys sylvatica*	6.33	80.09	*Silene dioica*	5.71	91.43
4	*Silene dioica*	8.41	84.72	*Galium mollugo*	3.62	83.71	*Calystegia sepium*	2.86	94.29
5	*Chaerophyllum temulum*	6.99	91.71	*Teucrium scorodonia*	3.62	87.33	*Convolvulus arvensis*	2.86	97.14

Midlands: Willow & Controls									
Rank	*Episyrphus balteatus*			*Bombus terrestris* agg			*Maniola jurtina*		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Cirsium dissectum*	18.95		*Cirsium vulgare*	26.79		*Cirsium vulgare*	31.43	
2	*Convolvulus arvensis*	16.29	35.24	*Cirsium dissectum*	13.21	40.00	*Trifolium repens*	20.71	52.14
3	*Rubus fruticosus* agg.	15.97	51.21	*Rubus fruticosus* agg.	16.60	56.60	*Rubus fruticosus* agg.	14.29	66.43
4	*Epilobium hirsutum*	9.03	60.24	*Heracleum sphondylium*	16.23	72.83	*Heracleum sphondylium*	9.29	75.71

Midlands: Willow & Controls cont.									
Rank	*Episyrphus balteatus*			*Bombus terrestris* agg			*Maniola jurtina*		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
5	*Cirsium vulgare*	8.71	68.95	*Trifolium repens*	9.06	81.89	*Cirsium dissectum*	7.14	82.86

Appendix 15: Treatment specific breakdown (i.e. *Miscanthus x giganteus*, adjacent cereal, distant cereal) of the relative percentage and cumulative relative percentage of flower visits by the three most recorded flower visitors from each of the focal pollinator taxa to the top 5 most visited plants in southwest England (2012, 2014, 2015).

Miscanthus									
Rank	*Episyrphus balteatus*			*Bombus terrestris agg.*			*Pyronia tithonus*		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Heracleum sphondylium*	8.14		*Rubus fruticosus agg.*	66.23		*Rubus fruticosus agg.*	81.48	
2	*Galium mollugo*	12.79	20.93	*Silene dioica*	14.29	80.52	*Galium mollugo*	7.41	88.89
3	*Rubus fruticosus agg.*	36.05	56.98	*Stachys sylvatica*	-	-	*Silene dioica*	7.41	96.30
4	*Silene dioica*	23.26	80.23	*Galium mollugo*	1.30	81.82	*Calystegia sepium*	-	-
5	*Chaerophyllum temulum*	5.81	86.05	*Teucrium scorodonia*	9.09	90.91	*Convolvulus arvensis*	-	-
Adjacent Cereal									
Rank	*Episyrphus balteatus*			*Bombus terrestris agg.*			*Pyronia tithonus*		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Heracleum sphondylium*	31.23		*Rubus fruticosus agg.*	63.10		*Rubus fruticosus agg.*	71.43	
2	*Galium mollugo*	30.04	61.26	*Silene dioica*	5.95	69.05	*Galium mollugo*	-	-
3	*Rubus fruticosus agg.*	13.83	75.10	*Stachys sylvatica*	13.10	82.14	*Silene dioica*	-	-
4	*Silene dioica*	8.30	83.40	*Galium mollugo*	1.19	83.33	*Calystegia sepium*	14.29	85.71
5	*Chaerophyllum temulum*	7.51	90.91	*Teucrium scorodonia*	1.19	84.52	*Convolvulus arvensis*	14.29	100.00

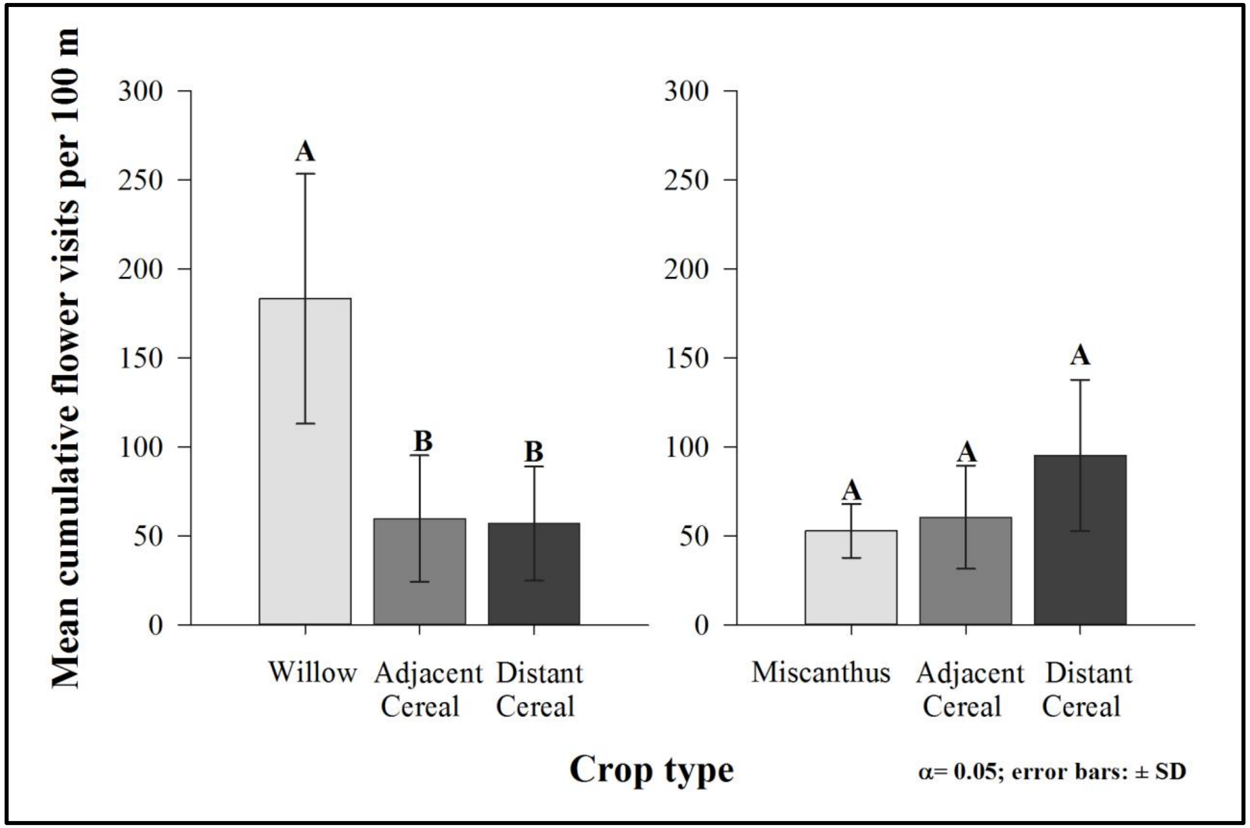
Distant Cereal									
Rank	*Episyrphus balteatus*			*Bombus terrestris agg.*			*Pyronia tithonus*		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Heracleum sphondylium*	46.14		*Rubus fruticosus* agg.	61.67		*Rubus fruticosus* agg.	-	
2	*Galium mollugo*	28.51	74.65	*Silene dioica*	10.00	71.67	*Galium mollugo*	-	-
3	*Rubus fruticosus agg.*	5.54	80.20	*Stachys sylvatica*	5.00	76.67	*Silene dioica*	-	-
4	*Silene dioica*	5.94	86.14	*Galium mollugo*	10.00	86.67	*Calystegia sepium*	-	-
5	*Chaerophyllum temulum*	6.93	93.07	*Teucrium scorodonia*	-	-	*Convolvulus arvensis*	100.00	100.00

Appendix 16: Treatment specific breakdown (i.e. willow short-rotation coppice, adjacent cereal, distant cereal) of the relative percentage and cumulative relative percentage of flower visits by the three most recorded flower visitors from each of the focal pollinator taxa to the top 5 most visited plants in Nottinghamshire and Lincolnshire, England, 2015.

Willow									
Rank	*Episyrphus balteatus*			*Bombus terrestris agg.*			*Maniola jurtina*		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Cirsium dissectum*	6.59		*Cirsium vulgare*	33.33		*Cirsium vulgare*	39.05	
2	*Convolvulus arvensis*	23.05	29.64	*Cirsium dissectum*	2.92	36.26	*Trifolium repens*	27.62	66.67
3	*Rubus fruticosus agg.*	11.27	40.90	*Rubus fruticosus agg.*	7.02	43.27	*Rubus fruticosus agg.*	1.90	68.57
4	*Epilobium hirsutum*	17.50	58.41	*Heracleum sphondylium*	20.47	63.74	*Heracleum sphondylium*	8.57	77.14
5	*Cirsium vulgare*	10.92	69.32	*Trifolium repens*	14.04	77.78	*Cirsium dissectum*	3.81	80.95
Adjacent Cereal									
Rank	*Episyrphus balteatus*			*Bombus terrestris agg.*			*Maniola jurtina*		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Cirsium dissectum*	5.48		*Cirsium vulgare*	2.33		*Cirsium vulgare*	4.00	
2	*Convolvulus arvensis*	10.62	16.10	*Cirsium dissectum*	9.30	11.63	*Trifolium repens*	-	-
3	*Rubus fruticosus agg.*	41.10	57.19	*Rubus fruticosus agg.*	67.44	79.07	*Rubus fruticosus agg.*	72.00	76.00
4	*Epilobium hirsutum*	-	-	*Heracleum sphondylium*	6.98	86.05	*Heracleum sphondylium*	12.00	88.00
5	*Cirsium vulgare*	2.74	59.93	*Trifolium repens*	-	-	*Cirsium dissectum*	-	-

Distant Cereal									
Rank	*Episyrphus balteatus*			*Bombus terrestris agg.*			*Maniola jurtina*		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Cirsium dissectum*	48.79		*Cirsium vulgare*	25.49		*Cirsium vulgare*	20.00	
2	*Convolvulus arvensis*	10.24	59.03	*Cirsium dissectum*	50.98	76.47	*Trifolium repens*	-	-
3	*Rubus fruticosus* agg.	3.50	62.53	*Rubus fruticosus* agg.	5.88	82.35	*Rubus fruticosus* agg.	-	-
4	*Epilobium hirsutum*	2.96	65.50	*Heracleum sphondylium*	9.80	92.16	*Heracleum sphondylium*	10.00	30.00
5	*Cirsium vulgare*	9.97	75.47	*Trifolium repens*	-	-	*Cirsium dissectum*	60.00	90.00

Appendix 17: Treatment specific (*Miscanthus x giganteus* or willow short-rotation coppice, adjacent cereal, distant cereal) differences in combined focal taxa flower visitation counts. Different letters indicate significant difference based on Tukey HSD post-hoc tests, α= 0.05. Error bars ± 1 SD.



Appendix 18: Region specific, relative percentage and cumulative relative percentage flower availability for those plant species accounting for 80% of visits from each of the three focal taxa.

Southwest: *Miscanthus* & Controls									
Rank	Hoverfly			Bumblebee			Lepidoptera		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Heracleum sphondylium*	0.67		*Rubus fruticosus* agg.	4.34		*Rubus fruticosus* agg.	4.34	
2	*Rubus fruticosus* agg.	4.34	5.01	*Silene dioica*	4.32	8.66	*Silene dioica*	4.32	8.66
3	*Galium mollugo*	69.67	74.68	*Stachys sylvatica*	1.54	10.20	*Galium mollugo*	69.67	78.33
4	*Silene dioica*	4.32	79.00	*Teucrium scorodonia*	1.28	11.48	*Chaerophyllum temulum*	1.18	79.51
5	*Chaerophyllum temulum*	1.18	80.19	*Centaurea nigra*	0.01	11.50	*Geranium robertianum*	9.53	89.05
6	-	-	-	-	-	-	*Ranunculus repens*	0.15	89.19

Midlands: Willow & Controls									
Rank	Hoverfly			Bumblebee			Lepidoptera		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Cirsium dissectum*	3.14		*Cirsium vulgare*	14.79		*Cirsium vulgare*	14.79	
2	*Rubus fruticosus* agg.	7.52	10.66	*Cirsium dissectum*	3.14	17.93	*Trifolium repens*	8.36	23.15
3	*Convolvulus arvensis*	2.46	13.12	*Rubus fruticosus* agg.	7.52	25.45	*Rubus fruticosus* agg.	7.52	30.66
4	*Cirsium vulgare*	14.79	27.92	*Heracleum sphondylium*	15.32	40.77	*Heracleum sphondylium*	15.32	45.98

Midlands: Willow & Controls cont.									
Rank	Hoverfly			Bumblebee			Lepidoptera		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
5	*Heracleum sphondylium*	15.32	43.23	*Trifolium repens*	8.36	49.12	*Cirsium dissectum*	3.14	49.12
6	*Epilobium hirsutum*	10.59	53.82	-	-	-	*Senecio jacobaeae*	1.83	50.95
7	*Senecio jacobaea*	1.83	55.65	-	-	-	-	-	-
8	*Chaerophyllum temulum*	4.22	59.87	-	-	-	-	-	-

Appendix 19: Treatment specific breakdown (i.e. *Miscanthus x giganteus*, adjacent cereal, distant cereal) of relative percentage and cumulative relative percentage flower availability (based on estimates of margin flower number) for plants receiving 80% of focal pollinator visits in southwest England. Post-hoc output based on Tukey tests, comparisons indicate no significant difference (*ns*) between crop treatments, α= 0.05.

Miscanthus									
Rank	Hoverfly^{ns}			Bumblebee^{ns}			Lepidoptera^{ns}		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Heracleum sphondylium*	0.19		*Rubus fruticosus* agg.	7.07		*Rubus fruticosus* agg.	7.07	
2	*Rubus fruticosus* agg.	7.07	7.26	*Silene dioica*	5.74	12.81	*Silene dioica*	5.74	12.81
3	*Galium mollugo*	75.72	82.79	*Stachys sylvatica*	1.08	13.89	*Galium mollugo*	75.72	88.54
4	*Silene dioica*	5.74	81.47	*Teucrium scorodonia*	2.64	16.53	*Chaerophyllum temulum*	1.09	89.63
5	*Chaerophyllum temulum*	1.09	6.84	*Centaurea nigra*	0.04	16.57	*Geranium robertianum*	2.15	91.78
6	-	-	-	-	-	-	*Ranunculus repens*	0.18	91.95
Adjacent Cereal									
Rank	Hoverfly^{ns}			Bumblebee^{ns}			Lepidoptera^{ns}		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Heracleum sphondylium*	0.49		*Rubus fruticosus* agg.	4.08		*Rubus fruticosus* agg.	4.08	
2	*Rubus fruticosus* agg.	4.08	4.57	*Silene dioica*	3.64	7.72	*Silene dioica*	3.64	7.72
3	*Galium mollugo*	79.38	83.95	*Stachys sylvatica*	2.05	9.77	*Galium mollugo*	79.38	87.10

Adjacent Cereal cont.									
Rank	Hoverfly^{ns}			Bumblebee^{ns}			Lepidoptera^{ns}		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
4	*Silene dioica*	3.64	87.59	*Teucrium scorodonia*	0.97	10.75	*Chaerophyllum temulum*	1.35	88.45
5	*Chaerophyllum temulum*	1.35	88.94	*Centaurea nigra*	0.00	10.75	*Geranium robertianum*	1.22	89.67
6	-	-	-	-	-	-	*Ranunculus repens*	0.16	89.84

Distant Cereal									
Rank	Hoverfly^{ns}			Bumblebee^{ns}			Lepidoptera^{ns}		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Heracleum sphondylium*	1.25		*Rubus fruticosus* agg.	2.52		*Rubus fruticosus* agg.	2.52	
2	*Rubus fruticosus* agg.	2.52	3.77	*Silene dioica*	3.99	6.51	*Silene dioica*	3.99	6.51
3	*Galium mollugo*	53.95	57.71	*Stachys sylvatica*	1.31	7.82	*Galium mollugo*	53.95	60.45
4	*Silene dioica*	3.99	61.70	*Teucrium scorodonia*	0.59	8.41	*Chaerophyllum temulum*	1.06	61.51
5	*Chaerophyllum temulum*	1.06	62.76	*Centaurea nigra*	0.00	8.41	*Geranium robertianum*	24.70	86.21
6	-	-	-	-	-	-	*Ranunculus repens*	0.10	86.31

Appendix 20: Treatment specific breakdown (i.e. willow short-rotation coppice, adjacent cereal, distant cereal) of relative percentage and cumulative relative percentage flower availability (based on estimates of margin flower number) for plants receiving 80% of focal pollinator visits. Nottinghamshire and Lincolnshire, central England. Post-hoc output based on Tukey tests, different letters indicate significant difference, α=0.05.

Willow									
Rank	Hoverfly^{a}			Bumblebee^{a}			Lepidoptera^{a}		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Cirsium dissectum*	0.34		*Cirsium vulgare*	20.73		*Cirsium vulgare*	20.73	
2	*Rubus fruticosus* agg.	5.12	5.46	*Cirsium dissectum*	0.34	21.07	*Convolvulus arvensis*	13.19	33.92
3	*Convolvulus arvensis*	2.37	7.83	*Rubus fruticosus* agg.	5.12	26.19	*Rubus fruticosus* agg.	5.12	39.04
4	*Cirsium vulgare*	20.73	28.56	*Heracleum sphondylium*	17.16	43.35	*Epilobium hirsutum*	17.16	56.20
5	*Heracleum sphondylium*	17.16	45.73	*Trifolium repens*	13.19	56.54	*Cirsium dissectum*	0.34	56.54
6	*Epilobium hirsutum*	10.88	56.60	-	-	-	*Senecio jacobaeae*	1.65	58.19
7	*Senecio jacobaea*	1.65	58.25	-	-	-	-	-	-
8	*Chaerophyllum temulum*	1.48	59.73	-	-	-	-	-	-
Adjacent Cereal									
Rank	Hoverfly^{a}			Bumblebee^{b}			Lepidoptera^{b}		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Cirsium dissectum*	7.10		*Cirsium vulgare*	3.63		*Cirsium vulgare*	3.63	
2	*Rubus fruticosus* agg.	14.42	21.52	*Cirsium dissectum*	7.10	10.74	*Convolvulus arvensis*	14.42	18.05

Adjacent Cereal cont.									
Rank	Hoverfly[a]			Bumblebee[b]			Lepidoptera[b]		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
3	*Convolvulus arvensis*	2.79	24.31	*Rubus fruticosus* agg.	14.42	25.16	*Rubus fruticosus* agg.	14.99	33.04
4	*Cirsium vulgare*	3.63	27.95	*Heracleum sphondylium*	14.99	40.15	*Epilobium hirsutum*	7.10	40.15
5	*Heracleum sphondylium*	14.99	42.94	*Trifolium repens*	-	-	*Cirsium dissectum*	2.72	42.87
6	*Epilobium hirsutum*	14.39	57.33	-	-	-	*Senecio jacobaeae*	-	-
7	*Senecio jacobaea*	2.72	60.06	-	-	-	-	-	-
8	*Chaerophyllum temulum*	6.82	66.87	-	-	-	-	-	-
Distant Cereal									
Rank	Hoverfly[a]			Bumblebee[b]			Lepidoptera[b]		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Cirsium dissectum*	9.06		*Cirsium vulgare*	5.66		*Cirsium vulgare*	5.66	
2	*Rubus fruticosus* agg.	8.23	17.29	*Cirsium dissectum*	9.06	14.72	*Convolvulus arvensis*	0.04	5.71
3	*Convolvulus arvensis*	2.41	19.70	*Rubus fruticosus* agg.	8.23	22.96	*Rubus fruticosus* agg.	8.23	13.94
4	*Cirsium vulgare*	5.66	25.37	*Heracleum sphondylium*	8.61	31.56	*Epilobium hirsutum*	8.61	22.55
5	*Heracleum sphondylium*	8.61	33.97	*Trifolium repens*	0.04	31.61	*Cirsium dissectum*	9.06	31.61
6	*Epilobium hirsutum*	4.80	38.77	-	-	-	*Senecio jacobaeae*	1.41	33.02
7	*Senecio jacobaea*	1.41	40.18	-	-	-	-	-	-
8	*C. temulum*	11.60	51.78	-	-	-	-	-	-

Appendix 21: Region specific relative percentage and cumulative relative percentage flower availability for the plant species receiving 80% of visits from each of the three most recorded insect visitors belonging to each focal taxon.

Southwest: *Miscanthus* & Controls									
Rank	*Episyrphus balteatus*			*Bombus terrestris* agg.			*Pyronia tithonus*		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Heracleum sphondylium*	0.67		*Rubus fruticosus* agg.	4.34		*Rubus fruticosus* agg.	4.34	
2	*Galium mollugo*	4.34	5.01	*Silene dioica*	4.32	8.66	*Galium mollugo*	69.67	74.01
3	*Rubus fruticosus* agg.	69.67	74.68	*Stachys sylvatica*	1.54	10.20	*Silene dioica*	4.32	78.33
4	*Silene dioica*	4.32	79.00	*Galium mollugo*	69.67	79.87	*Calystegia sepium*	0.03	78.36
5	*Chaerophyllum temulum*	1.18	80.19	*Teucrium scorodonia*	1.28	81.16	*Convolvulus arvensis*	0.03	78.39
Midlands: Willow & Controls									
Rank	*Episyrphus balteatus*			*Bombus terrestris* agg			*Maniola jurtina*		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Cirsium dissectum*	3.14		*Cirsium vulgare*	14.79		*Cirsium vulgare*	14.79	
2	*Cirsium arvensis*	2.46	5.61	*Cirsium dissectum*	3.14	17.93	*Cirsium dissectum*	3.14	17.93
3	*Rubus fruticosus* agg.	7.52	13.12	*Rubus fruticosus* agg.	7.52	25.45	*Rubus fruticosus* agg.	7.52	25.45
4	*Epilobium hirsutum*	10.59	23.71	*Heracleum sphondylium*	15.32	40.77	*Heracleum sphondylium*	15.32	40.77
5	*Cirsium vulgare*	14.79	38.51	*Trifolium repens*	8.36	49.12	*Trifolium repens*	8.36	49.12

6	*Heracleum sphondylium*	15.32	53.82	-	-	-	-	-	-
Midlands: Willow & Controls cont.									
Rank	*Episyrphus balteatus*			*Bombus terrestris* agg			*Maniola jurtina*		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
7	*Chaerophyllum temulum*	4.22	58.05	-	-	-	-	-	-

Appendix 22: Relative percentage and cumulative relative percentage flower availability for the plant species receiving 80% of visits from the most recorded flower visiting insect species from each focal taxon in southwest England (2012, 2014, 2015). Post-hoc output based on Tukey tests & pairwise comparisons indicate no significant difference (*ns*) between crop treatments (i.e. *Miscanthus*, adjacent cereal, distant cereal), α=0.05.

Miscanthus									
Rank	*Episyrphus balteatus*^{ns}			*Bombus terrestris* agg.^{ns}			*Pyronia tithonus*^{ns}		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Heracleum sphondylium*	0.19		*Rubus fruticosus* agg.	7.07		*Rubus fruticosus* agg.	7.07	
2	*Galium mollugo*	75.72	75.92	*Silene dioica*	5.74	12.81	*Galium mollugo*	75.72	82.79
3	*Rubus fruticosus* agg.	7.07	82.99	*Stachys sylvatica*	1.08	13.89	*Silene dioica*	5.74	88.54
4	*Silene dioica*	5.74	88.73	*Galium mollugo*	75.72	89.62	*Calystegia sepium*	0.00	88.54
5	*Chaerophyllum temulum*	1.09	89.83	*Teucrium scorodonia*	2.64	92.26	*Convolvulus arvensis*	0.01	88.56
Adjacent Cereal									
Rank	*Episyrphus balteatus*^{ns}			*Bombus terrestris* agg.^{ns}			*Pyronia tithonus*^{ns}		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Heracleum sphondylium*	0.49		*Rubus fruticosus* agg.	4.08		*Rubus fruticosus* agg.	4.08	
2	*Galium mollugo*	79.38	79.87	*Silene dioica*	3.64	7.72	*Galium mollugo*	79.38	83.46
3	*Rubus fruticosus* agg.	4.08	83.95	*Stachys sylvatica*	2.05	9.77	*Silene dioica*	3.64	87.10
4	*Silene dioica*	3.64	87.59	*Galium mollugo*	79.38	89.15	*Calystegia sepium*	0.01	87.11
5	*Chaerophyllum temulum*	1.35	88.94	*Teucrium scorodonia*	0.97	90.12	*Convolvulus arvensis*	0.00	87.11

Distant Cereal									
Rank	*Episyrphus balteatus*^{ns}			*Bombus terrestris* agg.^{ns}			*Pyronia tithonus*^{ns}		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Heracleum sphondylium*	1.25		*Rubus fruticosus* agg.	2.52		*Rubus fruticosus* agg.	2.52	
2	*Galium mollugo*	53.95	55.19	*Silene dioica*	3.99	6.51	*Galium mollugo*	53.95	56.47
3	*Rubus fruticosus* agg.	2.52	57.71	*Stachys sylvatica*	1.31	7.82	*Silene dioica*	3.99	60.45
4	*Silene dioica*	3.99	61.70	*Galium mollugo*	53.95	61.77	*Calystegia sepium*	0.07	60.52
5	*Chaerophyllum temulum*	1.06	62.76	*Teucrium scorodonia*	0.59	62.36	*Convolvulus arvensis*	0.08	60.61

Appendix 23: Relative percentage and cumulative relative percentage flower availability for the plants accounting for 80% of visits by the most recorded flower visiting insect species from each focal taxon in Nottinghamshire and Lincolnshire, 2015. Post-hoc output based on Tukey tests, different letters indicate significant difference between crop treatments (i.e. willow short-rotation coppice, adjacent cereal, distant cereal), α=0.05.

Willow									
Rank	*Episyrphus balteatus*[a]			*Bombus terrestris* agg.[a]			*Maniola jurtina*[a]		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Cirsium dissectum*	0.34		*Cirsium vulgare*	20.73		*Cirsium vulgare*	20.73	
2	*Cirsium arvensis*	2.37	2.71	*Cirsium dissectum*	0.34	21.07	*Cirsium dissectum*	0.34	21.07
3	*Rubus fruticosus* agg.	5.12	7.83	*Rubus fruticosus* agg.	5.12	26.19	*Rubus fruticosus* agg.	5.12	26.19
4	*Epilobium hirsutum*	10.88	18.71	*Heracleum sphondylium*	17.16	43.35	*Heracleum sphondylium*	17.16	43.35
5	*Cirsium vulgare*	20.73	39.44	*Trifolium repens*	13.19	56.54	*Trifolium repens*	13.19	56.54
6	*Heracleum sphondylium*	17.16	56.60	-	-	-	-	-	-
7	*Chaerophyllum temulum*	1.48	58.08	-	-	-	-	-	-

Adjacent Cereal									
Rank	*Episyrphus balteatus*[a]			*Bombus terrestris* agg.[b]			*Maniola jurtina*[b]		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Cirsium dissectum*	7.10		*Cirsium vulgare*	3.63		*Cirsium vulgare*	3.63	
2	*Cirsium arvensis*	2.79	9.89	*Cirsium dissectum*	7.10	10.74	*Cirsium dissectum*	7.10	10.74
3	*Rubus fruticosus* agg.	14.42	24.31	*Rubus fruticosus* agg.	14.42	25.16	*Rubus fruticosus* agg.	14.42	25.16

Adjacent Cereal									
Rank	*Episyrphus balteatus*[a]			*Bombus terrestris* agg.[b]			*Maniola jurtina*[b]		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
4	*Epilobium hirsutum*	14.39	38.71	*Heracleum sphondylium*	14.99	40.15	*Heracleum sphondylium*	14.99	40.15
5	*Cirsium vulgare*	3.63	42.34	*Trifolium repens*	-	-	*Trifolium repens*	-	-
6	*Heracleum sphondylium*	14.99	57.33	-	-	-	-	-	-
7	*Chaerophyllum temulum*	6.82	64.15	-	-	-	-	-	-
Distant Cereal									
Rank	*Episyrphus balteatus*[a]			*Bombus terrestris* agg.[b]			*Maniola jurtina*[b]		
	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %	Species	Relative %	Cumulative Relative %
1	*Cirsium dissectum*	9.06		*Cirsium vulgare*	5.66		*Cirsium vulgare*	5.66	
2	*Cirsium arvensis*	2.41	11.47	*Cirsium dissectum*	9.06	14.72	*Cirsium dissectum*	9.06	14.72
3	*Rubus fruticosus* agg.	8.23	19.70	*Rubus fruticosus* agg.	8.23	22.96	*Rubus fruticosus* agg.	8.23	22.96
4	*Epilobium hirsutum*	4.80	24.50	*Heracleum sphondylium*	8.61	31.56	*Heracleum sphondylium*	8.61	31.56
5	*Cirsium vulgare*	5.66	30.16	*Trifolium repens*	0.04	31.61	*Trifolium repens*	0.04	31.61
6	*Heracleum sphondylium*	8.61	38.77	-	-	-	-	-	-
7	*Chaerophyllum temulum*	11.60	50.37	-	-	-	-	-	-

Appendix 24: Output of statistical analyses of mean crop treatment differences of total number of floral units at sites in southwest England (2012, 2014, 2015) and central England (2015), alongside standard error of the mean (S.E.). Superscript letters indicate treatment differences based on Tukey post-hoc tests, α= 0.05.

Total Flowers				
	Mean	**S.E.**	**Test Output**	***P***
Miscanthus	24248.52	10052.79		
Adjacent Cereal	30878.28	13025.31	$H_{2,15}$= 0.082	P = 0.96
Distant Cereal	22475.47	8145.50		
	Mean	**S.E.**	**Test Output**	***P***
Willow^{a}	9002.28	1771.66		
Adjacent Cereal^{b}	2883.01	773.49	$F_{2,9}$= 9.001	P = 0.007
Distant Cereal^{b}	2334.66	910.59		

Appendix 25: Output of statistical analyses of mean crop treatment differences in the total number of floral units for plants receiving 80% of hoverfly visits at sites in southwest England (2012, 2014, 2015) and central England (2015), alongside standard error of the mean (S.E.). α= 0.05.

Hoverfly Flower Preference				
	Mean	**S.E.**	**Test Output**	***P***
Miscanthus	21369.712	10519.141		
Adjacent Cereal	27650.616	12707.745	$F_{2,15}$= 0.052	P = 0.95
Distant Cereal	14067.626	3988.1737		
	Mean	**S.E.**	**Test Output**	***P***
Willow	4916.25	2737.62		
Adjacent Cereal	1512.50	421.21	$H_{2,9}$= 2.346	P = 0.309
Distant Cereal	1016.50	263.97		

Appendix 26: Output of statistical analyses of mean crop treatment differences in the total number of floral units for plants receiving 80% of bumblebee visits at sites in southwest England (2012, 2014, 2015) and central England (2015), alongside standard error of the mean (S.E.). Superscript letters indicate treatment differences based on Tukey post-hoc tests, α= 0.05.

Bumblebee Flower Preference				
	Mean	**S.E.**	**Test Output**	***P***
Miscanthus	3527.11	1295.80		
Adjacent Cereal	3175.56	956.46	$F_{2,15}$= 0.298	P = 0.747
Distant Cereal	2068.33	493.30		
	Mean	**S.E.**	**Test Output**	***P***
Willow^{a}	5089.75	2244.17		
Adjacent Cereal^{b}	1157.50	164.40	$F_{2,9}$= 11.259	P = 0.004
Distant Cereal^{b}	737.75	243.21		

Appendix 27: Output of statistical analyses of mean crop treatment differences in the total number of floral units for plants receiving 80% of Lepidoptera visits at sites in southwest England (2012, 2014, 2015) and central England (2015), alongside standard error of the mean (S.E.). Superscript letters indicate treatment differences based on Tukey post-hoc tests, α= 0.05.

Lepidoptera Flower Preference				
	Mean	**S.E.**	**Test Output**	***P***
Miscanthus	21806.92	10513.36		
Adjacent Cereal	27917.79	12671.66	$F_{2,15}$= 0.007	$P = 0.993$
Distant Cereal	18598.04	7602.42		
	Mean	**S.E.**	**Test Output**	***P***
Willow^{a}	5238.00	2194.95		
Adjacent Cereal^{b}	1236.00	201.24	$F_{2,9}$= 11.401	$P = 0.003$
Distant Cereal^{b}	770.75	240.79		

Appendix 28: Output of statistical analyses of mean crop treatment differences in the total number of floral units for plants receiving 80% of visits from the most frequent hoverfly species at sites in southwest England (2012, 2014, 2015) and central England (2015), alongside standard error of the mean (S.E.). α= 0.05.

***Episyrphus balteatus* Flower Preference**				
	Mean	**S.E.**	**Test Output**	***P***
Miscanthus	21369.71	10519.14		
Adjacent Cereal	27650.62	12707.74	$F_{2,15}$= 0.052	P = 0.95
Distant Cereal	14067.63	3988.17		
Willow	5,089.89	2,244.04		
Adjacent Cereal	1,157.41	164.31	$F_{2,5.568}$= 0.989	P = 0.429
Distant Cereal	737.91	243.24		

Appendix 29: Output of statistical analyses of mean crop treatment differences in the total number of floral units for plants receiving 80% of visits from the most frequent bumblebee taxa at sites in southwest England (2012, 2014, 2015) and central England (2015), alongside standard error of the mean (S.E.). Superscript letters indicate treatment differences based on Tukey post-hoc tests, α= 0.05.

***Bombus terrestris* agg. Flower Preference**				
	Mean	**S.E.**	**Test Output**	***P***
Miscanthus	22487.28	10148.39		
Adjacent Cereal	27979.83	12793.34	$F_{2,15}$= 0.095	P = 0.91
Distant Cereal	14015.78	3893.76		
Willow^{a}	5089.75	2244.17		
Adjacent Cereal^{b}	1157.50	164.40	$F_{2,9}$= 11.259	P = 0.004
Distant Cereal^{b}	737.75	243.21		

Appendix 30: Output of statistical analyses of mean crop treatment differences in the total number of floral units for plants receiving 80% of visits from the most frequent Lepidopteran species at sites in southwest England (2012, 2014, 2015) and central England (2015), alongside standard error of the mean (S.E.). Superscript letters indicate treatment differences based on Tukey post-hoc tests, α= 0.05.

***Pyronia tithonus* Flower Preference**				
	Mean	**S.E.**	**Test Output**	***P***
Miscanthus	21168.17	10531.44		
Adjacent Cereal	27174.83	12672.82	$F_{2,15}$= 0.07	P = 0.932
Distant Cereal	13587.50	3958.74		
***Maniola jurtina* Flower Preference**				
	Mean	**S.E.**	**Test Output**	***P***
Willow^{a}	5376.94	2799.70		
Adjacent Cereal^{b}	1928.00	623.68	$F_{2,9}$= 11.256	P = 0.004
Distant Cereal^{b}	1208.96	309.84		

Appendix 31: Flower visitation network legend

Flower Visitor		Plant	
Taxa	Code	Taxa	Code
Aglais io	A1	Angelica archangelica	B1
Aglais urticae	A2	Arctium minus	B2
Anthocharis cardamines	A3	Ballota nigra	B3
Aphantopus hyperantus	A4	Bellis perennis	B4
Aricia agestis	A5	Bryonia alba	B5
Autographa gamma	A6	Calystegia sepium	B6
Baccha elongata	A7	Cardamine flexuosa	B7
Bombus hortorum	A8	Centaurea nigra	B8
Bombus hypnorum	A9	Chaerophyllum temulum	B9
Bombus lapidarius	A10	Chamerion angustifolium	B10
Bombus pascuorum	A11	Cirsium arvense	B11
Bombus pratorum	A12	Cirsium dissectum	B12
Bombus ruderatus	A13	Cirsium vulgare	B13
Bombus sylvarum	A14	Clematis vitalba	B14
Bombus sylvestris	A15	Clinopodium vulgare	B15
Bombus terrestris agg.	A16	Conium maculatum	B16
Bombus vestalis	A17	Convolvulus arvensis	B17
Brachypalpoides lentus	A18	Crataegus monogyna	B18
Chrysogaster solstitialis	A19	Crepis spp.	B19
Criorhina floccosa	A20	Digitalis purpurea	B20
Depressaria daucella	A21	Epilobium hirsutum	B21
Epistrophe eligans	A22	Epilobium leptophyllum	B22
Epistrophe grossulariae	A23	Eupatorium cannabinum	B23
Episyrphus balteatus	A24	Filipendula ulmaria	B24
Eristalis sp.	A25	Galeopsis tetrahit	B25
Eupeodes sp.	A26	Galium mollugo	B26
Gonepteryx rhamni	A27	Geranium columbinum	B27
Leucozona glaucia	A28	Geranium dissectum	B28
Leucozona lucorum	A29	Geranium robertianum	B29
Macroglossum stellatarum	A30	German chamomile	B30
Maniola jurtina	A31	Heracleum mantegazzianum	B31
Melangyna umbellatorum	A32	Heracleum sphondylium	B32
Melanostoma scalare	A33	Hieracium spp.	B33
Meligramma cincta	A34	Impatiens glandulifera	B34
Meliscaeva cinctella	A35	Jacobaea vulgaris	B35
Myathropa florea	A36	Lapsana communis	B36
Ochlodes sylvanus	A37	Ligustrum vulgare	B37
Pararge aegeria	A38	Lonicera periclymenum	B38
Pieris sp.	A39	Myosotis arvensis	B39
Platycheirus sp.	A40	Oenanthe crocata	B40
Polyommatus icarus	A41	Potentilla reptans	B41
Pseudopanthera macularia	A42	Ranunculus repens	B42
Pyronia tithonus	A43	Rosa arvensis	B43
Rhingia campestris	A44	Rosa canina	B44
Scaeva pyrastri	A45	Rubus fruticosus agg.	B45
Sericomyia silentis	A46	Sambucus spp.	B46
Sphaerophoria sp.	A47	Silene dioica	B47
Synanthedon myopaeformis	A48	Silene latifolia	B48
Syritta pipiens	A49	Sisymbrium officinale	B49
Syrphus sp.	A50	Solanum dulcamara	B50
Thymelicus sylvestris	A51	Sonchus spp.	B51
Tyria jacobaeae	A52	Stachys sylvatica	B52
Vanessa atalanta	A54	Stellaria graminea	B53
Vanessa cardui	A55	Taraxacum officinale agg.	B54
Volucella bombylans	A56	Teucrium scorodonia	B55
		Trifolium campestre	B56
		Trifolium dubium	B57
		Trifolium repens	B58
		Tripleurospermum inodorum	B59
		Unidentified Species 1	B60
		Unidentified Species 2	B61
		Urtica dioica	B62
		Veronica chamaedrys	B63
		Vicia cracca	B64
		Vicia sativa	B65

Appendix 32: The location of *Miscanthus x giganteus* and bean (*Vicia faba*) field sites used for phytometer seed set experiments in 2016, alongside their corresponding cereal controls.

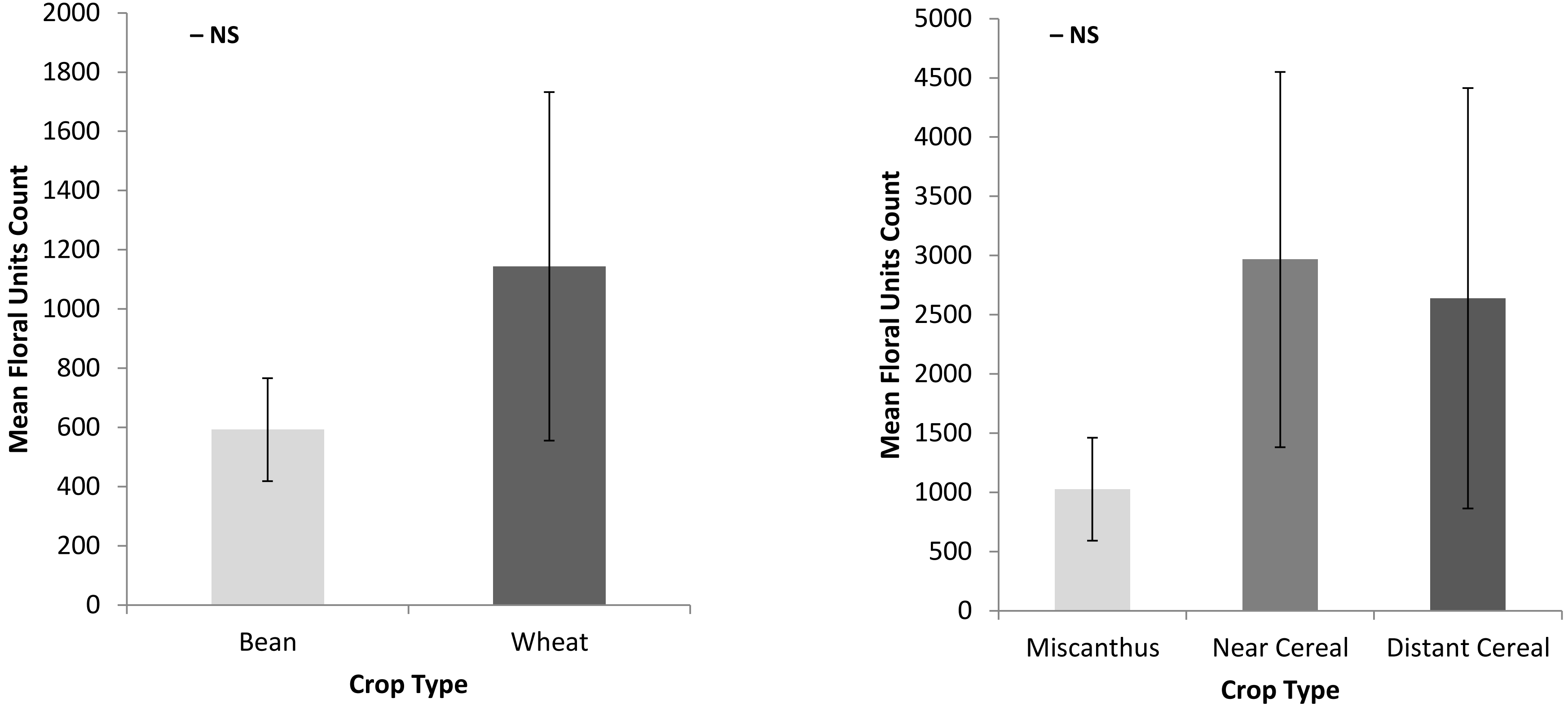
Perennial energy crop & controls

Crop	Site	Lat.Lon.
Miscanthus	Coldrenick	50°30.2616'"N; 4°41.1261'"W
	Lostwithiel	50°25.6738'"N; 4°37.8046'"W
	St Minver	50°33.0877'"N; 4°51.3017'"W
	Yarford	51°3.7935'"N; 3°8.6699'"W
Adjacent Cereal	Coldrenick	50°30.3220'"N; 4°41.0489'"W
	Lostwithiel	50°25.8209'"N; 4°37.4237'"W
	St Minver	50°33.2042'"N; 4°51.2695'"W
	Yarford	51°3.8816'"N; 3°8.5656'"W
Distant Cereal	Coldrenick	50°31'8.81"N; 4°45'10.08"W
	Lostwithiel	50°25.7457'"N; 4°35.4594'"W
	St Minver	50°33.1876'"N; 4°50.1079'"W
	Yarford	51°0.5046'"N; 2°59.8898'"W

Mass-flowering crop & controls

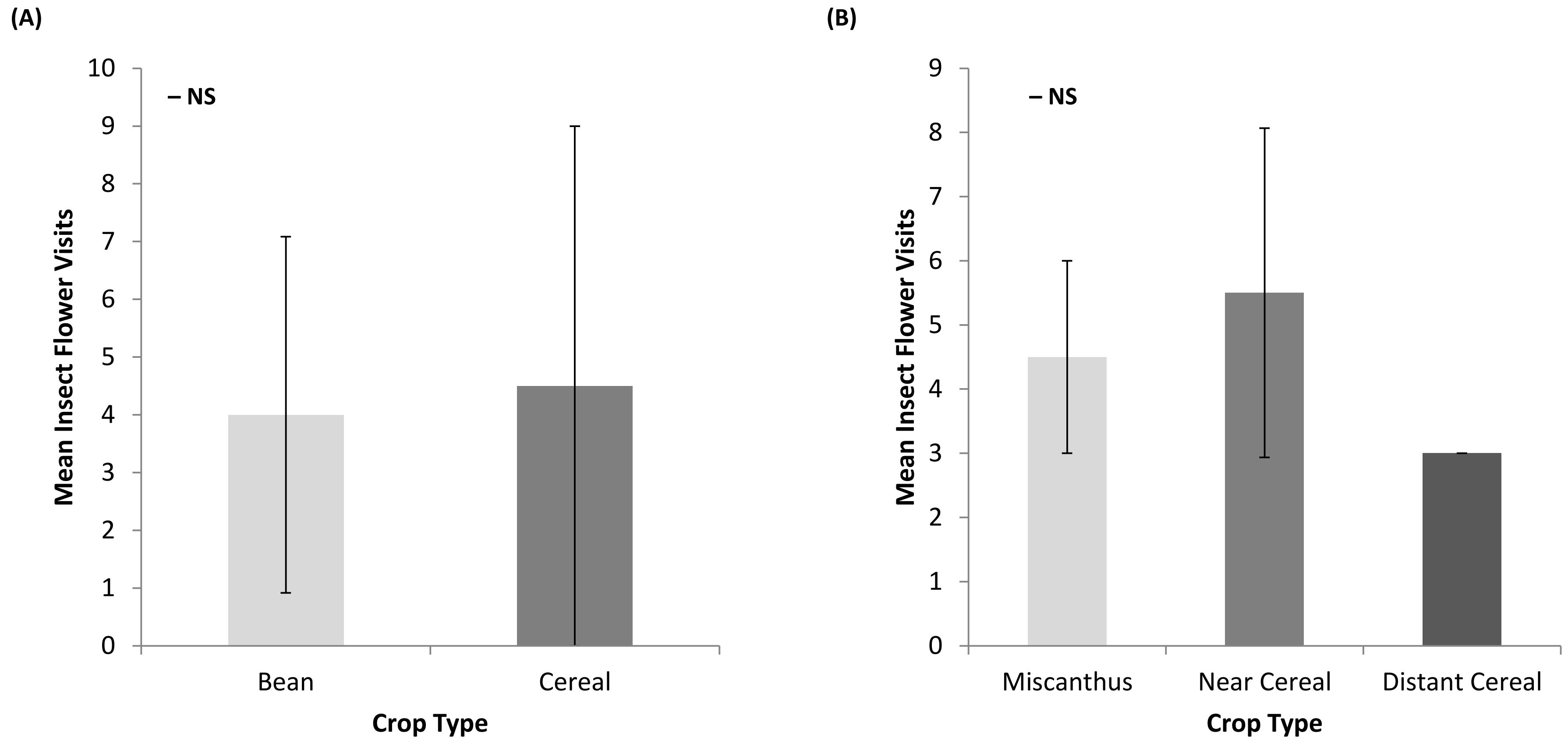
Crop	Site	Lat./Lon.
Field Bean	Merton	50°53.9973'"N; 4°5.6248'"W
	Plympton	50°21.5907'"N; 4°1.8050'"W
	Quethiock	50°27.9167'"N; 4°22.2284'"W
	Sowton	50°43.5710'"N; 3°26.4522'"W
Cereal	Merton	50°52.2952'"N; 4°3.7837'"W
	Plympton	50°22.2357'"N; 4°2.3600'"W
	Quethiock	50°26.7122'"N; 4°20.7822'"W
	Sowton	50°42.4481'"N; 3°24.2860'"W

Appendix 33: Mean floral unit counts at bean (*Vicia faba*) and wheat (*Triticum* spp.) sites (A) and *Miscanthus x giganteus* and cereal control sites (B) and in southwest England, 2016; including hedge bedstraw counts. Error bars indicate ± 1 S.E. α= 0.05. NS= Non-significant.

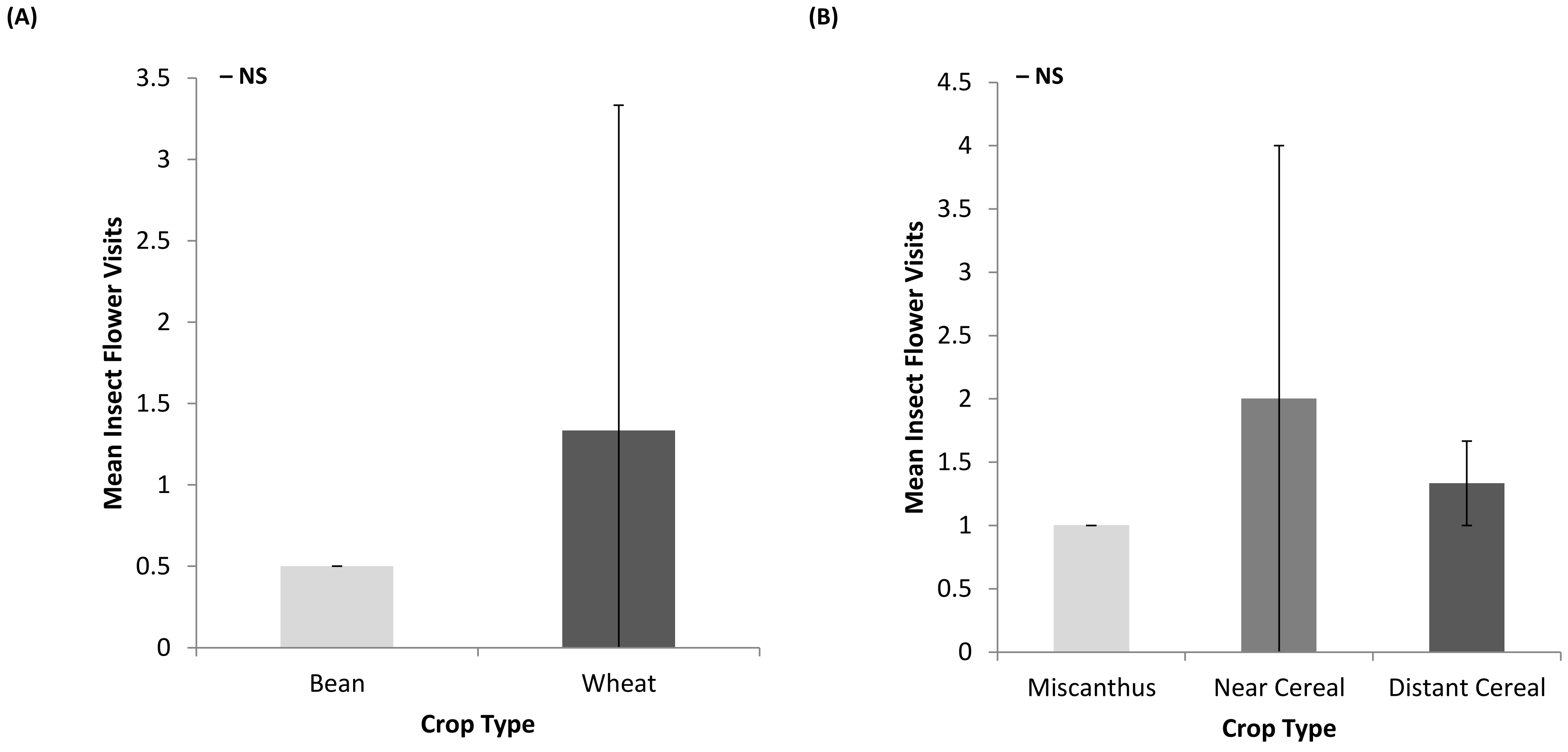

(A)

(B)

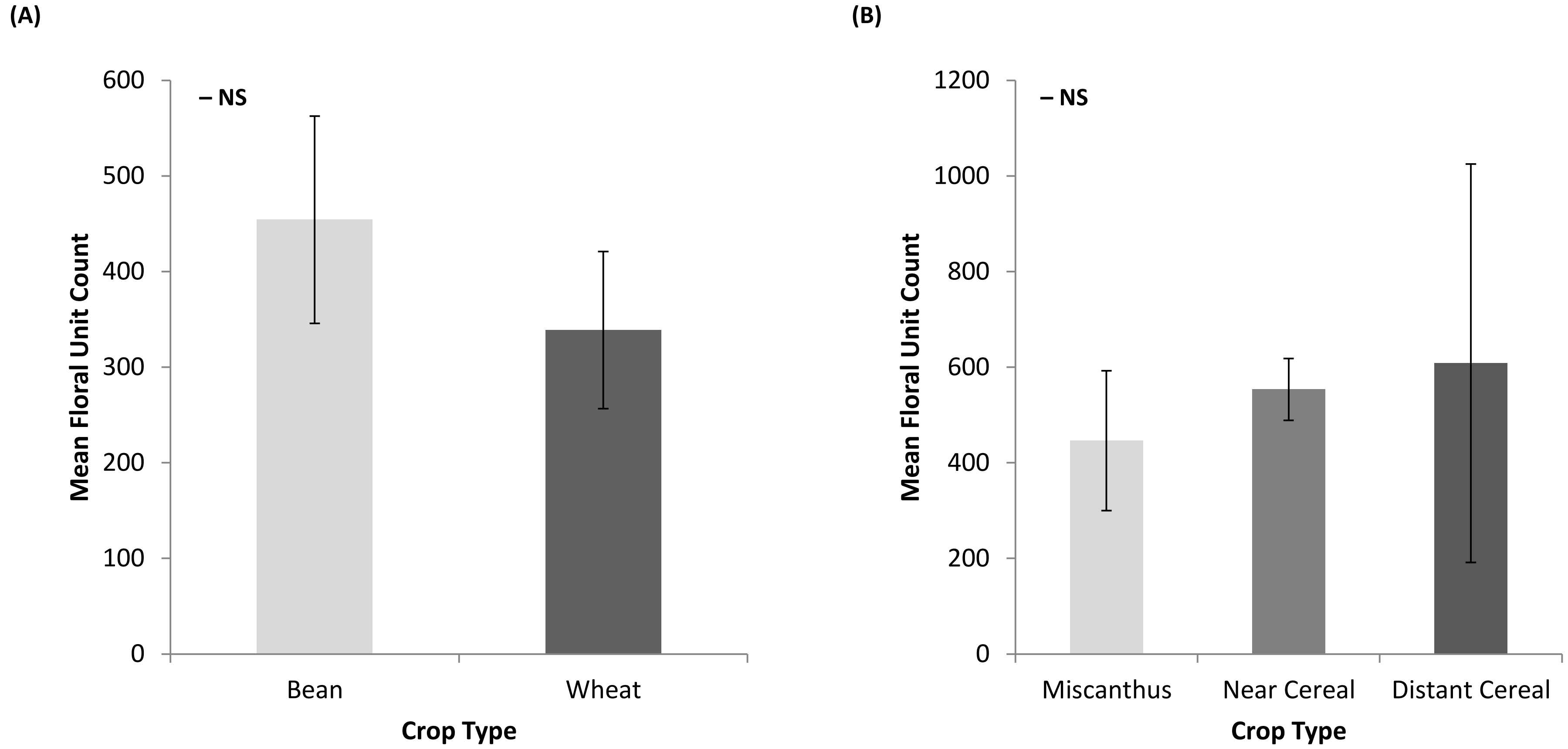
Appendix 34: Mean insect visitation to California poppy (*Eschscholzia californica*) flowers at bean and wheat sites (A) and *Miscanthus x giganteus* and cereal control sites (B) in southwest England, 2016. Error bars indicate ±1 S.E. α= 0.05. NS= Non-significant.



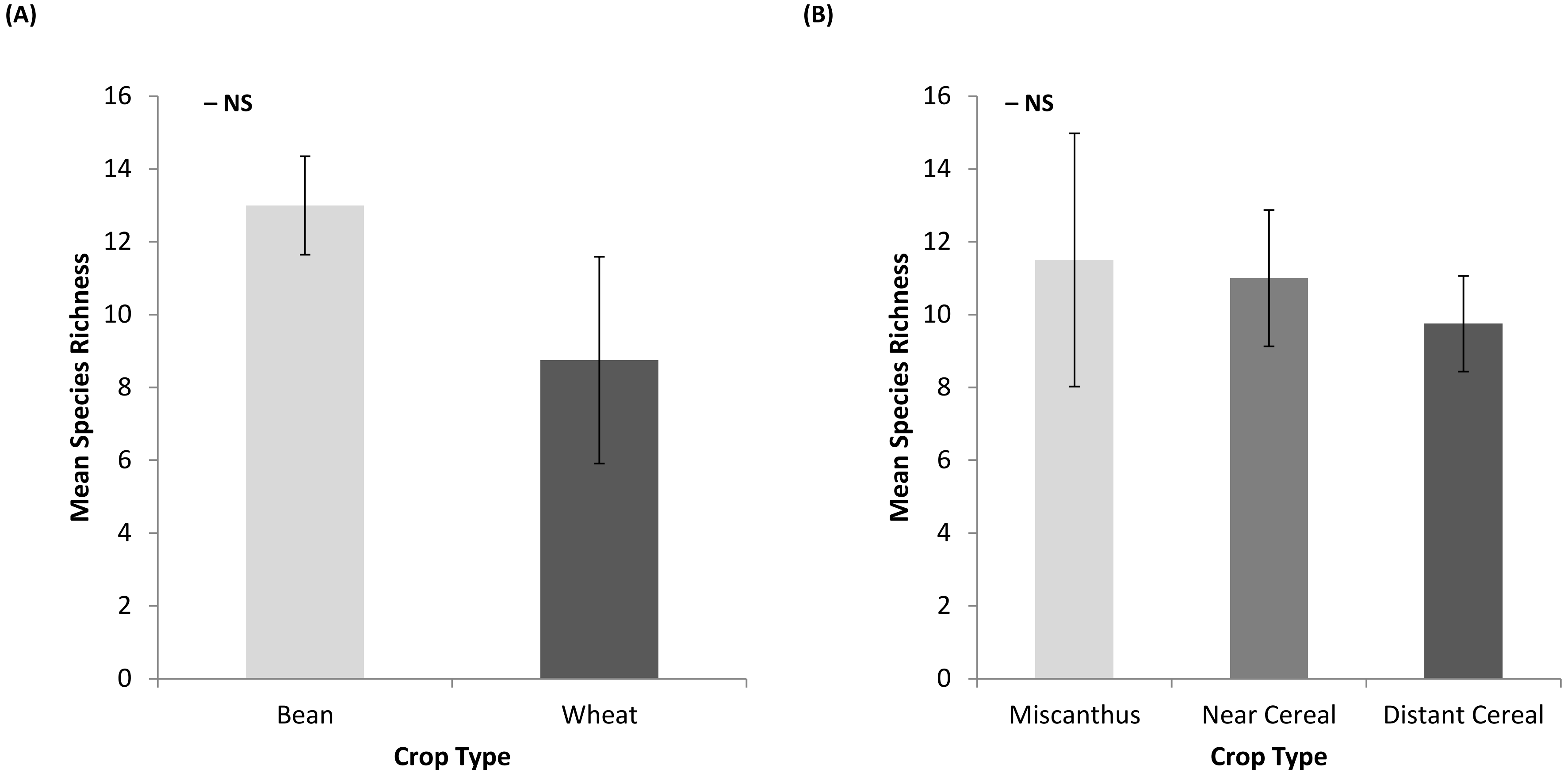
Appendix 35: Mean insect visitation to clover flowers at bean (*Vicia faba*) and wheat (*Triticum* spp.) fields (A) and *Miscanthus x giganteus* and cereal control sites (B) in southwest England, 2016. Error bars indicate ±1 S.E. α= 0.05. NS= Non-significant.



Appendix 36: Mean floral unit counts at bean (*Vicia faba*) and wheat (*Triticum* spp.) fields (A) and *Miscanthus x giganteus* and cereal control fields (B) in southwest England, 2016; excluding hedge bedstraw. Error bars indicate ± 1 S.E. α= 0.05. NS= Non-significant.



Appendix 37: Mean species richness of margin wildflowers at bean (*Vicia faba*) and wheat (*Triticum* spp.) sites (A) and *Miscanthus x giganteus* and cereal control sites (B) in southwest England, 2016. Error bars indicate ±1 S.E. α= 0.05. NS= Non-significant.



Appendix 38: Mean ‘most bioaccessible’ CISED concentration (nmol g^{-1}) of each element (washes 1-4) and standard error (S.E.) of the mean within the margin region of *Miscanthus x giganteus* and cereal controls. < LOD = below Limit Of Detection. α= 0.05.

Element	**Al**		**As**		**B**		**Ba**		**Ca**		**Co**		**Cr**	
Land use	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**
Mean	403.68	625.46	< LOD	0.55	22.02	11.90	1.47	1.47	6953.97	5957.26	< LOD	< LOD	< LOD	< LOD
S.E.	63.26	139.56	< LOD	0.33	3.63	4.60	0.58	0.27	346.57	249.22	< LOD	< LOD	< LOD	< LOD
Analysis	U(6)= 12; P= 0.343		N/A		t(3.755)= 1.685; P= 0.172		t(4.206)= 0.008; P= 0.994		t(6)= 2.335; P= 0.058		N/A		N/A	
Element	**Cu**		**Fe**		**K**		**Mg**		**Mn**		**Na**		**Ni**	
Land use	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**
Mean	1.25	0.66	121.97	195.48	1796.01	996.71	1724.86	1143.50	462.40	358	747.95	626.77	0.132	0.201
S.E.	0.44	0.09	23.88	31.34	814.16	221.32	224.63	96.65	168.03	140.59	34.45	94.01	0.132	0.067
Analysis	t(3.265)= 1.323; P= 0.271		t(6)= 1.865; P= 0.111		t(6)= 0.947; P= 0.38		t(6)= 2.377; P= 0.055		t(6)= 0.543; P= 0.607		U(6)= 4; P= 0.343		t(6)= 0.46; P= 0.662	
Element	**P**		**Pb**		**S**		**Si**		**Ti**		**V**		**Zn**	
Land use	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**
Mean	10.05	3.479	< LOD	< LOD	59.29	38.31	475.79	556.61	1.99	1.81	< LOD	7.46E-02	3.22	5.93
S.E.	5.633	1.65	< LOD	< LOD	6.44	2.06	90.66	174.95	0.80	0.64	< LOD	7.46E-02	1.33	3.21
Analysis	t(3.511)= 1.119; P= 0.334		N/A		t(6)= 3.103; P= 0.021		t(6)= 0.41; P= 0.696		t(6)= 0.181; P= 0.863		N/A		t(6)= 0.782; P= 0.464	

Appendix 39: Mean total CISED concentration (nmol g^{-1}) of each element (washes 1-4) and standard error (S.E.) of the mean within the margin region of *Miscanthus x giganteus* and cereal controls. α= 0.05.

Element	**Al**		**As**		**B**		**Ba**		**Ca**		**Co**		**Cr**	
Land use	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**
Mean	49906.4	48669.22	40.83	41.83	397.08	550.57	82.33	65.07	28165.49	19398.05	29.68	35.54	39.16	44.59
S.E.	11826.34	11652.04	9.86	8.85	64	101.65	18.23	19.21	7627.81	4975.46	11.31	14.13	7.77	10.59
Analysis	U(6)= 10; P= 0.686		t(6)= 0.076; P= 0.942		t(6)= 1.278; P= 0.249		t(6)= 0.652; P= 0.539		t(6)= 0.963; P= 0.373		U(6)= 9; P= 1		t(6)= 0.414; P= 0.693	
Element	**Cu**		**Fe**		**K**		**Mg**		**Mn**		**Na**		**Ni**	
Land use	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**
Mean	399.03	260.50	50554.79	55519.52	5480.51	3378.06	5430.61	4709.98	10217.73	9248.31	950.13	925.27	29.71	27.26
S.E.	320.78	146.73	6522.44	7422.45	2326.55	630.09	1558.05	1936.89	3578.43	3673.31	37.00	216.35	9.44	10.64
Analysis	t(6)= 0.029; P= 0.978		t(6)= 0.502; P= 0.633		t(6)= 0.872; P= 0.427		t(6)= 0.617; P= 0.56		t(6)= 0.189; P= 0.856		t(6)= 113; P= 0.914		t(6)= 0.39; P= 0.71	
Element	**P**		**Pb**		**S**		**Si**		**Ti**		**V**		**Zn**	
Land use	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**	**M**	**C**
Mean	981.04	799.95	55.25	55.05	405.71	300.45	7867.35	7637.10	226.95	251.25	75.69	78.29	325.33	214.39
S.E.	298.78	276.73	13.40	16.06	60.91	38.32	2012.72	2867.38	54.88	70.32	16.32	26.72	174.36	98.28
Analysis	t(6)= 0.62; P= 0.558		t(6)= 0.01; P= 0.993		t(6)=1.463; P= 0.194		t(6)= 0.066; P= 0.95		t(6)= 0.272; P= 0.794		t(6)= 0.128; P= 0.903		t(6)= 0.554; P= 0.599	

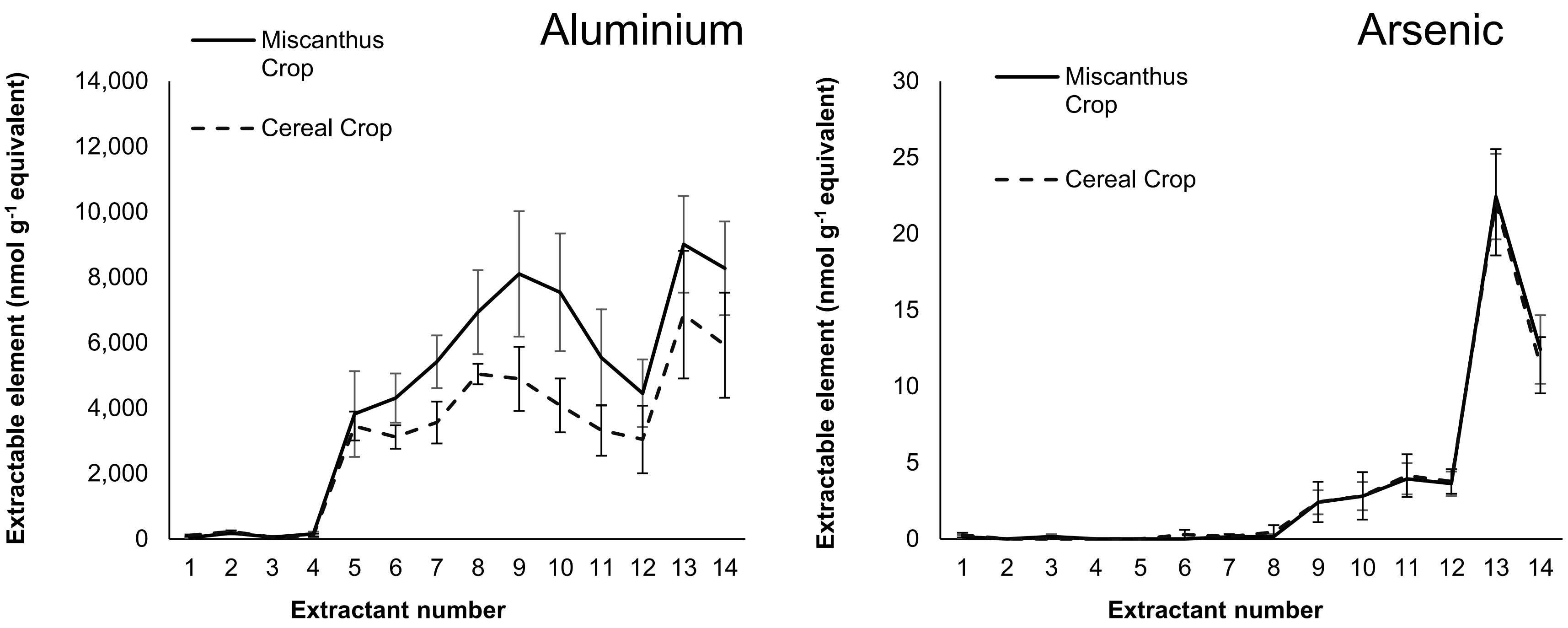
Appendix 40: Mean ‘most bioaccessible’ CISED concentration (nmol g^{-1}) of each element (washes 1-4) and standard error (S.E.) of the mean within the margin region of willow short-rotation coppice and cereal controls. LOD = Limit Of Detection. α= 0.05.

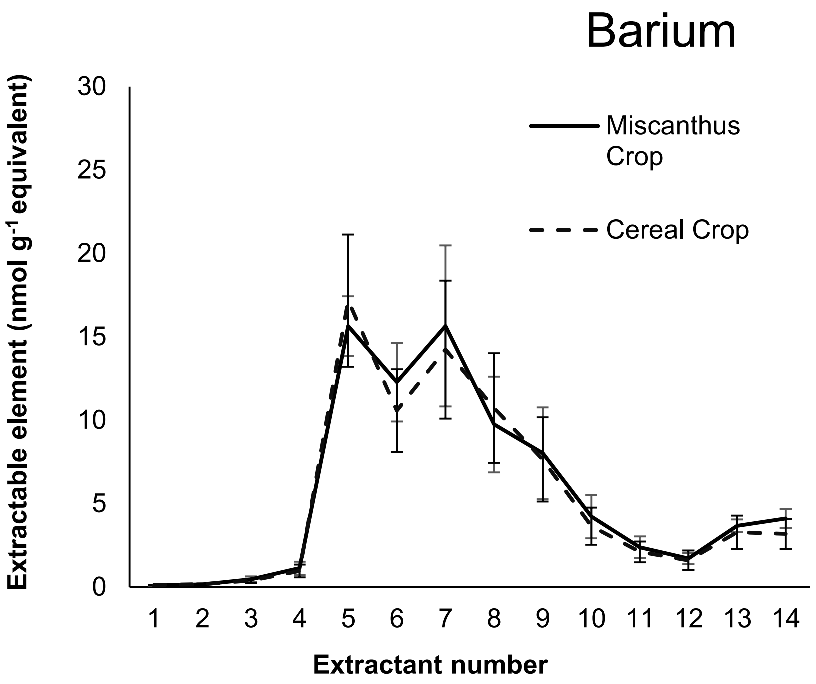
Element	**Al**		**As**		**B**		**Ba**		**Ca**		**Co**		**Cr**	
Land use	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**
Mean	1406.99	1017.68	< LOD	3.63E-01	32.41	20.15	5.14	6.05	8240.56	6846.65	< LOD	< LOD	0.87	0.43
S.E.	657.74	380.27	< LOD	2.13E-01	14.55	8.89	1.67	0.29	1176.95	1664.09	< LOD	< LOD	0.56	0.43
Analysis	t(6)= 0.512; P= 0.627		N/A		U(6)= 5; P= 0.486		t(3.187)= 0.541; P= 0.624		t(6)= 0.684; P= 0.52		N/A		t(6)= 0.631; P= 0.551	
Element	**Cu**		**Fe**		**K**		**Mg**		**Mn**		**Na**		**Ni**	
Land use	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**
Mean	1.79	0.72	515.49	276.98	1409.22	1473.70	2815.66	1861.09	86.11	126.31	350.10	246.97	0.58	0.74
S.E.	0.79	0.24	227.14	97.57	177.22	419.16	1069.75	775.49	27.75	78.30	212.23	88.26	0.24	0.15
Analysis	t(3.55)= 1.296; P= 0.273		t(4.071)= 0.965; P= 0.388		t(6)= 0.142; P= 0.892		t(6)= 0.761; P= 0.476		t(6)= 0.069; P= 0.947		t(6)= 0.144; P= 0.89		t(6)= 0.647; P= 0.542	
Element	**P**		**Pb**		**S**		**Si**		**Ti**		**V**		**Zn**	
Land use	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**
Mean	21.69	27.80	< LOD	< LOD	104.09	37.51	2926.59	1615.81	12.39	7.40	1.23	0.68	3.78	4.67
S.E.	9.35	12.81	< LOD	< LOD	64.15	12.03	1555.69	805.62	6.32	3.78	0.72	0.47	1.57	1.77
Analysis	U(6)= 10; P= 686		N/A		t(6)= 0.973; P= 0.368		t(6)= 0.748; P= 0.483		U(6)= 6; P= 0.686		t(6)= 0.637; P= 0.548		t(6)= 0.377; P= 0.719	

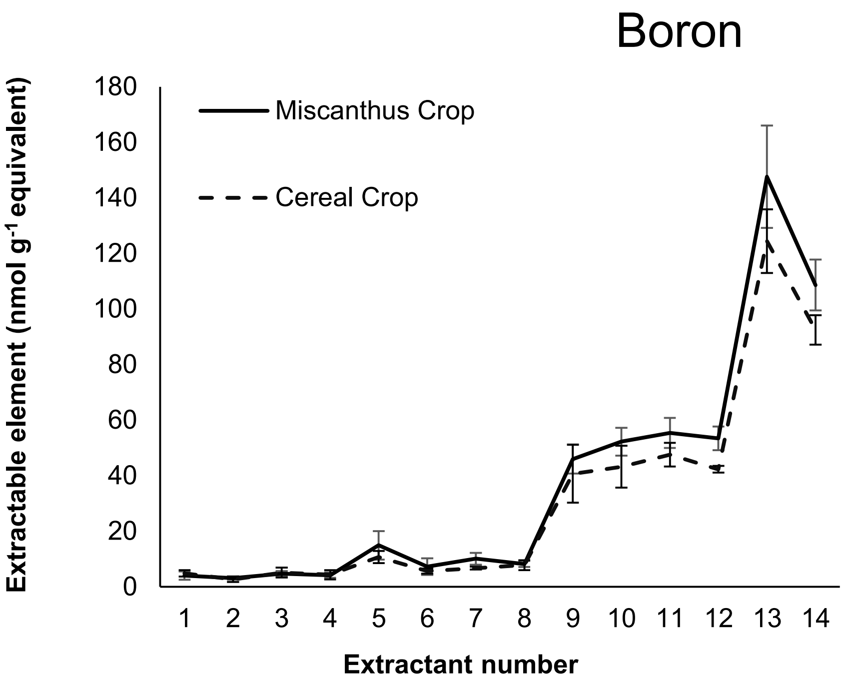
Appendix 41: Mean total CISED concentration (nmol g^{-1}) of each element (washes 1-4) and standard error (S.E.) of the mean within the margin region of willow short-rotation coppice and cereal controls. α= 0.05.

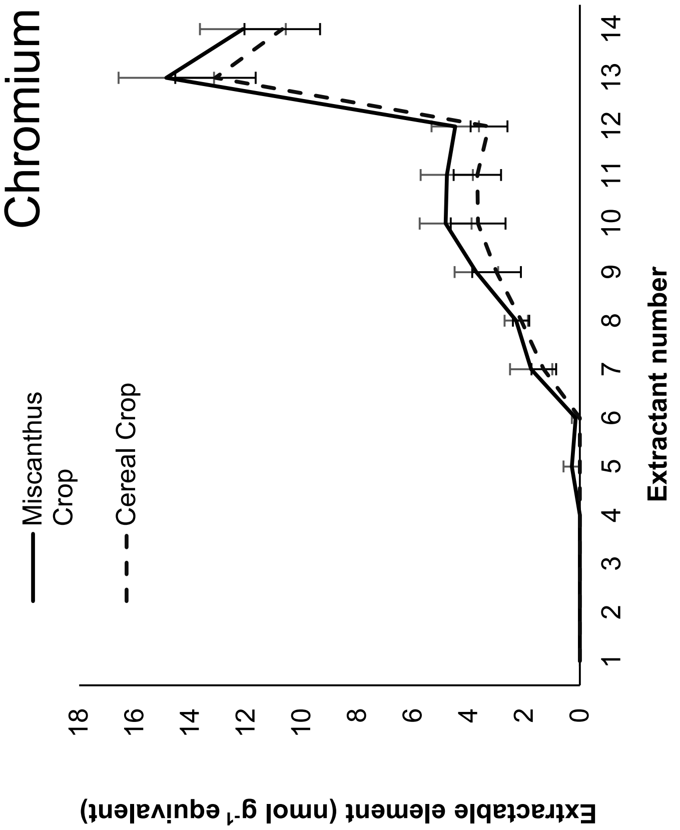
Element	**Al**		**As**		**B**		**Ba**		**Ca**		**Co**		**Cr**	
Land use	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**
Mean	30756.22	20652.19	25.23	17.84	215.85	184.28	320.72	245.1	80541.30	88172.60	10.34	11.42	5.16	3.04
S.E.	6543.63	4723.33	10.70	7.60	98.27	92.59	123.8	95.88	32044.41	66158.18	6.58	10.26	5.16	3.04
Analysis	t(6)= 1.228; P= 0.266		t(6)= 0.585; P= 0.58		t(6)= 0.436; P= 0.678		t(6)= 0.483; P= 0.646		t(6)= 0.413; P= 0.694		t(6)= 0.34; P= 0.746		t(6)= 0.981; P= 0.364	
Element	**Cu**		**Fe**		**K**		**Mg**		**Mn**		**Na**		**Ni**	
Land use	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**
Mean	159.87	182.73	37019.33	23968.99	17479.26	12057.36	15763.07	27152.29	3808.34	3011.11	1147.68	1099.66	42.48	30.48
S.E.	56.81	111.21	16254.65	10578.87	6941.53	3911.37	6262.41	17053.54	1209.59	1144.46	630.08	679.61	16.56	12.03
Analysis	t(6)= 0.077; P= 0.941		t(6)= 0.709; P= 0.505		t(6)= 0.709; P= 0.505		t(6)= 0.627; P= 0.554		t(6)= 0.479; P= 0.649		t(6)= 0.114; P= 0.913		t(6)= 0.114; P= 0.913	
Element	**P**		**Pb**		**S**		**Si**		**Ti**		**V**		**Zn**	
Land use	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**	**W**	**C**
Mean	994.28	670.88	72.16	58.81	1019.04	471.7	14049.18	10195.20	295.48	165.11	93.81	66.67	179.35	210.30
S.E.	303.46	205.11	37.77	31.01	401.84	156.67	4310.73	3415.85	112.35	46.19	34.81	25.26	65.30	57.30
Analysis	t(6)= 0.883; P= 0.411		U(6)= 6; P= 0.686		t(3.891)= 1.269; P= 0.275		t(6)= 0.701; P= 0.51		t(6)= 1.073; P= 0.324		t(6)= 0.631; P= 0.551		t(6)= 0.356; P= 0.734	

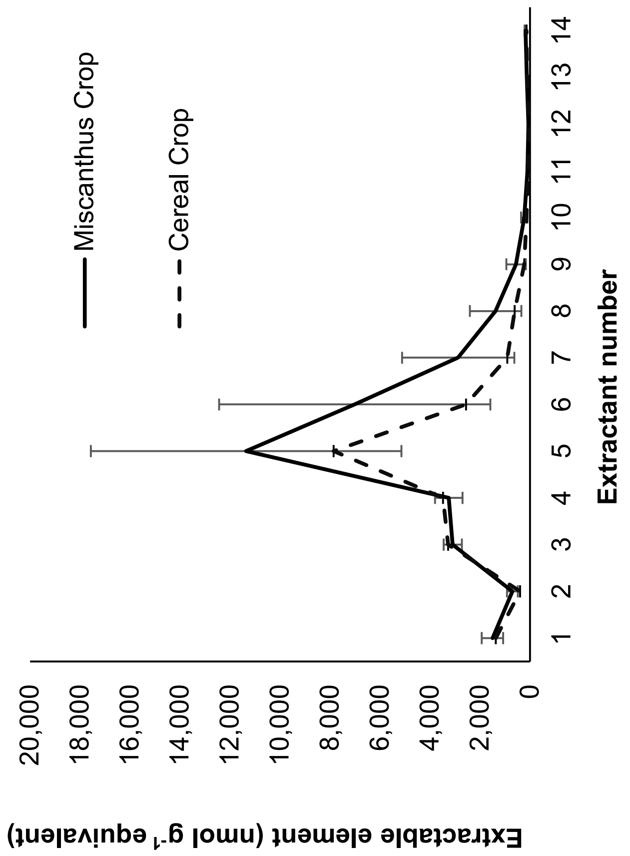
Appendix 42: Mean site total CISED concentrations (washes 1-14) in the cropped region of *Miscanthus x giganteus* and cereal control fields in southwest England for each of 21 analysed elements.

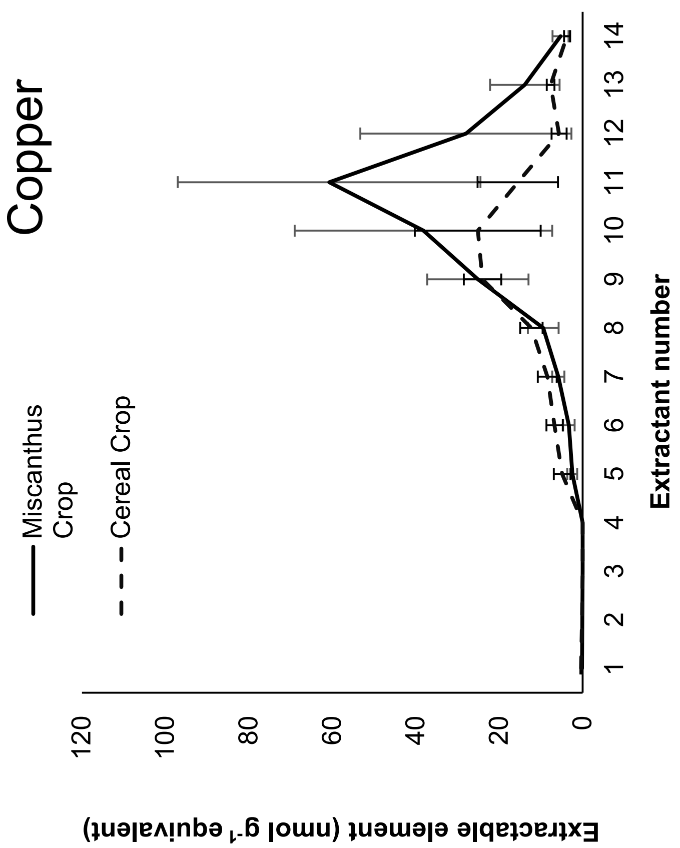


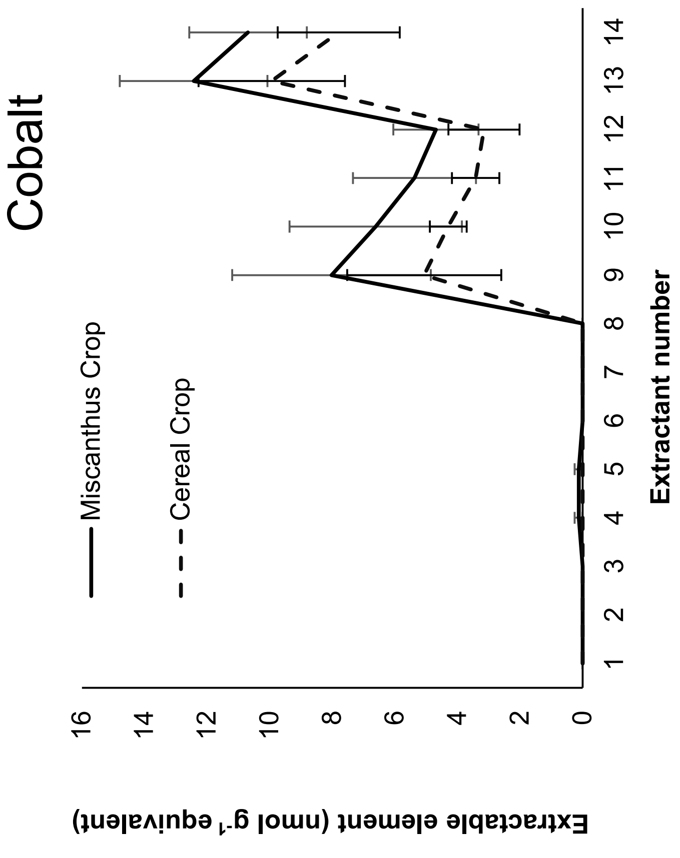

Barium
Extractable element (nmol g-1 equivalent)
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number
Miscanthus Crop
Cereal Crop

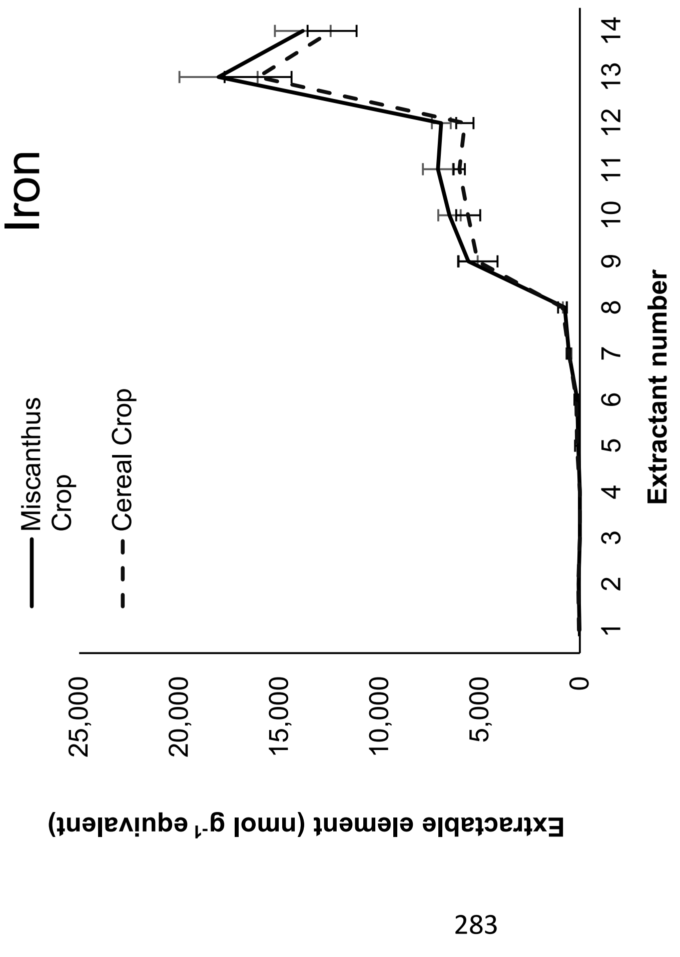

Boron
Extractable element (nmol g-1 equivalent)
180
160
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number
Miscanthus Crop
Cereal Crop

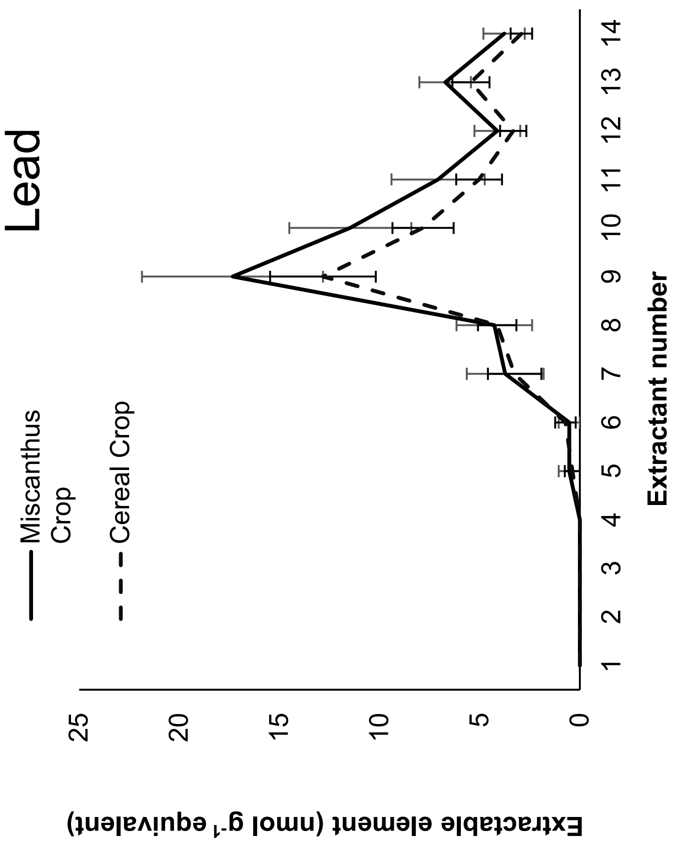

Chromium
Miscanthus Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
18
16
14
12
10
8
6
4
2
0
Extractant number
1
2
3
4
5
6
7
8
9
10
11
12
13
14

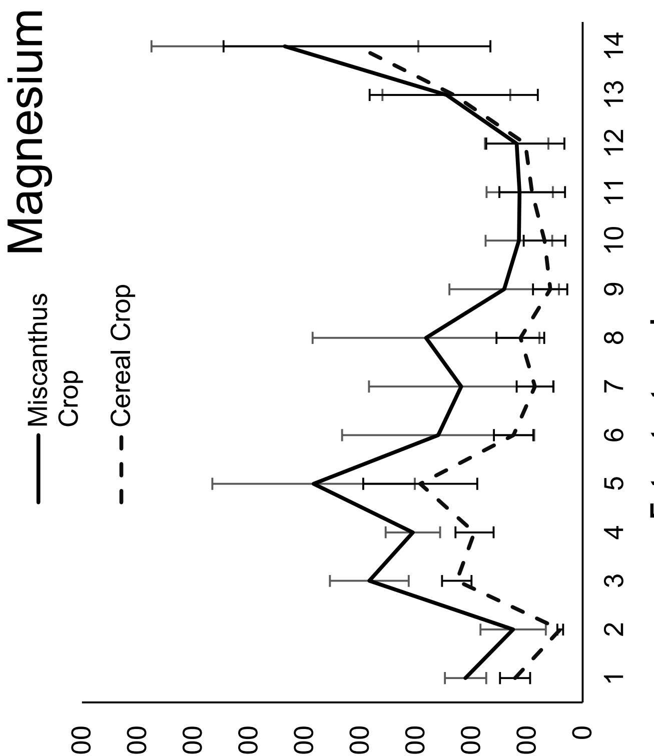

Calcium
Miscanthus Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
20,000
18,000
16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
Extractant number
1
2
3
4
5
6
7
8
9
10
11
12
13
14

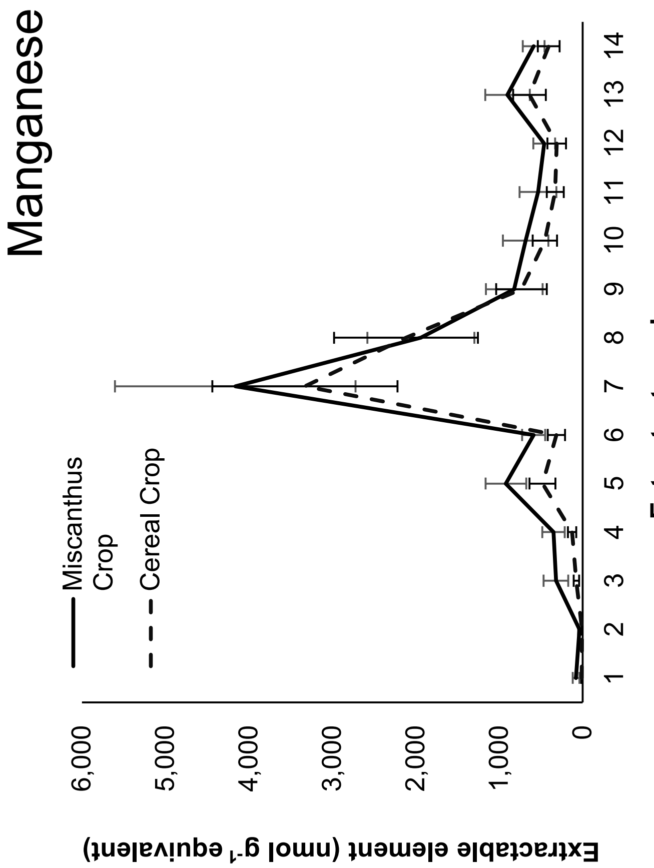

Copper
Miscanthus Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
0
20
40
60
80
100
120
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

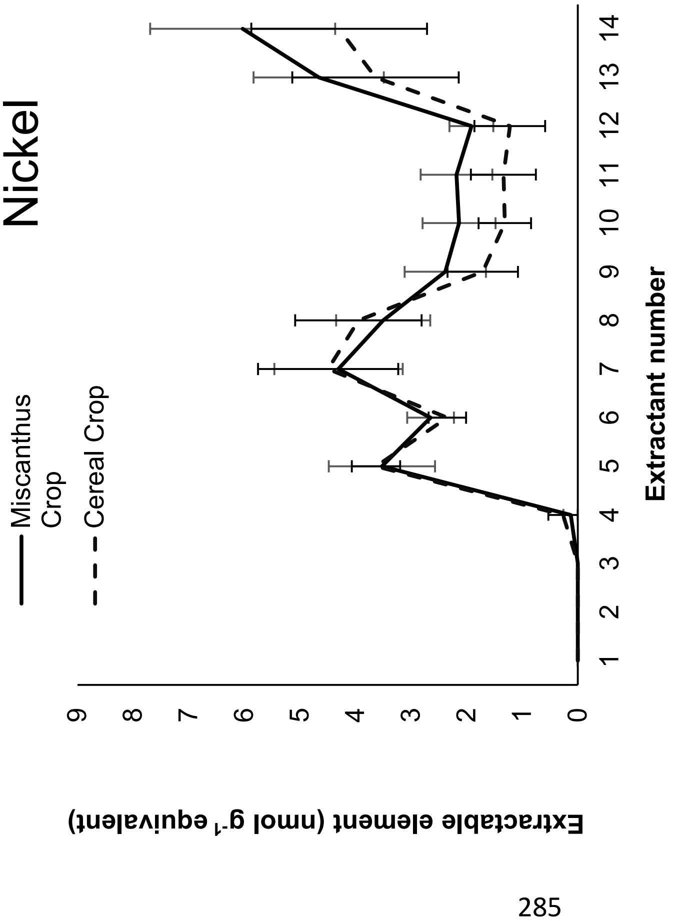

Cobalt
Miscanthus Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
0
2
4
6
8
10
12
14
16
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

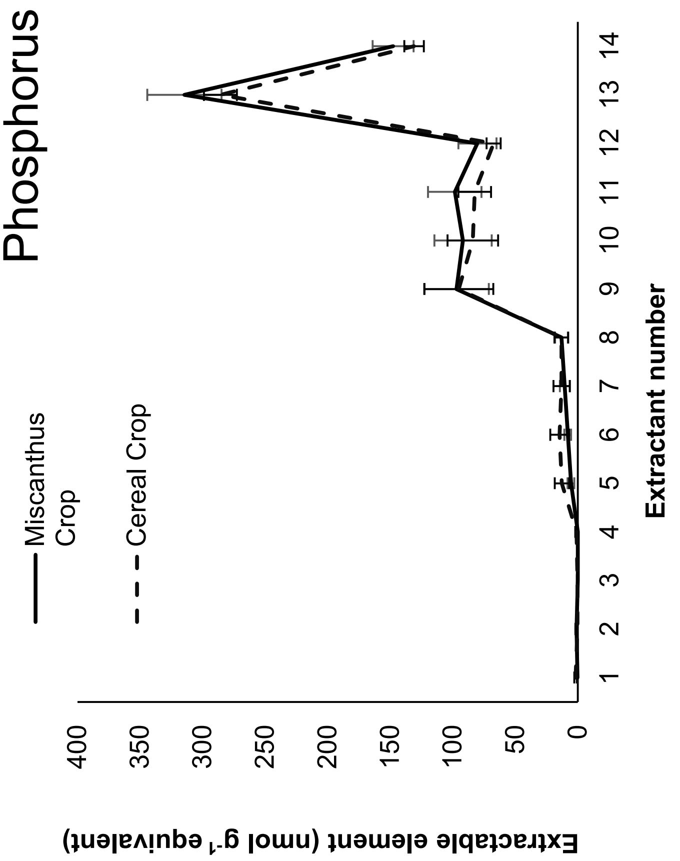

Iron
Miscanthus Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
25,000
20,000
15,000
10,000
5,000
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

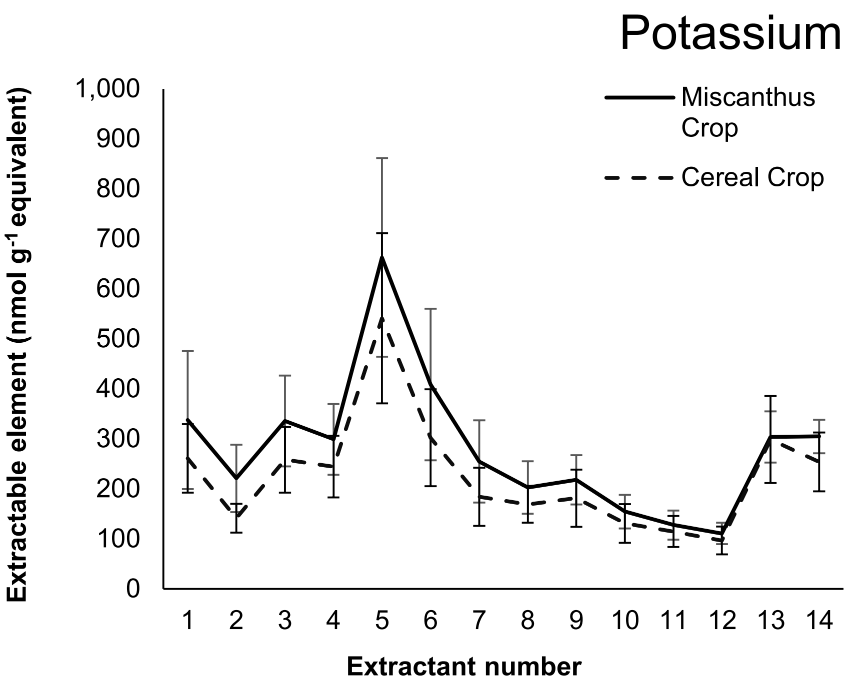

Lead
Miscanthus Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

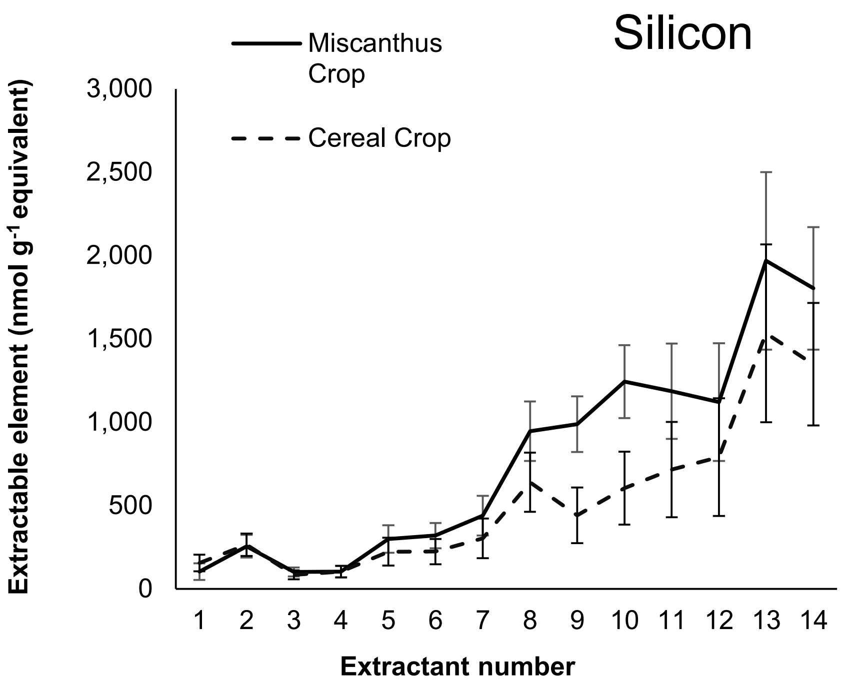

Magnesium

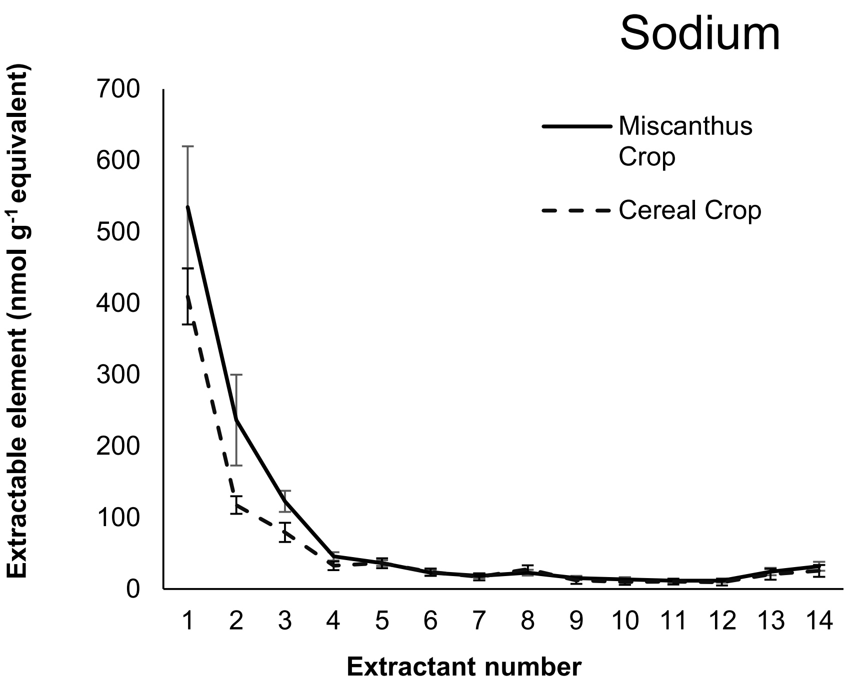

Manganese

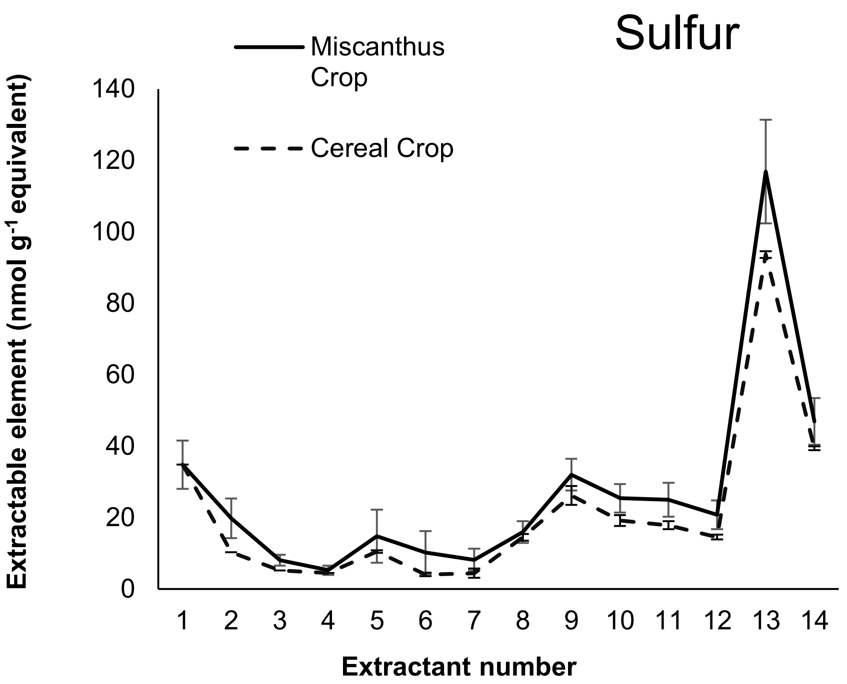

Nickel
Miscanthus Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
0
1
2
3
4
5
6
7
8
9
Extractant number
1
2
3
4
5
6
7
8
9
10
11
12
13
14

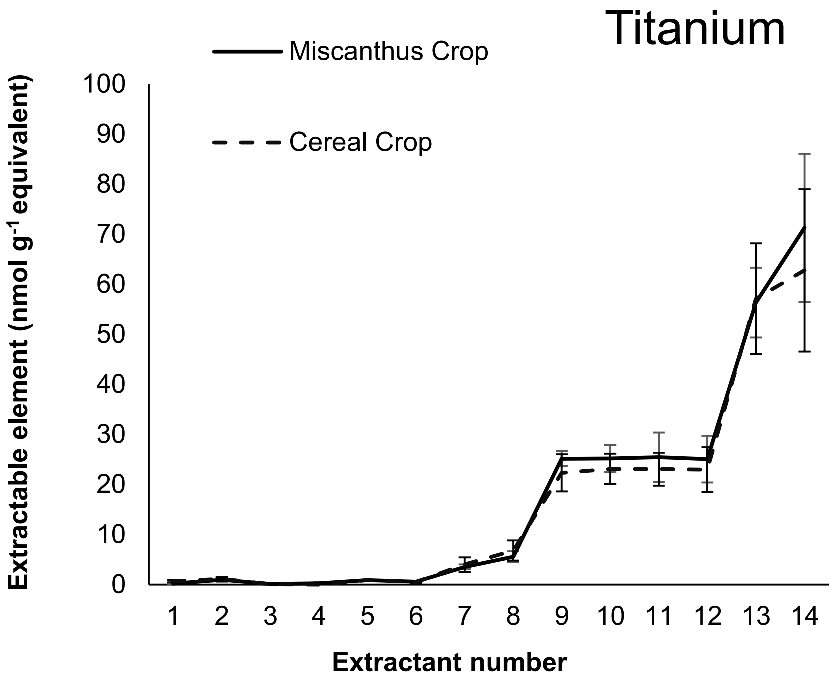

Phosphorus
Miscanthus Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
0
50
100
150
200
250
300
350
400
Extractant number
1
2
3
4
5
6
7
8
9
10
11
12
13
14

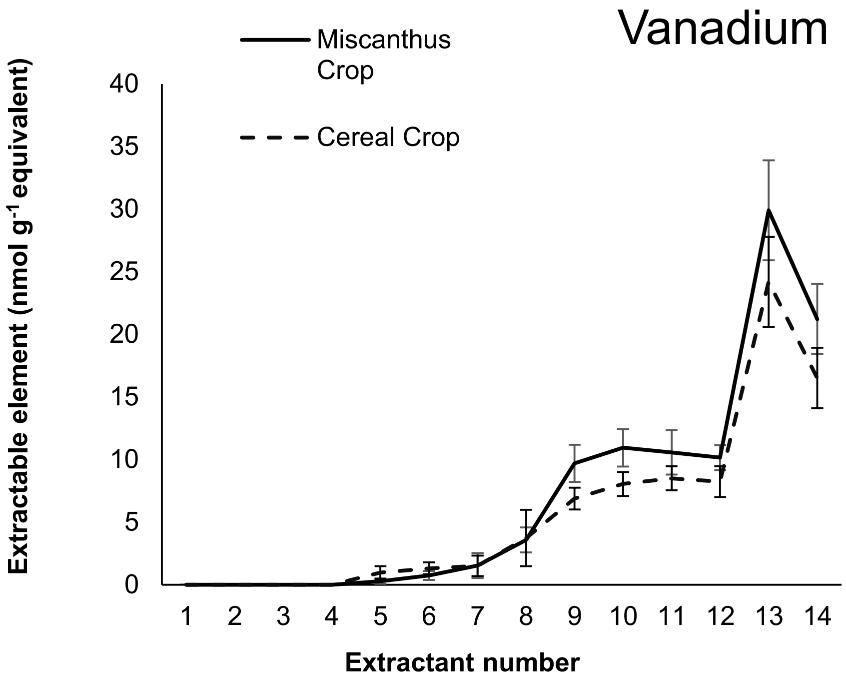

Potassium
Miscanthus Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
1,000
900
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

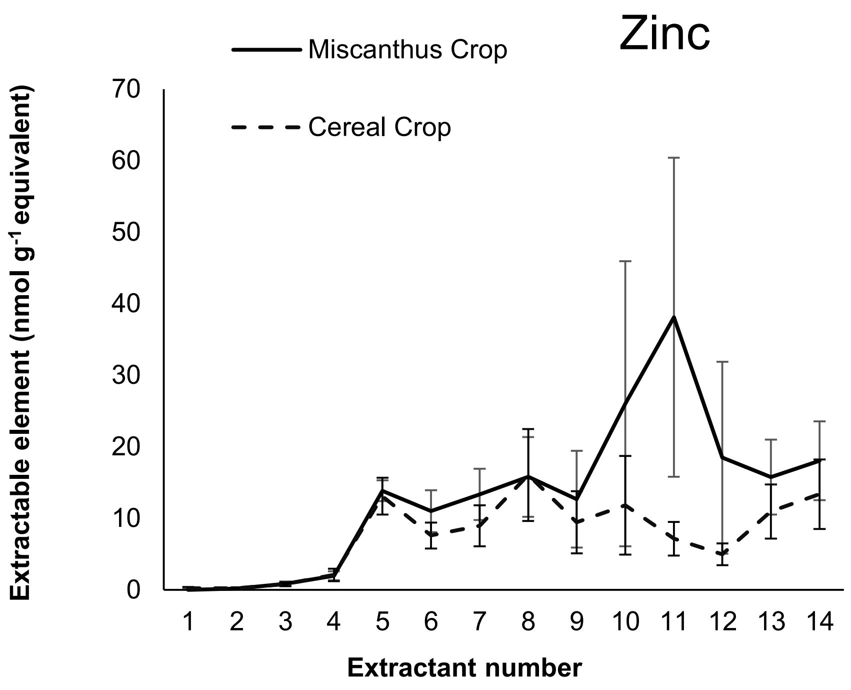

Silicon
Miscanthus Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
3,000
2,500
2,000
1,500
1,000
500
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number


Sodium
Extractable element (nmol g-1 equivalent)
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number
Miscanthus Crop
Cereal Crop

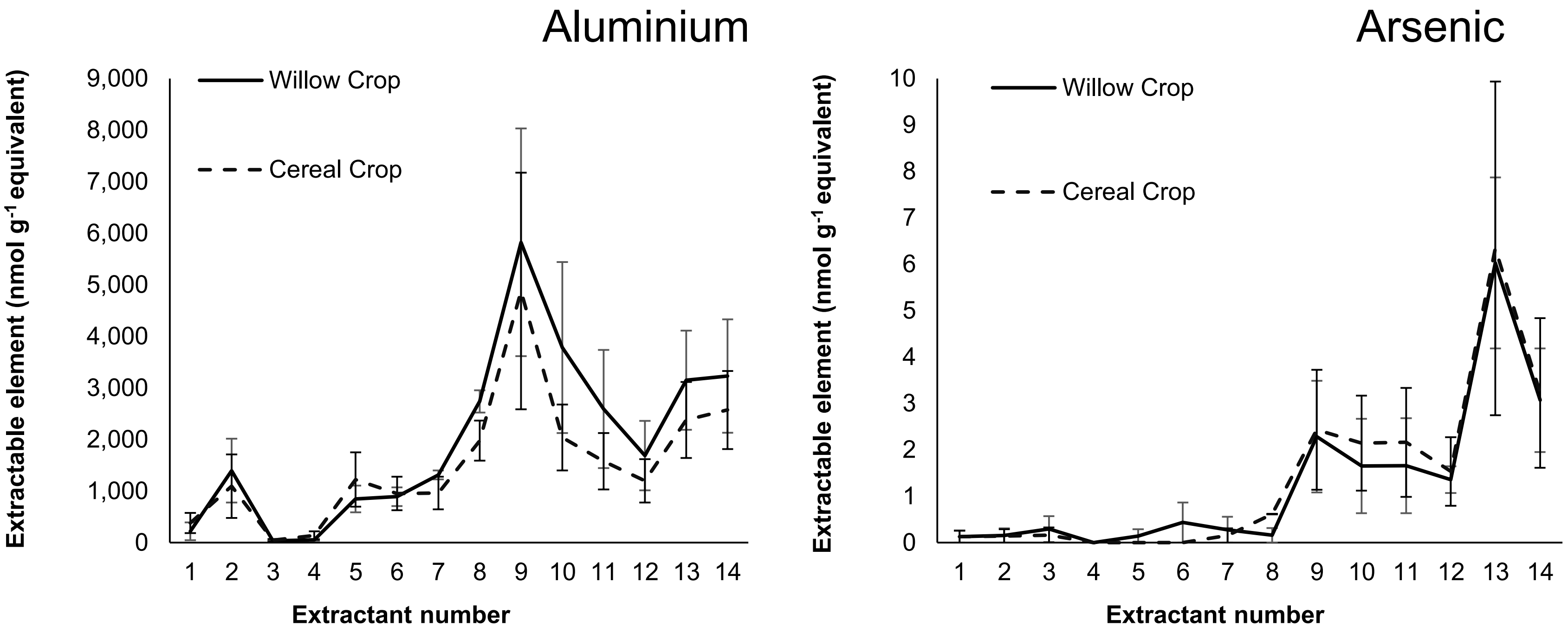

Sulfur
Extractable element (nmol g-1 equivalent)
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number
Miscanthus Crop
Cereal Crop

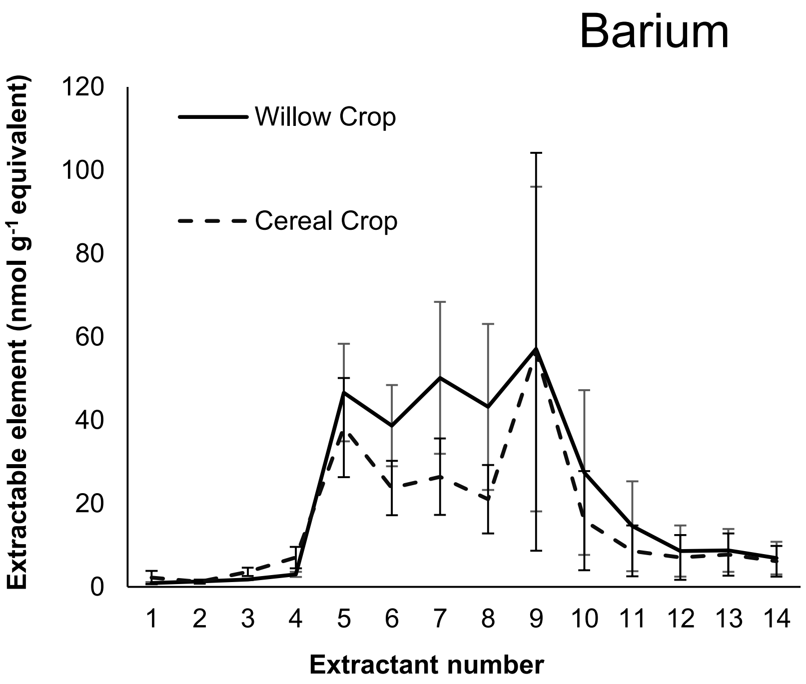

Titanium
Miscanthus Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

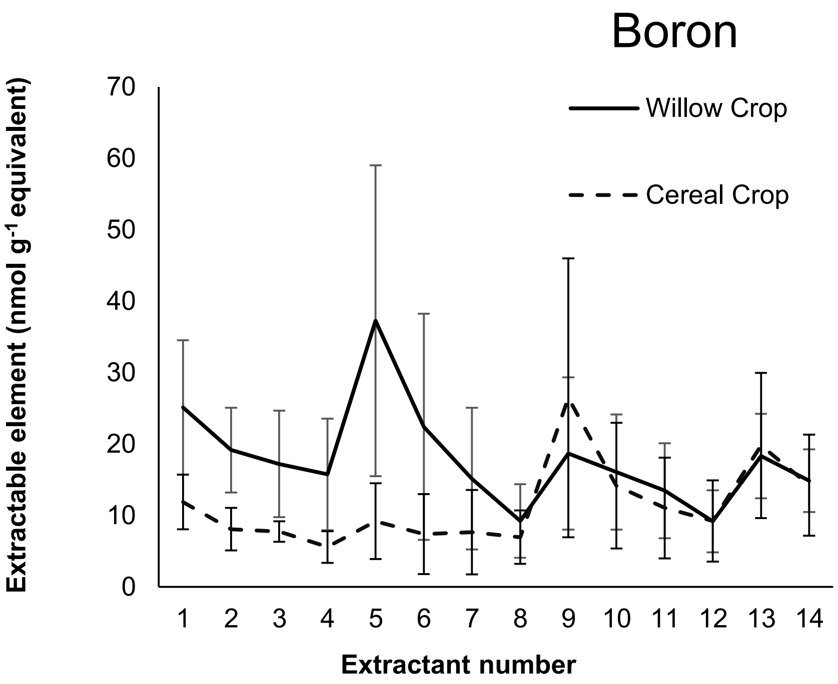

Vanadium
Miscanthus Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
40
35
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

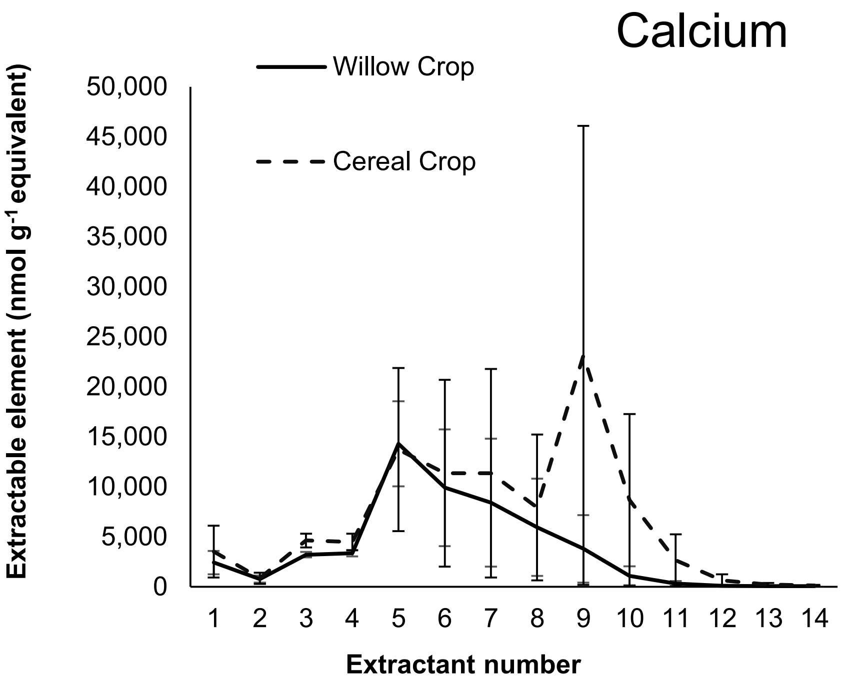

Zinc
Miscanthus Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

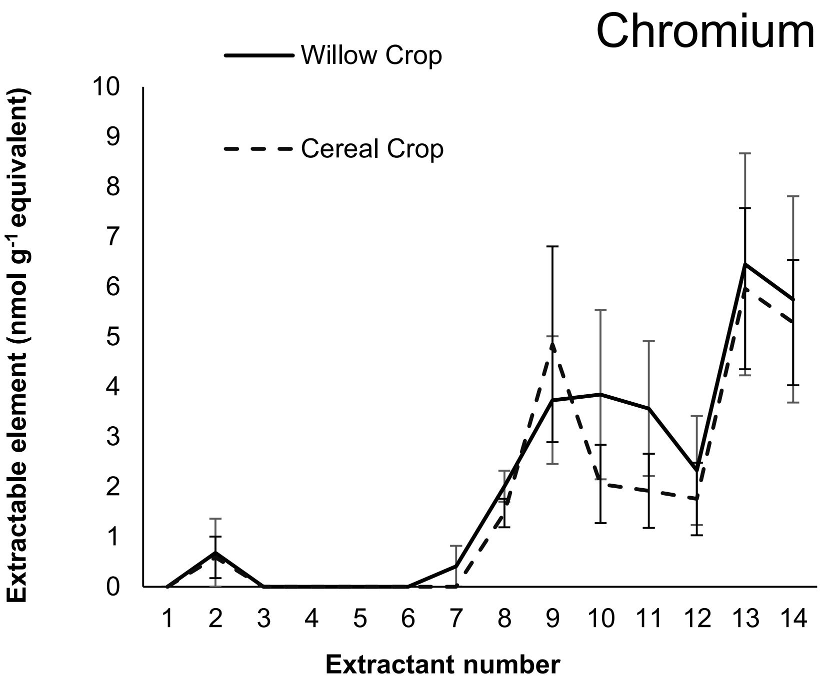
Appendix 43: Mean site total CISED concentrations (washes 1-14) in the cropped region of willow short-rotation coppice and cereal control fields in central England for each of 21 analysed elements.

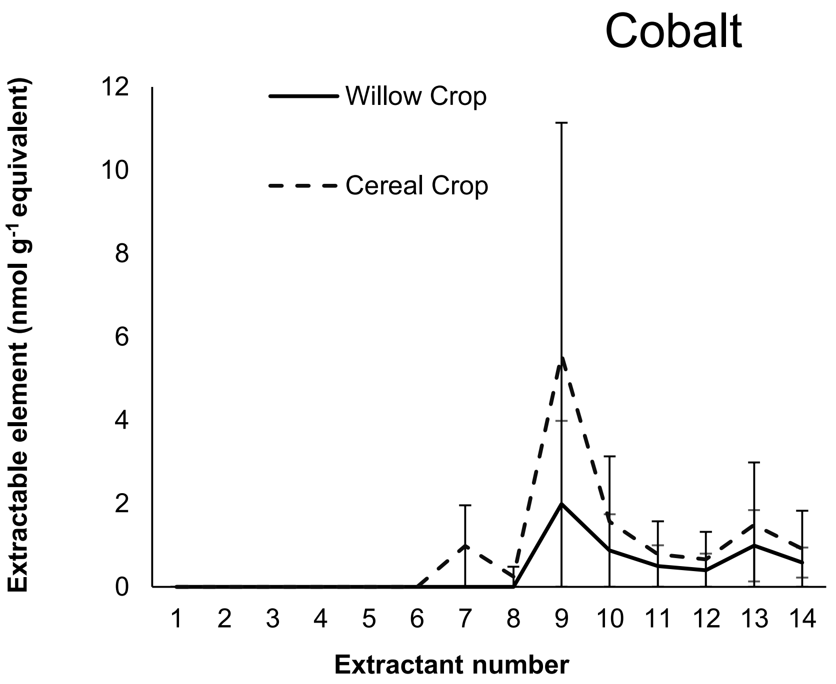


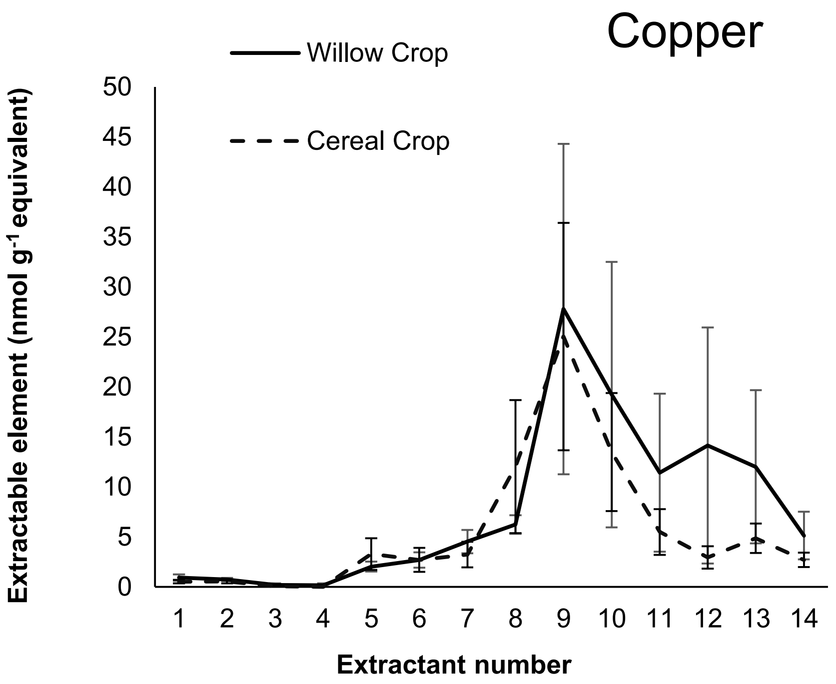

Barium
Willow Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
0
20
40
60
80
100
120
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

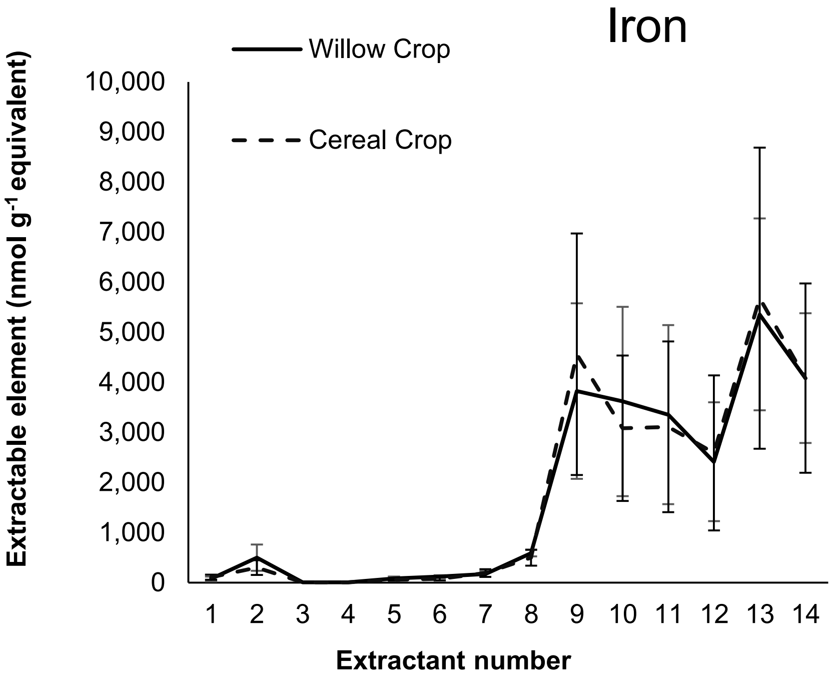

Boron
Willow Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
0
10
20
30
40
50
60
70
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

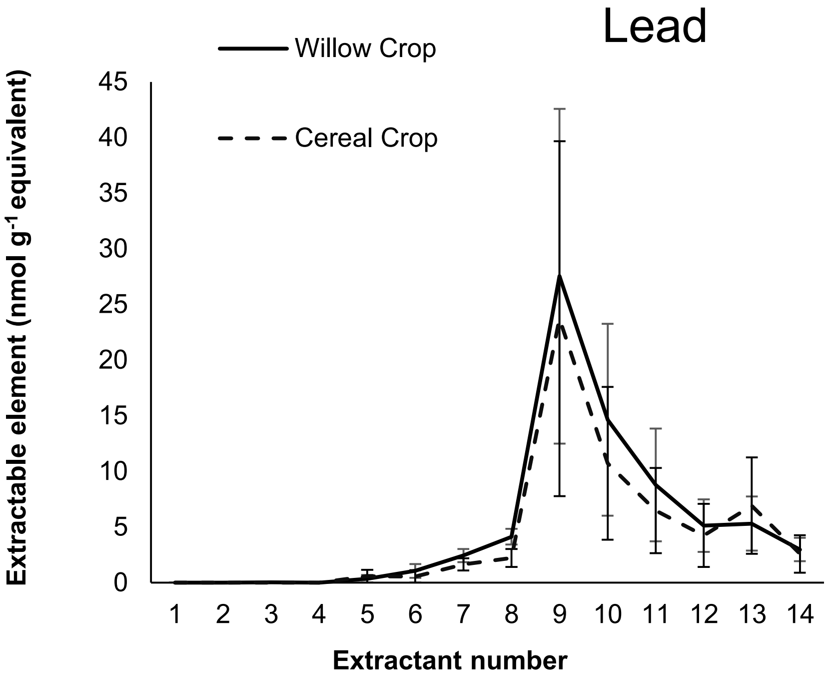

Calcium
Willow Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
50,000
45,000
40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

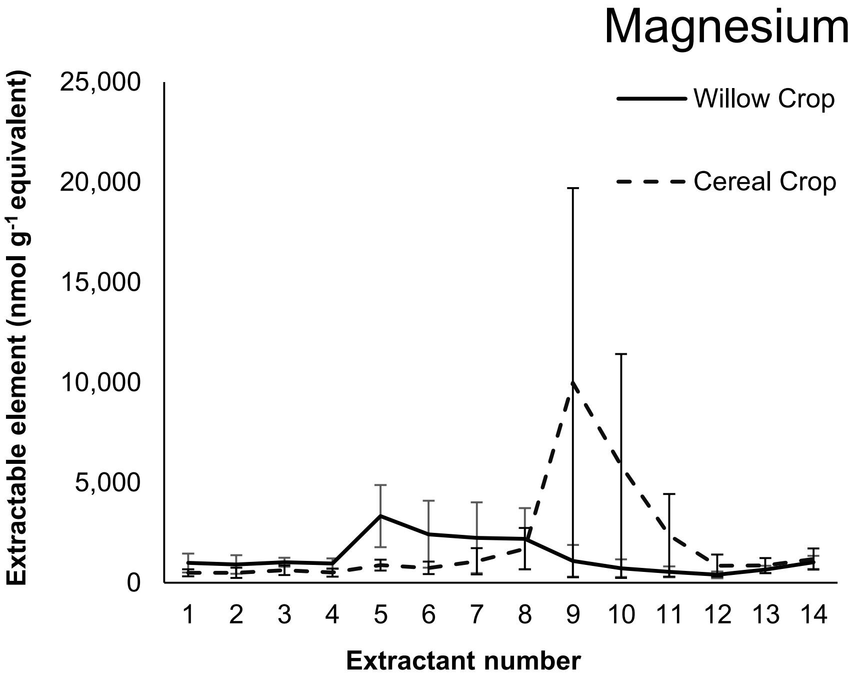

Chromium
Willow Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
10
9
8
7
6
5
4
3
2
1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

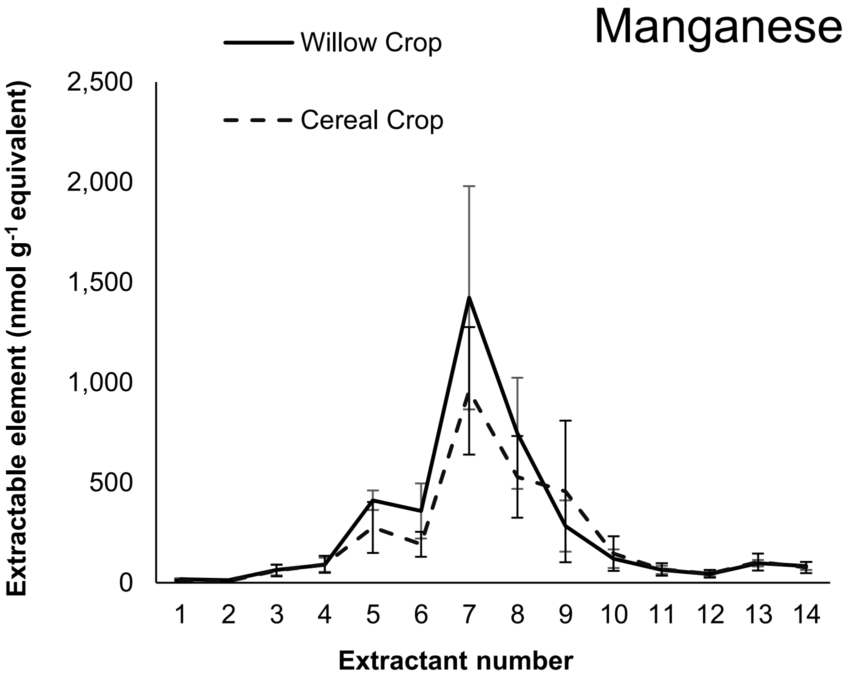

Cobalt
Willow Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
0
2
4
6
8
10
12
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Extractant number

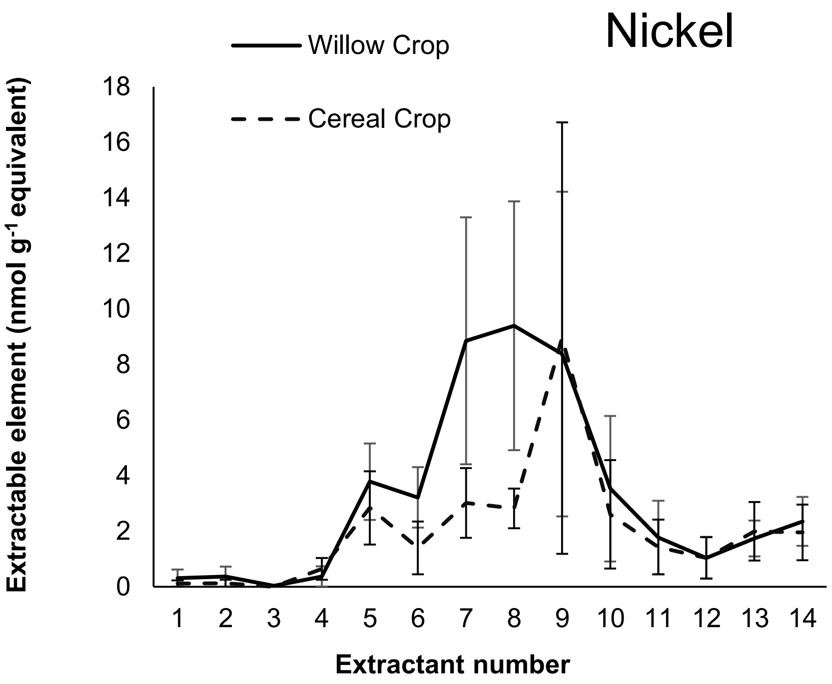

Copper
Willow Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
0
5
10
15
20
25
30
35
40
45
50
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Extractant number

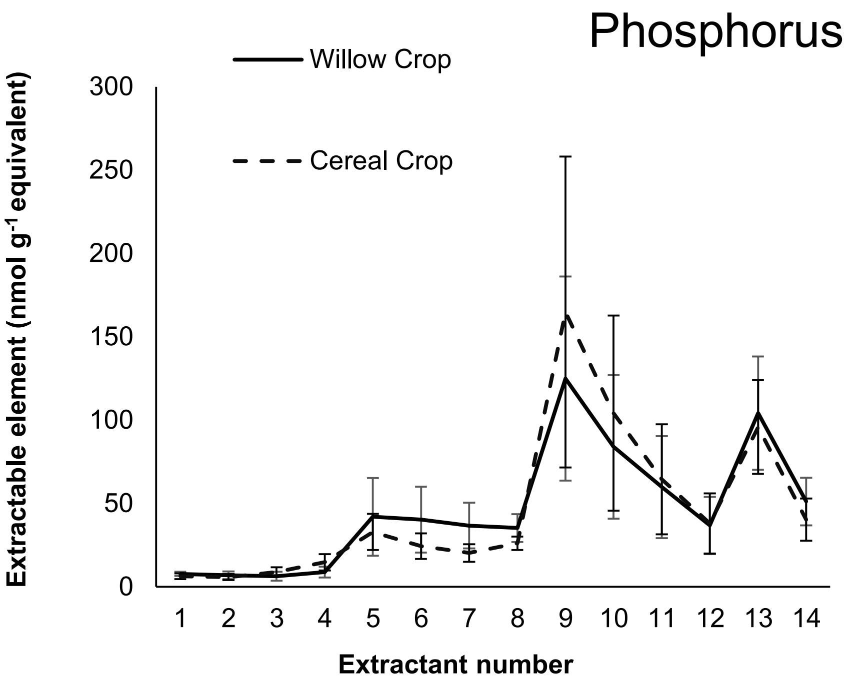

Iron
Willow Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

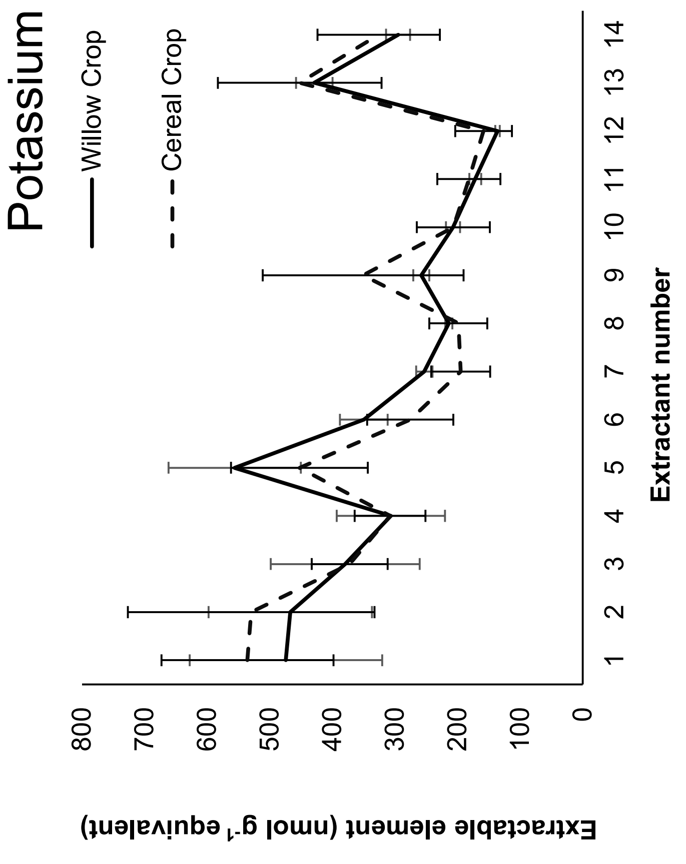

Lead
Willow Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
45
40
35
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

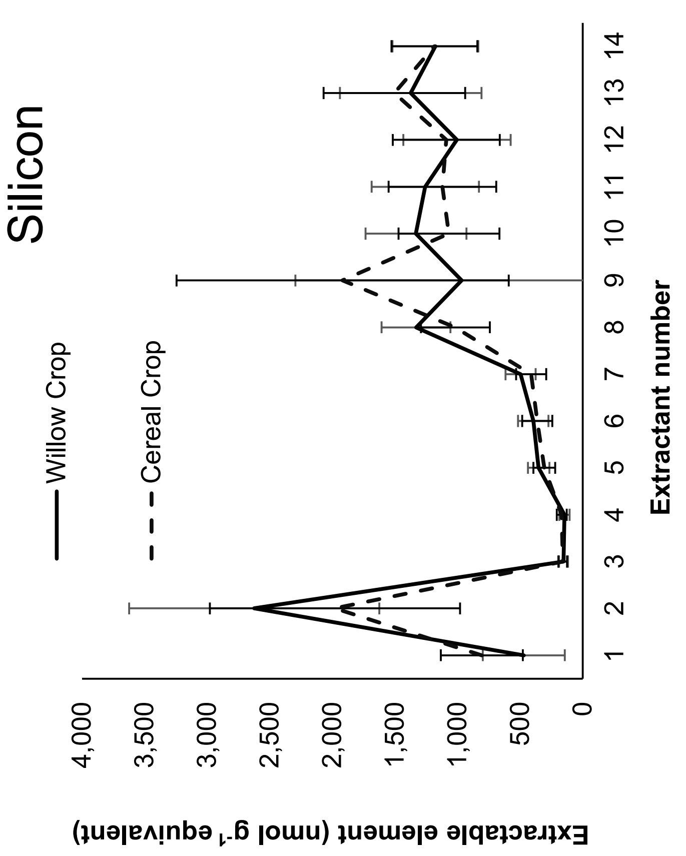

Magnesium
Willow Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
25,000
20,000
15,000
10,000
5,000
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

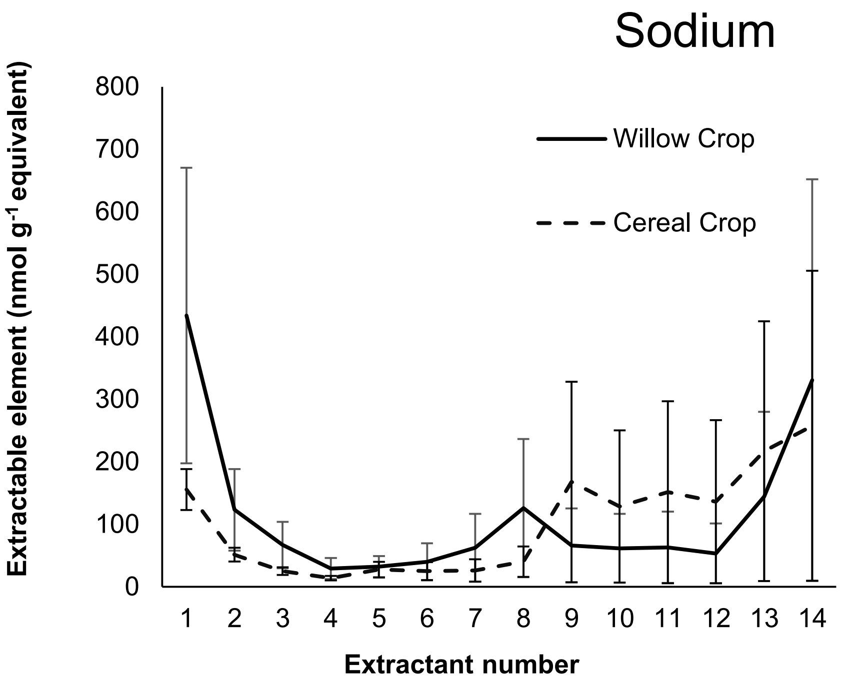

Manganese
Willow Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
2,500
2,000
1,500
1,000
500
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

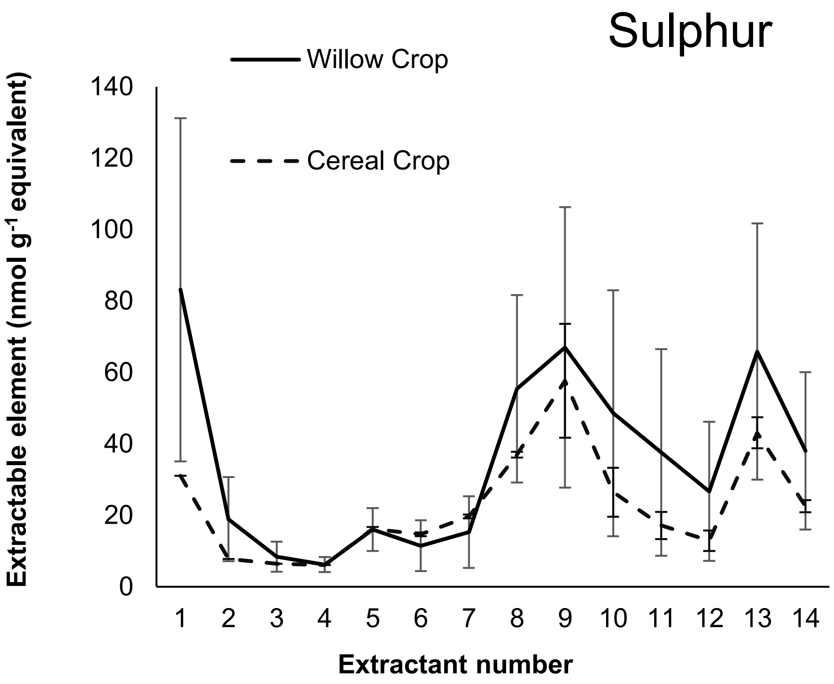

Nickel
Willow Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
0
2
4
6
8
10
12
14
16
18
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

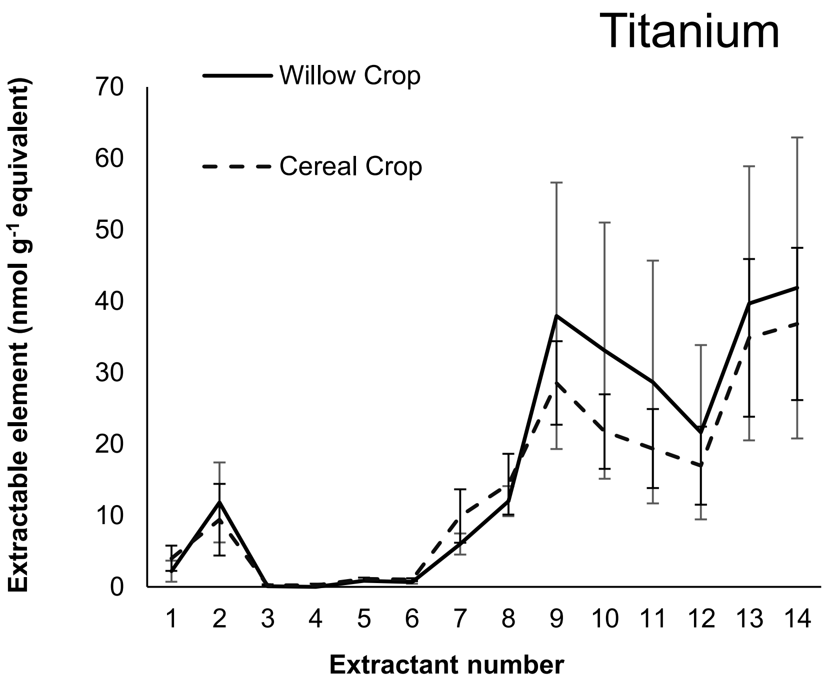

Phosphorus
Willow Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
0
50
100
150
200
250
300
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

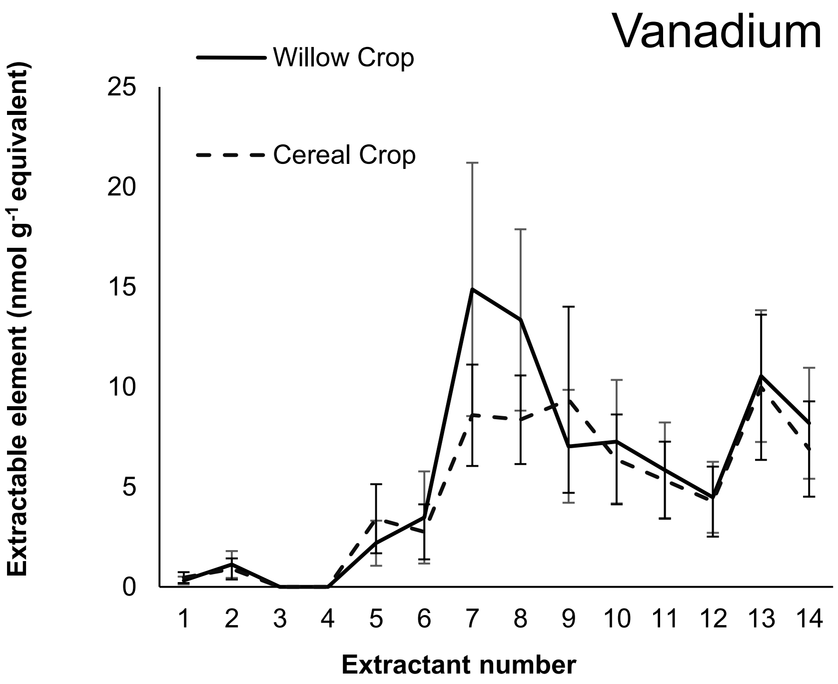

Potassium
Willow Crop
Cereal Crop
Extractable element (nmol g⁻¹ equivalent)
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

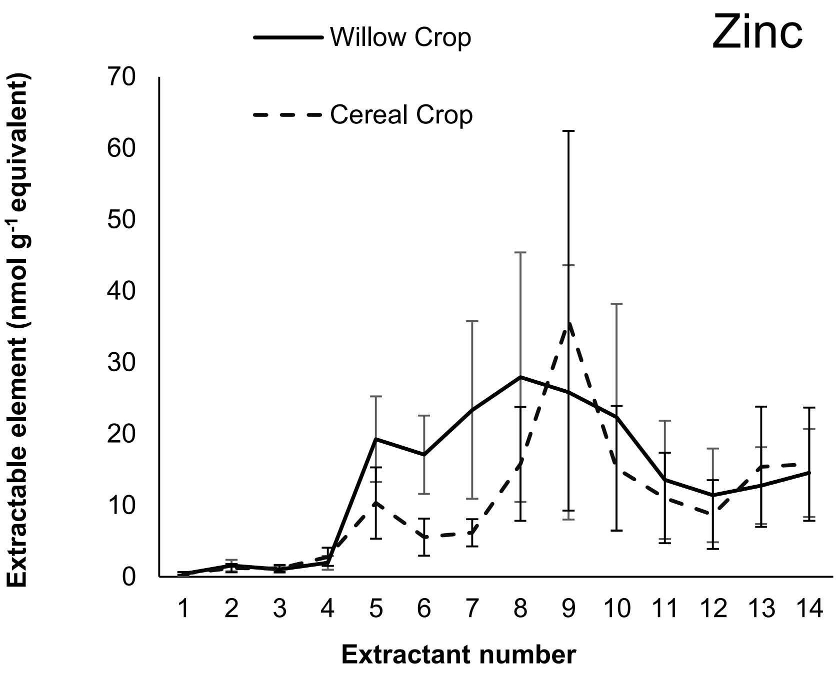

Silicon
Willow Crop
Cereal Crop
Extractable element (nmol g⁻¹ equivalent)
4,000
3,500
3,000
2,500
2,000
1,500
1,000
500
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number


Sodium
Willow Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number


Sulphur
Willow Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number


Titanium
Willow Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
0
10
20
30
40
50
60
70
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

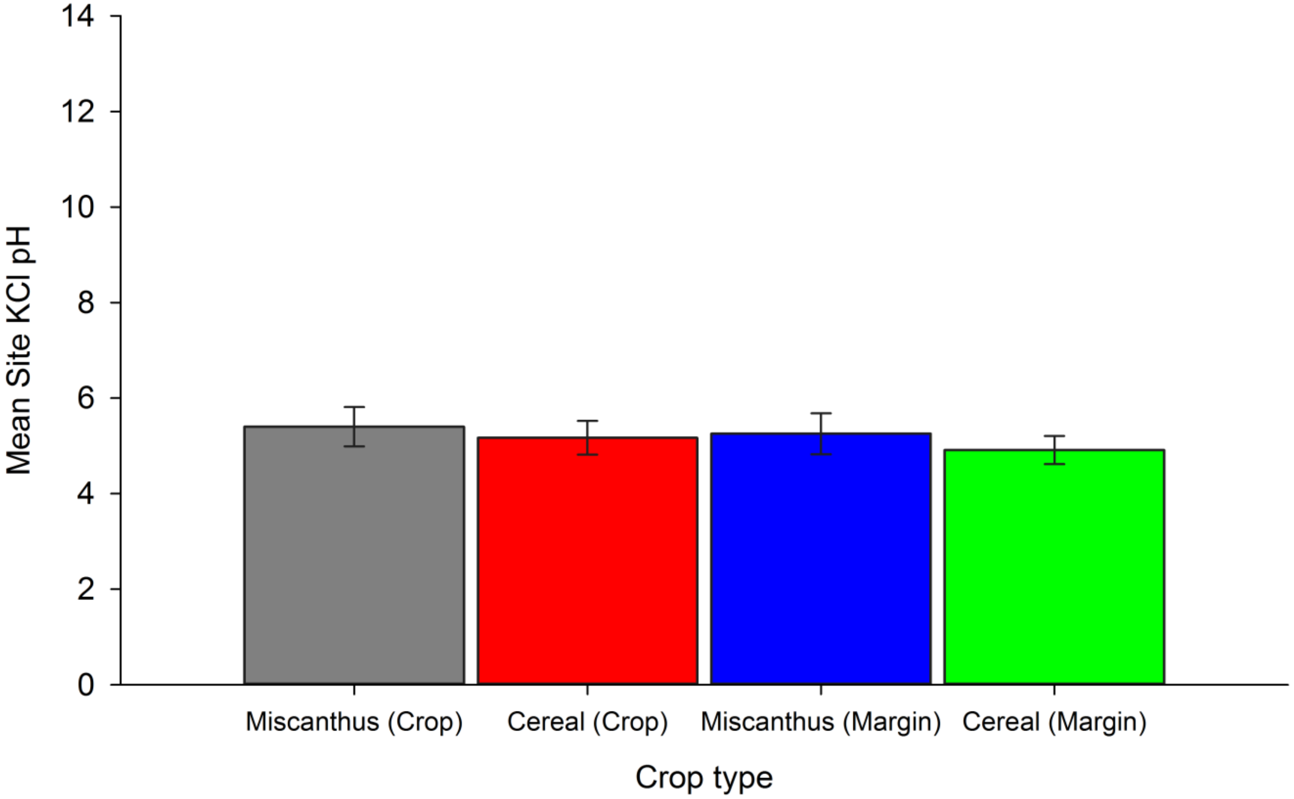

Vanadium
Willow Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
0
5
10
15
20
25
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number


Zinc
Willow Crop
Cereal Crop
Extractable element (nmol g-1 equivalent)
0
10
20
30
40
50
60
70
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Extractant number

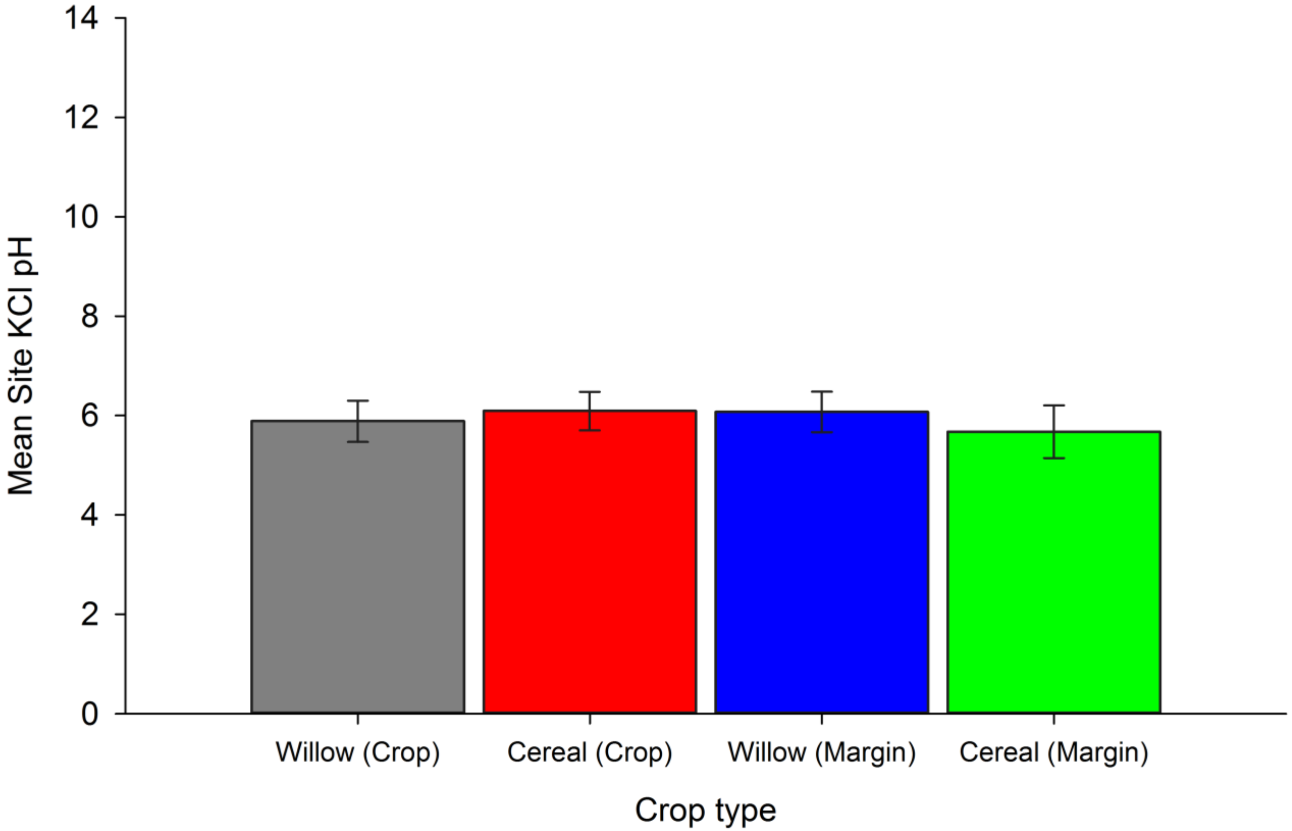
Appendix 44: Salt (1:2.5 KCl) and international ISO standard water (1:5) pH of soil samples in bioenergy crop (*Miscanthus x giganteus* or willow short-rotation coppice) and cereal controls (wheat (*Triticum* spp.) or barley (*Hordeum vulgare*) in cropped and margin regions of each field.

Site	**Crop**	**Field Position**	**Salt pH**	**H_2O pH**
Lostwithiel	Miscanthus	Crop	4.51	5.63
Pelynt	Miscanthus	Crop	5.61	6.72
Wadebridge	Miscanthus	Crop	6.43	7.41
St Minver	Miscanthus	Crop	5.05	6.19
Lostwithiel	Miscanthus	Margin	4.26	5.34
Pelynt	Miscanthus	Margin	4.83	5.97
Wadebridge	Miscanthus	Margin	6.1	7.14
St Minver	Miscanthus	Margin	5.82	6.91
Lostwithiel	Cereal	Crop	4.27	5.35
Pelynt	Cereal	Crop	5.18	6.32
Wadebridge	Cereal	Crop	5.22	6.36
St Minver	Cereal	Crop	6	7.06
Lostwithiel	Cereal	Margin	4.08	5.12
Pelynt	Cereal	Margin	4.96	6.10
Wadebridge	Cereal	Margin	5.17	6.31
St Minver	Cereal	Margin	5.44	6.57
Newark	Willow	Crop	6.14	7.18
Gainsborough	Willow	Crop	6.77	7.67
Stapleford	Willow	Crop	4.77	5.91
Whatton	Willow	Crop	5.86	6.94
Newark	Willow	Margin	5.86	6.94
Gainsborough	Willow	Margin	6.41	7.39
Stapleford	Willow	Margin	5.05	6.19
Whatton	Willow	Margin	6.97	7.81
Newark	Cereal	Crop	5.36	6.49
Gainsborough	Cereal	Crop	7.17	7.96
Stapleford	Cereal	Crop	5.75	6.85
Whatton	Cereal	Crop	6.08	7.13
Newark	Cereal	Margin	5.6	6.71
Gainsborough	Cereal	Margin	7.09	7.90
Stapleford	Cereal	Margin	4.52	5.64
Whatton	Cereal	Margin	5.48	6.61

Appendix 45: Treatment averages for soil KCl pH measured at sites in Devon and Cornwall, southwest England. Error bars ± S.E.



Appendix 46: Treatment averages for soil KCl pH measured at sites in Nottinghamshire and Lincolnshire, central England. Error bars ± S.E.



Appendix 47: Parent rock and soil type properties at field sites used for leaf litter assays, Cornwall, southwest England.

Location	Crop	Lat. Lon.	Parent Rock	Soil Type
Coldrenick, Cornwall	Miscanthus	50° 30.290'N, 4°41.1850'W	**Trevose Slate Formation & Rosenum**- Slate & Siltstone. Sedimentary bedrock formed 374-398 MYA in Devonian. Local environment previously dominated by shallow seas with pelagite deposits	**Arable & horticulture.** Depth: intermediate-shallow. Organic matter: low. pH: neutral. Temperature av. 11.57C yr-1. Parent material: argillite-slate. Texture: Clayey loam to silty loam.
	Adjacent Cereal	50° 30.365'N, 4°41.1180'W	**Trevose Slate Formation & Rosenum**- Slate & Siltstone. Sedimentary bedrock formed 374-398 MYA in Devonian. Local environment previously dominated by shallow seas with pelagite deposits	**Arable & horticulture.** Depth: intermediate-shallow. Organic matter: low. pH: neutral. Temperature av. 11.63C yr-1. Parent material: argillite-slate. Texture: Clayey loam to silty loam.
Lostwithiel, Cornwall	Miscanthus	50° 25.704'N, 4° 37.887'W	**Staddon Formation**- Sandstone, Siltstone & Mudstone. Sedimentary Bedrock formed ~398-407 MYA in Devonian. Local environment previously dominated by shorelines.	**Arable & horticulture.** Depth: intermediate. Organic matter: low. pH: neutral. Temperature av. 11.68C yr-1. Parent material: sandstone & mudstone. Texture: silty loam.
	Adjacent Cereal	50° 25.857'N, 4° 37.483'W	**Staddon Formation**- Sandstone, Siltstone & Mudstone. Sedimentary Bedrock formed ~398-407 MYA in Devonian. Local environment previously dominated by shorelines.	**Arable & horticulture.** Depth: intermediate. Organic matter: low. pH: neutral. Temperature av. 11.8C yr-1. Parent material: sandstone & mudstone. Texture: silty loam.
Pelynt, Cornwall	Miscanthus	50° 21.886'N, 4° 31.222'W	**Bovisand Formation**- Sandstone. Sedimentary Bedrock. Formed ~ 398-411 MYA in the late Devonian. Local environment previously dominated by shallow seas.	**Arable & horticulture.** Depth: intermediate-shallow. Organic matter: low. pH : neutral. Temperature av. 11.87C yr-1. Parent material: sandstone. Texture: sandy loam.
	Adjacent Cereal	50° 21.620'N, 4°31.110'W	**Bovisand Formation**- Sandstone. Sedimentary Bedrock. Formed ~ 398-411 MYA in the late Devonian. Local environment previously dominated by shallow seas.	**Arable & horticulture.** Depth: intermediate. Organic matter- low. pH: neutral. Temperature av. 11.48C yr-1. Parent material: Claystone/Mudstone. Texture: Loam to Silty Loam.
St Minver, Cornwall	Miscanthus	50° 33.076'N, 4° 51.233'W	**Polzeath Slate Formation**- Mudstone, Siltstone & Sandstone. Sedimentary Bedrock formed ~359-385 MYA in Devonian. Local environment previously dominated by shallow seas with pelagite deposits.	**Arable & horticulture.** Depth: intermediate. Organic matter- low. pH: neutral. Temperature av. 12.04C yr-1. Parent material: clastic rocks. Texture: loam to silty.
	Adjacent Cereal	50° 33.240'N, 4° 51.334'W	**Polzeath Slate Formation**- Mudstone, Siltstone & Sandstone. Sedimentary Bedrock formed ~359-385 MYA in Devonian. Local environment previously dominated by shallow seas with pelagite deposits.	**Arable & horticulture.** Depth: intermediate. Organic matter: low. pH: neutral. Temperature. av. 12.04C yr-1. Parent material: clastic rocks. Texture: loam to silty.

Appendix 48: Mean remaining mass of common nettle (*Urtica dioica*) leaf-litter present at sites across all seasons, crops and field positions in southwest England. Error bars show ±1 S.E. Non-significant, α= 0.05. NS= Non-significant.