The Effect of Phosphorus Addition and Cutting Date on the Nutrient Dynamics and Species Composition of Floodplain Meadows

Thesis

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The effect of phosphorus addition and cutting date on the nutrient dynamics and species composition of floodplain meadows

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A thesis submitted for the degree of Doctor of Philosophy

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Abstract

Many studies have investigated the impact of nitrogen (N), phosphorus (P) and potassium (K) on grassland in terms of vegetation production, composition and species richness, however few have investigated the impact of P addition on floodplain meadows and none have addressed the effect of P availability on N mineralization and N uptake. It has been suggested by several researchers that net N mineralization increases with increasing P availability in the soil, which implies the effects of P on vegetation could be in part mediated by N, however this has never been tested directly.

This thesis tests the hypothesis that net nitrogen mineralization increases with increasing phosphorus availability in the soil. A replicated P addition experiment in the field has been used in conjunction with performing multiple cuts throughout the growing season to investigate the allied effect of nutrient removal. The biomass harvested was analysed for N and P content to estimate N and P uptake by the vegetation. A laboratory incubation experiment was also used to investigate the effect on N-mineralization rate of adding P to soil cores.

The field experiment revealed that the addition of P in combination with N produced a significant increase in vegetation N uptake, and that P was also significant in explaining plant species composition. However, addition of nutrients N and P had no effect on species richness and vegetation community change was driven by temporal changes in water regime. The incubation experiment demonstrated that P addition increased N mineralization in the soil cores where significantly increased levels of NH_4^+ were detected with increasing P concentration. Above-ground biomass was primarily limited by N, although the increase in N offtake in the treatment that added both P and N indicates co-limitation. The cutting trial revealed that peak nutrient offtake occurred later

in the growing season with the addition of P; maximum nutrient removal was mid-June for unfertilized plots and mid-July for P fertilized plots indicating that cutting date may need to be flexible on this vegetation type.

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Chapter 1

Introduction

1.1 Biodiversity

Biodiversity is the genetic and phenotypic variation within ecosystems and communities consisting of the sum total of all the plants, animals, microbes and fungi on Earth (Dirzo & Raven 2003). The five major drivers of biodiversity loss on a global scale according to Sala *et al.* (2000) are land-use, climate, atmospheric CO_2, nitrogen deposition and acid rain, and biotic exchanges (deliberate or accidental introduction of plants or animals to an ecosystem). They identified that the highest driver of biodiversity loss in grasslands would be land use changes, and the highest driver of biodiversity loss in northern temperate areas would be land use changes and atmospheric nitrogen deposition.

When averaged across biomes, land use change is the driver thought to have the most impact on biodiversity loss by the year 2100 (Sala *et al.* 2000), this is thought to be due to the changes in habitat availability and consequent habitat loss. Tilman *et al.* (2001) have predicted that 10^9 hectares of natural ecosystems will be converted to agricultural land by 2050, if past human activities of food consumption, population growth and dependence on agricultural land continue. This would also be accompanied by increases in nitrogen and phosphorus driven eutrophication of terrestrial, aquatic, and near shore marine ecosystems. Therefore, large-scale nutrient enrichment is considered to be an important threat to biodiversity conservation.

International conventions seek to minimise biodiversity loss, climate change, CO_2 emission and nitrogen deposition. In 2002 at the Johannesburg World Summit on Sustainable Development, 192 countries committed to a significant reduction in the current rate of biodiversity loss at the global, regional and national level by 2010 (Convention on Biological Diversity, 2010). This was termed the Convention on Biological Diversity (CBD). However despite limited local successes, the rate of biodiversity loss did not slow down (Butchart *et al.* 2010). Following on from this, at its tenth meeting in Nagoya in Japan in 2010, the CBD adopted a revised and updated plan for biodiversity for the period from 2011 to 2020 including the Aichi biodiversity targets, which are a set of strategic goals and targets to drive action on biodiversity.

In response to the global commitments made in Nagoya, the UK government published the UK 'biodiversity 2020' which is a national strategy for wildlife and ecosystem services. It sets out the Government's ambition to halt overall loss of England's biodiversity by 2020 (DEFRA 2011a). One of the targets is to improve wildlife habitats and to create quality goals for sites of special scientific interest (SSSI). The UK biodiversity action plan (BAP) priority habitats are those that were identified as being the most threatened, and requiring conservation action. The list was first published in 2007 and then updated in 2011, and includes 1149 species and 65 habitats which span terrestrial, freshwater and marine ecosystems (DEFRA 2011b).

1.2 Floodplain meadow decline

One of the habitats under threat from land-use change, and a UK BAP priority habitat, are floodplain meadows. Floodplain meadows are a type of wet semi-natural grassland that are cut annually for hay in midsummer followed by aftermath grazing in August. They are internationally recognised as a habitat worthy of conservation; some are designated as Special Areas for Conservation (SAC) under the habitats directive as they support rare and unique plant communities, large populations of breeding, migratory and wading birds and are important for invertebrates such as dragonflies and water beetles (Joyce & Wade 1998). The high alpha diversity of these habitats gives them interest in terms of their conservation value, ecology, and aesthetics thus many of these sites have been designated Sites of Special Scientific Interest (SSSI).

Throughout the last century, floodplain meadows have severely declined in the UK and Europe. The main reasons for this decline are agricultural intensification, such as fertilizer application, and gravel extraction, urban development and water abstraction (Fuller 1987). The most typical plant community which is restricted to lowland floodplain habitats soils is the *Alopecurus pratensis- Sanguisorba officinalis* (MG4) grassland as classified by the National Vegetation Classification (Rodwell 1992). This grassland type has undergone severe decline over the last century with less than 1500 hectares remaining in England and over 90% is nationally or internationally designated (Jackson & McLeod 2000). MG4 grasslands are productive species-rich communities (up to 38 species per m^2) sensitive to changes in hydrology, management and nutrient inputs, which require agricultural management in order to maintain their productivity and diversity (Bakker & Society 1994; Smith *et al.* 2000). Plant communities in floodplain meadows are strongly influenced by fluctuations in water-table depth (Gowing *et al.* 1997), with experimental field studies demonstrating that minor shifts in hydrological regime can alter species assemblages and diversity (Gowing & Spoor 1998; Gowing *et al.* 1998; Leyer 2005; Beltman, Willems & Güsewell 2007). These grasslands have also been subjected to increasing management intensity since the 1940s following the increased use of fertilizers to increase hay productivity for livestock (Hopkins & Hopkins 1994). Many grassland and floodplain sites have been abandoned across Europe due to alterations to agricultural policies and changes in hay trading (Cop, Vidrih & Hacin 2009) which can lead to change in community composition by reduction in species richness and dominance of taller forbs and graminoids (Jensen & Schrautzer 1999). Mowing is one of the most commonly applied techniques to restore species-rich grasslands on floodplains subject to abandonment and nutrient enrichment (Grootjans *et al.* 2002). By removing taller species, slower growing herbs are encouraged by through a reduction in the competition for light (Bobbink & Willems 1991; Hellstrom *et al.* 2006). Cutting also removes nutrients from the system which accumulate in the standing crop, and can lead to a net export of nitrogen and phosphorus (Koerselman & Verhoeven 1995).

Changes in nutrient availability to grassland plant communities, either by reduction or increase, can be damaging to both the vegetation community and the hay crop by reducing species richness, which has both conservation and economic implications such as decreases in overall site diversity, and a reduction in the size of the hay crop (Manchester *et al.* 1999). To maintain the productivity of MG4 grassland, it requires inputs of nutrients to replace those harvested in the annual hay-crop (Gowing *et al.* 2002). Typically floodplain meadow communities have an intermediate soil fertility (5-15 mg kg $^{-1}$Olsen extractable phosphorus) (Gilbert, Gowing & Wallace 2009). This is due to inputs of nutrients such as phosphorus in floodwaters from point sources such as sewage works, but also via diffuse pollution from phosphorus bound to soil particles being washed into water courses and subsequently deposited by floodwaters (Mainstone & Parr 2002). The main input of particulate phosphate, however, is from agriculture (Mainstone, Parr & Day 2000). Major sources of nitrogen inputs are by deposition of atmospheric nitrogen through traffic pollution and combustion of fossil fuels (predominantly NO_3^-) or in the reduced form of ammonia (NH_4^+) mainly from agriculture (Bobbink, Hornung & Roelofs 1998; Stevens *et al.* 2004; Venterink *et al.* 2006). However, some sites have also experienced nutrient deficiency as a result of flood protection measures restricting flooding and hence replacement of nutrients which could lead to a decline in the quality and quantity of hay yield (van Oorschot, Hayes & van Strien 1998).

1.3 Historical nutrient studies

Many studies have investigated the effect of nutrients on composition of neutral semi-natural grasslands by means of field fertilization experiments. It is well documented that the addition of inorganic fertilizers can cause significant botanical change when applied to diverse grasslands, increasing the dominance of some species whilst decreasing species richness overall (Lawes, Gilbert & Masters 1882).

Nutrient addition experiments are a far from new approach in studying how nitrogen (N), phosphorus (P) and potassium (K) affect the botanical composition of grasslands. Lawes and Gilbert started nine field experiments on arable systems between 1843 and 1856 which are now known as the 'Classical' experiments (Catt & Henderson 1993). Their main objectives were to measure the effects on crop yields of inorganic compounds containing nitrogen, phosphorus, potassium, sodium and magnesium. Initially these experiments were set up to investigate crop yields which had important economic implications. Today, with certain habitats under threat, their effect on species richness as well as yield is also of primary importance to ecologists, land managers and farmers alike (Poulton 2006). The Park Grass Experiment was started in 1856 and is the oldest experiment on permanent grassland in the world. Initially it was designed to investigate the effect of manure and inorganic fertilizers on crop yield, but within 2-3 years it was evident that such treatments were having an effect on the species composition of the vegetation. The treatments consisted of various combinations of P, K, Mg, Na, N and incorporated controls (nil plots) where no fertilizer or manure was applied. Comprehensive species surveys have been done annually on the treatment plots between 1991 and 2000 (Poulton 2006) displaying marked differences; all the original treatments imposed in 1856 have caused a decline in species number compared to the original sward due to changes in soil fertility, management and annual nutrient inputs. The main botanical effects were as follows: 1) the most diverse flora was on the nil plots 2) applying P alone has decreased the number of species, but no more than any other treatment. 3) supplying N with P has greatly reduced the number of species with fewer forbs and larger proportions of grasses such as *Festuca rubra* and *Anthoxanthum odoratum* 4) applying K with P increased the amount of legumes (Poulton 2006; Silvertown *et al.* 2006).

The first significant papers were published by Lawes & Gilbert (1863), Lawes & Gilbert, (1880) and Lawes, Gilbert & Masters (1882) which described the results of plant species abundance, hay yield and soil chemistry. Since then, multiple papers have been published as a result of the Park Grass Experiment using more recent data (Silvertown 1980; Dodd *et al.* 1994; Tilman *et al.* 1994; Silvertown *et al.* 2006) Silvertown *et al.* (1994) investigated rainfall, biomass variation and community composition in the Park Grass Experiment. They found that biomass was a better prediction of compositional variation than rainfall due to a) rainfall not being the exclusive influence on plant growth b) rainfall selectively favoured grasses in the community which competed asymmetrically for light when rainfall was high, therefore the magnitude of the competition depended more on biomass than upon rainfall directly. Some studies have also investigated herbage yield in the Park Grass Experiment (Thurston, Williams & Johnston 1976; Jenkinson *et al.* 1994; Hill & Carey 1997) where yields of hay varied between treatments from the start of the experiment, although declined on most plots in the first 60 years. The largest yield was on the plots receiving the most N plus P, K, Na, Mg, although analysis in long-term trends in yield between 1891 and 1992 showed no significant trends with time. The Park Grass experiment had no replication, but provides a template for nutrient addition experiments on other sites and habitats which can be applied to this study. Results indicated that P increased legumes (Silvertown *et al.* 2006) and therefore suggests further research is necessary in relation to the effects of P on species composition.

More recently, a number of nutrient-addition trials have been carried out on grasslands to establish the extent of botanical change at different levels of nutrient application. Fertilizer experiments at Tadham Moor Site of Special Scientific Interest (SSSI) in the south-west of England have investigated the effects of nutrients on grassland diversity and community composition (Mountford, Lakhani & Kirkham 1993; Tallowin *et al.* 1994; Kirkham, Mountford & Wilkins 1996; Stevens *et al.* 2012). Kirkham, Mountford & Wilkins (1996) applied different levels of N, P and K to diverse grasslands coupled with a cutting experiment to ascertain the effect of cutting date and N application on species diversity and abundance. They investigated if the effects of fertilizer application could be mitigated by applying most of the annual N application after hay cutting. They found an individual species response where *Trifolium pratense* became abundant where P and K were applied with nil or a low level of N (similar to that found in the Park Grass study), but all legumes were suppressed at high rates of N application, particularly in conjunction with P application. They also found that P was more important than N in determining biomass production and botanical change. This is another example of where P has been shown to have significant effects on grassland vegetation, but is not the primary aim of the study. Thus further work is needed to focus on P as a principal hypothesis.

In 1990 a field trial began at Colt Park on the Ingleborough National Nature Reserve in northern England to investigate the multiple effects of management such as different types of grazing, cutting date, fertilizer application and seed application on plant species diversity and productivity of a species poor agriculturally improved upland meadow (Smith *et al.* 2000). Species number increased in plots which received no mineral fertilizer, when the sward was cut in mid-July and when it was grazed in both Spring and Autumn. They determined that this was the best management regime for the restoration of high plant species diversity in combination with sowing seed of absent species. The effect of management on soil microbial community was also investigated at Colt Park (Bardgett & McAlister 1999; Smith *et al.* 2003). Management changes were found to induce shifts in the soil microbial community, and were indicative of improvements in the efficiency of nutrient cycling in these grasslands. Despite these studies, there is a lack of management experiments on grasslands in the UK; especially with regards to altering cutting date within growing seasons. There has been no cutting management trial on MG4 grassland to establish optimum mowing time in terms of nutrient removal and vegetation diversity.

The Rengen Grassland Experiment (RGE) in Germany is one of the oldest continuously managed experiments worldwide, which was established in 1941 in the Eifel Mountains of Germany on low productive grassland (Hejcman *et al.* 2010a). It consists of five fertilizer treatments, and an unfertilized control on a mountainous hay meadow which is cut twice annually (Schellberg, Moseler & Kuhbauch 1999). The treatments were applied annually and were a combination of Ca, N, P, and K. Results of the treatment applications found that P addition caused the greatest difference in vegetation structure and composition with tall grasses dominating such as *Alopecurus pratensis, Arrhenatherum elatius* and *Trisetum flavescens*, N enrichment was not found to be detrimental to plant species richness if applied on its own (Hejcman *et al.* 2007). They also found that biomass was most positively affected by P and K soil contents under N application and the most powerful predictors of plant species composition were soil P, K, Mg contents and biomass production (Hejcman, Schellberg & Pavlu 2010). The biomass N:P ratios were consistent with N or P limitation, with the simultaneous application of NPK decreasing N concentration in above-ground biomass indicating that there is not necessarily a positive relationship between applied nutrients and their plant biomass element concentrations (Hejcman *et al.* 2010b).

The above studies are representative of keystone nutrient addition experiments across Europe and the UK, and demonstrate that the addition of N and P have effects on the vegetation composition, species richness and biomass production of grasslands. However, there is no published work from MG4 floodplain meadows and controlled nutrient addition experiments within this habitat would be necessary to demonstrate which of the major nutrients limit productivity of this particular sward. These studies focus mainly on the effects of N addition on grasslands, or a combination of nutrients; effects of P have been documented as a subsidiary part of the experiments, therefore it would be beneficial to investigate P addition individually, its influence on the vegetation dynamics, but also on nutrient cycling in floodplain meadows. These studies identified P as causing significant botanical change (Silvertown *et al.* 2006; Hejcman *et al.* 2007) and above-ground production changes (Kirkham, Mountford & Wilkins 1996), which warrant further research.

1.4 Limiting nutrients

Plant production is usually limited by the availability of nutrients N, P and less frequently K or a combination of these nutrients (Verhoeven, Koerselman & Meuleman 1996). Vegetation responds to nutrient availability rather than the total nutrient in the soil, therefore it is important to understand what controls the availability of these nutrients. Nutrient limitation is one of the most important factors influencing the structure of plant communities, as species richness tends to decline with increasing nutrient availability which favours growth of competitive species capable of rapid resource acquisition and biomass production (Al-Mufti *et al.* 1977; Grime 1979). Grassland systems are mainly thought to be N limited, although there have been some clear observations made by a number of studies that P is also important in limiting the productivity of the vegetation (Kirkham & Wilkins 1994; Janssens *et al.* 1998; Wassen *et al.* 2005; Ceulemans *et al.* 2011). In the majority of terrestrial ecosystems, nitrogen and phosphorus are the main limiting nutrients controlling plant productivity and microbial functioning (Vitousek & Howarth 1991; Elser *et al.* 2007). However, both P and N cycles do not necessarily exist independently of one another; and studies have attempted to investigate the dynamics of P and N and their possible interactions (Pastor *et al.* 1984; Oorschot *et al.* 1997; Janssens *et al.* 1998). These interactions may influence the vegetation by increasing biomass and playing an important role in grassland diversity. Furthermore the availability of N and P due to fertilization can strongly influence microbial activity (Olander & Vitousek 2000).

The concept of N:P ratios originated from many of the Dutch studies on grasslands (Koerselman & Meuleman 1996; Verhoeven, Koerselman & Meuleman 1996; Güsewell, Koerselman & Verhoeven 2003). A cost effective tool to determine if above-ground production is limited by N or P was developed, without the need to perform nutrient addition experiments, by assaying the N and P concentrations of herbaceous plant material and combining these into a ratio (Verhoeven, Koerselman & Meuleman 1996). Koerselman & Meuleman (1996) suggested that plant populations with high N:P ratios (>16) would be enhanced by fertilization with P, and populations with low N:P ratios (<14) would be enhanced by fertilization with N. However, contrary to these findings Güsewell, Koerselman & Verhoeven (2003) found that N:P ratios of co-limited vegetation or plant populations were not confined to the narrow range of 14 to 16 and concluded that N:P ratios were a useful tool, although not exclusively suitable for predicting how changed nutrient supply will affect plant species composition. Alternatively they can be used to investigate how the relative availability of N and P influence ecological processes and can assist in monitoring and evaluating the effects of conservation management (Güsewell, Koerselman & Verhoeven 2003).

The availability of nitrogen and phosphorus to the vegetation is affected by the microbial processes occurring in the soil. Nitrogen mineralization is the conversion of organic N to NH_4^+ (ammonium) by decomposers in the soil and is the process in the soil that controls the N availability to plants (Harmsen 1955; Abbasi, Shah & Adams 2001). Once in the form of ammonium, N is available either for direct use by plants or for further transformation into nitrate via nitrification. Some researchers have suggested that net N mineralization increases with increasing P availability in grasslands (Ruess & Seagle 1994; Oorschot *et al.* 1997). Janssens *et al.,* (1998) suggest a relationship between soil available P and organic matter N mineralization which can influence soil available N. Thus plants may increase uptake of N in response to P addition. This theory suggests that P is important for plant productivity even in N limited systems and suggests an indirect effect on N availability. However, this is yet to be tested experimentally in the field. If P influences the rate of N mineralization, this could change how much N is taken up by the vegetation and stored, which could be investigated by adding P to experimental plots and harvesting the above ground vegetation biomass at regular intervals and analysing the harvested biomass for N. A few studies have also found a link between available P and ammonium production in soils (Troelstra *et al.* 1990; Ruess & Seagle 1994). Similarly fertilizer experiments on grasslands in the UK have indicated that N availability is increased by the application of P (Kirkham & Wilkins 1994). However, this has never been tested as an independent hypothesis on UK floodplain soils.

Despite numerous studies into N and P enrichment and broad literature on this subject, nutrient addition experiments are still relevant in establishing the effects of N and P on plant species composition in grasslands. As MG4 grassland is a species-rich community, it is likely to be sensitive to changes in nutrient availability and therefore a useful system to test whether N or P limit the productivity of the vegetation. Based on the current literature (especially many of the Dutch studies) and on the findings of (Verhoeven, Koerselman & Meuleman 1996) and (Güsewell, Koerselman & Verhoeven 2003), they are more likely to be N limited. Furthermore data from current MG4 hay analysis suggests the MG4 N:P ratio is approximately 8 (therefore below 14) which supports this (Gowing *et al.* 2002). Controlled fertilization on a stand of MG4 vegetation would provide further insight into nutrient limitation, biomass production and species composition on wet meadows. Testing the type of nutrient limitation is secondary to the hypothesis of P addition increasing M mineralization. It is also of nature conservation importance, so the results have practical application for floodplain meadow conservation.

1.5 Management

The availability of N and P on grasslands can be controlled to some extent by vegetation management. The traditional management of MG4 floodplain meadows consists of a midsummer hay cut, followed by aftermath grazing in the autumn and winter. This maintains high species diversity and prevents accumulation of nutrients in the soil which can lead to changes in the productivity, composition and diversity of grasslands (Bakker 1989). Generally cutting and removal of the hay-crop with aftermath grazing is more successful in nutrient removal than either cutting or grazing alone (Smith *et al.* 2000). This increases reductions in soil fertility and opens up germination gaps in the sward for seedling establishment (Walker *et al.* 2004). In the Netherlands, it has been demonstrated that regular vegetation harvesting creates a net loss of P from the system relative to N (Koerselman, Bakker & Blom 1990), therefore wet grasslands with a long history of regular cutting would be P limited and irregularly mown fens would be N limited

(Verhoeven, Koerselman & Meuleman 1996). MG4 floodplain communities are relatively productive typically yielding 4 t ha^{-1} y^{-1}, requiring a mesotrophic soil to support this productivity. Nutrient availability at these sites needs to be managed in order to maintain productivity and species diversity. This can potentially be achieved by altering the timing and frequency of hay cutting; as more frequent mowing can result in net export of nutrients from the system.

1.6 The need for this study

Findings from these long-term studies both in the UK and Europe indicate that there is still disparity in determining which nutrients have most effect on plant communities in terms of species richness and composition. Previous studies (Verhoeven & Schmitz 1991; Verhoeven, Koerselman & Meuleman 1996; Olde Venterink *et al.* 2003) have attempted to identify which nutrients are most important in limiting vegetation biomass, diversity and species-composition in grasslands, which are important when considering conservation of these habitats.

The primary focus of this thesis is on fertilization by P, and hypothesises that P addition may enhance the rate of N mineralization in the soil, and therefore increase the uptake of N by the vegetation but never been tested experimentally on UK floodplain meadows. P addition may also affect the plant community and above-ground biomass of the vegetation (Tilman *et al.* 1994; Kirkham, Mountford & Wilkins 1996; Ceulemans *et al.* 2011). Effects of nutrient addition on the plant community may also be mitigated by altering cutting dates or increasing cutting frequency, which is also a principal objective of this thesis. Further details and background description can be found in the introduction of each chapter relevant to specific research questions.

1.6.1 Research Aims

This study aims to investigate the effects of P addition on N mineralization and thus on, biomass production and ultimately plant-community composition. Investigations will be made into whether the addition of P increases N mineralization by means of laboratory experiments, and field nutrient addition experiments.

A second aim is to assess the effects of different cutting dates on the nutrient balance of meadows. This aspect of the study may give insight into the conservation and restoration management of grasslands in terms of optimising guidance on cutting regime.

The following research objectives will be experimentally tested in this project.

1.6.2 Objectives

- To determine if P addition increases N mineralization.
- To determine if N offtake increases in response to P addition.
- To determine if N offtake peaks earlier in the growing season in response to P addition.
- To determine if functional composition of the vegetation changes in response to P addition.
- To determine if altering cutting date alters the species richness and composition of the vegetation.
- To determine if changes to N offtake are a result of biomass changes or tissue composition.
- To determine if forb cover increases with more frequent cutting.

1.7 Thesis structure

Within this thesis there are four experimental chapters, which are self-contained experimental studies. Chapter 2 and chapter 3 have the same experimental setup, which consists of a cutting experiment, the design of the experiment is described in full in chapter 2 and includes the methodology for both chapters. Chapter 4 and chapter 5 have a different experimental design to the previous chapters, which is described fully in chapter 4. This part of the thesis is a factorial nutrient addition experiment. Control plots from this experiment are also utilised in chapters 2 and 3 as part of the cutting experiment, thus there is a two-fold experimental design which is linked between chapters. The experimental methodology is described in full in chapter 2 and chapter 4, and only summarised in other chapters.

Chapter 2 investigates the effect of cutting date on hay yield and nutrient off-take from MG4 grassland, and investigates the optimum time to cut a meadow in terms of hay yield, but also removing nutrients from the system. The addition of P on vegetation above-ground biomass and its effects on nutrient off-take and peak standing biomass is also studied. Weather conditions and the effect on above-ground biomass are also explored by attempting to correlate rainfall, soil moisture deficit and hay yield. Chapter 3 considers the effect of cutting date on the botanical composition of floodplain meadows, in terms of species richness and also community composition.

Chapter 4 focusses on nutrient addition to floodplain meadows and the effect of nitrogen (N) and phosphorus (P) on the nutrient content and yield of the above-ground biomass, and the effect on N:P ratios and nutrient limitation in floodplain soils. It also includes the results of laboratory incubation experiments and the *in-situ* use of plant-root-simulator probes (PRS-probes) to test the effect of P addition on nitrogen mineralization.

Chapter 5 investigates the effect of nutrient addition on the botanical composition of floodplain meadows, and any temporal changes occurring over the duration of the project.

Chapter 2

The effect of cutting date on hay yield and nutrient offtake

2.1 Introduction

2.1.1 Traditional hay cutting in meadows

Floodplain meadows are traditional agricultural systems which have been managed over hundreds of years. Historically, they were cut for hay when the grass reached its optimal state in terms of feed value, typically the last week in June or the first week in July or 'midsummer' (Baker 1937; Brian 1993). Cattle, or other livestock, then grazed the land throughout the autumn, and were removed in the winter and spring to allow the hay to grow once more (Brian 1993). In many cases, this is still the same today, although there are other external influences which have altered the thinking on deciding when to cut a meadow. Not all meadows are managed in a traditional manner anymore, due to variation in land ownership and a variety of uses for meadows and their hay. Many meadows have their cut delayed or fixed to a set date in July in response to agri-environment scheme agreements (such as Environmental Stewardship or Higher Level Stewardship) which can encourage less variation in management (Warren, Lawson & Belcher 2007), whilst others are cut much later to allow species either to set seed for aesthetic reasons or to encourage certain species of flora or fauna. In addition, if the ground is too wet or flooded it can be impossible to cut the hay at the desired date. Therefore management of these once traditional systems is not a simple matter (Gowing *et al.* 2010).

The diagram below illustrates the annual hay cycle (figure 2.1). With more hours of daylight and increasing temperatures in the spring and summer, the vegetation grows to support a wide variety of invertebrates and birds. When the hay is at its maximum productivity, it is cut in 'midsummer', which removes nutrients from the system. After the hay cut, animals graze the meadow or grasslands ingesting further nutrients, but returning them to the system via waste and excretion. The animals are removed from the meadow as conditions get wetter through the autumn and winter. Low rates of evapotranspiration over winter lead to a rise in the water table and river levels, often resulting in flooding. Deposition of nutrient rich sediment which may be rich in P by floodwaters, replaces nutrients which are lost from the system by cutting. Nitrogen is added to the system in small quantities throughout the year by atmospheric deposition. The cycle of nutrients in the management of a hay meadow is critical to maintain the high diversity of grasses and plants. If nutrient levels become too high or even too low, then the diversity of plants will change to reflect the changing conditions. As weather cycles vary in different years, management of meadows need to be flexible in order to maintain a balance of nutrients that will maximise vegetation diversity (Gowing *et al.* 2010).

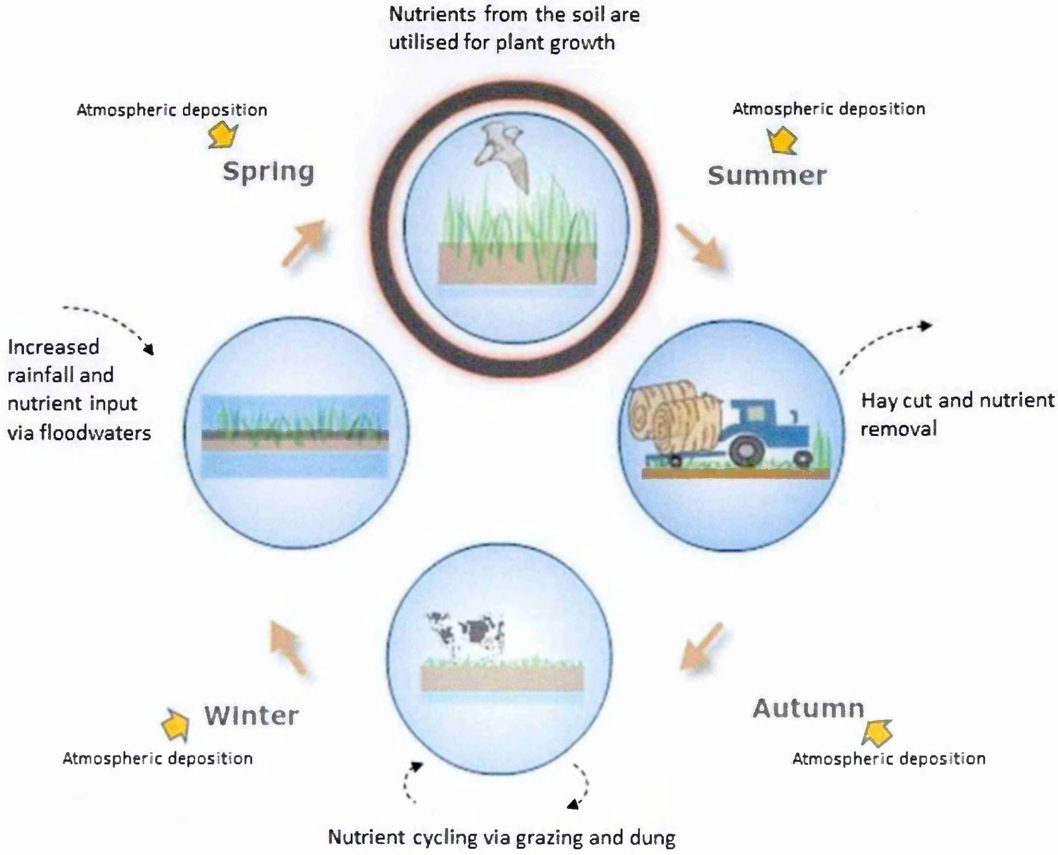


Figure 2.1 Typical hay cycle in one year on a floodplain meadow (Floodplain meadows website; Gowing *et al.* 2010)

2.1.2 Nutrient cycling

Nutrients particularly nitrogen (N), phosphorus (P) and potassium (K) can enter floodplain meadows from numerous sources; atmospheric deposition, farmyard manure, and flood-deposited nutrient enriched silts. Removal of the annual hay crop in the form of biomass can balance these inputs providing the nutrient input does not exceed the output.

Plant growth on floodplain meadows and other wetlands is commonly limited by the availability of N, P and K, or a combination of these elements, and biomass production can therefore be enhanced by the addition of one or more of these nutrients (Verhoeven & Schmitz 1991; Verhoeven, Koerselman & Meuleman 1996). Nitrogen exists in the atmosphere in the form of N_2 and can become available to plants by the process of nitrogen fixation by rhizobia bacteria that nodulate the roots of legume plants (Bardgett 2005). The majority of soil N is in the form of organic nitrogen compounds which are not directly available to plants, but can be converted to available ammonium ions (NH_4^+). Ammonium ions not immobilized or taken up by higher plants are often converted to nitrate ions (NO_3^-) by the process of nitrification, which occurs readily in agricultural soils (Abbasi, Shah & Adams 2001). Nitrate ions are very soluble and can be lost easily from the system due to leaching in drainage waters or by denitrification, which occurs when soils become warm and saturated and nitrates are lost to the atmosphere as N_2. Crop removal represents a loss and therefore output of N; inputs are a result of atmospheric N deposition and particulate N in floodwaters (Abbasi, Shah & Adams 2001; Bardgett 2005).

In contrast to N, most P in the soil exists as insoluble forms that are unavailable to plants. Phosphate (PO_4^{3-}) is the main inorganic form of P which is available to plants.

This phosphate can be divided into two forms, firstly the labile P pool which consists of phosphates on mineral surfaces which can be easily absorbed by plants roots and is immediately available to microbes (Bardgett 2005). Secondly, P can precipitate within calcium, iron and aluminium compounds that are insoluble and unavailable to plants (Simpson *et al.* 2012). Inputs of P in most systems is due to weathering of bedrock, however on floodplain meadows the majority of P input comes from sediment deposition in floodwaters (Berendse *et al.* 1994) (figure 2.2). P and N are removed from the system in the annual hay cut, which is the main nutrient output (Verhoeven, Koerselman & Meuleman 1996).

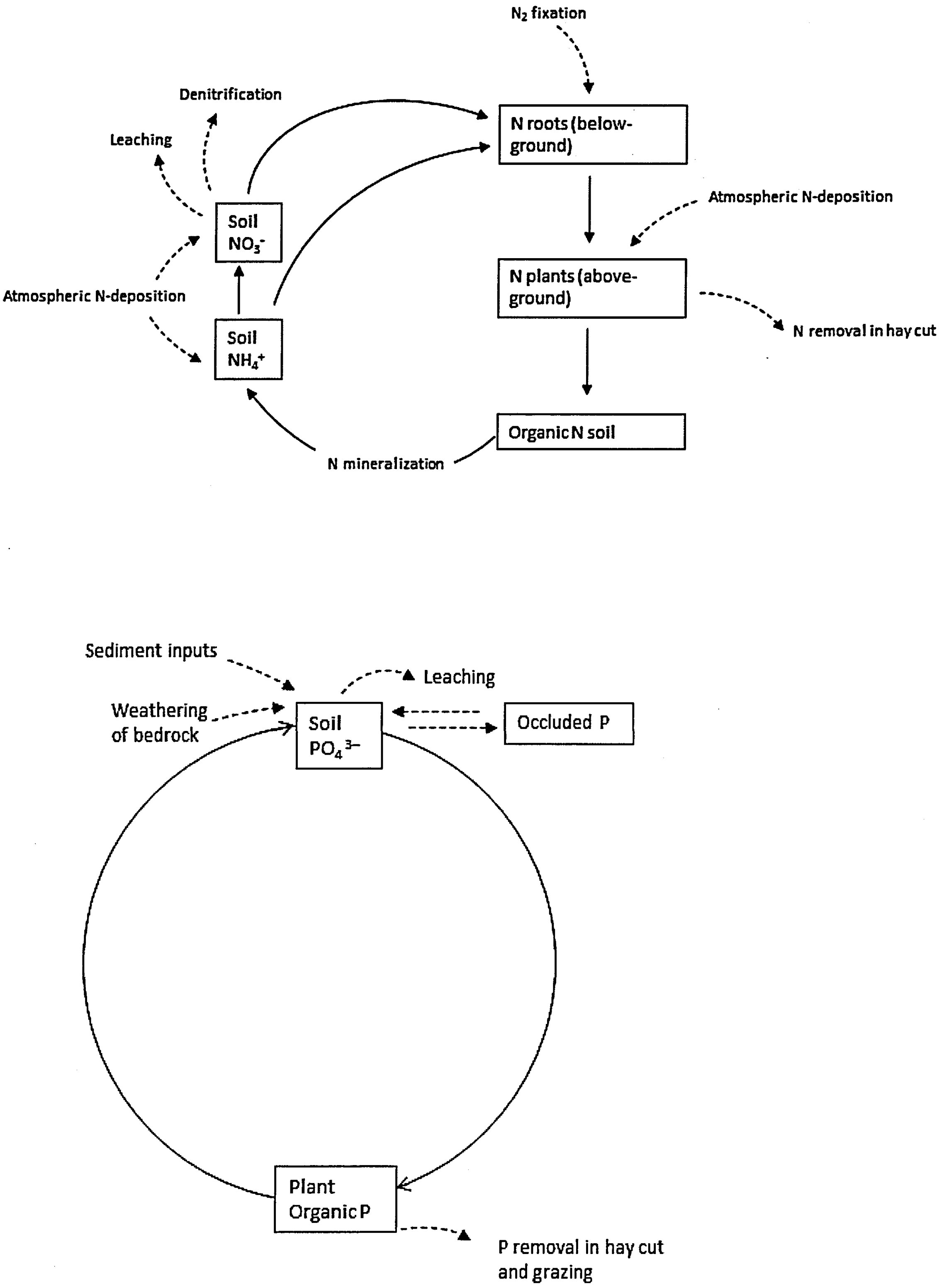


Figure 2.2 Schematic diagram of the N and P cycles (Berendse *et al.* 1994)

In the majority of terrestrial ecosystems, nitrogen and phosphorus constrain plant productivity and microbial functioning (Vitousek & Howarth 1991; Elser *et al.* 2007). However, both P and N cycles do not necessarily exist independently of one another, studies have attempted to investigate the dynamics of P and N and their possible interactions (Pastor *et al.* 1984; Oorschot *et al.* 1997; Janssens *et al.* 1998), which influence the vegetation by increasing biomass and playing an important role in grassland diversity. Furthermore, the availability of N and P due to fertilization can strongly influence microbial activity (Olander & Vitousek 2000). This study primarily focusses on fertilization by P addition and hypothesises that P addition may further enhance the effects of N on microbial processes by increasing the rate of N mineralization in the soil, and therefore increasing the uptake of N by the vegetation. This can be measured by analysing plant nutrient content for N and multiplying this percentage with the above ground biomass weight of the vegetation to give rise to a plant N offtake value. Janssens *et al.* (1998) have suggested that soil available P can limit organic matter mineralization by the depression of micro-organisms which affect the nitrification and mineralization processes. Similarly other studies have found that N mineralization is positively correlated with the available soil P pool (Troelstra *et al.* 1990; Cadisch, Schunke & Giller 1994; Ruess & Seagle 1994). This central hypothesis is discussed in more depth in chapter 4 of this study, but is also relevant to this current chapter as we will investigate N offtake in floodplain meadows after the addition of P. Increased rate of N uptake (measured by plant N offtake) by the vegetation may occur earlier in the growing season with the addition of P if N mineralization is increased (figure 2.3), because a general characteristic of unfertilized species rich grasslands is that they have a considerably lower growth rate in the Spring and early Summer compared with agriculturally improved grasslands (Tallowin 1997). No such studies have been carried out on MG4 floodplain communities to investigate herbage production and N offtake of the vegetation after P addition. Multiple cuts made throughout the growing season and subsequent analysis of the vegetation samples for N, could establish if P has increased vegetation above-ground biomass, and if N offtake has been increased. The timing of the hay cuts in the growing season can also be investigated in conjunction with this.

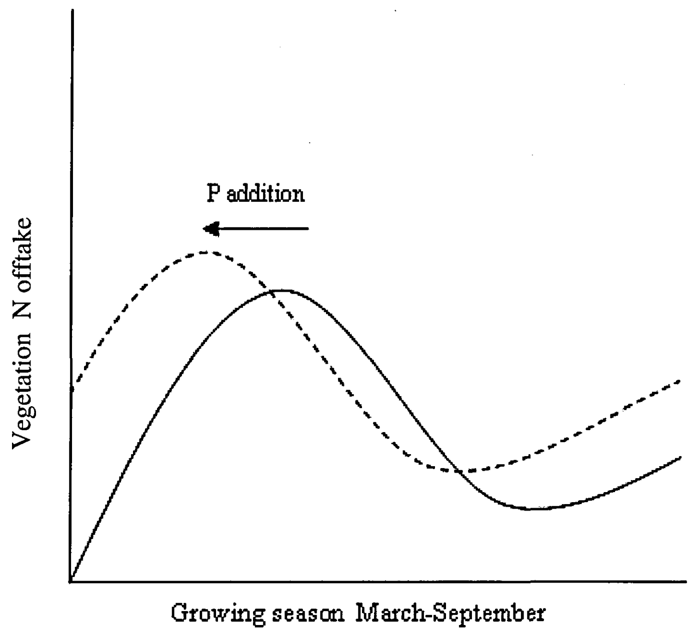


Figure 2.3 Theoretical N offtake curve produced over time and brought forward with the addition of P.

Cutting date on floodplain meadows is critical to the development of the vegetation (Smith & Jones 1991). Cutting too early prevents some species from setting seed, which could lead to their disappearance. However cutting too late, can encourage tall growing bulky species to dominate, shading out other species and gaining a strong competitive advantage (Bobbink & Willems 1991). Aside from maintaining species richness and vegetation structure, hay cutting also influences soil nutrient concentrations by the removal of minerals accumulated in the standing crop (Oomes 1990), and a net export of P and K from the system (Koerselman & Verhoeven 1995). Land abandonment and failing to cut a meadow promotes the occurrence of competitive species, while regular mowing removes a disproportionately higher proportion of tall competitors (Buckland *et al.* 2001; Gerard *et al.* 2008). It also reduces the competition for light, giving the opportunity for smaller competitors to establish (Leps 1999; Kotowski & van Diggelen 2004).

2.1.3 Hay yield

Floodplain meadows are very productive systems, with a net annual above-ground primary production across Europe (NAPP) ranging from 300 to more than 4000 g dry weight (DW) m $^{-2}$ year $^{-1}$, depending on the dominant vegetation species (Tetter *et al.* 1988; Kaplova, Edwards & Kvet 2011). Although a review of hay production from lowland grasslands in the UK (Tallowin & Jefferson 1999) suggest that the dry matter yields from different unfertilized agriculturally unimproved grasslands in the UK is much lower than this, ranging from 150 g m^{-2} to 600 g m^{-2} when first cut in late June or early July. Different national vegetation community (NVC) grassland and floodplain meadow communities (Rodwell 1992) will give rise to different biomass yields ranging from 200 g m^{-2} to 800 g m^{-2} (Peeters & Janssens 1998), although data available for some NVC communities is limited. Tallowin & Jefferson (1999) collated dry matter yields from different NVC types from numerous studies in order to compare the yields; these included MG1, MG5, MG6, MG7, MG8, MG10, MG13, M16, M23 and M24. Dry matter yields obtained from a single hay cut in mid-summer from a sample of semi-natural grassland containing MG4 vegetation community, ranged from 300-600 g m^{-2} (Gowing *et al.* 2002). On grassland sites where fertilizer has been applied (levels of 100 kg N ha y^{-1} and 37.5 kg P ha y^{-1}) the dry matter yield can increase by 50-100% (Kirkham & Wilkins 1994; Kirkham & Tallowin 1995) and their botanical composition may change towards species poorer communities such as agriculturally improved grasslands *Lolium perenne-Cynosurus cristatus* grassland (MG6) (Mountford, Lakhani & Kirkham 1993; Kirkham, Mountford & Wilkins 1996). Although grassland production on various NVC communities may differ, there is also variability within communities and between different seasons, due to different soil properties between sites, and climatic conditions between years. The variation in hay yield may be lower in more species-rich than in species- poor swards due to species richness providing a buffer against climatic variation (Dodd *et al.* 1994).

The biomass production in floodplain meadows and grassland systems is very sensitive to changes in weather, particularly rainfall (Smith 1960; Dodd *et al.* 1994). Smith (1960) established a relationship between meadow hay yields, and estimated ‘actual’ transpiration during the growing season, which in turn is related to soil moisture deficit. In his study, data for actual hay yield and transpiration were combined to calculate the predicted yield for specific areas across the UK. It could also be used to explain the variability in dry matter yields between years on the same site.

Traditionally, hay meadows have been cut in late June or early July, which gives rise to the highest hay yield. Generally unfertilized grasslands have a considerably lower growth rate in the spring and early summer than that of agriculturally improved or fertilized grasslands, which means that the peak yield may be achieved later in the season in unfertilized grasslands (Robson 1981). Tallowin & Jefferson (1999) plotted dry matter data from various nutrient addition experiments from across the UK, which were cut at different times in the growing season (figure 2.4). Improved and unimproved grasslands were compared; fertilized plots represent a mixture of N, P and K additions ranging from 44-200 kg ha y $^{-1}$ of N, 21-150 kg ha y $^{-1}$ of P and 52-390 kg ha y^{-1} of K (graph from Tallowin and Jefferson (1999), data source from (Tallowin 1997)). Dry matter yield was highest in the agriculturally improved grassland, and reached its ceiling earlier than in the unfertilized semi-natural grasslands. Another much earlier experiment by Gately, Ryan & Doyle (1972) investigated the effect of applying high amounts of N on the yield, total-N and nitrate-N content of herbage over the growing season, from data collated from 24 sites across Ireland. The amount of N applied ranged from 0-672 kg ha y^{-1}. They found the dry matter yield was greatest on the plots cut on 14th July and increased with a higher concentration of N fertilizer application (figure 2.5).

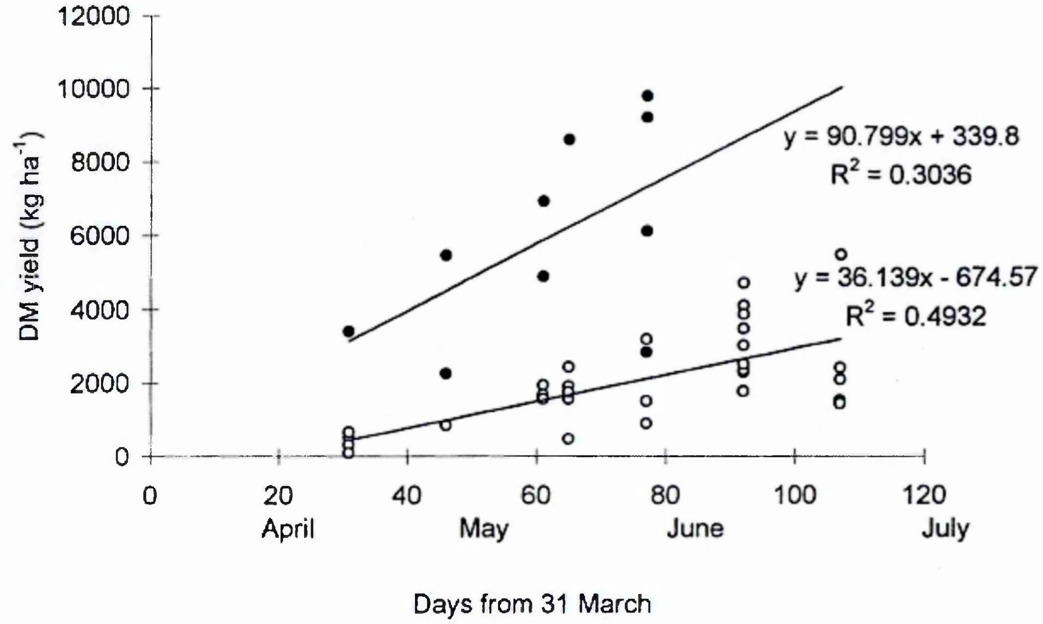


Figure 2.4 Changes in dry-matter yield (DM) during the early summer in unimproved, unfertilized semi-natural grasslands (○) and agriculturally improved and/or fertilized grasslands (●). (Tallowin and Jefferson, 1999).

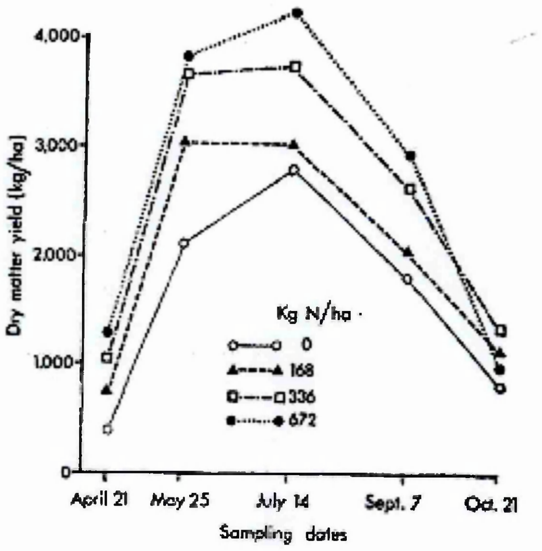


Figure 2.5 Seasonal effect of N fertilizer on the mean dry matter yields of herbage (Gately, Ryan & Doyle 1972)

They also measured the total N percentage in the dry matter from each hay-cut to investigate the effect of increased N fertilizer on the critical concentration of nitrate in the hay. The peak for the total N in the dry matter was in April and October (figure 2.6) based on single cuts from March to October; this was attributed to peak N mineralization levels and therefore increased nitrate offtake in April and a 'late season effect' which could be due to the slowing down of growth rate with reduced light intensity in October.

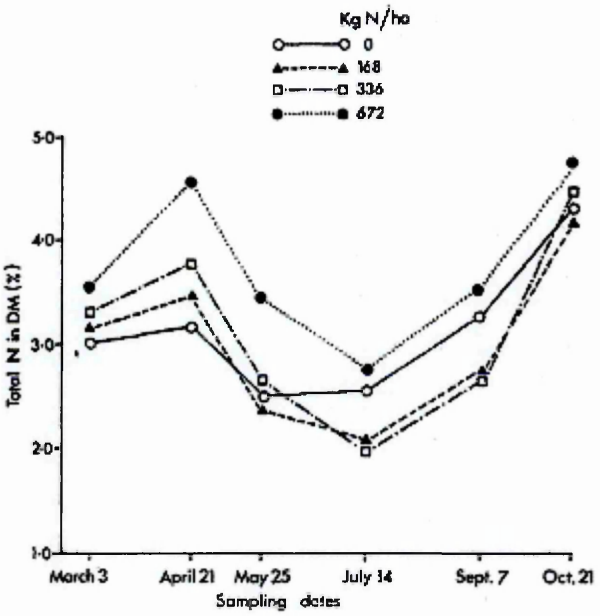


Figure 2.6 Effect of sampling dates on the mean total-N content of herbage (Gately, Ryan and Doyle, 1972)

In a more recent study on a wet grasslands Kaplova, Edwards & Kvet (2011) found that above-ground biomass was twice as large in the high nutrient area (1689.5 g m^{-2} y^{-1}) compared with a low nutrient area (874.4 m^{-2} y^{-1}) within their study in the growing season. The high nutrient area had 1.16% of total nitrogen and 0.18% of total phosphorus in the soil, compared with the low nutrient area which had a total nitrogen percentage of 0.87 and total P of 0.21, therefore the high nutrient area was driven by increased N rather than increased P. They also found that biomass increased in both areas until the middle of July (200 days from 1st Jan), after which it declined in the low nutrient area, but continued to increase until September in the high nutrient area (see figure 2.7). Their study site however was in the Czech Republic with different climatic conditions from the UK. This pattern of biomass removal within the growing season has not been investigated in the UK on MG4 floodplain meadows, from cuts made at the same site.

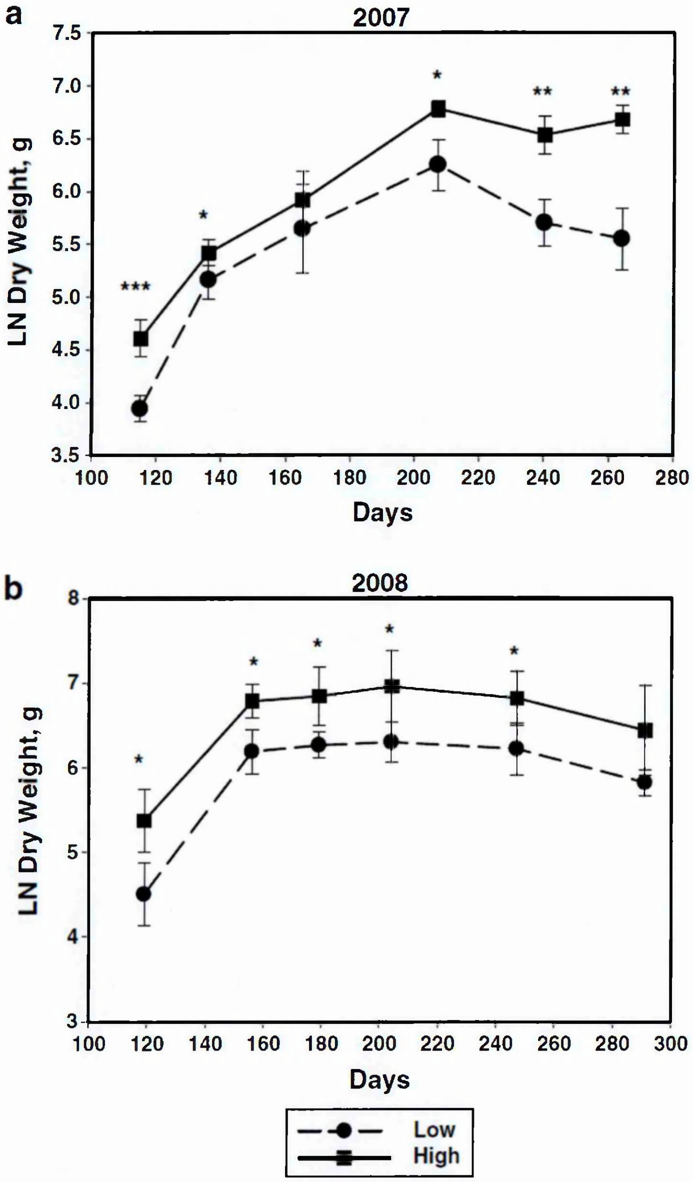


Figure 2.7 From Kaplova, Edwards and Kvet, (2011). Log and standard error monthly total biomass (g DW m^{-2}) in the low and high nutrient areas for 2007 and 2008 growing seasons. *$p<0.05$, ** $p<0.01$, ***$p<0.001$

Although there have been many studies investigating disturbance of meadows and grasslands through cutting, there have been a limited number investigating mowing regime within a growing season, and specifically the effect of cutting date itself on the nutritional value of the hay crop in terms of nutrient removal from the system. There have been studies investigating hay yield of various vegetation communities, although data have been collated and compared from different sites rather than sequential cuts from the same site (Tallowin 1997; Tallowin & Jefferson 1999; Gowing *et al.* 2002). The aim of this study was to investigate the pattern of sequential biomass harvests through the growing season, with and without P addition; the biomass yield (with and without P addition) and the nutrient content of the hay crop through the growing season. In turn this should provide useful information with regards to optimum cutting date in terms of maximum nutrient removal from floodplain grasslands and maximum hay crop yield. Interaction between biomass production and soil-moisture deficit is also investigated in order to produce a model to predict and explain variation in hay yield from soil-moisture conditions, between the same vegetation communities. This chapter addresses the following questions:

1. What is the effect of P addition on the hay yield in floodplain meadow MG4 vegetation? (Page 45)
2. To determine if hay yield can be predicted based on rainfall using the Smith calculation (Page 50)
3. What is the effect of P addition on the peak offtake of N from floodplain meadows? (Page 54)

2.2 Methods

2.2.1 Study site

Leaches Meadow is situated close to the Oxfordshire-Buckinghamshire border, near Bicester in the UK (figure 2.8a). The study site is an undesignated MG4 site owned by Berkshire, Buckinghamshire and Oxfordshire Wildlife Trust (BBOWT). It is part of the Upper Ray Meadows nature reserve which is a core part of BBOWT's Upper River Ray project area, one of their 'Living Landscapes' schemes. The River Ray lies within the Upper Thames catchment area. The land around it is a broad flat valley with Oxford clay, and is liable to flooding. The Upper Ray Meadows Nature Reserve is located along the A41, two miles west of Kingswood in Buckinghamshire and consists of an area of 144 hectares (BBOWT 2008). The site is managed in the manner of a traditional unimproved hay meadow, with a hay cut in mid-July followed by aftermath grazing by sheep in the autumn (Mick A'Court, pers. comm.). The vegetation is typical of an MG4 community; the dominant forb is *Sanguisorba officinalis* with patches of *Carex riparia* and *Carex disticha* within lower lying areas within the meadow. Dominant grasses are *Alopecurus pratensis*, *Cynosurus cristatus*, and *Agrostis stolonifera.*

The field study was conducted during the 2010, 2011, 2012 and 2013 growing seasons. Climate conditions differed between the years with the main differences occurring in rainfall and mean air temperature during this period. 2012 was considerably wetter than the other years, although had a slightly colder growing season (figure 2.9 and table 2.1). Evapo-transpiration was greatest overall in 2011, but was highest in July 2013, and lowest in the growing season of 2012 (figure 2.10).

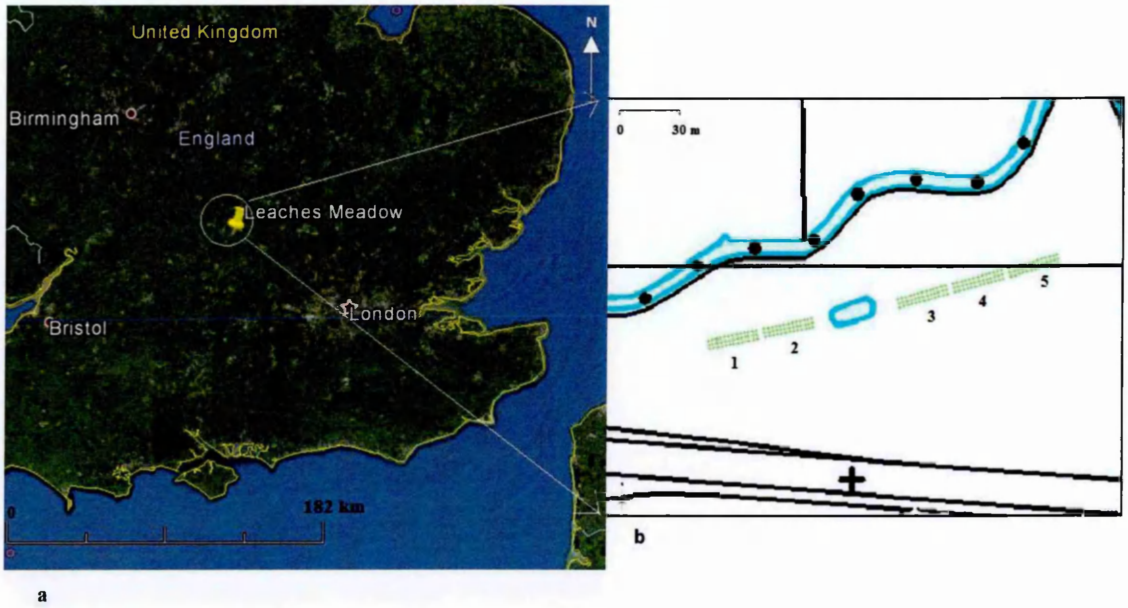


Figure 2.8a) Location map of Leaches Meadow and b) alignment of blocks at the site labelled 1-5

	2010	2011	2012	2013
Annual rainfall (mm)	582.4	467.6	892.4	639.2
April-July rainfall (mm)	109.8	141.1	407.5	150.5
Mean air temp (°C)	8.47	10.09	8.99	8.99
April-July mean air temp (°C)	12.4	12.75	11.75	12.02
Annual ET (mm)	495.2	531.9	473.0	495.4

Table 2.1 Annual and seasonal rainfall and temperature means for 2010, 2011, 2012 and 2013

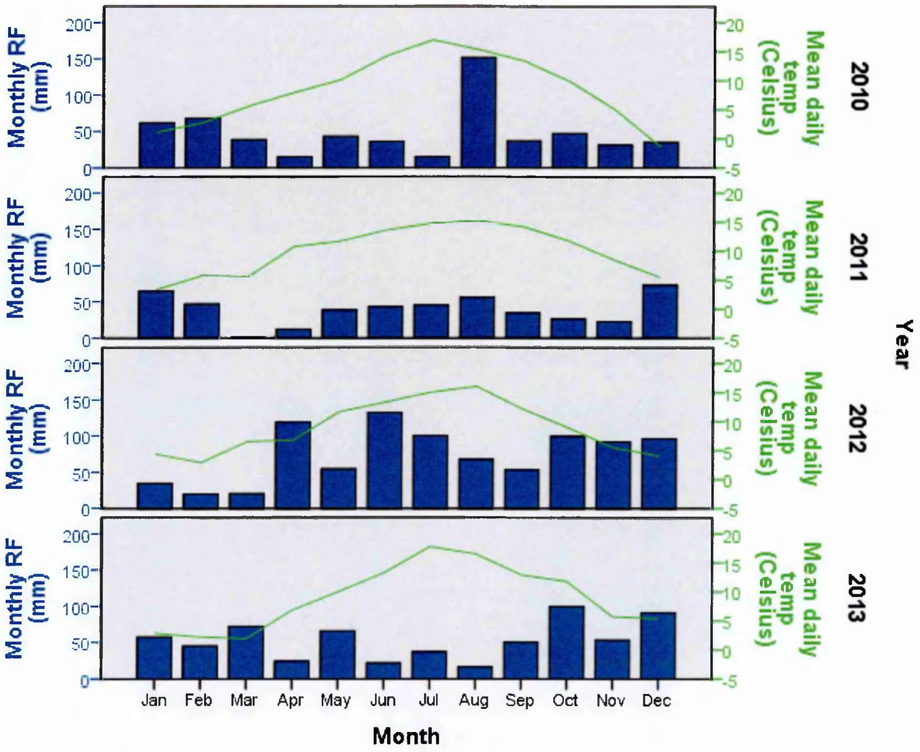


Figure 2.9 Mean monthly rainfall and temperature for 2010, 2011, 2012 and 2013

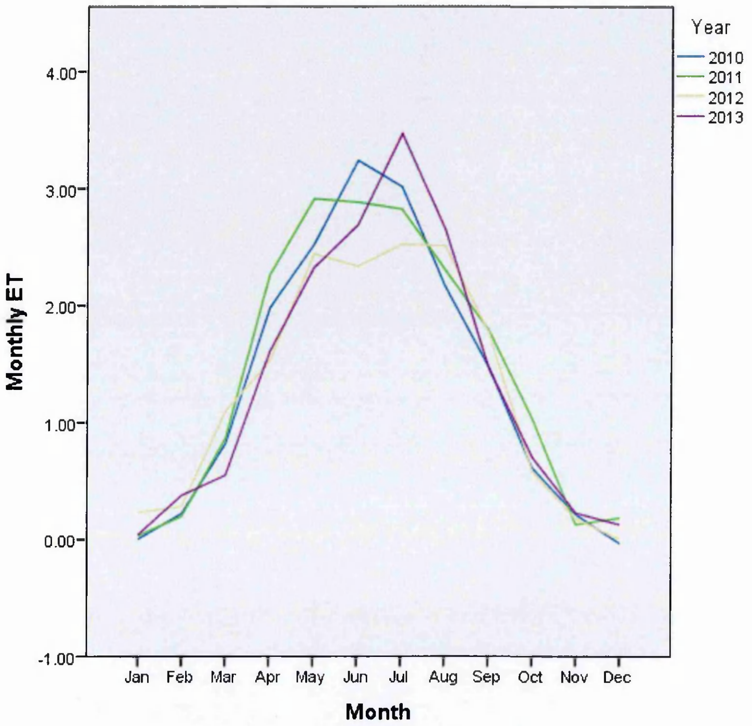


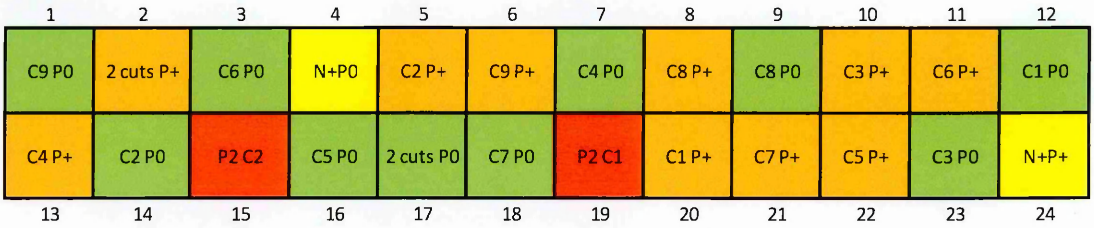
Figure 2.10 Evapotranspiration per month for each year.

2.2.2 Experimental design and treatment additions

In April 2010, experimental plots were laid out using a randomized block design, each block consisting of twenty-four 2 m x 2 m plots. Five replicate blocks were set up aligned adjacent to one another in order for the grazier to avoid the experimental area when cutting the majority of the meadow (figure 2.8 b). After initial soil analysis, all blocks were uniform in terms of Olsen extractable P level and appeared homogenous in terms of vegetation community. Each 2 x 2 m plot was marked out with wooden tent pegs, using a GPS system (Leica RX1200) that is accurate to 2 cm for horizontal distances, to record the coordinates of each point so the plots could be relocated in subsequent years, even if pegs were lost (Dodd 2011).

Different treatments were applied to each of the 24 plots as follows (figure 2.11): 10 plots received 25 kg P ha y^{-1} (P+), two plots received 75 kg P ha y^{-1} (hereafter referred to as P2), 10 plots were controls thus received no P (P0), and two plots received 50 kg N ha y^{-1} (N+). The reason for N addition in conjunction with P is to determine whether N availability is limiting the productivity of the P plots. The treatments were applied in three doses 2 weeks apart at the end of April 2010 and again in April 2011, in order to give the vegetation and microbial communities an opportunity to absorb the nutrients, thus avoiding losses through runoff and leaching. P added to the plots was in the dissolved form of sodium dihydrogen phosphate (NaH_2PO_4), which was applied directly onto the 2 x 2 m vegetation plot dissolved in 1 litre of water (table 2-2). N was applied in the same way to two additional plots within each block, one receiving P and one not, in the dissolved form of ammonium nitrate (NH_4NO_3). Deionised water was added to the control plots to account for the difference in moisture.

The plot and block design incorporated two major field experiments. Firstly, a cutting experiment where biomass cuts were made at nine specific dates throughout the growing season in 2010, 2011 and 2012. These cuts were timed to reflect a consistent increment in biomass for each cut (table 2.3). On each cutting date, the treatment plots (P+) and the control plots (P0) were cut, in order to examine the difference in biomass and vegetation composition between the two treatment groups. Secondly, a factorial experiment was incorporated into the block design, in order to investigate the effects of N, N and P together, and P at a higher dose. Two of the plots within each block were also cut twice in order to investigate the effects on the vegetation composition of greater nutrient removal. The first two chapters of this thesis include results from the cutting experiment only. The factorial experiment will be described in more detail in chapters four and five.



1	2	3	4	5	6	7	8	9	10	11	12
C9 P0	2 cuts P+	C6 P0	N+P0	C2 P+	C9 P+	C4 P0	C8 P+	C8 P0	C3 P+	C6 P+	C1 P0
C4 P+	C2 P0	P2 C2	C5 P0	2 cuts P0	C7 P0	P2 C1	C1 P+	C7 P+	C5 P+	C3 P0	N+P+
13	14	15	16	17	18	19	20	21	22	23	24

Figure 2.11 Treatment block consisting of 24 randomly allocated plots.

				Per plot 2 x 2 m (4m^2)			
Treatment	P/N form	Annual dose (kg ha^{-1})	Annual dose g/m^2	Water (L)	g of N/P per year	g per application	g of chemical per application (Ammonium nitrate or sodium dihydrogen phosphate)
P0	Control	0	0	1	0	0.0	0
P+	NaH_2PO_4	25	2.5	1	10	3.3	12.91
N+	NH_4NO_3	50	5	1	20	6.7	19.05
P2	NaH_2PO_4	75	7.5	1	30	10.0	38.73

Table 2.2 P and N addition treatments per plot and per dose.

2.2.3 Cutting treatments

In 2010 and 2011, above ground biomass cuts were made on nine dates throughout the growing season (table 2.3). The cut vegetation was split into functional groups (graminoids, forbs and legumes) per plot, oven-dried and weighed to establish the dry weight biomass, before testing for N and P to obtain the nutrient content of each functional group over time. Each 1 m^2 area in the centre of a 2 x 2 m plot was cut to 4 cm above the ground surface using electric clippers, this being the average cutting height of most conventional cutter bar and agricultural mowers (Tallowin & Jefferson, 1999), after which the rest of the remaining 'buffer' area of the plot was cut to the same height using electric and petrol strimmers. In 2012, cuts were repeated at similar dates, but due to access problems caused by prolonged flooding, some dates were modified. In 2013, the final year of the experiment, only the factorial plots were cut in order to obtain difference in biomass values between treatment N and P treatment plots (see chapter 4 for further detail).

	Cut date 2010	Cut date 2011	Cut date 2012
C1	26-Apr-10	27-Apr-11	18-May-12
C2	10-May-10	13-May-11	19-May-12
C3	24-May-10	28-May-11	25-May-12
C4	07-Jun-10	08-Jun-11	07-Jun-12
C5	14-Jun-10	16-Jun-11	21-Jun-12
C6	21-Jun-10	22-Jun-11	26-Jun-12
C7	28-Jun-10	28-Jun-11	05-Jul-12
C8	12-Jul-10	14-Jul-11	19-Jul-12
C9	26-Jul-10	26-Jul-11	02-Aug-12

Table 2.3 Cutting date for each of the nine plots in 2010, 2011 and 2012

2.2.4 Vegetation sampling and nutrient analysis

The 1 m^2 biomass samples collected from each plot were stored at -14°C (which preserves the nutrient concentrations of vegetation samples, R. Bobbink, personal communication), after which it was sorted into functional groups (graminoids, forbs and legumes) and dried at 55°C for 3 days. The dry weight of the total biomass and of each functional group was then measured. The vegetation samples were then ground to less than 1 mm (Retsch ZM200 centrifugal mill) and analysed for P and K using a dry ashing method modified from Chapman & Pratt (1961) followed by acidification with 1% HNO_3 and analysis with ICP-AES (Leeman Prodigy). Total C and N were determined using the LECO-2000® Elemental Auto-analyser.

Species cover-abundance was obtained for the P2 plots, N plots and C6 plots in the first two years of the experiment (6 plots per block, 30 plots in total). All 120 plots were surveyed in this way in the fourth and final year of the experiment in 2013. Relative abundance was obtained using a visual scoring method for the percentage vegetation cover of all species present in the 1m^2 sampling plot. Species dominance data were also obtained in these same plots using a vertical point quadrat to record 'first hit' at 50 points per plot, in 2010. This method was not used in subsequent years as correlation between cover and point quadrat values were high enough to justify use of one method only.

Photograph 2.1 2 m x 2 m plots within a block. Cut 29/06/2010.

Photograph 2.2 1 x 1 m plot cut for biomass in June 2013

2.2.5 Water Table Monitoring

Soil-water levels were monitored from November 2010 to July 2013, using three automated pressure-transducing loggers known as ‘divers’ (Divers, Eijkelkamp NL), which were installed between blocks one and two, blocks three and four, and blocks four and five. The wells were 120 cm deep and 5 cm diameter, dug using a Dutch Auger. Each diver was enclosed within the well of perforated plastic pipe, and fitted with a cap to prevent direct entry of surface water, precipitation and debris. Each diver recorded the pressure and temperature within the well at three hour intervals. A barometer placed nearby the site was used to record atmospheric pressure in order to calibrate each diver to give a water-table depth. Each diver was downloaded every year onto a handheld data storage device. Diver-Office (Schlumberger Water Services) was then used to combine the diver data and barometer data to give a water-table depth.

2.2.6 Data analyses

The above-ground biomass data were natural log transformed to achieve normality and homogeneity of variance. Then the data were analysed using repeated measures ANOVA to determine if changes in biomass levels between plots receiving P (P+) and controls (P0) changed differently over time throughout the growing seasons. Statistical differences between biomass data, offtake N and tissue content data from P0 and P+ plots within each year were determined using General Linear Models in SPSS 21 using P treatment, block and year as independent variables.

2.2.7 Cumulative dry weight and growing day degrees

Cumulative growing degree days (CGDD) were calculated for each year of the experiment (figure 2.12) using the following equation (McMaster & Wilhelm 1997). The base temperature used was 4°C:

Equation 1

$$GDD = \frac{T_{\mathrm{max}} + T_{\mathrm{min}}}{2} - T_{\mathrm{base}}$$

A mean for each cut date was calculated and then plotted against the mean dry weight of each cut (C1, C2 etc.), in plots which received P and the controls. A one way ANOVA showed no significant difference in the cumulative dry matter yield (incorporating 2010, 2011 and 2012 figure 2.19) between the plots receiving P and the control plots. CGDD was used rather than a mean cutting date for the three years to take into account the difference in temperature between the three years and eradicating any difference in dry matter yield caused by the seasonality.

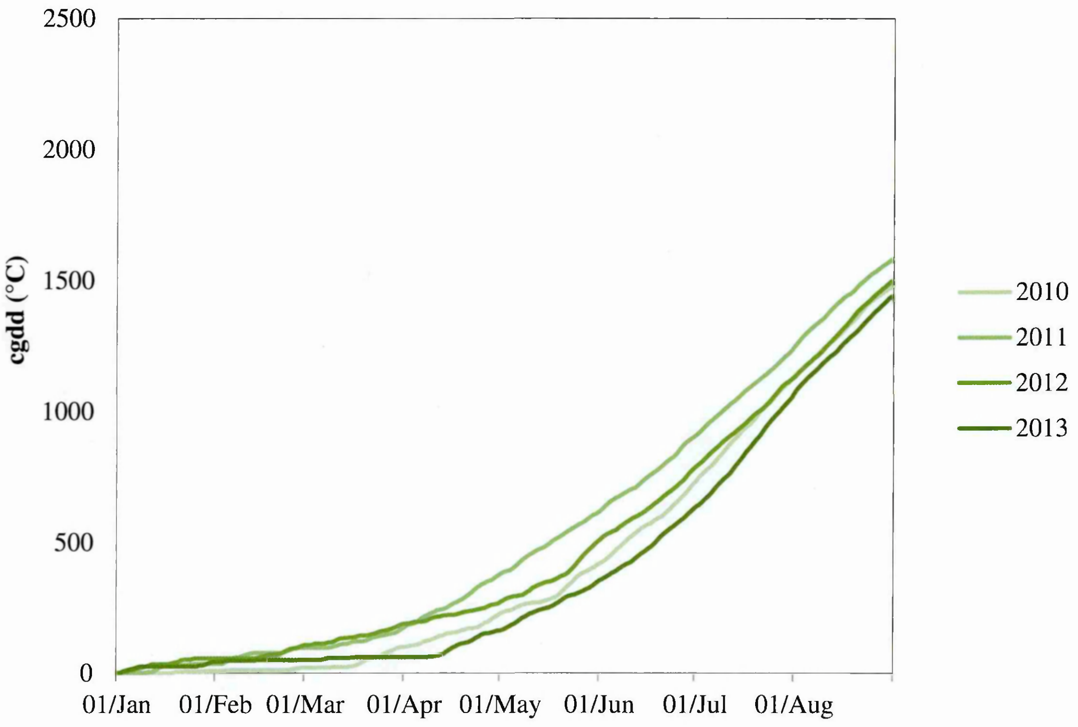


Figure 2.12 Cumulative growing day degrees for 2010, 2011, 2012 and 2013

2.28 Smith Calculation

Soil is at field capacity when it is holding the maximum amount of water against the pull of gravity. When it starts to dry, it is said to be below capacity; the quantity of water needed to bring the soil back to field capacity is known as the soil moisture deficit. Large soil moisture deficits lead to wilting plants and the cessation of transpiration. Actual transpiration has been calculated from potential evapotranspiration figures (obtained from the MET office). For calculation purposes, Smith (1960) assumed that the soil was at field capacity at the start of the growing season each year. If the rainfall exceeds transpiration, then the soil remains at field capacity and the actual transpiration (AT) is equal to potential evapotranspiration (ET); if the rainfall is less than ET, a potential soil moisture deficit develops. In this way using data from 2010 to 2013, actual transpiration has been calculated (equation 2).

Sum of AT has also been calculated for each yield harvest date (C1-C9) and then plotted against yield for each year. Incorporating meteorological data in order to predict yield has been used as per Smith (1960) using the following equation. T represents the estimated actual transpiration and N is the number of years since 1946.

Equation 2

$$\text{Calculated Yield} = 7.05 + 1.56T + 0.27N$$

2.3 Results

2.3.1 Dry weight through the growing season (2010, 2011, 2012)

There were nine harvests in each year timed from April until August. Each cut was performed at the same time or as close as possible to a set date in each year, with the exception of 2012 when summer flooding prevented access to the site.

Overall 2012 was the most productive year with a mean total above-ground biomass for all plots (C1-C9) of 2682.4 g/m^2, compared to 1976.6 g/m^2 in 2010 and 1547.5 g/m^2 in 2011 which is significant ($p<0.05$) (figure 2.13). Maximum above-ground biomass occurred in mid July in 2010, the end of June in 2011 and the end of July in 2012. No cuts were made in August due to site restrictions.

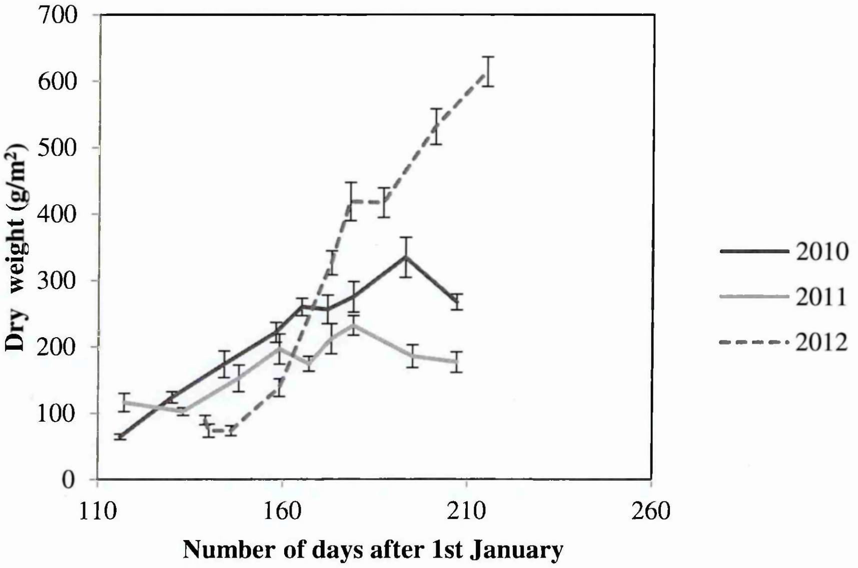


Figure 2.13 Mean and standard error dry weight per cut in 2010, 2011, 2012 on days since 1st January in each year. Values represent mean of P+ and P0 treatments combined for each cut.

Aboveground biomass levels increased through the growing season in 2010 for both control plots (P0) and treatment plots (P+), with maximum biomass occurring in mid-July for both groups, and then decreasing in the C9 plots in the final cut (figure 2.14). Biomass was greater in the plots receiving P, with this trend become more obvious from cut 5 in June, although this was not statistically significant. The greatest difference in total biomass between the treatment plots and controls was in the C8 cuts on 14th July 2010. Aboveground biomass in 2011 was much more erratic (figure 2.15), especially in the control plots, and was a less productive year compared with 2010. The biomass between treatments and controls was also not significant for 2011. Above-ground biomass levels between the treatment and control plots was not significant in 2012 either (figure 2.16), however there was a steadier increase throughout the growing season, with a small plateau in June. P+ plots were slightly more productive than the controls, except for the final C9 cut. There was no block effect in 2010 or 2012, but block was significant for dry weight in 2011.

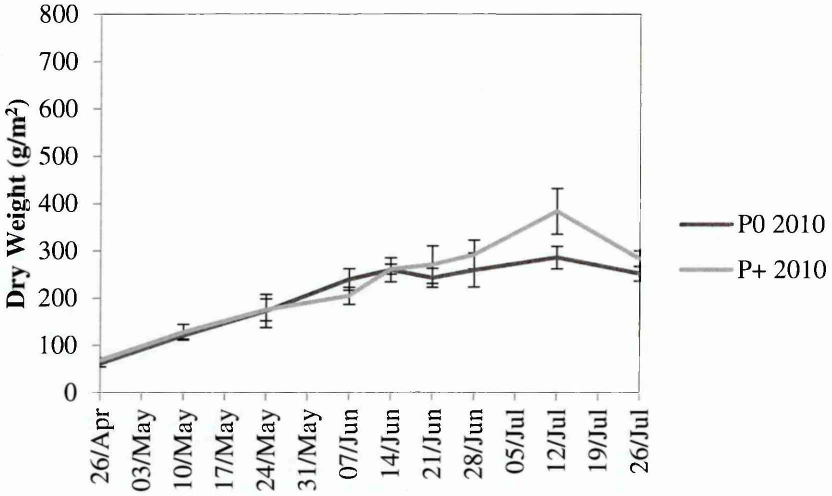


Figure 2.14 Mean and standard error 2010 above-ground biomass dry weight from each cut for treatment plots and control plots

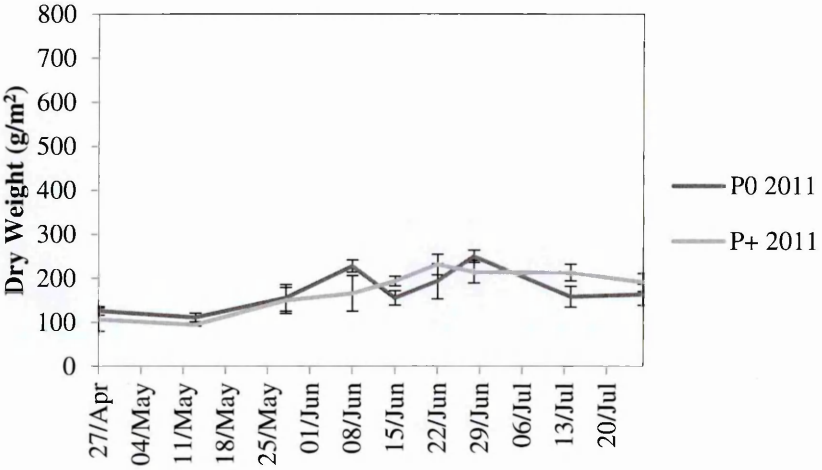


Figure 2.15 Mean and standard error 2011 above-ground biomass dry weight from each cut for treatment plots and control plots

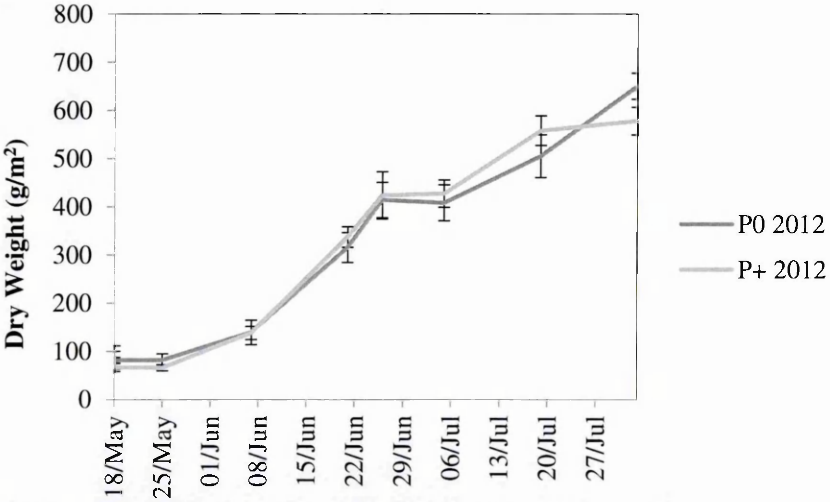


Figure 2.16 Mean and standard error 2012 above-ground biomass dry weight from each cut for treatment plots and controls

Water-table depth was monitored from November 2010 until the end of the experiment (see appendices for raw output from divers). Output from divers is in the form of pressure which has to be converted into water-table depth by compensating raw data with atmospheric pressure, and then subtracting elevation obtained by using the GPS Leica RX1200 for each plot. The mean water-table depth between each year was significantly different ($p<0.001$); with 2012 being the wettest year (figure 2.17). Within years, the water-table depth was significantly different between all blocks in 2010 with the exception of block 1 and block 3. Water-table depth was significantly different between all blocks in 2011 with the exception of block 1 and block 5. Water-table depth was significantly different between all blocks in 2012. According to figure 2.17, block 3 represents the wettest block and block 2 the driest. Negative values do not represent permanently wet plots, as the values used are mean water-table depths.

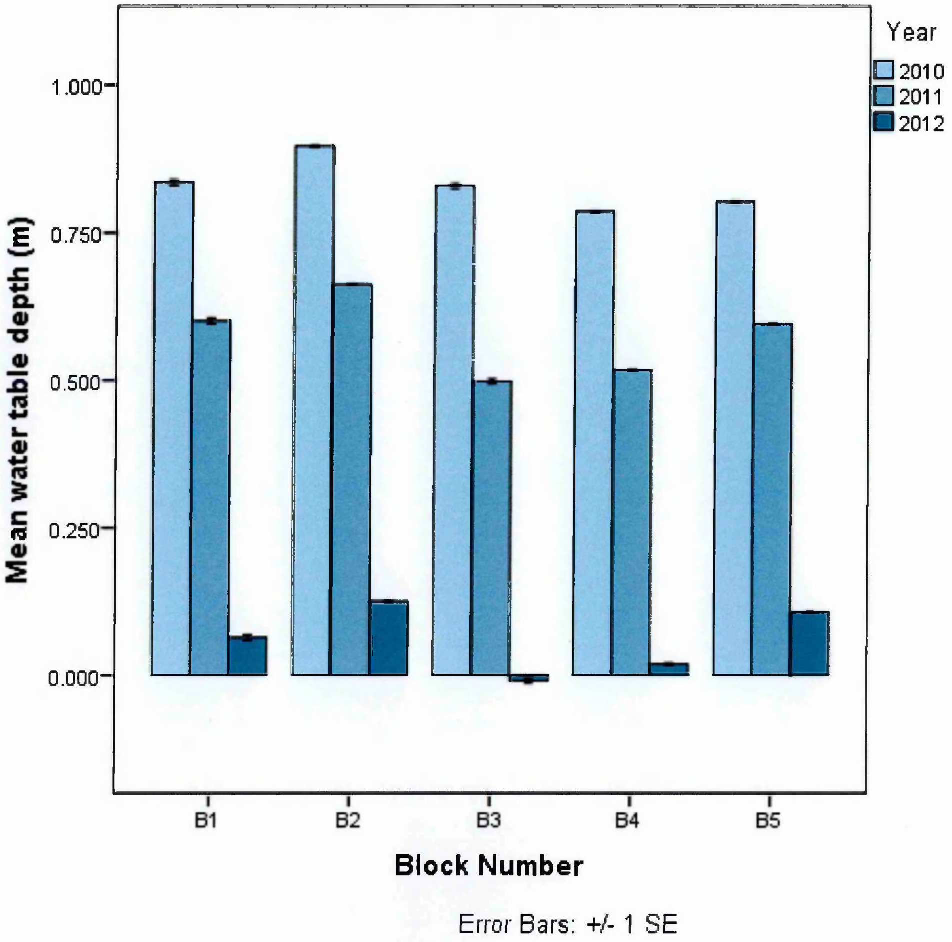


Figure 2.17 Mean and standard error water-table depth per block, per year. 0.00 represents ground level, positive values represent below ground and negative values represent above ground i.e. flood events. NB 2010 represents only data from November and December divers

Water-table depth was built into the general linear model for testing significance of above-ground biomass values between treatments, and used as a covariate in the analysis. Although, this made no difference to the outcome in terms of significance in dry weight between treatments.

Calculated growing day degrees (cgdd) was used instead of mean cutting date to take into account the difference in temperature between the three years and to eradicate any difference in dry matter yield caused by the seasonality (figure 2.18). However, regardless of cutting date and cgdd, there was still no significant difference between the yield of plots receiving P and the controls (p=0.730) (figure 2.19).

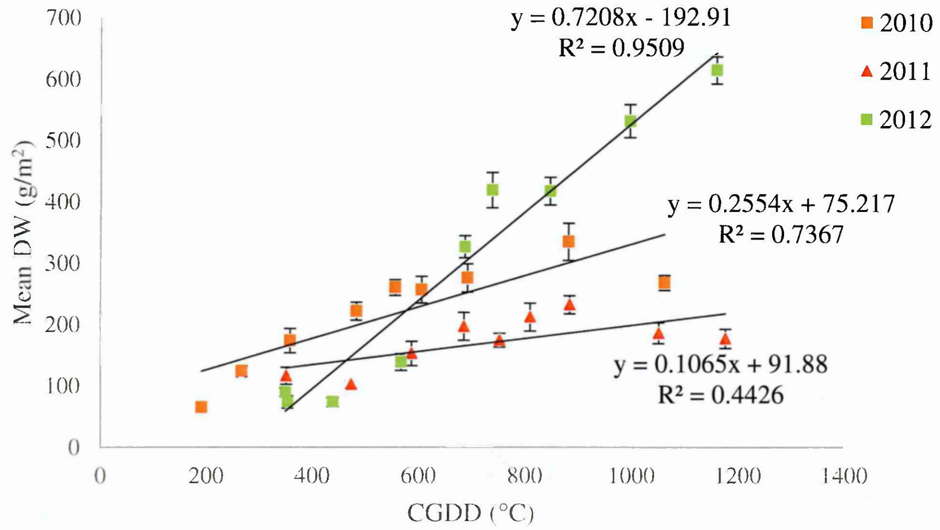


Figure 2.18 Mean and standard error DW per cut per year against cumulative growing day degrees for 2010, 2011 and 2012

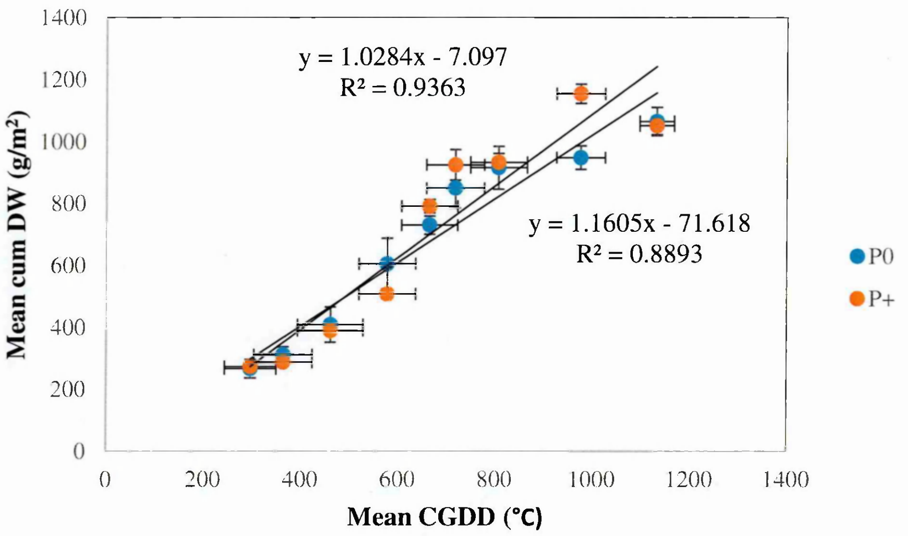


Figure 2.19 Mean and standard error cumulative dry weight (sum of dry weight for 2010, 2011 and 2012) against mean cumulative growing day degrees for P0 and P+ plots combining all experimental years

The mean error between calculated yield and actual yield according to Smith (1960) was 2.1% with a correlation coefficient of 0.95 when total annual yield was plotted for each year between 1940 and 1955. Applying the same equation to yields obtained for 2010, 2011, 2012 and 2013 produced a greater mean error, with actual yields exceeding that of calculated yield (figure 2.20). Mean percentage error between actual and calculated yield including 2013 was 10.8% (figure 2.20). The Pearson correlation between calculated and actual yield for all years was 0.86.

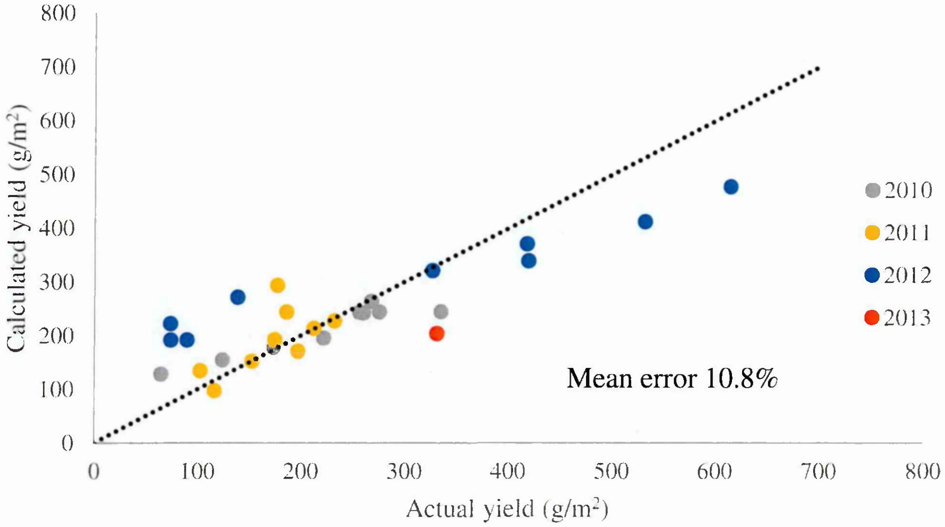


Figure 2.20 Actual yield and calculated yield for each experimental year

2.3.3 Plant tissue nutrient content and offtake.

Total nitrogen percentage (N %) and total phosphorus percentage (P %) in the biomass samples from each cut decreased gradually through the duration of the growing season (figure 2.21 and 2.22). There was a significant difference in P% between treatment plots and control plots, but not for N%. The N% and P% values were used with the total biomass DW from each cut to produce an offtake value for each plot (kg ha^{-1} y $^{-1}$) (figure 2.24 and 2.25).

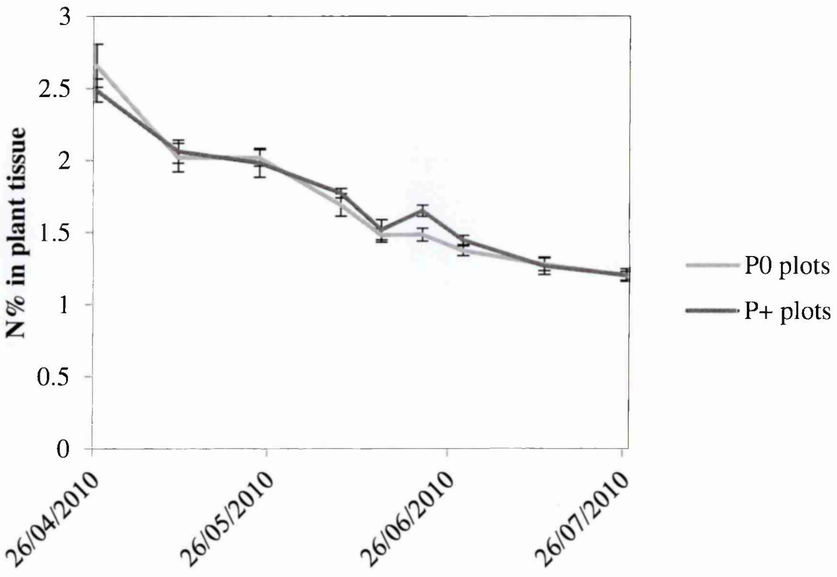


Figure 2.21 Mean and standard error N% in above-ground biomass samples through the season in P+ and P0 plots in 2010

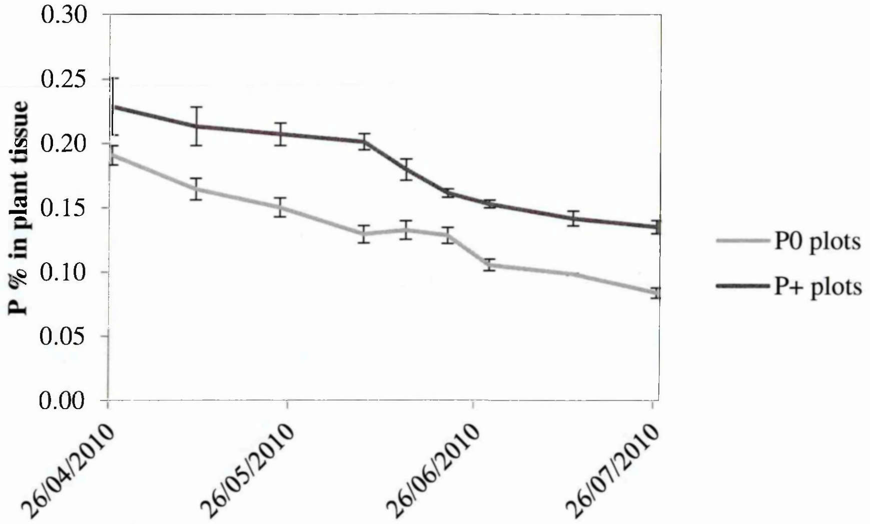


Figure 2.22 Mean and standard error P% in above-ground biomass samples through the season in P+ and P0 plots in 2010

Mean N and P offtake for each cut has been plotted for the growing season of 2010 (figures 2.24 and 2.25). Both N and P offtake were greater overall in the treatment plots. This is significant for P offtake overall (one way ANOVA between groups, $p<0.001$) and post hoc significant differences between treatment and control for cuts C5, C7, C8 and C9. There was no significant difference between treatments for N offtake. N offtake peaked in the P0 plots in early June (C4), and in the P+ plots in the middle of July (C8). P offtake peaked in the P0 plots in the middle of June (C5) and in the P+ plots in the middle of July (C8). The N:P ratio of the vegetation biomass from each plot was calculated (figure 2.23) and was significantly different between treatment and control plots ($p<0.001$), in every cut except for C1, N:P ratio was greater in the control plots than the treatment plots. The mean N:P ratio for the controls was 12.7, and for the P+ plots 9.7 which was significantly different ($p<0.01$).

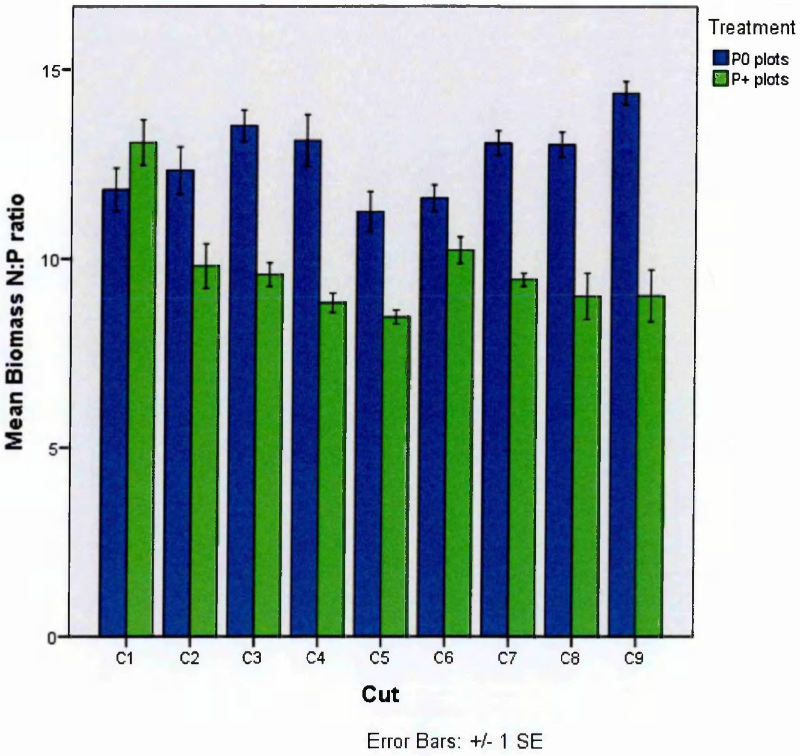


Figure 2.23 Mean and standard error biomass N:P ratio for each cut for P+ and P0 plots

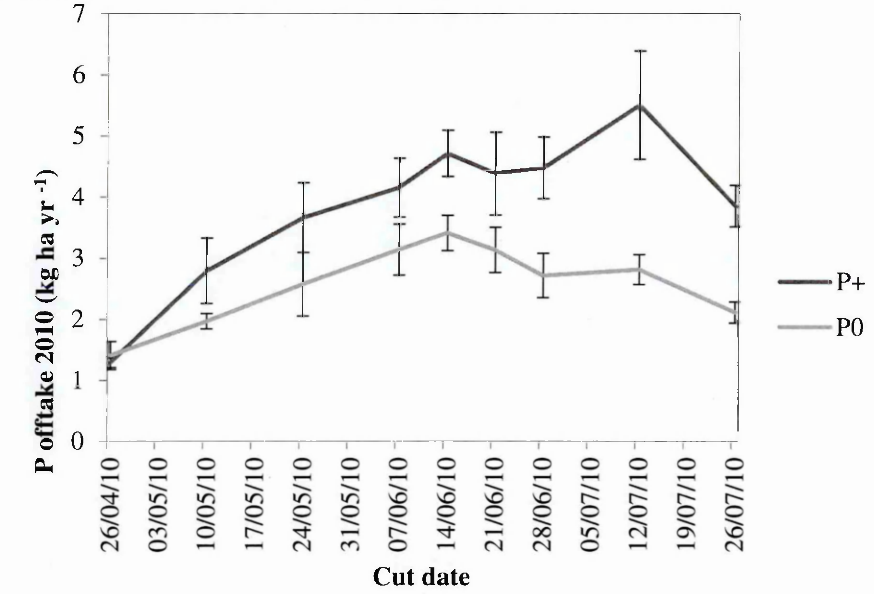


Figure 2.24 Mean and standard error 2010 P offtake per cut in P+ and P0 plots

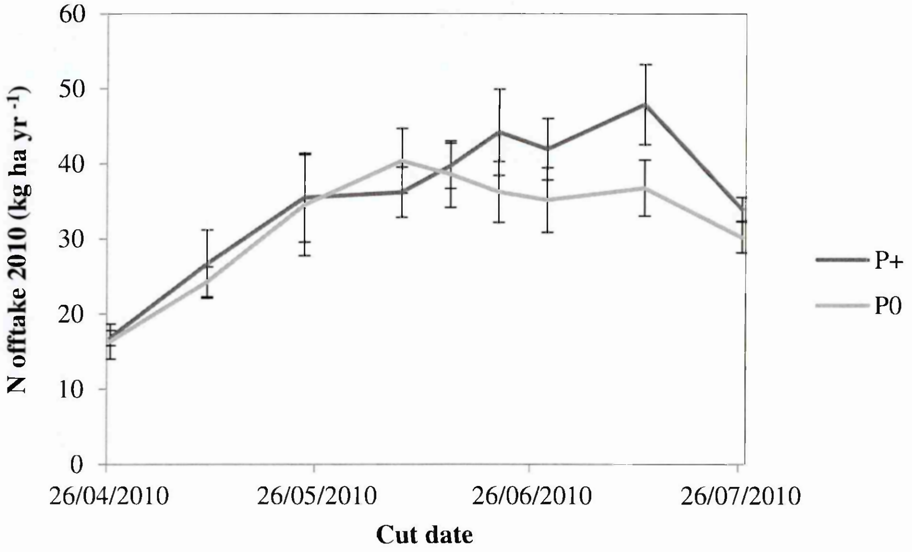


Figure 2.25 Mean and standard error 2010 N offtake per cut in P+ and P0 plots

2.4 Discussion

2.4.1 Above-ground biomass yield

Floodplain meadows containing the MG4 community can be productive (Gowing *et al.* 2002). The yield achieved from plots cut in this experiment was similar to that of other MG4 sites within the UK. Plots cut in July in this experiment yielded the equivalent range from 2319 kg ha^{-1} y^{-1} to 4172 kg ha^{-1} y^{-1} of hay, 2011 being the least productive year and 2012 being the most productive year (almost double the hay yield of 2011 from the same plots). These figures are similar to other UK MG4 sites such as Cricklade North meadow, which had a mean hay yield over several years data of 3617 kg ha^{-1} y^{-1} (David Massen, English Nature pers comm), Mottey meadow which had a mean yield of 4600 kg ha^{-1} y^{-1} (Tim Coleshaw, English Nature pers comm) and the River Ray, Oxon which had a yield ranging from 3700-5000 kg ha^{-1} y^{-1} (R Lambourne pers comm) (Gowing *et al.* 2002). The variability in yield data achieved from the same plots in different years of this experiment is very likely to be related to the variation in rainfall. 2012 was the most productive year, and it was also the wettest receiving 892.4 mm of rainfall almost double that of 467.6 mm in 2011. Therefore in this case, the productivity of the vegetation in 2010 and 2011 was very likely to be limited by the very dry conditions in the growing seasons of 2010 and 2011. This is reinforced by plotting dry weight against calculated growing day degrees for each year, unsurprisingly 2012 exhibited a much higher R^2 than either 2010 or 2011 and a higher Pearson correlation co-efficient, even without taking rainfall into account. This demonstrates that above-ground production was highly correlated with temperature in 2012 (r=0.98).

The cutting dates spread throughout the growing season in this experiment, aimed to show the optimal time to cut a hay meadow in terms of removing the most nutrients from the system. A secondary aim to this experiment was to investigate the effect of P addition on the pattern of nutrient removal, namely to see if peak nutrient removal was brought forward by the addition of P alleviating limitation on plant growth. The addition of P made no significant difference to the above-ground biomass in any year of this experiment, although notable trends were that P did increase above-ground biomass in 2010 and 2012, and that peak above-ground biomass occurred at the same hay cut whether P was added or not. For 2010 this was around 12th July and 22nd-29th June in 2011, biomass continued to increase beyond the end of July in 2012. Therefore, in this case P appears not to be limiting above-ground production despite often being reported as a limiting nutrient of species-rich grassland (Janssens *et al.* 1998; Critchley *et al.* 2002; Wassen *et al.* 2005).

The N:P ratios reported in this experiment lie between 7 and 14; P plots had a mean of 9.7, and P0 plots had a mean of 12.7. Whilst significantly different, both values lie well below 14 and therefore according to Koerselman & Meuleman (1996), they are indicative of N limitation. Those authors suggested communities with N:P ratios above 16 would be P limited, whereas those falling below 14 would be N limited, and those lying between the two would be co-limited. This suggests that the findings from this experimental study site are in fact either N limited or co-limited, so the addition of N rather than P in this case would be more likely to increase vegetation productivity. This is contrary to the findings of other studies where P was found to increase productivity of grasslands (Kirkham, Mountford & Wilkins 1996; Harpole & Tilman 2007; Hejcman *et al.* 2007), although this is not necessarily the case in systems limited solely by nitrogen (Venterink *et al.* 2006; Semmartin *et al.* 2007). In 2012, above-ground biomass kept increasing steadily throughout the growing season, whereas in 2010 it reached its peak around 12th July (Cut 8) and around 28th June in 2011. Both 2010 and 2011 were exceptionally dry years and therefore plant growth was severely limited by moisture deficit. This may have been responsible for the huge variation in yield between plots in 2011 as species favouring wetter climatic conditions were unable to thrive, hence plant growth was restricted regardless of nutrient input. The variation in water-table depth between years is also compatible with meteorological data, with 2012 having a much shallower water table than 2010 or 2011. The variation in water-table depth between blocks is significantly different, which could lead to a variation in plant community and the subsequent dry weights of each plot, depending on the baseline existing plant community in each plot, prior to the start of the experiment and treatment additions. Block and water-table depth were built into the general linear model when testing for significant difference between treatment plots, although made no difference to the outcome.

In order to remove some of the variability between years, suspected to be due to differences in rainfall, the 'Smith' calculation was employed (Smith 1960; see equation 2). His paper states that yield can be predicted based on a calculation taking rainfall, sum of AT and soil moisture deficit into account. This theory was applied to meteorological data obtained for Leaches Meadow in order to explain the variability in productivity between years. Calculated yield was then plotted against actual yield for each year, and for each plot cut within each year. When initially plotted, the calculated yield severely overestimated the actual yield achieved, therefore the equation was adjusted to remove '*N*', which represented number of years since 1946, as it is unlikely the author of the paper anticipated its use 60 years into the future. Following the alteration, the Smith calculation appears to only slightly underestimate actual yield, with a mean error resulting in 10.8%, much higher than 2.1% that the paper suggests. Although there is still a linear relationship between actual and calculated yield, an R^2 of 0.9448, and a strong positive correlation of 0.86. This suggests there is definite relevance to using the Smith calculation when predicting yield in hay meadows, but it should be used with caution and to identify an estimate of yield rather than to predict accurate yields. Furthermore, the Smith calculation does not take water-table depth into account when predicting yield, which may account for the percentage error difference between the results of this study and the published work in Smith (1960). Including water-table depth into the equation would involve water table modelling, and would be site-specific rather than regional, as suggested by Smith, which could also account for some of the error. It may also be the case that Smith had not accounted for atmospheric N deposition, which would result in greater actual yield than calculated yield, especially if the site is N limited, as suspected.

2.4.2 Plant tissue nutrient content and offtake

Both P% and N% in plant biomass reduced gradually through the growing season, giving rise to less nutrient rich hay in the July cuts compared with the plots cut in May. This is similar to the findings of other studies where nitrogen content in herbage falls at a similar rate in both species-rich unimproved sites, and fertilized grasslands (Gately, Ryan & Doyle 1972; Tallowin 1997). This is due to a dilution effect where N and P are diluted by the increased growth that occurs in the vegetation later on in the season (the ‘Steenbbjerg effect’ Weetman 1989), and the tendency of grass to form seedheads in midseason with a consequent lower N content (Gately, Ryan & Doyle 1972). Gately, Ryan & Doyle (1972) also reported a ‘late season effect’ where higher tissue N content was found in some of the sites in October, which was attributed to the slowing in growth rate of the vegetation, and lower light intensity reducing nitrate reductase activity and promoting nitrate accumulation.

With the addition of P, plant tissue N% did not show any increase or significant difference from the control plots. However, as expected with the addition of P, plant P% was significantly different from the controls indicating that the plants were taking up the additional P supplied to them, but not using it for growth. This suggests luxury consumption of P by the vegetation (Chapin 1980). To calculate nutrient offtake for both P and N, the above-ground biomass yields were multiplied by the tissue N and P percentage. Again, the addition of P was not significant in increasing N offtake, although P offtake was significantly increased. This was driven by the increase in tissue P percentage taken up by vegetation in the treatment plots. However, there was a trend in increased N offtake with the addition of P, just not significantly. Peak N offtake in the control plots was on 7th June in 2010 (cut C4), but on 12th July (C8) for the treatment plots, and for P offtake peak was on 14th June (C5) for control plots, and 12th July (C8) for treatment plots. Therefore, hay cut earlier in the growing season is more nutrient rich, but greater nutrients are actually removed from the system if the hay cut is left until late June or early July. The results also suggest that sites with elevated soil P or soil N can be cut slightly later (second week in July) in the season to achieve maximum nutrient removal if desired. This was driven by above-ground biomass data, which increased in P+ plots in 2010 compared to the controls for every cut date after 21st June, therefore later in the season. The nutrient additions were not complete until the end of May in 2010, so the vegetation response to the available nutrients may have been delayed in this case.

The response of the vegetation to P, could arise from the trade-offs associated with P acquisition. N is required to produce phosphatases, which are released by plants into the soil to increase P availability, therefore P limited plants could increase P uptake with increases in P availability or N availability, because in turn increasing N availability could allow plants to produce more phosphatases. This implies that P limited systems could also respond to N deposition in terms of increased productivity (Treseder & Vitousek 2001). Alternatively, low P availability might inhibit soil microbial activity which breaks down soil organic matter to release available N (Craine & Jackson 2010). However in the case of this experiment, N offtake (and assumed N uptake by plants) was not increased significantly by the addition of P and made no significant difference to productivity, which would certainly be the case if microbes were stimulated by the addition of P alone to increase N supply. Chapter 4 investigates the addition of P and N in more detail, by means of a factorial experiment and the subsequent response of the vegetation in terms of productivity and nutrient offtake.

2.5 Conclusion

The effect of P addition on the hay yield of floodplain meadow vegetation from this study site was minimal. Although there were slight increases in the yield mid-way through the growing season in 2010 and 2011, this increase was not significant. Off-take N was also increased with P addition in 2010, but again this was not significant. Changes in the offtake of N and P were driven by the variation in above-ground biomass in 2010, and the limitation of the site by reduced rainfall and water-table depth. Had biomass not been limited by the dry conditions in the growing season of 2010 (and 2011), the difference in nutrient offtake may have been significant with the addition of P. However, it is very likely that the site vegetation biomass is N limited, and therefore would respond only to nitrogen fertilization or a combination of N and P together. This is tested in chapter 4. Peak nutrient offtake occurred later in the growing season with the addition of P, for both P offtake and N offtake. Optimum cutting date in terms of maximum nutrient removal was therefore mid-June for unfertilized plots, and mid-July for P fertilized plots, although in a year with more average rainfall conditions, optimum cutting date would probably be slightly later as the vegetation productivity would not be limited by dry conditions. Cutting date on this type of vegetation should therefore be flexible and based on a combination of factors. This experiment also re-iterated the findings of Smith (1960), that hay yield can be predicted by using meteorological data and soil-moisture deficits, but must be used with caution, especially in years of atypical rainfall.

Chapter 3

The effect of cutting date on the botanical composition of floodplain meadows

3.1 Introduction

Regular management of wet grasslands and floodplain meadows favours species-rich, low-growing herbaceous vegetation which support large populations of invertebrates and ground nesting birds, which are important for nature conservation (Joyce & Wade 1998). Species rich wet grasslands are one of the most threatened types of habitat across Europe including the UK, and a recent decline in the last century is likely to have been a result of changes in management regimes, such as agricultural intensification or abandonment (Tallowin *et al.* 1994; Tallowin & Jefferson 1999; Berg, Joyce & Burnside 2012). These habitats are sensitive to changes in hydrological regime, management practices and nutrient inputs which can result in a change in community composition and reduction in species richness. Typically, floodplain meadows in the UK which have an MG4 NVC community (*Alopecurus pratensis- Sanguisorba officinalis*) are very species rich in terms of their plant communities with up to 39 species per m^2 (Gowing *et al.* 2002), which is under threat if these habitats are not managed appropriately. It has been suggested that species-rich communities have a greater capability to buffer environmental variability than species-poor communities, and that increased biodiversity gives rise to greater resistance against ecological disturbance, (Naeem *et al.* 1994, 2000; Tilman 1999; Foster *et al.* 2002).

3.1.1 Cutting and abandonment

Abandonment or neglect of floodplain meadows can lead to more competitive tussock-forming species of larger stature, competing for light and forming dense canopies which displace smaller species (Bobbink & Willems 1991; Hellstrom *et al.* 2006). Abandonment issues on floodplain meadows and grasslands are becoming more common due to changing economic climate within Europe and uncertainty of funding or ownership. Throughout Europe approximately 15 million hectares of formerly managed grassland have been abandoned, threatening biodiversity and longevity of these important ecosystems (Joyce & Wade 1998) and an urgent need to implement restoration.

Mowing is the most commonly applied technique to restore species richness in grasslands which have been subject to nutrient enrichment or abandonment (Grootjans *et al.* 2002). Taller graminoids and more robust plants are removed, allowing smaller herbs to compete for light and become established (Bobbink & Willems 1991; Mountford, Lakhani & Kirkham 1993). Cutting is preferred to grazing when restoring abandoned grasslands as it induces a decrease in nutrients within the biomass more quickly than grazing, which returns nutrients back to the soil through livestock excreta (Kayser & Isselstein 2005). Grazing also encourages growth of legumes such as *Trifolium repens* which contributes to N input through N_2 fixation (Pavlu *et al.* 2007). A few recent studies have investigated the effect of reinstating cutting on abandoned grasslands or wet meadows: Billeter, Peintinger & Diemer (2007) investigated restoration of abandoned fen meadows in Switzerland and whether reinstated mowing techniques after 4-35 years of abandonment could increase species richness. They found that after two years of mowing, plant species richness was 11% higher in mown plots than in unmanaged plots, and specifically fen indicator species and herbs were increased, whilst graminoids were unaffected. Similarly Huhta *et al.* (2001) investigated the timing and number of cuts within a year to assess the effect on the vegetation of two abandoned meadows. They surveyed changes in plant cover and species composition of permanent plots in a formerly grazed meadow over a period of six years, in plots that were mown and unmown. They found that abandonment led to a decrease in the cover of small forbs, a decrease in the cover of grasses and an increase in the cover of tall forbs. The mown plots showed little change with respect to the cover of small herbs. This study shows that mowing late in the season can be used as a management tool for the maintenance of existing species composition and diversity, but not necessarily the control of abandoned grass-dominated grasslands.

3.1.3 Cutting frequency

Increasing the number of cuts to a grassland or floodplain within the same growing season could potentially lead to a decrease in grassland productivity (Vinther 2006) in favour of species richness. This is due to the concentration of nutrients being higher in the above-ground biomass at the start of the growing season (April-May) than later on (July-August) (Kirkham & Tallowin 1995; Pontes *et al.* 2007) hence more potential for nutrient removal with increasing the cutting frequency throughout the growing season. Increasing the number of cuts within a growing season has been investigated for restoration purposes after the cessation of fertilizer application (Bakker, Elzinga & de Vries 2002; Hejcman, Schellberg & Pavlu 2010), finding that increasing the number of cuts made little or no difference to species richness in these grasslands, but has affected species composition overall with some species being more tolerant to defoliation and gaining a competitive advantage over others.

3.1.4 Cutting and hydrological regime

Hydrological regime is considered to be one of the most important factors in determining the vegetation composition of floodplain meadows (Gowing & Spoor 1998; Grevilliot, Krebs & Muller 1998; Casanova & Brock 2000; van Eck *et al.* 2004). Flooding is one of the major sources of nutrient inputs (particularly P), whereas mowing is the predominate method of nutrient export. Investigating cutting regime in conjunction with hydrological regime would determine the nutrient mitigation potential of hay removal in terms of species composition and species richness. A study by Gerard *et al.* (2008) explored the interaction between flood frequency and mowing in determining species composition of temperate lowland floodplain meadows in Belgium. They compared the composition of annually mown and non-mown vegetation within floodplain sites of differing flood frequencies and found that mowing was more important than flood regime in maintaining higher numbers of smaller species. Non-mown sites supported higher numbers of taller graminoid species. The combination of frequent flooding with annual mowing increased species richness attributed to the fact that mowing provides gaps for germination of flood imported seeds.

Similarly, Berg, Joyce & Burnside (2012) studied the effect of reinstating mowing on two abandoned coastal wet grasslands in Estonia to assess their restoration potential. One site was a species-poor lower shore grassland, the other a more diverse tall grassland. They found that the lower shore community responded more positively to mowing than the tall grassland community, and produced greater changes in composition and species abundance. The sites differed in hydrological regime, with the taller grassland being more stable in terms of water regime, which suggests that hydrological regimes should also be considered when considering restoration of wet grasslands.

3.1.5 Cutting effects on species composition

In terms of individual species response to cutting, *Phleum pratense* and *Agrostis capillaris* have been found to increase in abundance with later cutting dates, and *Anthoxanthum odoratum* was most abundant in plots cut in August, however *Ranunculus* *repens* showed a linear trend of declining abundance with date of cut (Kirkham & Tallowin 1995). Although Hellstrom *et al.* (2006) found that late cutting reduced the abundance of *Agrostis capillaris*. Cutting twice was found to control *Carex acuta* and *Carex acutiformis* on floodplain meadows, with a double cut within the growing season being more effective than the timing of the individual cuts (Newman 2013). When comparing cut and uncut swards rather than timing of cut, *Agrostis stolonifera* had a competitive advantage over other species and increased in abundance after five years of abandonment (therefore not favoured by cutting) (Berg, Joyce & Burnside 2012), and cutting stimulated the abundance of *Festuca arundinacea* and *Festuca rubra.* In contrast early cutting favoured *Poa pratensis, Holcus lanatus* and *Dactylis glomerata* with the majority of species favouring being cut in late July on an upland site (Smith *et al.* 2000).

This part of the overall experiment investigates the effect of cutting at different times in the growing season and cutting frequency on species richness and composition in floodplain meadows. To date, there have been very few studies investigating nutrient addition on floodplain meadow MG4 grassland, alongside a cutting experiment to investigate mitigation of the added nutrients in terms of species composition, on the same sward within the growing season. Most studies have concentrated on the effect of cutting and mowing frequency for restoration of grasslands following cessation of fertilizer application, and have been long-term experiments, rather than short-term mitigation experiments for nature conservation. In addition to the timing of the hay cut, the frequency of cutting will also be investigated by cutting certain permanent plots twice; once in May and once in August. There were nine different cutting dates, and therefore ten different treatments (including the plots that were cut twice). This chapter addresses the following questions:

1. Does altering the cutting date of MG4 vegetation change the species composition? (Page 73-75)
2. Does altering the cutting date change the abundance of individual species? (Page 76-78)
3. Which environmental variables are significant in explaining any variation in species composition? (Page 82-83)

3.2 Methods

3.2.1 Cutting treatments

Details of the experimental set up are given in Chapter 2. In 2010 and 2011, above-ground biomass cuts were made on nine dates throughout the growing season (table 3.1). The cut vegetation was split into groups (graminoids, forbs and legumes) per plot, oven dried and weighed to establish the dry weight biomass, before testing for N and P to obtain the nutrient content of each functional group over time (see Chapter 2). Each 1 m^2 area in the centre of a 2 x 2 m plot was cut to 4 cm above the ground surface using electric clippers, this being the average cutting height of most conventional cutter bar and agricultural mowers (Tallowin & Jefferson 1999), after which the rest of the remaining 'buffer' area of the plot was cut to the same height using electric and petrol strimmers. In 2012, cuts were made throughout the growing season, but at different times, due to the summer flooding and consequent difficulty in accessing and cutting the relevant plots. In 2013, the final year of the experiment, only the factorial plots were cut in order to obtain difference in biomass values between treatment N and P treatment plots (discussed in chapter 4).

	Cut date 2010	Cut date 2011	Cut date 2012
C1	26-Apr-10	27-Apr-11	18-May-12
C2	10-May-10	13-May-11	19-May-12
C3	24-May-10	28-May-11	25-May-12
C4	07-Jun-10	08-Jun-11	07-Jun-12
C5	14-Jun-10	16-Jun-11	21-Jun-12
C6	21-Jun-10	22-Jun-11	26-Jun-12
C7	28-Jun-10	28-Jun-11	05-Jul-12
C8	12-Jul-10	14-Jul-11	19-Jul-12
C9	26-Jul-10	26-Jul-11	02-Aug-12
Cut twice	14-Jun-10 and 02-Aug-10	16-Jun-11 and 02-Aug-11	Not cut

Table 3.1 Cutting date for each of the nine plots in 2010, 2011 and 2012

The plot design was the same as that described in Chapter 2, with five replicated treatment blocks, therefore each cutting treatment was repeated five times. Five controls and five treatments receiving P within each cut. The cut dates were deliberately timed to incorporate a hay cut for each week in June, and then two weeks interval either side of June to give rise to early, mid and late cutting dates in terms of when the hay cut is usually timed for wet grasslands. Growing day degrees were used to calculate when the first treatment cut should take place, the last treatment cut was planned for later in the season, however this had to be brought forward due to animals being brought onto the site to graze. One set of plots were cut twice in 2010 and 2011 (see table 3.1) in order to investigate the effects of a double cut on the vegetation community.

3.2.2 Vegetation survey

All treatment plots were surveyed between 21st and 29th June 2013. The percentage cover for each plant species present, as well as bare ground and litter were visually estimated. For each cut (C1-C9) there were five controls which received no P treatment, and five P treatment plots. No cutting regime was implemented in 2013 to allow the species survey to take place. Data for species richness, diversity indices and functional group percentage in this chapter are all taken from the vegetation survey data of 2013. The factorial treatment plots will be investigated in chapters 4 and 5.

3.2.3 Statistical analysis

Changes in the floristic composition between each treatment plot were described using species richness and abundance for each treatment plot. Multivariate analysis was performed using Canoco 4.5 (Braak & Smilauer 2002) including all the species and without down-weighting. Detrended Correspondence Analysis (DCA) was used to provide a summary of the variation and plant community dynamics, and Redundancy Analysis (RDA) was used to test treatment effects on plant community composition. Community diversity was assessed using species richness and the Shannon-Wiener diversity and evenness indices. Significance between treatment groups and cut was assessed using General Linear Models (GLM), incorporating analysis of variance with Tukey post-hoc tests to detect any significant differences between treatment groups, with significance set at $p < 0.05$. Differences in percentage cover values between species was assessed using General Linear Model multivariate analysis in SPSS 21. Water-table depth was used as a co-variable in the analysis, and block as a random factor as there was a 'block effect' for all variables, although no interaction effects between variables.

3.3 Results

3.3.1 Species richness and diversity indices

The results for species richness and Shannon-Wiener diversity were calculated using data from the 2013 final year vegetation survey (table 3.2, figures 3.1 and 3.2). There was no significant difference in species richness and Shannon-Wiener diversity (SW) between cutting dates and the plots that were cut twice (p=0.592 and p=0.311). There was also no difference in species richness and SW between plots receiving P (P+) and the controls (P0). The mean species richness per quadrat overall was 16.17 species. The mean for P0 plots was 16.14 and the mean for P+ plots was 16.20. The plots that were cut twice had the lowest mean species richness of 14.40 per m^2, although this was not significantly different from the mean.

Factor	Df	F value	p value
Species Richness	9	0.828	0.592
SW diversity	9	1.194	0.311
Graminoid %	9	1.59	0.133
Forb %	9	1.464	0.176
Legume %	9	0.87	0.556

Table 3.2 ANOVA table of significance of species richness, SW diversity, and functional group percentage

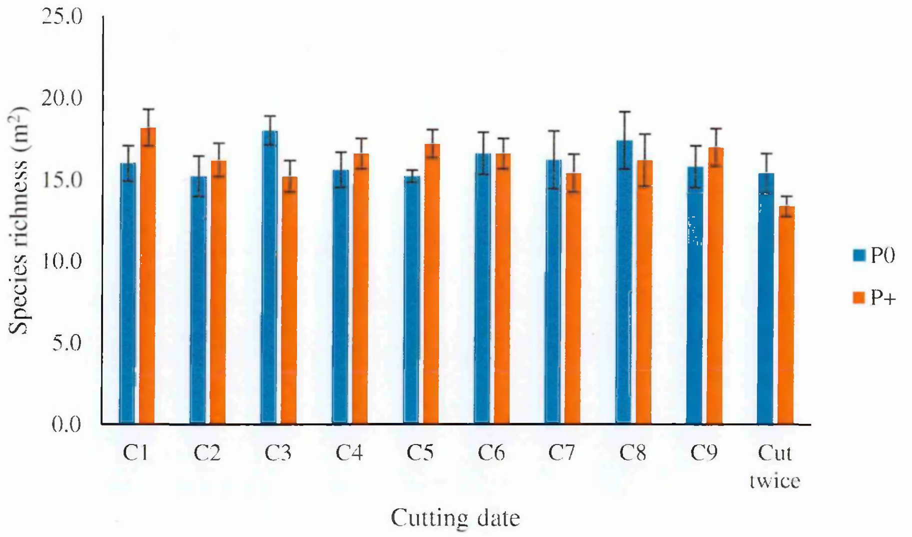


Figure 3.1 Mean and standard error species richness in P0 and P+ plots at each cutting date (C1-C9)

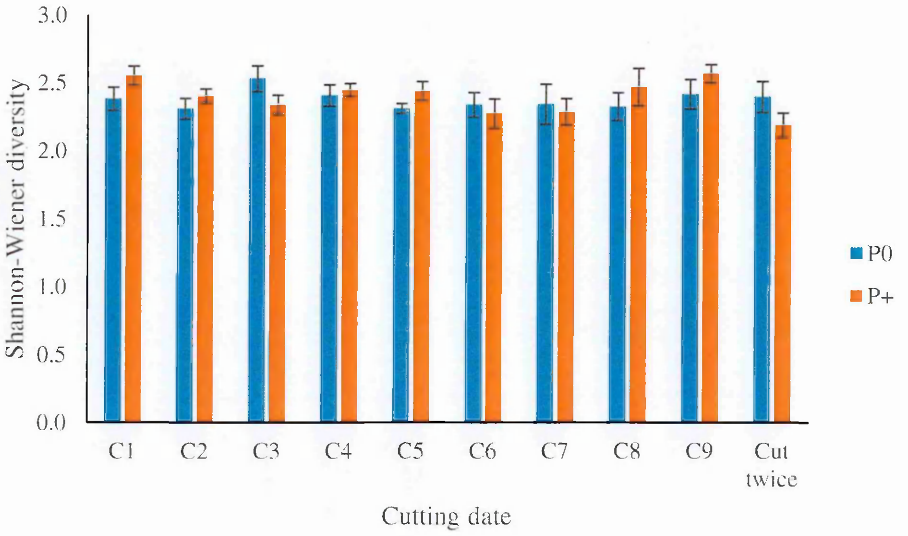


Figure 3.2 Mean and standard error Shannon-Wiener diversity indices in P0 and P+ plots at each cutting date

3.3.2 Functional group percentage

Vegetation percentage cover in each plot was used to calculate functional group percentage for 2013 data (figure 3.3). Mean functional group percentage including all 100 plots was 81.5% graminoids, 15.7% forbs and 1.8% legumes. There was no significant difference between functional group percentage between control plots and P+ plots (table

3.2) or between different cutting dates, or plots that were cut twice (figures 3.4, 3.5, 3.6, 3.7 and 3.8).

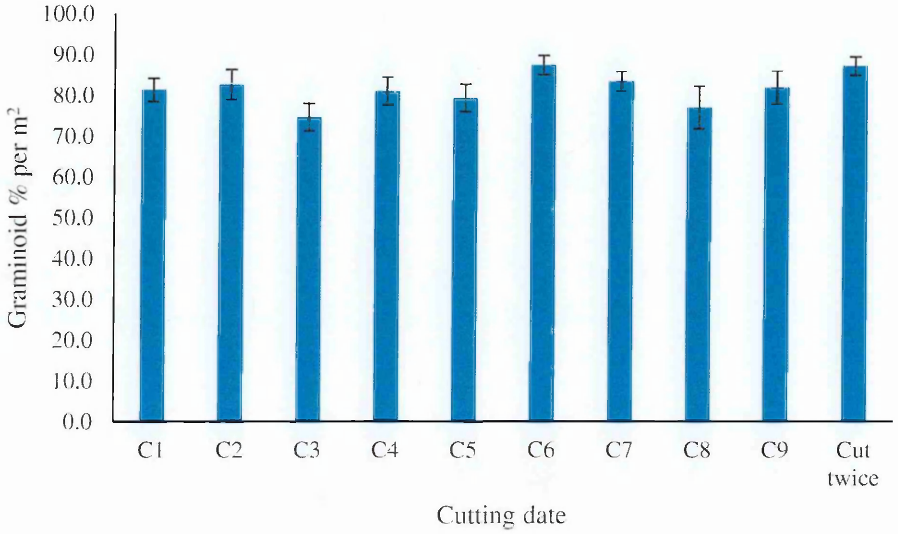


Figure 3.3 Mean and standard error graminoid percentage between cutting dates

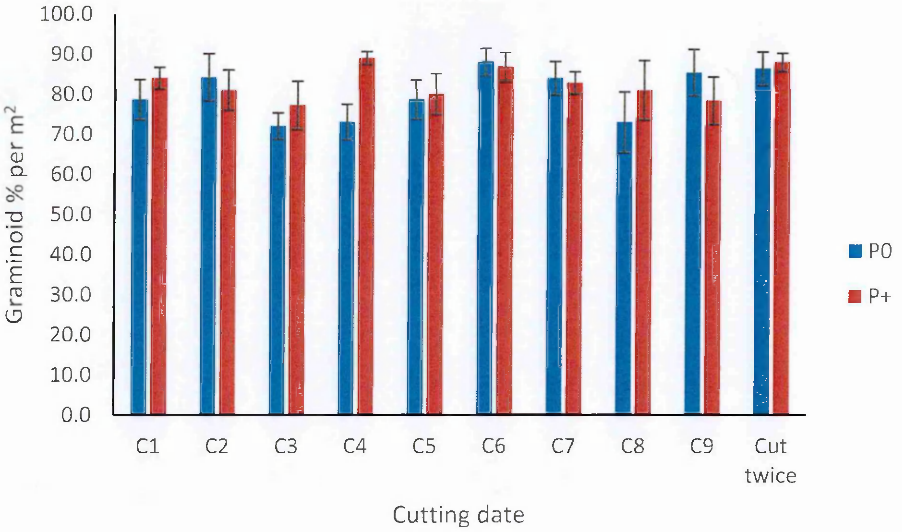


Figure 3.4 Mean and standard error graminoid percentage between P+ and P0 plots for each cutting date

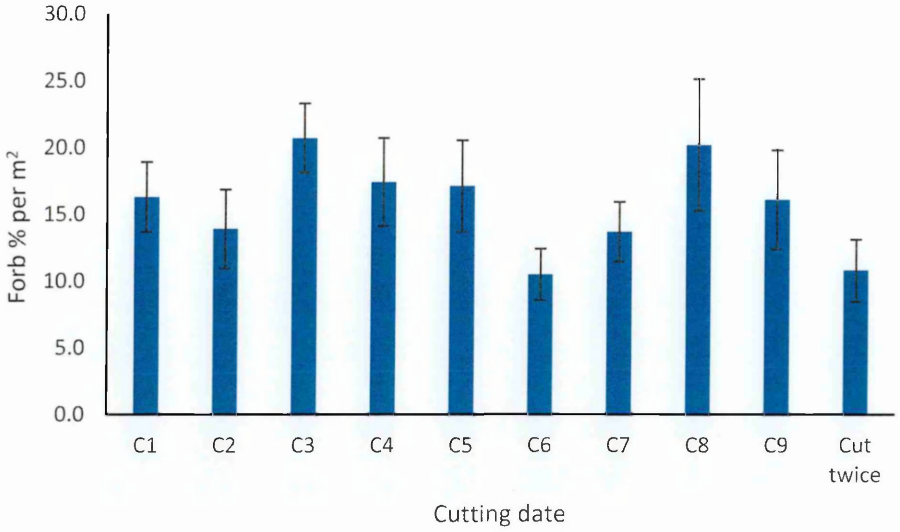


Figure 3.5 Mean and standard error forb percentage between cutting dates

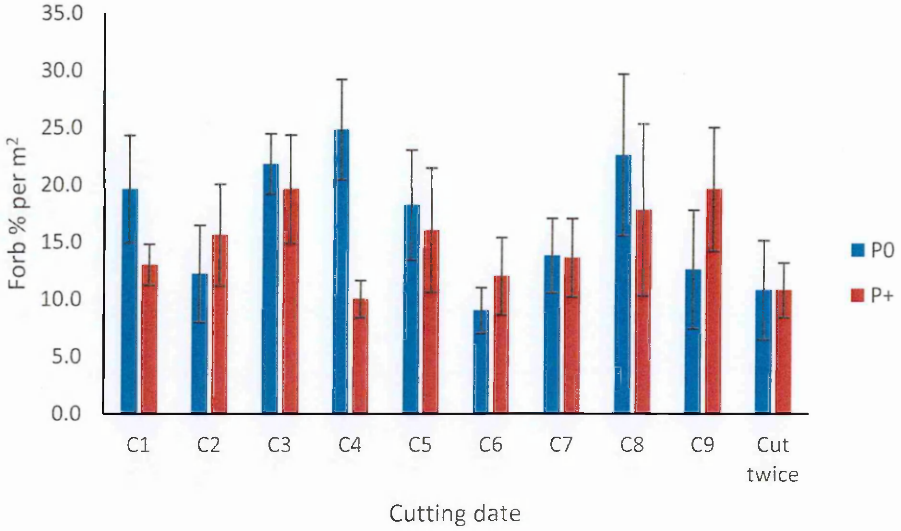


Figure 3.6 Mean and standard error forb percentage between P0 and P+ plots for each cutting date

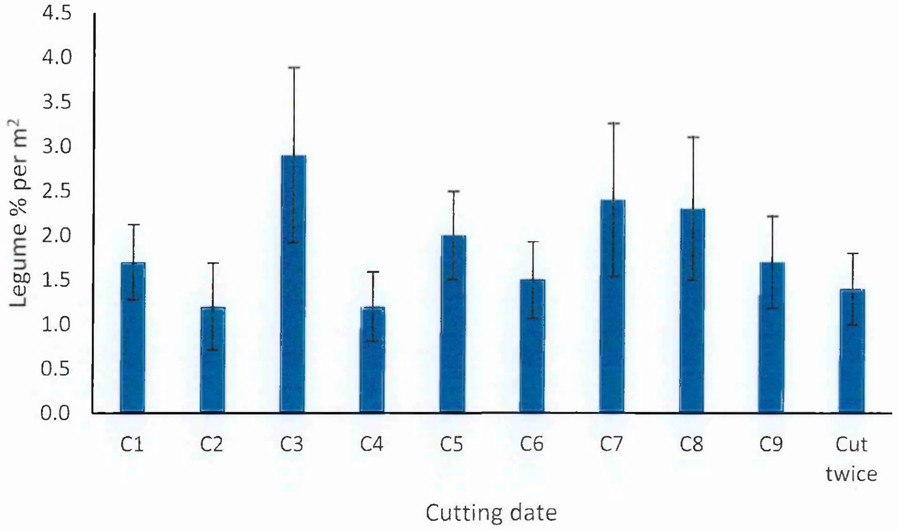


Figure 3.7 Mean and standard error legume percentage for each cutting date

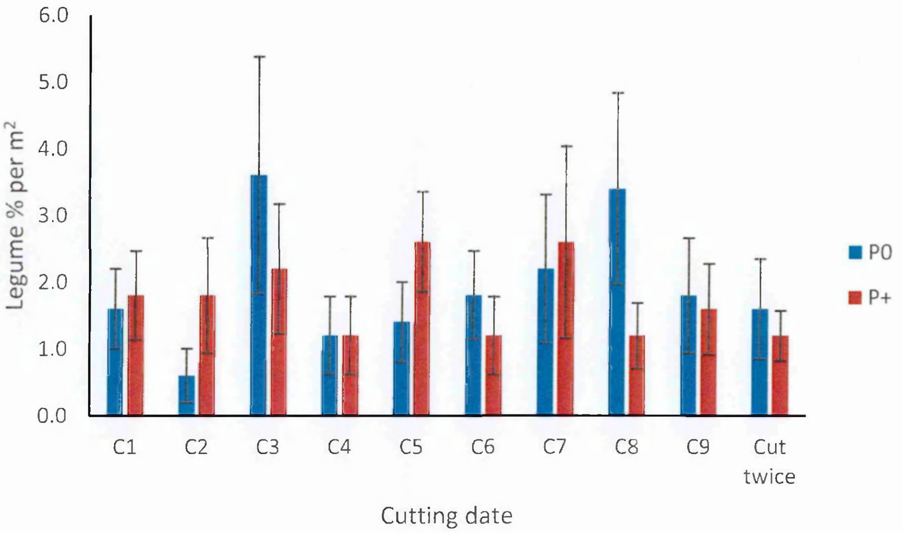


Figure 3.8 Mean and standard error legume percentage for P0 and P+ plots for each cutting date

3.3.3 Species cover abundance

In total 38 species were found at the site with the most frequent graminoids occurring in the plots being *Agrostis stolonifera*, *Agrostis canina*, *Carex disticha*, *Alopecurus pratensis* and *Carex riparia*. The most abundant forbs were *Sanguisorba officinalis*, *Ranunculus repens, Ranunculus acris, Filipendula ulmaria*, and *Centaurea nigra*. Figure 3.9 describes the individual mean abundance for each species. Figure 3.10 describes the mean species abundance for each cutting date for the most abundant species. There was no significant difference between mean species abundance for cut or treatment group for any species, however if the cut dates were consolidated into early, mid, late and cut twice groups there was a significant difference overall for mean species abundance between cut group (p=0.023) figure 3.11). Water-table depth was also significant (p<0.001) and therefore used as a covariate in the analysis. The species which showed significant difference in mean abundance percentage cover between cutting treatments (early, mid, late and cut twice) were *Agrostis stolonifera, Anthoxanthum odoratum, Festuca pratensis, Phleum pratense, Centaurea nigra, Oenanthe fistulosa* and *Trifolium repens* (p<0.05). The top twelve most abundant species at the site were *Agrostis stolonifera, Agrostis canina, Carex disticha, Alopecuris pratensis, Carex riparia, Holcus lanatus, Cynosurus cristatus, Sanguisorba officinalis, Poa trivialis, Festuca rubra, Ranunculus repens and Ranunculus acris.*

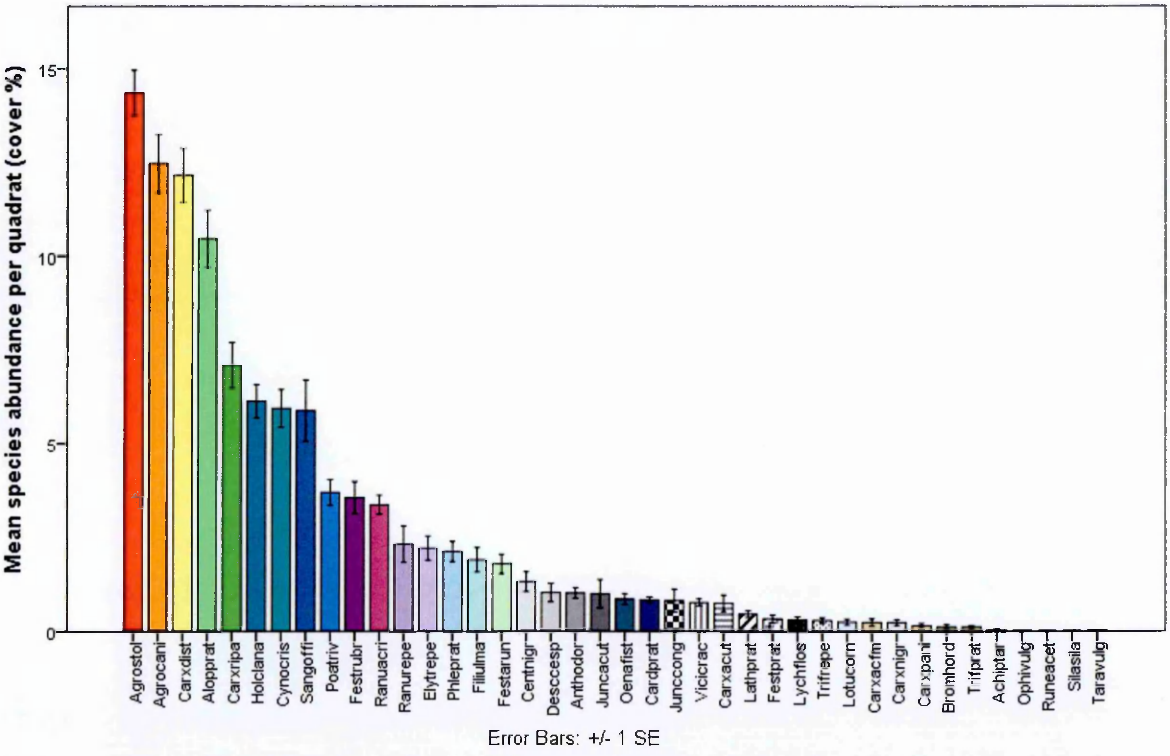


Figure 3.9 Mean and standard error species cover abundance per quadrat

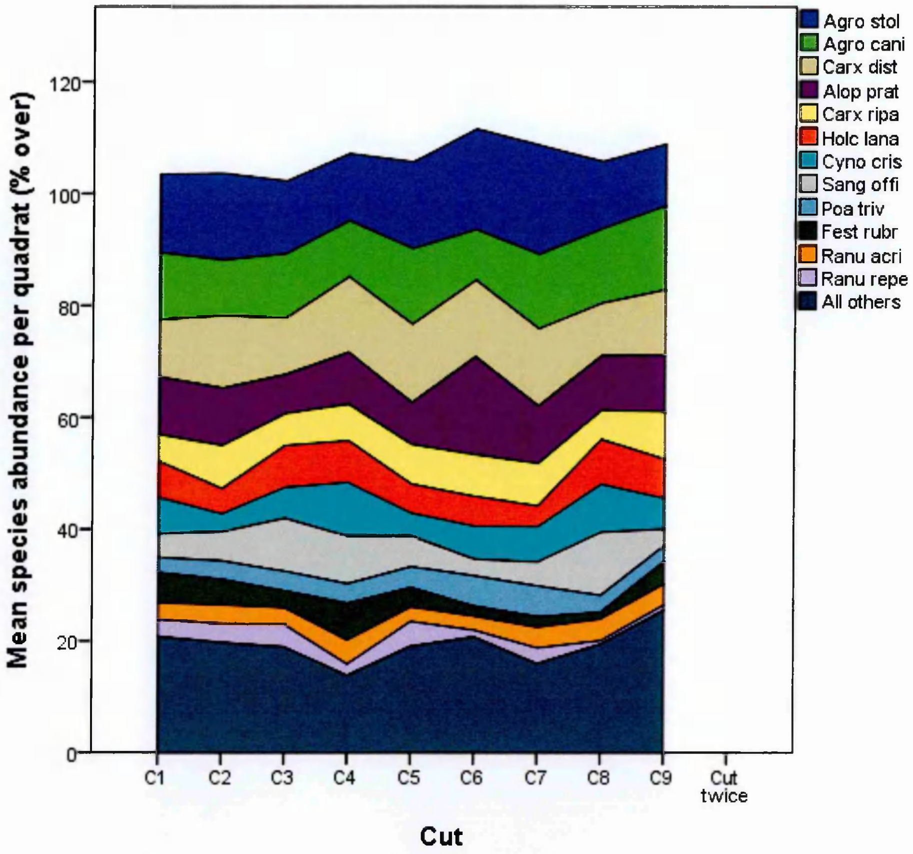


Figure 3.10 Mean species cover abundance for each cut date C1-C9

Agrostis stolonifera was most abundant in mid-cut plots, which was significantly different to early (p=0.042) and late (p=0.003) cuts. *Poa trivialis* was also most abundant in the mid-cuts, although this was not significantly different. Species most abundant in the plots cut later were *Phleum pratense*, *Anthoxanthum odoratum*, *Centaurea nigra* and *Oenanthe fistulosa*. Species reduced significantly by cutting twice were *Trifolium repens* (p=0.019), *Festuca pratensis* (p=0.026), and *Centaurea nigra* (p=0.035), however the species increased significantly by an extra cut was *Phleum pratense* (p=0.016). (figure 3.11). Figure 3.12 describes the mean abundance of *Trifolium repens* individually, due to loss of clarity on figure 3.11 because of the larger scale.

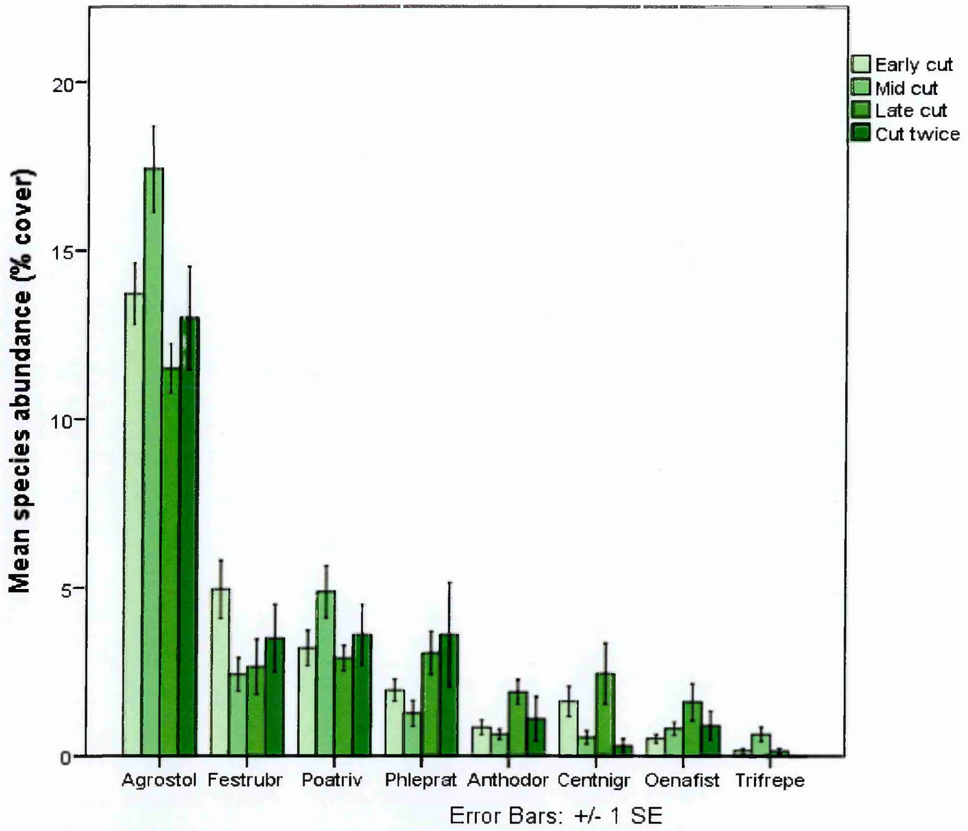


Figure 3.11 Mean and standard error species cover for early, mid, late and plots cut twice for species which were significantly different between cuts (with the exception of *Poa trivialis* which was not significant). Data based on 2013 vegetation survey

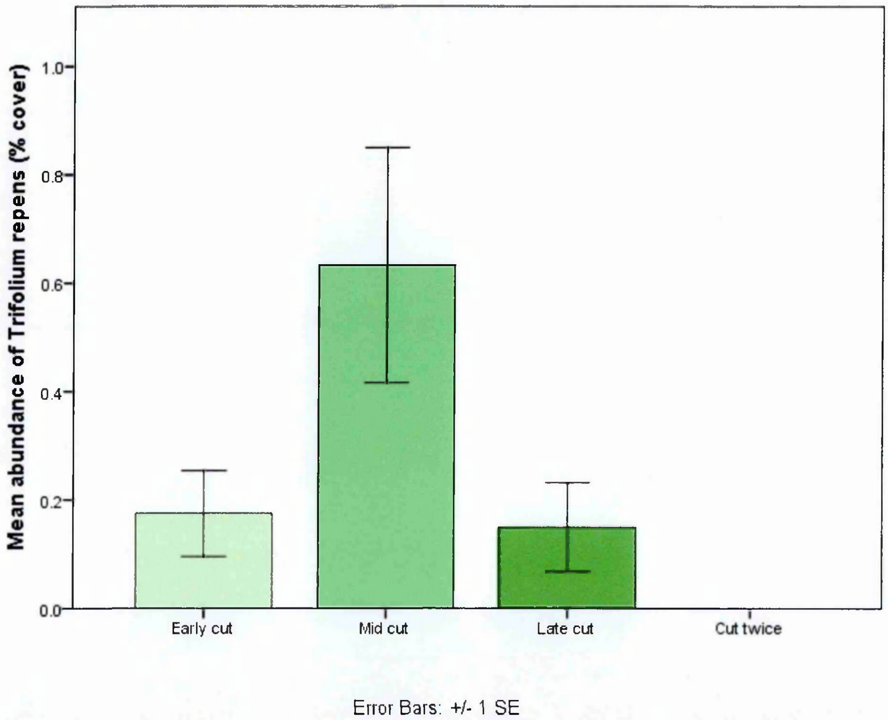


Figure 3.12 Mean and standard error for cover of Trifolium repens for early, mid, late and cut twice plots

3.3.4 Ordinations

An initial detrended correspondence analysis (DCA) of the total vegetation data for 2013 resulted in the longest gradient in the data being 1.975, indicating that that a linear ordination technique would be most appropriate for investigating the community composition. Principle components analysis (PCA) was then used as the ordination method for displaying species data alone (figure 3.13) and redundancy analysis for species and environmental variables (figure 3.14 and 3.15). The PCA of the species composition data showed that axis 1 accounted for 23.7% of the total inertia, and axis 2 accounted for 14.3% of the total inertia. Species positively correlated with the first PCA axis were *Sanguisorba offinalis*, *Filipendula ulmaria, Centaurea nigra, Cynosurus cristatus* and *Bromus hordeaceous* and negatively correlated with the first axis were *Oenanthae fistulosa*, *Carex acuta* and *Carex riparia.* Axis 1 also had a significant correlation with species Ellenberg F values for moisture ($p<0.05$). As such, axis 1 of the PCA seemed to delineate a moisture gradient. The same PCA diagram was reproduced to code different treatment plots (P0 and P+) and cutting plots (C1-C9) which revealed scattered points and no obvious pattern. Axis 2 of the PCA was correlated positively with species *Ranunculus repens, Phleum pratense, Festuca arundinacea* and *Elytrigia repens*, and negatively with *Festuca pratensis.*

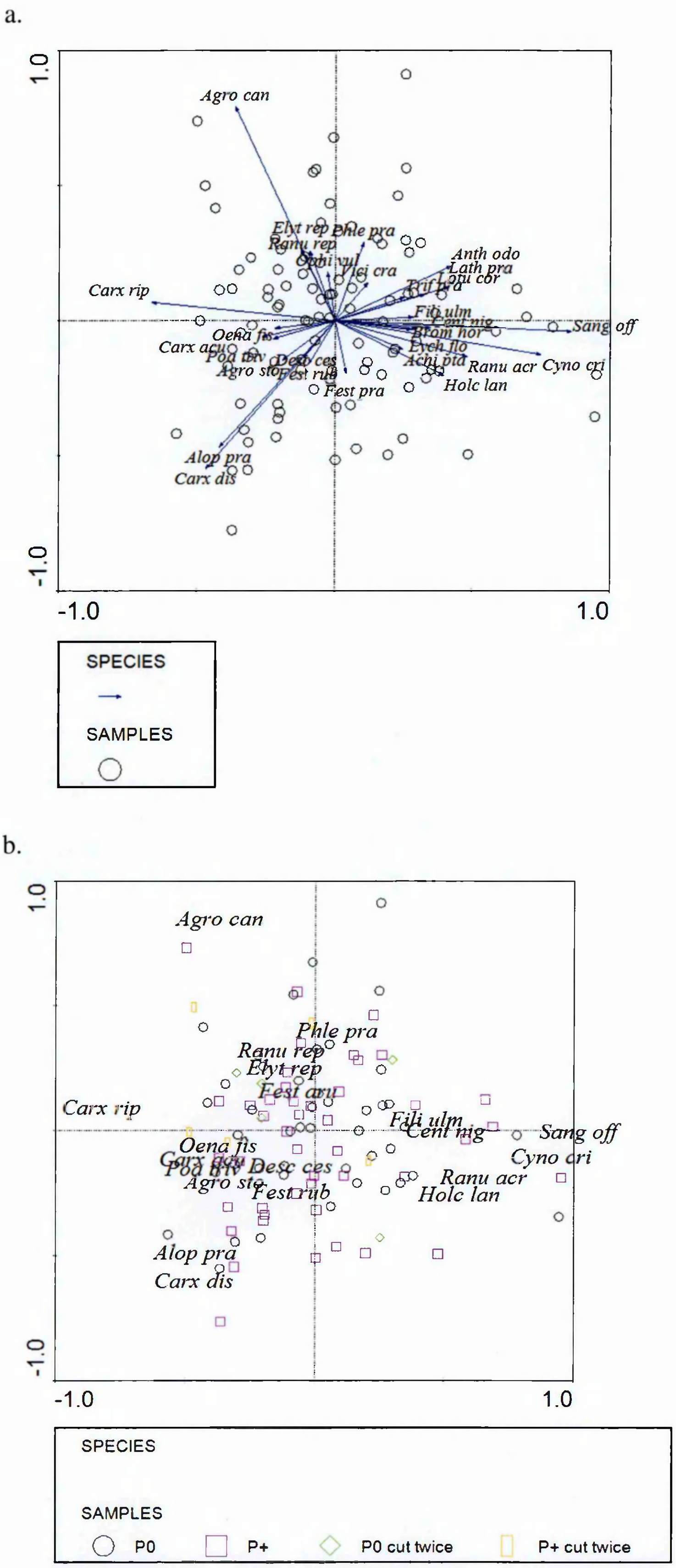


Figure 3.13 PCA of 2013 vegetation species and samples data a) species and samples b) species and samples with colour coded treatment plots

Environmental variables and species were plotted using an RDA. 21.5% of species variation can be explained by the environmental variables. The statistical significance of the effects of the environmental variables was tested using Monte Carlo permutation tests, where water-table depth and cut 6 were significant ($p<0.05$) (table 3.3). The addition of P and cutting twice were not significant in explaining the species variation. Water-table depth correlated positively with *Centaurea nigra, Filipendula ulmaria* and *Holcus lanatus*, but negatively with *Carex acutiformis*, *Carex acuta, Poa trivialis* and *Juncus conglomeratus*. Water-table depth increase is associated with drier species (in the direction of the arrow variable), and decrease with wetter species. An additional RDA was plotted where cutting dates were grouped into early (C1-C4), mid (C5-C7) and late (C8 and C9) cuts to identify any broader trends among the species variation (figure 3.15). *Phleum pratense* was positively correlated with later cuts, *Agrostis canina, Oenanthe fistulosa* and *Ophioglossum vulgare* were positively correlated with plots that were cut twice, and *Carex riparia, Carex acuta*, *Alopecurus pratensis* and *Elytrigia repens* were all negatively correlated with water-table depth (and therefore positively correlated with water table height ergo the wetter plots). Monte Carlo permutation tests revealed water-table depth and mid cut to be significant in explaining the species variation (table 3.4). Species positively correlated with mid cutting were the majority of the species, which reflects conventional management regimes on this habitat; but particularly *Agrostis stolonifera, Festuca pratensis, Trifolium repens* and *Cardamine pratensis.*

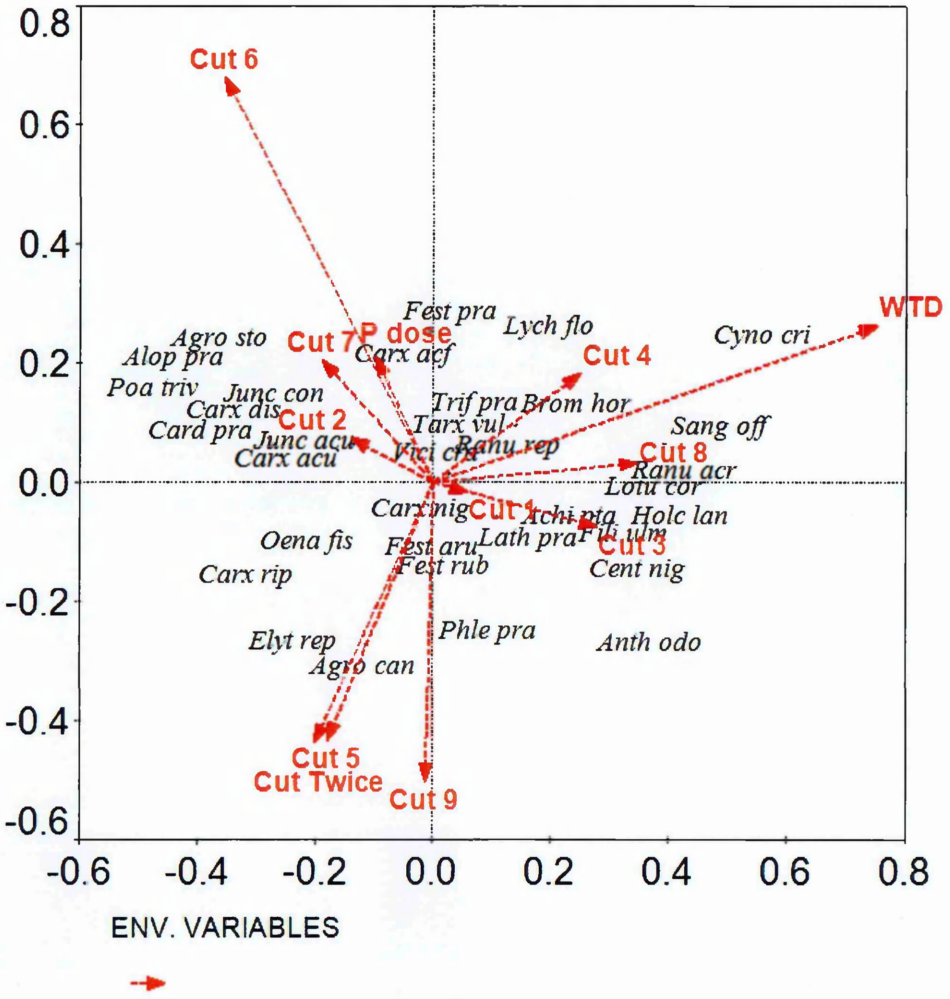


Figure 3.14 RDA of 2013 vegetation species and environmental data

Variable	LambdaA	P-value	F
WTD	0.05	0.002	4.74
Cut 6	0.02	0.012	2.57
Cut 8	0.02	0.086	1.61
Cut 4	0.01	0.114	1.5
Cut 7	0.01	0.224	1.33
P dose	0.01	0.384	1.06
Cut Twice	0.01	0.43	0.97
Cut 9	0.01	0.222	1.24
Cut 1	0.01	0.698	0.71
Cut 3	0.01	0.748	0.68
Cut 2	0	0.962	0.41

Table 3.3 RDA Monte Carlo permutation test summary

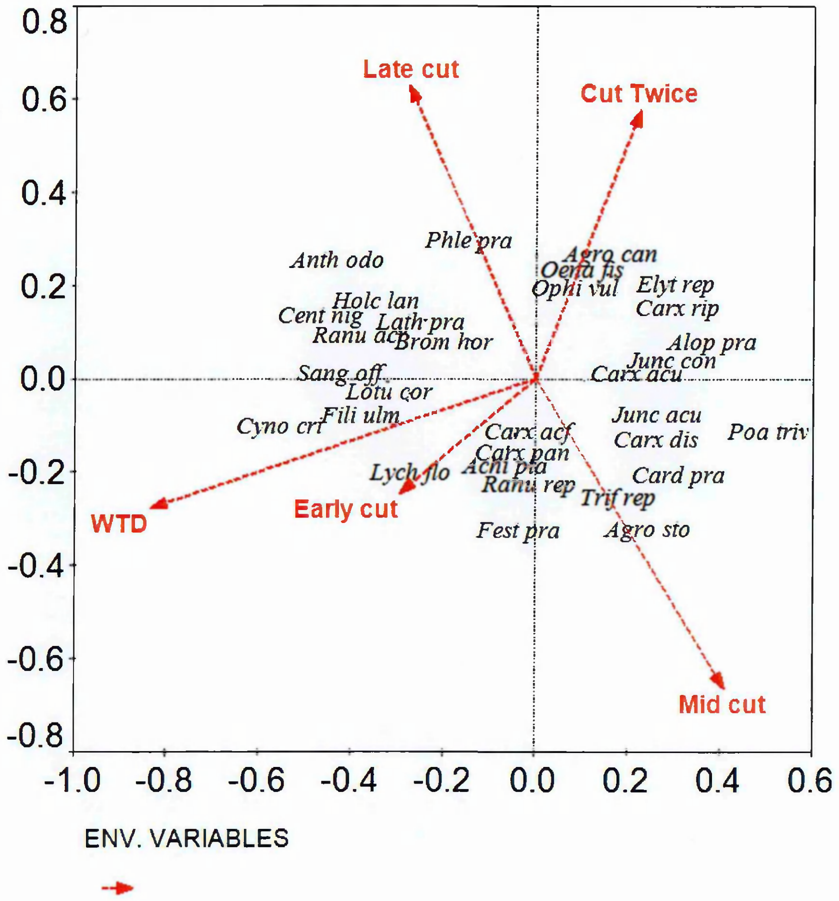


Figure 3.15 RDA of species and environmental variables with cuts split into early, mid, late and cut twice

Variable	LambdaA	P-value	F
WTD	0.05	0.002	4.74
Mid cut	0.02	0.004	2.77
Early cu	0.02	0.098	1.66
Late cut	0	0.746	0.69

Table 3.4 RDA Monte Carlo permutation test summary

3.4 Discussion

The addition of P and varying the cutting date within the growing season did not show any significant difference in relation to species richness, Shannon-Wiener diversity or functional group percentage from the results of the species survey conducted in the final year of the experiment (2013). There were trends and significant differences associated with individual species. The study site was subjected to multiple flood events for the duration of the experiment, typically floodplain meadows are inundated periodically during winter and spring (van Eck *et al.* 2004). Following the exceptional rainfall in 2012, water-level data from Leaches meadow (see appendix 1) indicates that the site was flooded four times between April and August of 2012, and inundated for long periods. Many studies agree that the hydrological regime of a meadow is the most important factor to influence its floristic composition (Gowing & Spoor 1998; Gowing *et al.* 1998; Grevilliot, Krebs & Muller 1998; Casanova & Brock 2000; van Eck *et al.* 2004) therefore the summer flooding frequency and periods of inundation during 2012 might be expected to have more of an effect on the plant community than the experimental cutting date. Furthermore Monte Carlo permutation tests and multivariate analysis of variance on the data show that water-table depth was significant in explaining the variation of the plant community. Ellenberg F scores for moisture also correlated with the first axis of the PCA.

The RDA of the species data and cutting dates as environmental variables showed that only cut 6 (plots cut on 21st/22nd June) was significant in explaining any variation in species composition, reflected in the second RDA which showed that many of the species were positively correlated with the mid cutting dates (from 14th-28th June). Furthermore when the cuts were grouped into early, mid and late, only 'mid cuts' were significant in explaining the variation in species composition (*p*=0.002). In this case, it is probably not a co-incidence that this reflects conventional management cutting regimes for this type of habitat, although many sites are cut later than June to favour and encourage nesting of birds (Nocera *et al.* 2005), which could be detrimental to vegetation species composition.

Only one species was significantly positively affected by cutting earlier, which was *Festuca rubra.* Increase of *Festuca rubra* with cutting compared to abandoned meadows has been reported in the literature; especially in temperate grasslands in mountainous areas (Huhta *et al.* 2001; Krahulec *et al.* 2001; Hellstrom *et al.* 2006). But in terms of timing of hay cut Kirkham & Tallowin (1995) reported that *Festuca rubra* was not particularly susceptible to variations in cutting date. The colonization of *Festuca rubra* is often slower than other grasses (Grime, Hodgson & Hunt 1988), and may have thrived in plots cut earlier in this experiment due to the reduction in canopy height and removal of other more competitive species which then gained advantage in the later cut plots.

Species that thrived in the plots cut later were *Phleum pratense, Anthoxanthum odoratum, Centaurea nigra* and *Oenanthe fistulosa. Phleum pratense* was also the only species to increase significantly by cutting twice. Other studies have found cutting twice increased dominance of finer species and increased species richness overall (Wells 1980; Parr & Way 1988; Hejcman, Schellberg & Pavlu 2010), although Bissels *et al.* (2006) found that cutting twice also increased the cover of only one species (*Serratula tinctoria*), attributed to lowering the amount of above-ground biomass and thus reducing the competitive effects of established species and a higher incidence of light for seedling establishment. In agreement with this study Parr and Way (1988) also found that *Phleum pratense* increased with cutting twice on one of two study sites on road verges in the UK.

Some species may increase in plots cut later in response to other species decreasing. This could be the case with *Anthoxanthum odoratum* which increased significantly in the late cuts. This is also in agreement with Pavlu, Schellberg & Hejcman (2011) and Hansson & Fogelfors (2000) who reported an increase in *Anthoxanthum odoratum* with cutting due to its high sensitivity to shading in tall grasslands. It is also an efficient colonizer and spreads rapidly, so may have taken advantage by colonizing gaps made available by other species reduced by late cutting, as its growth rate can increase later in the season (Lambrechtsen 1968). Dominant grasses which decreased in later cut plots were *Agrostis stolonifera* and *Poa trivialis*, which could explain the significant increase in *Anthoxanthum* in those plots. Taller growing forbs would also favour a later cut due to their competitive advantage over smaller rosette forming species (Wahlman & Milberg 2002; Jantunen 2003; Hellstrom *et al.* 2006), which is demonstrated by the increased cover of *Oenanthe fistulosa*, and *Centaurea nigra* in the later cut plots.

Cutting twice significantly decreased the cover of *Centaurea nigra* and *Trifolium repens*, which disappeared altogether in these plots. This is contrary to the findings of other studies which found *Trifolium repens* to increase in cut plots and plots cut twice or more than twice (Parr & Way 1988; Pavlu, Schellberg & Hejcman 2011) due to its intolerance of shade (Grime, Hodgson & Hunt 1988). However Stampfli (1992) found that *Trifolium repens* can behave differently depending on the ecological conditions of the sward. In the case of this experiment, *Trifolium repens* may have been outcompeted by other taller growing species which responded positively to a second cut, such as *Alopecurus pratensis, Carex riparia* and *Phleum pratense*. Pavlu, Schellberg & Hejcman (2011) found an increase in *Trifolium repens* only after five years of cutting, which goes beyond the time constraints of this investigation. Similarly, other studies investigating cutting have been longer-term (Bakker, Elzinga & de Vries 2002; Hejcman, Schellberg & Pavlu 2010) allowing time for effects of cutting to impact the species composition.

3.4.1 Conservation implications

Altering the timing of the hay cut caused no significant increase or decrease in species diversity in this short term experiment, although there was an effect on species composition when the cutting date was changed. This has implications for management of floodplain meadows as later mowing favoured some taller herbs such as *Centaurea nigra* and *Oenanthe fistulosa,* which could be due to the individual plants setting seed in

July and therefore increasing in abundance in the later cut plots as both plants have very little vegetative spread (Grime, Hodgson & Hunt 1988). However, early cutting can favour smaller growing species rather than species with taller-growing and leafier stems. This is because the former grow closer to the ground and mowing removes a smaller proportion of their above-ground biomass (Hellstrom *et al.* 2006). There was no trend in this direction observed at Leaches, with the exception of the slow-growing grass *Festuca rubra* favouring earlier cutting dates due to the reduction of other species responding positively to later cutting. According to this study, in terms of maximum nutrient removal (discussed in chapter 2), it is better to cut no later than the end of June for unimproved meadow grasslands, and mid-July for improved sites as peak nutrient removal occurred at these times in fertilized and unfertilized plots. Cutting later than this could therefore lead to increased fertility of the soil, as the nutrient value of the hay crop reduces later on in the season (Kirkham & Tallowin 1995). Nutrient build up in the soil could potentially lead to a reduction in species richness over time (Kirkham, Mountford & Wilkins 1996; Silvertown *et al.* 2006; Dupre *et al.* 2010). Thus cutting date on floodplain meadows should not necessarily be a fixed date, but needs flexibility depending on the overall nutrient status of the site, the dominant vegetation species which may (or may not) be desirable, the control of aggressive species which favour multiple cutting dates (Newman 2013), and the individual species composition of floodplain meadow sites which may favour either an earlier or later cut depending on the phenology of specific desirable species.

3.5 Conclusion

The addition of 25 kg P ha yr^{-1} to treatment plots, was not significant in altering the species composition or species richness of the vegetation. Cutting dates were not significant in changing the species richness of the vegetation between plots, but showed significance in partly explaining the species composition in the redundancy analysis and multivariate analysis of variance. The plots cut in 'mid' growing seasons and more specifically cut 6, were significant in explaining some of the species composition variation, but no other cuts were significant. However the cutting regimes were only implemented in 2010, 2011 and partly in 2012 which may not have been long enough to induce an effect on the vegetation at this site. More relevant is perhaps the fluctuating dynamics of the water table from 2010 to 2013 which would have driven a change in species composition at the site, regardless of cutting date (van Eck *et al.* 2004; Jung, Hoffmann & Muller 2009). However, changes to the site over time are discussed in chapter 5. The gaps between cutting dates may have been too small to elicit an effect, making it impossible to separate the effects of cutting between 14th June and 21st June for example, both in terms of species richness and composition. Grouping the cuts into early, mid and late produced a significant result in terms of individual species dynamics, with mid cutting significantly explaining the variation in species data on the RDA, reflecting current floodplain meadow management regime and reinforcing timing the cutting for late June rather than July for this type of vegetation community (see chapter 2). It is not possible to say that cutting later in the season favoured tall growing species in general, as dominant taller species such as *Alopecurus pratensis, Sanguisorba officinalis, Carex riparia, Poa trivialis, Ranunculus repens* and *Ranunculus acris* were not increased significantly by later cuts. Nor did cutting twice within the season significantly reduce the dominance of coarser species such as *Carex* sp or taller growing herbs such as

Sanguisorba officinalis, which was reduced by an additional later cut, but not significantly. Perhaps an extension in the duration of the cutting experiment would have induced a more pronounced species response, which would give further indications for nature conservation and floodplain meadow MG4 management.

Chapter 4

The effect of P and N addition on above-ground production, nitrogen mineralization, nutrient content and offtake: A factorial experiment

4.1 Introduction

Deficiency of resources limits the productivity of plants, which encourages competition between species and consequently shapes the composition and interactions between and within plant communities (Farrior *et al.* 2013). Limitation of plant growth by a nutrient is caused by a low availability of that nutrient to the plant roots, to the extent that the growth demands of the plants cannot be fulfilled (Verhoeven, Koerselman & Meuleman 1996). The majority of models of plant resource limitation are based on Liebig's conceptual model of the law of the minimum (Liebig's Minimum Hypothesis, LMH) where a plant will always be limited by a single resource because resources are not supplied to plants in equal proportions (Liebig & Gregory 1842). This idea was originally developed to understand agricultural systems, but has been used by ecologists to explain nutrient limitation across aquatic and terrestrial ecosystems (Elser *et al.* 2007) to which it is not necessarily relevant, due to the diverse communities existing within these ecosystems which will adjust their stoichiometry to that of their resources (Danger *et al.* 2008). Several ecologists have adapted Liebig's law of the minimum to a Multiple Limitation Hypothesis (MLH) whereby plants adjust uptake of multiple resources to maintain limitation by all essential resources (Tilman 1982; Chapin *et al.* 1987) because by definition if plants are only limited by one resource at a given time, then plants are overinvesting in all other essential resources (Farrior *et al.* 2013).

4.1.1 N:P ratios

Plant growth in wet grasslands is controlled by the availability of nutrients, in particular nitrogen (N), phosphorus (P) and potassium (K) (Vitousek & Howarth 1991; Verhoeven, Koerselman & Meuleman 1996; Güsewell & Koerselman 2002). Usually biomass production is enhanced by the addition of a specific limiting nutrient whilst the addition of non-limiting nutrients have little or no effect on community biomass. Grime (1979) described the relationship between species richness and productivity as a hump-backed curve, demonstrating that maximum species richness and variation in species occurs at intermediate productivity levels. Species composition of the vegetation in wet grasslands can be affected by the addition of both limiting and non-limiting nutrients due to different species responses to the varying nutrients (Güsewell, Koerselman & Verhoeven 2003), suggesting that species differ in their nutrient requirements and may respond differently to enhanced or reduced nutrient supply (Garnier 1991).

Determining nutrient limitation on grasslands can be done by means of nutrient addition experiments. Koerselman & Meuleman (1996) attempted to develop a 'tool' in order to provide an alternative to this, which would provide the same information (establishing the limiting nutrient) but would be more efficient, cost effective, and cause less site disturbance. They hypothesized that the N:P ratio of the vegetation can demonstrate if the vegetation is N or P limited based on a review of 40 field fertilization experiments in wetlands, and suggested that the biomass production of the vegetation is limited by N if the N:P ratio of the above-ground biomass is low (<14) and by P if the N:P ratio is high (>16). Thus populations with high N:P ratios would be enhanced by P fertilizer, and populations with low N:P ratios would be enhanced by N fertilizer. However, contrary to these findings, Güsewell, Koerselman & Verhoeven (2003) found that N:P ratios of co-limited vegetation or plant populations were not confined to the narrow range of 14 to 16 and concluded that N:P ratios were a useful tool, although not exclusively suitable for predicting how changes in nutrient supply will affect plant species composition.

Availability of N and P can also be controlled by vegetation management; for example Koerselman, Bakker & Blom (1990) demonstrated that above-ground biomass harvesting in the Netherlands creates a strong net loss of P and a relatively small loss of N from the system. Therefore wet grasslands that have historically been managed by mowing would expected to be P limited, whereas fens which are mown infrequently would be N limited (Verhoeven, Koerselman & Meuleman 1996). Limited data is available from UK MG4 grassland, however samples obtained from the Derwent Ings suggest that the N:P ratio would indicate N limitation rather than P (Gowing *et al.* 2002). Although, in this grassland type there is evidence to suggest that community composition is linked to P availability where the most species-rich communities occurred on soils with the lowest available P following analysis of samples from eleven lowland grassland sites in England (Gilbert, Gowing & Wallace 2009). This has also been demonstrated in other studies; Wassen *et al.* (2005) examined N:P ratios in wetland vegetation across a wide geographical scale, rather than through nutrient addition experiments, and concluded that endangered plant species are more likely to exist under P limited rather than N limited conditions, thus species loss is likely to be due to enhanced P. More recently, Ceulemans *et al.* (2013) investigated the role of phosphorus versus nitrogen enrichment across North-west European grasslands and suggested that P enrichment was a more important driver of species loss from semi-natural grasslands than N enrichment. They found that irrespective of soil N levels, beyond 20 mg P kg^{-1}, species richness did not exceed 20 species per quadrat as observed in 132 grasslands surveyed across North-west Europe.

4.1.2 Microbial limitation

Nitrogen mineralization is the process that controls N availability to plants by the transformation of organic N to inorganic ammonium and then to nitrate by nitrification (Abbasi, Shah & Adams 2001). The N pool derived from mineralization is usually small compared to the total N pool within the soil but it forms the major source of N supply for plant uptake, thus plant N supply is strongly correlated with rates of N mineralization (Nadelhoffer, Aber & Melillo 1984). Understanding the dynamics of N mineralization and nitrification is important in the management of the nitrogen cycle within grasslands and may provide further insight into biomass production, species composition and species richness.

Release of N and P, and their availability to plants is affected by the microbial processes occurring in the soil (Harmsen & Van Schreven 1955; Abbasi, Shah & Adams 2001). Some researchers have suggested that net N mineralization increases with increasing P availability in grasslands (van Oorschot, Hayes & van Strien 1998; Changhui *et al.* 2014). Janssens *et al.* (1998) also hypothesised that available soil P could be a limiting factor of nitrate (NO_3) supply by mineralization of the soil organic matter, hence nitrogen would be the main element limiting plant diversity, but its availability controlled by P availability. If P influences the rate of N mineralization, this could change how much N is taken up by the vegetation and stored, which could be investigated by adding P to experimental plots and harvesting the above ground vegetation biomass at regular intervals and analysing the harvested biomass for N. Oorschot *et al.* (1997) aimed to analyse the reaction of a riverine ecosystem to nutrient enrichment and the effects of the N and P processes in the vegetation and soil by comparing a site which had received nutrient additions to one which had not. They found that N mineralization showed a strong negative correlation with the C:P ratio of soil organic matter, which suggests an interaction between P and N cycling for soil organic matter break-down at a low nutrient availability. A study by Ruess & Seagle (1994) on the Serengeti National Park in Tanzania, compared the different soil characteristics and microbial processes of 17 study sites within the area. They found that ammonium production was restricted with low P availability and that nitrogen mineralization was unrelated to microbial biomass while above-ground production was limited by nitrogen. Similarly, a study on 17 Dutch heathlands, (and then subdivided into 41 sites), found that N mineralization was positively correlated with the labile soil organic P-pool (Troelstra, Wagenaar & Boer 1990). In the UK, fertilizer experiments on the Somerset Levels at Tadham Moor (Kirkham & Wilkins 1994) indicated that the uptake and availability of N was increased by the application of inorganic K and P fertilizer and by the addition of inorganic P alone. In Western and central Europe a study by Janssens *et al.* (1998) on grasslands, found a hump-backed relationship between the soil available P and the percentage of legume cover per unit area. If the available P in the soil was greater than 5mg/100g, then legume cover declined. They suggest that this could be due to productive grasses being present, thus reducing the number of legumes through competition. Alternatively, they suggested that higher available N content could also influence the legume cover by the soil available P limiting the organic matter mineralization and influencing the soil available N quantities. They suggested that soil extractable P can indirectly influence grassland biodiversity by its control on the soil available N quantities by enhancing N mineralization. Similarly Cadisch, Schunke & Giller (1994) found a decrease in mineralization in plants experiencing a P deficiency, and therefore a lower release of available N.

These studies allude to a theory that increased soil P may stimulate microbial activity to increase N mineralization, and increasing the N made available to plants. Thus plants may increase uptake of N in response to P addition. This theory suggests that P is important for plant productivity even in N limited systems and suggests an indirect effect on N availability (figure 4.1). Supporting this idea, a very recent study (Changhui *et al.* 2014) investigated the effects of N and P additions on microbial N transformations and biomass on a saline-alkaline grassland in Northern China. They found that during the growing season, P addition significantly stimulated the soil inorganic N pool, and furthermore increased the soil net N mineralization rate, although did not increase peak above-ground biomass. They also found that P fertilization significantly decreased the ratio of microbial biomass carbon, to microbial biomass nitrogen (MBC/MBN) indicating a shift in the microbial community structure.

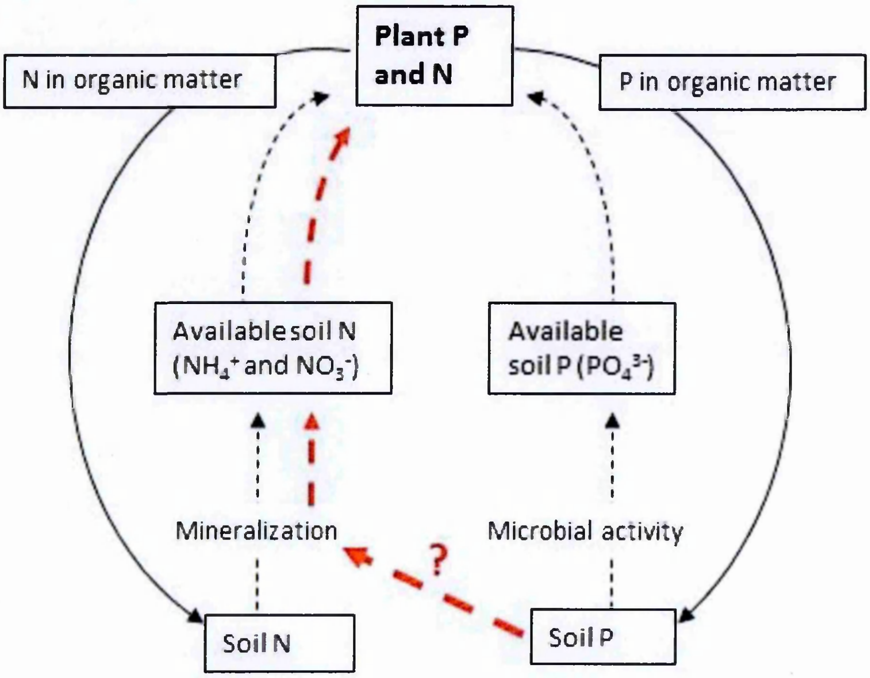


Figure 4.1 Schematic diagram of theoretical soil P effect on N mineralization. Bold dashed red arrows indicate potential pathway of increased P

4.1.3 Co-limitation

Numerous studies and long-term fertilization experiments have in fact demonstrated that the vegetation becomes dominated by different plant species under N-limited or P-limited conditions (Tilman 1982; Güsewell 2004; Hejcman *et al.* 2007) depending on the ability of the species to compete for N or P, which in turn is dependent upon the species traits and response to nutrient acquisition, nutrient retention and nutrient utilisation efficiency (Tilman 1988; Berendse *et al.* 1992). A recent study (Fay *et al.* submitted) investigated nutrient limitation of above-ground net primary productivity (ANPP) on 42 grasslands across five continents; they found that N and P synergistically co-limited global grasslands 1.4 times more than N or P independently.

More recently the paradigm of single resource limitation has changed towards concepts of co-limitation by multiple resources (Harpole *et al.* 2011). They reviewed 641 studies that applied N and P in a factorial design within freshwater, marine and terrestrial ecosystems and found that more than half of these displayed some type of synergistic response to N and P addition. Moreover 28% of the 641 studies showed strict definitions of co-limitation where the vegetation biomass for example, responded to only combined additions of N and P, rather than singularly to N or P independently. They suggest that there is a need for further studies that address the multiple mechanisms that could lead to different types of co-limitation.

Despite the extensive literature on N and P limitation in grasslands, from older studies which discuss resource limitation of either N or P, and using N:P ratios to describe nutrient limitation (Vitousek & Howarth 1991; Verhoeven, Koerselman & Meuleman 1996; Güsewell, Koerselman & Verhoeven 2003; Güsewell 2004), to more recent publications on multiple resource limitation (Harpole *et al.* 2011; Farrior *et al.* 2013), nutrient addition experiments are still relevant in establishing the effects of N and P. Not only on the productivity of the above-ground vegetation of MG4 communities, of which evidence is lacking for this specific community, but also on below-ground processes and limitation of the microbial community by either N and/or P. Farrior *et al.* (2013) suggests that multiple resources may be tied together, which would explain the difficulty in determining a general theory for plant limitation and the ongoing debate as to which nutrient N or P is most limiting (Wassen & Olde Venterink 2006; Ceulemans *et al.* 2013; Rowe, Smart & Emmett 2014).

Moreover there appears to be conflicting evidence in that according to the view of Verhoeven, Koerselman & Meuleman (1996), limitation of MG4 grassland would appear to be N limited and therefore P addition should have little effect on productivity and species richness. However, this is known to be incorrect as other studies have clear evidence for P limiting species richness (Wassen *et al.* 2005; Gilbert, Gowing & Wallace 2009; Ceulemans *et al.* 2013). This thesis examines the effect of P addition on the uptake of N by the vegetation, which would primarily question whether the microbial community is limited by P, and if P can operate by releasing N (Janssens *et al.* 1998), but also to identify which nutrient is most responsible for limitation of plant productivity on MG4 floodplain communities. This would support emerging theories of co-limitation by N and P on grasslands, and address the hypothesis that increased soil P availability can increase N mineralization. If this is the case then more N would become available for plant uptake; directly and indirectly increasing productivity of the vegetation. In turn this will contribute towards the understanding of P and N cycling, and help to explain the contradictory evidence on co-limitation. This chapter attempts to address the following questions:

1. Is the vegetation productivity limited by N or P? (Page 105)
2. Does the addition of P stimulate the vegetation to take up more N in the field? (Page 107)
3. Does the addition of P increase nitrogen mineralization in the soil of floodplain meadows? (Page 115)

4.2 Methods

4.2.1 Experimental design

The factorial component of this experiment consists of 8 treatments applied to randomly located plots within each of the five repetition blocks (figure 4.2 represents these plots, grouped together for purposes of explanation). Treatments were added to the plots in three doses between March and May in 2010 and 2011. Nitrogen was added to two of the plots as ammonium nitrate (NH_4NO_3) at a rate of 50 kg N ha^{-1} y^{-1} (referred to as N+). Phosphorus (P) was added as sodium dihydrogen phosphate (NaH_2PO_4) at rates of 25 kg P ha^{-1} y^{-1} to two of the plots (referred to as P+), and 75 kg P ha^{-1} y^{-1} to two of the plots (referred to as P2) and at 25 kg P ha^{-1} y^{-1} to one of the N+ plots (referred to as N+P+). The control plots (referred to as P0) received deionised water. The N+, one of the P0 and one of the P+ plots were cut in the third week of June each year (between 19th and 25th June), and the P2 plots, the other P0 plot and the other P+ plot were cut in the last week of June/first week in July each year (between 26th and 3rd July). Half of the P2 plots also received a second cut at the end of August each year in order to monitor the mitigation effects of a double cut. Nitrogen and phosphorus application rates attempted to represent the range of fertilizer additions typically added to improved grasslands. Treatment application was to the central metre square within each 2 by 2 metre plot, to allow for a buffer zone between treatments. Control treatments in this section are labelled C6P0 and C7P0 which refer to the date the plots were cut. So C6P0 are the control group for the N+P0 and N+P+ treatment groups, and C7P0 are the control group for the P2C1 and P2C2 treatments. This is because the N plots and P2 plots were cut in different weeks, so two control groups were needed (table 4.1).

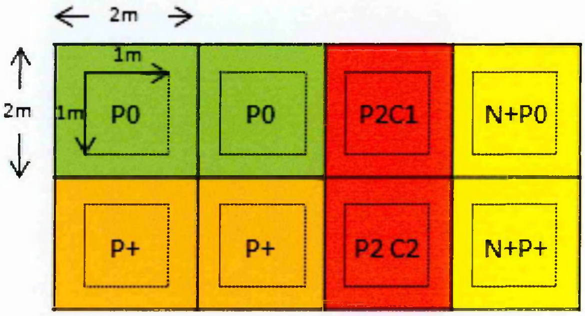


Figure 4.2 Factorial plot and block design

4.2.2 Vegetation sampling

The factorial treatments were surveyed for plant species composition in 2010, 2011 and 2013 in the same week as they were mown (table 4.1). Details of vegetation sampling can be found in chapter 2, and the results of the factorial vegetation composition are reported in chapter 5.

Plot Code	**Cut Date (2010, 2011, 2013)**	**Treatment**
C6 P+	21st June	25 kg P ha yr^{-1}
C6 P0	21st June	Control
C7 P+	28th June	25 kg P ha yr^{-1}
C7 P0	28th June	Control
N+P+	21st June	25 kg P ha yr^{-1} + 50 kg N ha yr^{-1}
N+P0	21st June	50 kg N ha yr^{-1}
P2 C1	28th June	75 kg P ha yr^{-1}
P2 C2	28th June/16th August	75 kg P ha yr^{-1}

Table 4.1 Plot code, treatment additions and cutting date of factorial plots

4.2.3 Soil sampling and analysis

Prior to commencing the field experiment in 2010, soil samples were collected from prospective locations within the field site and tested for Olsen extractable P (soil available P) and pH. Multiple soil cores from the upper 10 cm were collected from various locations within prospective sites for the treatment blocks (figure 4.3). Following analysis, the available P at the site for prospective blocks was less than 15 mg/kg PO4-P, thus suitable for a P addition experiment (table 4.2). Soil samples from the factorial plots were collected in 2011 and 2013, soil was oven dried at 40°C overnight, then ground to 4 mm. Extractable P was determined using an Olsen extract followed by colorometric analysis (MAFF 1986).

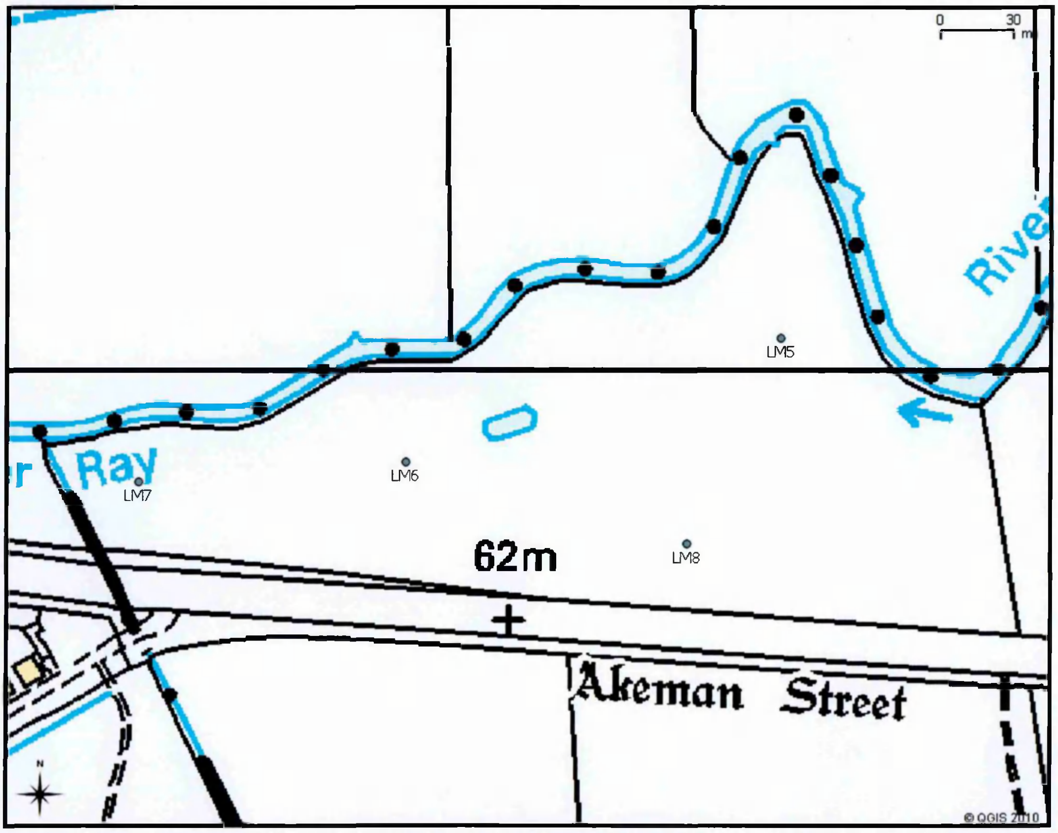


Figure 4.3 Initial soil sampling locations at Leaches meadow

Location Name	mg/kg PO4-P	pH
LM5	7.6	5.59
LM6	5.9	5.34
LM7	4.6	5.47
LM8	18.8	5.92

Table 4.2 Olsen P and pH results from soil sampling locations prior to starting the field experiment

4.2.4 PRS Probes

Measuring nutrient supply to plant roots can provide an *in-situ* measure of N flux over time in the soil (Sharifi *et al.* 2009). Nitrate and ammonium ion (NO_3^- and NH_4^+) supply to plants can be measured using Plant Root Simulator Probes (PRS^{TM}-probes) which consist of an ion exchange membrane encapsulated in a plastic casing. These membranes are chemically pre-treated to exhibit surface characteristics and nutrient sorption phenomena that resemble a plant root surface (Western Ag Innovations 2008). They can be inserted into the soil and left for a predetermined burial time to measure total nutrient supply to plant roots. The amount of nutrient ions absorbed onto the PRS^{TM}-probes at the end of the burial period, represents the nutrient supply rate to a plant root for the duration of the burial. The supply rate is expressed in units of micrograms of nutrient absorbed per 10 cm^2 of membrane surface over the burial time (Western Ag Innovations 2008).

For this study, the probes were used to measure the absorption of nitrate and ammonium ions in selected plots over a period of 8 weeks in 2011 and 2012. The plots selected for the experiment were those within the factorial design: P0, P+, N+P0, N+P+, P2C1 and P2C2 plots, from each experimental block. In 2011 the burial period was from May until July, and in 2012 the burial period was brought forward from March to May, to try to capture the spring growing season.

4.2.5 Laboratory incubation study to measure mineralization

The field P addition experiment was also simulated in the laboratory to investigate the effect of P on mineralization in the soil under laboratory conditions. For this study soil was collected from as near the experimental plots as possible at Leaches field study site from the top 20 cm of the soil profile. The soil was air dried for two weeks after which it was sieved and ground to 5 mm. The soil was then combined with equal weights of washed sand in order to improve drainage and avoid saturation and anoxia during the incubation phase of the experiment. Three soil cores of equal weight were made up from the soil-sand mix and placed on a water tension sand table in order to saturate for 24 hours. Each core was then weighed again to establish saturation weight, after which they were placed on the sand table adjusted to field capacity (a tension of 5 kPa) to equilibrate. The weight of the cores at field capacity was measured to enable a calculation of the exact volume of treatment solution required to add to each experimental core to obtain field capacity.

Twenty-five cores of equal weight were then made up and subjected to 4 different P treatments (P0, P1, P2, P3). P0 reflected the control and consisted of deionised water, P1 consisted of a solution of dihydrogen potassium phosphate, equivalent to adding 25 kg ha^{-1} of P fertilizer in the field. P2 was reflective of 75 kg ha^{-1} of P fertilizer and P3 was equivalent to 100 kg ha^{-1}. Each treatment was replicated 5 times, hence there were 5 cores for each treatment. The cores were covered with parafilm (Parafilm M® Pechiney Plastic Packaging, Chigago, IL) to ensure minimum evaporation and were incubated at 25 ° C for two weeks. Following incubation, the soil was removed from each core and mixed to ensure homogeneity. At this point 5 more cores were made up and treated with deionised water to represent treatment prior to incubation, these were labelled ‘P base’. 25 g of soil from each core was then shaken for 1 hour with 50 ml of 0.5M KCl extract. Extracts were then filtered through a Whatman No 1 filter paper (MAFF 1986). Each filtrate was then frozen at -80°C prior to analysis for nitrate and ammonium using an auto analyser (Braun Lubbe AutoAnalyser 3). This experiment was conducted in 2011 and then repeated in 2013.

4.2.6 Statistical analysis

Data were natural log transformed to achieve normality and homogeneity of variance where appropriate. Above-ground biomass data and nutrient data were then analysed using repeated measures ANOVA to determine if changes in between treatment plots were significant. Statistical differences were determined using General Linear Models in SPSS 21 using treatment, block and year as independent variables. Block effect and interaction effects were accounted for.

4.3 Results

4.3.1 Plant biomass and nutrient offtake

Above-ground biomass did not increase significantly with any of the treatment additions in 2010 or 2011 (figure 4.4). However, for both these years, the treatment which gave rise to the greatest biomass was the combined N and P treatment (N+P+). In 2013 above-ground biomass was significantly different between treatment groups (*p*=0.02) with post-hoc tests showing significance between C6P0 and N+P0 (*p*=0.05). Biomass overall in 2013 was significantly increased compared to that of 2010 and 2011, but 2010 and 2011 were not significantly different. Results are presented in table 4.3.

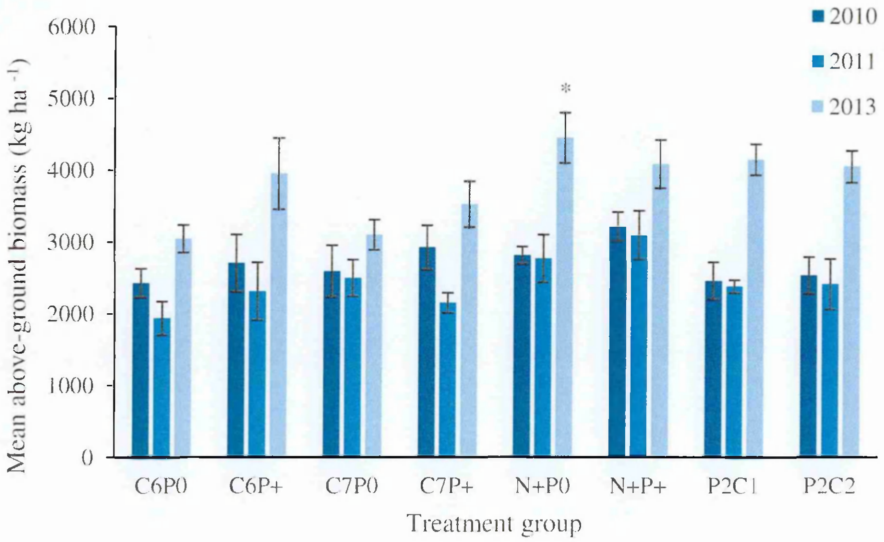


Figure 4.4 Mean and standard error for above-ground biomass for each treatment group in each year. * indicate significant difference from control groups (p<0.05)

4.3.2 Leaf tissue nitrogen content and offtake

Plant tissue N content (figure 4.5a) was significantly different between 2013 and the other years, but not between 2010 and 2011. Plant N tissue content was not significantly different between treatment groups in 2013 (p=0.28), but was significant between treatments in 2010 and 2011 (p<0.001). Although the overall model showed a significant effect of the treatment additions in 2010 and 2011, the only specific treatments showing a greater response than the controls was N+P0 and N+P+ in both years (p<0.001).

Nutrient offtake displayed similar treatment response to N tissue content (figure 4.5b). Offtake N differed significantly only between 2010 and 2013 (p=0.015) and 2011 and 2013 (p<0.001). 2010 and 2011 were not significantly different. Offtake N was significantly different between treatment groups within 2010 (p=0.004), and specifically between C6P0 and N+P+. P2C1 and N+P+ (p=0.015), and P2C2 and N+P+ (p=0.026). In 2011 offtake N was also significantly different between treatment groups (p=0.007) and specifically between C6P0 and N+P+ (p=0.02) and C7P+ and N+P+ (p=0.033). N offtake was not significantly different between treatment groups in 2013.

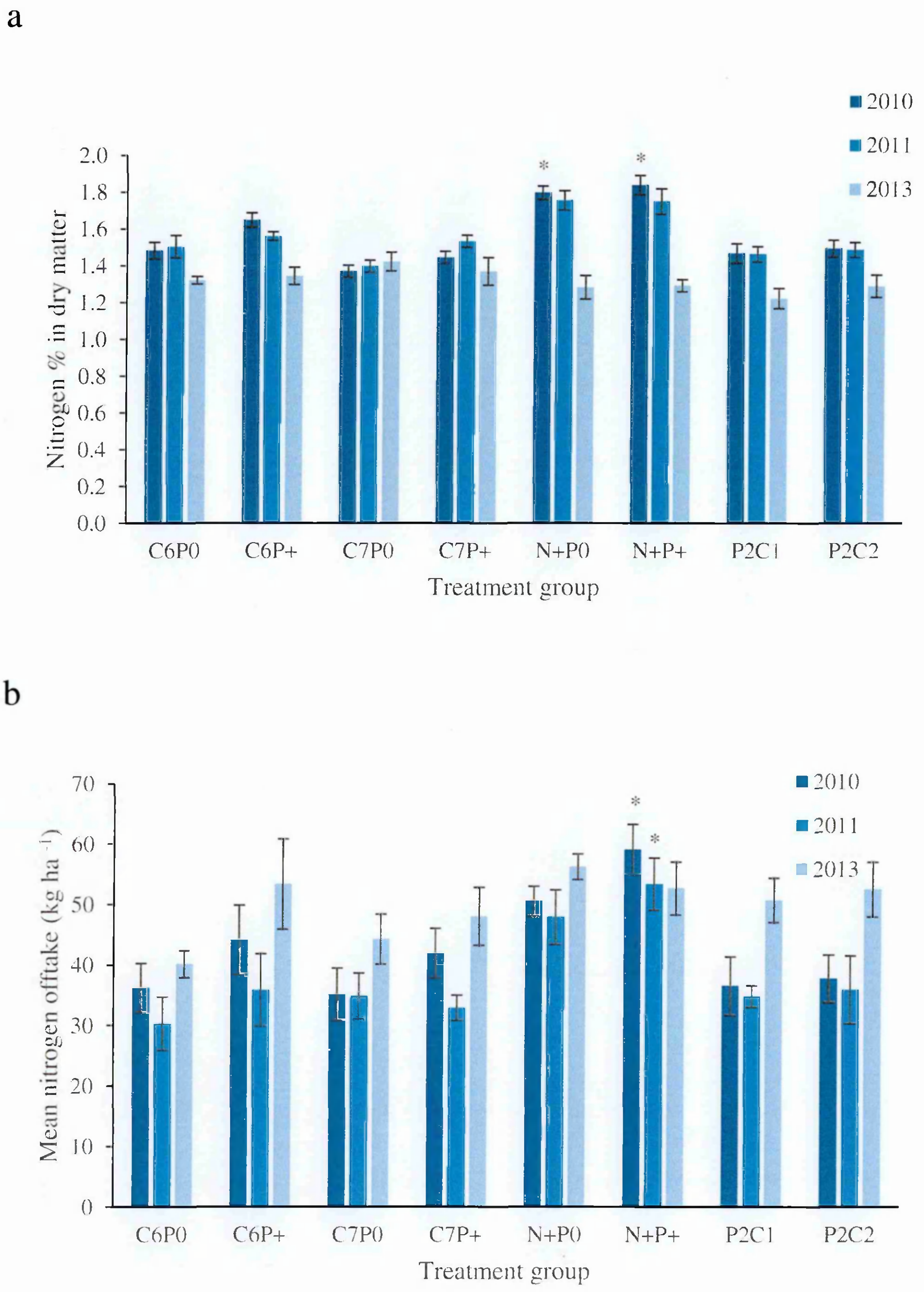


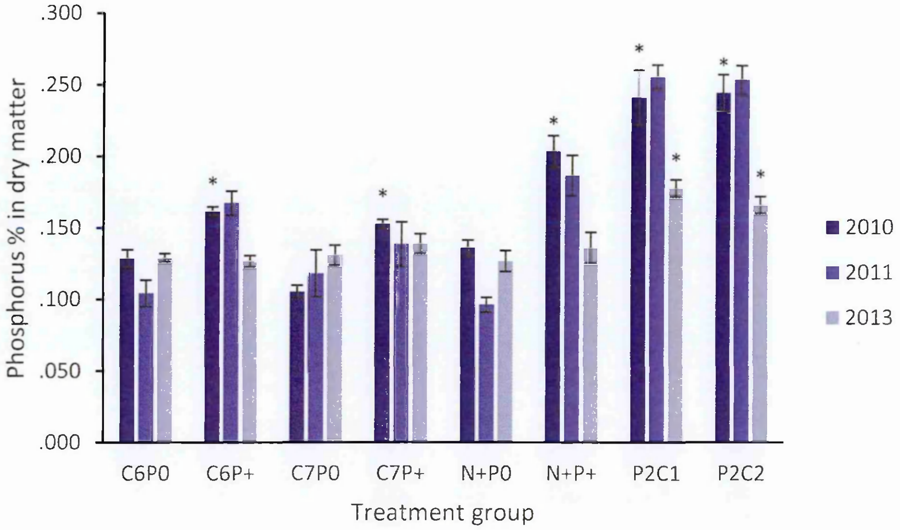
Figure 4.5 Mean and standard error for (a) nitrogen percentage in dry matter, (b) N offtake, for each treatment group. * indicate significant difference from control groups ($p<0.05$)

4.3.3 Leaf tissue phosphorus content and offtake

P % in dry matter was significantly different between years ($p<0.01$). Post-hoc tests showed significant difference between 2010 and 2013, and 2011 and 2013. P% between 2010 and 2011 did not differ significantly. Within all years, there was a significant difference of mean P% between treatments overall ($p<0.01$). In 2010 the treatments showing a significant difference from the controls were N+P+, P2C1 and P2C2. N+P0 and N+P+ were also significantly different. This was the same for 2011 but the controls and P+ were also significantly different. In 2013, post-hoc tests showed significance only between controls and the P2 treatments (figure 4.6 a).

P offtake was also significantly different between years, and post-hoc tests showed significant difference between all years. There was also a significant difference between the mean P offtake between treatment group in every year, post-hoc differences for P-offtake in each year are as follows. In 2010 and 2011 between the controls and N+P+ treatment, and the controls and the P2 treatments. In 2013 between the controls and the N+P0 and N+P+ treatments, and the controls and the P2 treatments (figure 4.6 b).

a



b

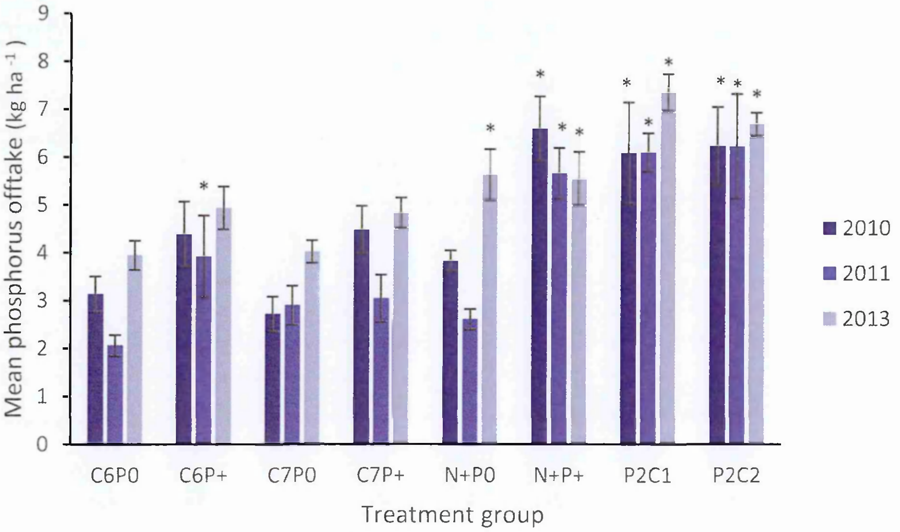


Figure 4.6 Mean and standard error for (a) P percentage in dry matter, (b) P offtake, for each treatment group. * indicate significant difference from control groups (p<0.05)

4.3.4 N:P ratios

Mean N:P ratios were significantly different across year overall ($p<0.001$) and post-hoc between all years except 2010 and 2013 (figure 4.7). Differences between treatments were significant within all years and *post-hoc* between the controls and the P2 treatments in all years, and the controls and the N+P0 and N+P+ treatments in 2010 and 2011. N:P ratios for controls in 2010 fell between 11.5 and 13.0, for 2011 they were between 12.7 and 14.6 and in 2013 they were between 10.2 and 10.9. There was no significant difference between the P2 plots which were cut once (P2C1) or cut twice (P2C2).

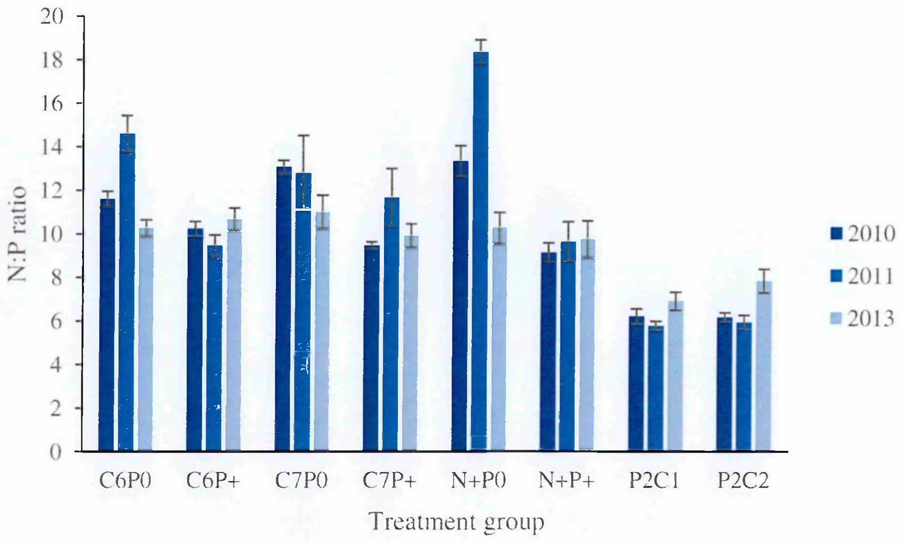


Figure 4.7 Mean and standard error N:P ratios for each treatment group in each year

Factor	Variable	df	F	P value
Treatment 2010	Offtake N	7	3.384	0.008
	N % in dry matter	7	15.816	<0.001
	Offtake P	7	6.729	<0.001
	P % in dry matter	7	39.657	<0.001
	NP ratio	7	55.39	<0.001
	Above-ground biomass	7	0.964	0.473
Treatment 2011	Offtake N	7	2.019	0.083
	N % in dry matter	7	5.108	<0.001
	Offtake P	7	9.484	<0.001
	P % in dry matter	7	3.782	0.004
	NP ratio	7	3.456	0.007
	Above-ground biomass	7	1.566	0.182
Treatment 2013	Offtake N	7	1.613	0.168
	N % in dry matter	7	1.348	0.261
	Offtake P	7	8.408	<0.001
	P % in dry matter	7	7.005	<0.001
	NP ratio	7	6.478	<0.001
	Above-ground biomass	7	3.134	0.012

Table 4.3 Anova table of significant differences between treatments in 2010, 2011 and 2013 for above-ground biomass, offtake N, N% in dry matter, offtake P, P% in dry matter and N:P ratio

4.3.5 Olsen extractable P and pH

Soil Olsen P between 2011 and 2013 did not differ significantly (figure 4.8). However in 2011 there was a significant difference between treatment groups P0 and P2C2 (p=0.048), and N+P+ and P2C2 (p=0.019). In 2013 there was also a significant difference between treatment groups P0, P+, N+P0, N+P+ and P2C1 ($p<0.05$), and treatment groups P0, N+P0, N+P+ and P2C2 ($p<0.05$). In summary; plots receiving 75 kg P ha yr^{-1} (P2 treatment plots) had significantly more extractable P than the other treatment groups. There was no significant difference between the plots receiving 25 kg P ha yr^{-1} and the controls. Soil pH (figure 4.9) was measured in 2013, there was no significant difference between treatment group, and the overall mean was 5.4.

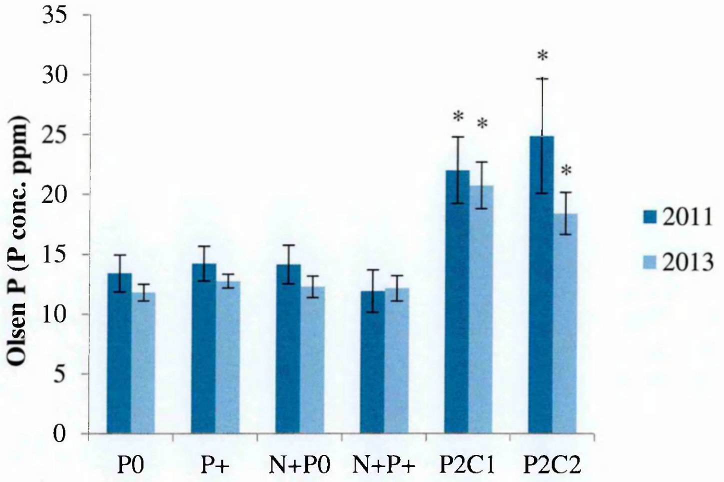


Figure 4.8 Mean and standard error for Olsen extractable P for each treatment group in 2011 and 2013. * indicates significant difference from control (P0).

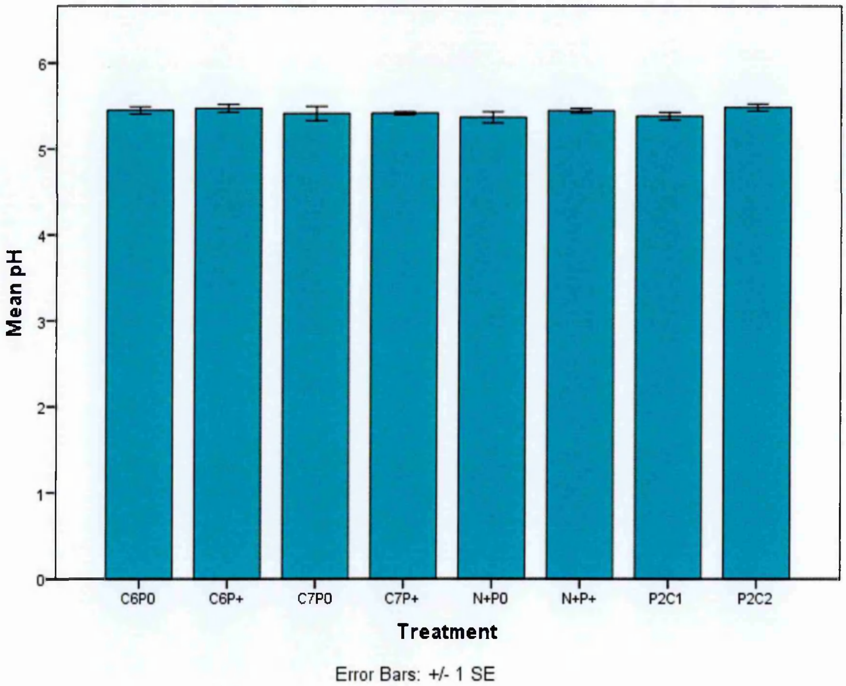


Figure 4.9 Mean soil pH and standard error between treatment groups in 2013

4.3.6 PRS probe data

The ion exchange resin results are presented below (figure 4.10). In 2011 generally more NO_3^- ions were adsorbed onto the probe membranes than NH_4^+ ions. The data were significant between treatment groups for NO_3-N overall but *post-hoc* only between N+P0 and P2C2 ($p=0.034$). NH_4-N was not significant between treatment groups. The ion exchange resin results were not significant in 2012 between any treatment group, although more NH_4-N was detected relative to NO_3-N in 2012 than in 2011.

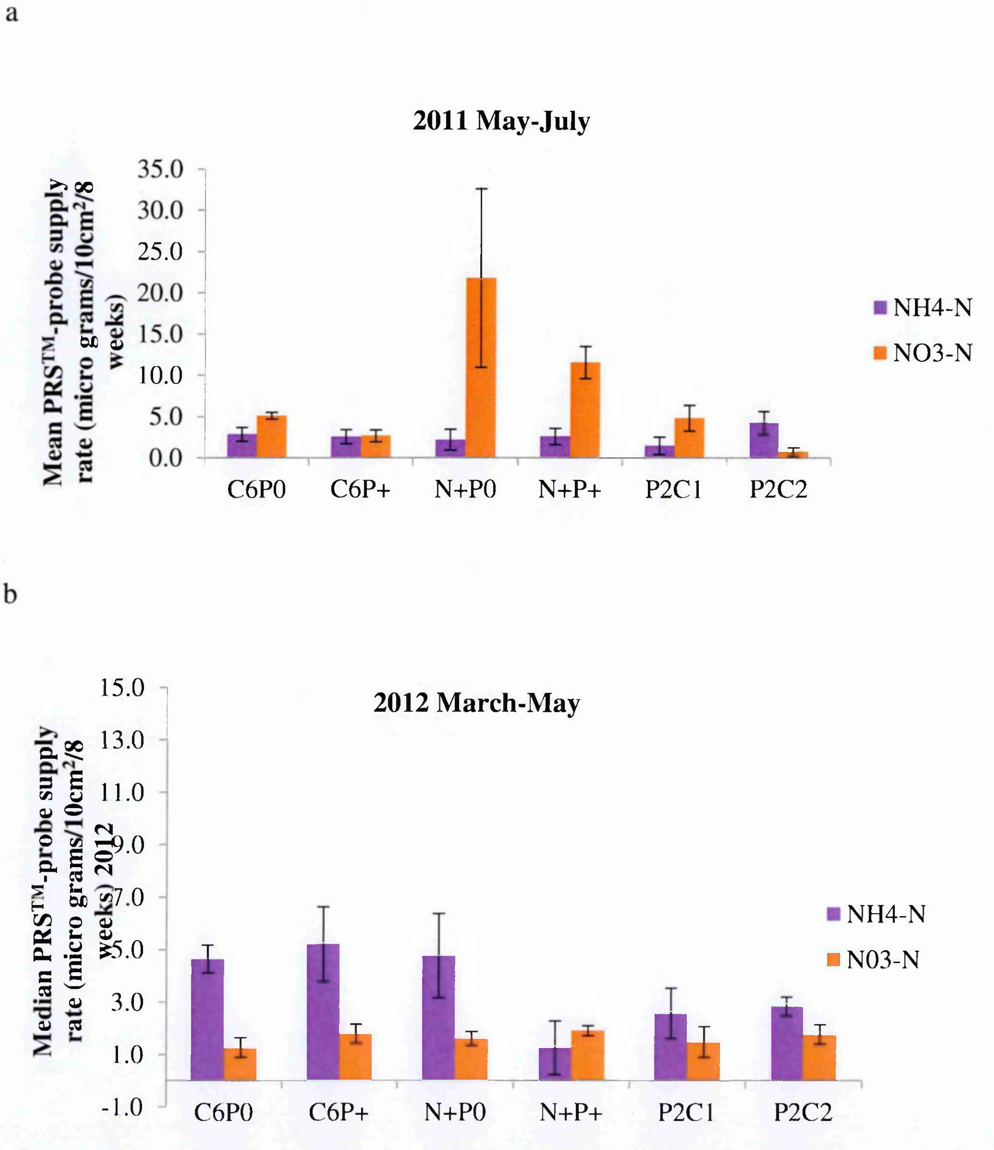


Figure 4.10 Mean and standard error PRS probe results for each treatment group

4.3.7 Incubation experiment

The concentration in extraction with KCl of ammonium ions in the first set of experiments, run in 2011 increased as the P treatment increased (figure 4.11a). However for treatments P2 and P3, the strength of dihydrogen sodium phosphate required for the correct application rates was so high that the sodium peak overlapped the ammonium peak on the ICP, so clarity was lost. However, the ammonium results were significant overall ($p<0.001$) and between P base and P1 ($p<0.001$), P0 and P1 ($p=0.012$). The nitrate extractions were also significant overall ($p<0.001$), and specifically between P base, P0, P1, P2 and P3 ($p<0.001$), and P2 and P3 ($p<0.001$). For the P nutrient additions for the experiment in 2013, dihydrogen potassium phosphate was used to avoid the problem of overlap between the ammonium peak and the sodium peak on the IC autoanalyzer. The results in 2013 exhibit the same pattern as the experiment in 2011 (figure 4.11b); as the P application strength increased, so did the concentration of ammonium ions in the extraction. This was significant overall and between P base and P0, P base and P1, P base and P2 and P base and P3 ($p<0.001$). And also between P0 and P2, P0 and P3 ($p<0.001$) (see figure 4.11 for post-hoc differences between P0 and P treatments). The nitrate results also showed significant differences overall, but this was mostly driven by the difference between the P base and treatment cores. No other treatments gave significant results. In both experiments, the concentration of ammonium ions was higher than nitrate ions in the extraction for the treatments than the P base samples post incubation, indicating that N mineralization had taken place.

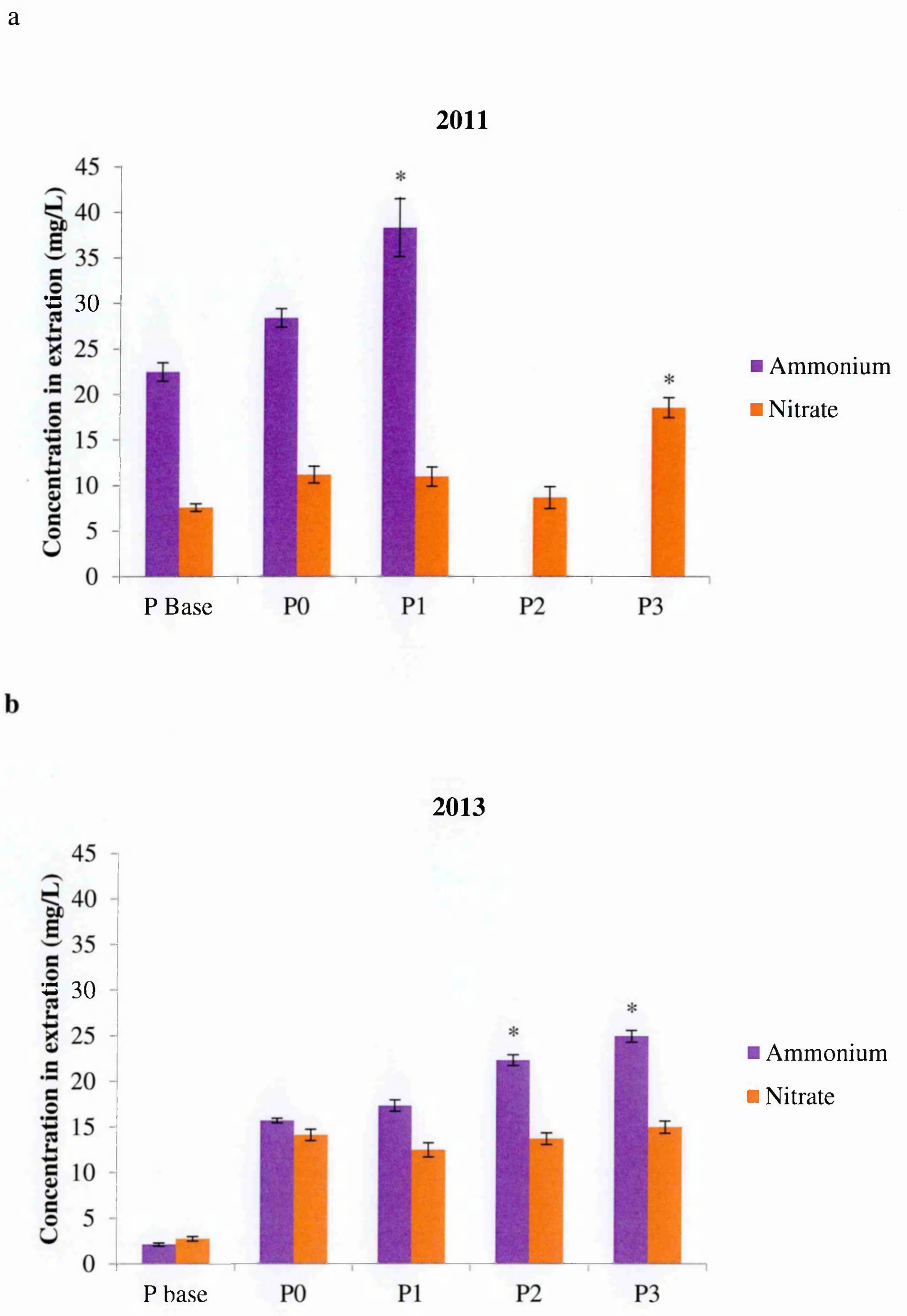


Figure 4.11 Mean and standard error of ammonium and nitrate ion concentration in extraction for each P treatment. * indicates significant difference from the control (P0) ($p<0.001$)

4.4 Discussion

4.4.1 Above-ground biomass results

The factorial nutrient addition experiment did not result in significantly increasing the above-ground biomass of any of the treatment groups within years 2010 and 2011 of the experiment, which was probably due to growth limitation by the lack of rainfall (see chapter 2). In 2013 this was not the case with biomass being significantly different between treatment groups over all and *post-hoc* differences between the controls and the plots receiving nitrogen alone. This supports the conclusions of chapter 2, in that the vegetation biomass on Leaches Meadow is N limited rather than P limited, and indeed the conclusion that nitrogen is the most frequent limiting nutrient in European herbaceous wetlands (Verhoeven, Koerselman & Meuleman 1996; Venterink, Vliet & Wassen 2001). Overall, 2010 and 2011 were not significantly different from each other in terms of vegetation productivity of above-ground biomass, but 2013 was significantly increased compared with 2010 and 2011, again probably down to increased rainfall in this year.

4.4.2 N:P ratios

In terms of biomass N:P ratios, the mean between the treatment plots in this experiment, differed between years and also within years. Significant differences were observed between controls and P2 treatments in every year, with the controls exhibiting a mean N:P ratio between 10 and 14, and the P2 plots between 5 and 7. In 2010 and 2011 there was a significant difference in mean N:P ratios between control plots, (which were in the range of 10-14) and the plots that received N alone (which were between 13 and 18), and the control plots and N+P+ plots, which had a mean N:P ratio of 9. All plots were therefore N limited (if using N:P ratio as a tool to assign limitation (Koerselman & Meuleman 1996)) due to the N:P ratios falling below 14, with the exception of the control plots in 2011, which had an N:P ratio of 14.6, and therefore co-limited, and N+P0 plots in 2011 which had a mean ratio of 18.3 and therefore P limited. Generally Leaches meadow had low N:P ratios which according to Koerselman & Meuleman (1996), suggests that the vegetation biomass is more likely to be enhanced by N fertilization. This critical ratio should however be used with caution as it can only be used to indicate the type of nutrient limitation when either N or P are limiting as for the majority of grasslands (Verhoeven, Koerselman & Meuleman 1996), rather than other factors, especially when light or water supply is limiting (Güsewell & Koerselman 2002).

4.4.3 Nutrient offtake results

In 2010 and 2011 P offtake and N offtake were significantly increased in the N+P+ treatment plots compared with control treatments, this appeared to be driven by the increase in tissue P content percentage, and N tissue content in these plots, rather than an increase in vegetation production. Interestingly, in this study P offtake was increased significantly compared to the controls in 2013 by the addition of N and P together, but also unexpectedly the addition of N on its own. This could be due to increased N stimulating phosphatase activity via N:P stoichiometry effects, which potentially increases plant P uptake (Fujita *et al.* 2010). N addition has also been found to stimulate root phosphatase activity in calcareous grassland in representatives of three major higher plant functional groups, although shoot N and P concentrations were not significantly affected (Phoenix *et al.* 2003). In the current study, root phosphatase activity was not measured, although significant increases in the plant nutrient offtake of P by the addition of N implies stimulation of phosphatases for direct P uptake by the vegetation, which supports the results of the findings of Johnson, Leake & Lee (1999), Phoenix *et al.* (2003), and Fujita *et al.* (2010). The nutrients taken up by the vegetation, (significant differences in P offtake between controls and P2 plots) were not necessarily utilised for plant growth, as demonstrated by the lack of difference in above-ground biomass between P0 and P+ and P2 plots in 2010 and 2011, although there was a non-significant increase by the addition of P in 2013. This suggests either luxury uptake (Chapin 1980), which was also the case in the experimental cutting plots within the growing season (Chapter 2) or limitation by another factor, such as drought (Weltzin *et al.* 2003; Harpole, Potts & Suding 2007). Plants are more prone to luxury uptake and internal storage of P than of N (Verhoeven & Schmitz 1991; Aerts & Chapin 1999) which could be why P was readily taken up by the vegetation, but not the limiting nutrient in terms of plant growth.

Similar to P offtake, N offtake was also significantly increased by the addition of N and P together (the N+P+ plots) in 2010 and 2011. In 2013 there was no significant difference in N offtake between treatment groups, however there was a *non-significant* increase in N offtake with the addition of P, N and P together and N alone, compared with the control plots. This signifies that the addition of P could increase N mineralization, which is the fundamental hypothesis of the study. The addition of 25 kg P ha yr^{-1} (P+ plots) produced no significant N offtake increase, which also supports the results of chapter 2. The increase in N offtake with the addition of N and P points towards an interaction between these two nutrients. Further evidence for P stimulating N mineralization follows from the incubation study.

4.4.4 Nitrogen mineralization

The addition of P to soil cores (made from soil extracted from the study site) significantly increased the concentration of ammonium ions in extraction, as the strength of P solution increased. This again suggests that P addition had positive effects on soil net N mineralization in this experiment. Supporting evidence for P addition increasing net N mineralization is from a very recent study by Changhui *et al.* (2014), who also demonstrated an increase in soil net N mineralization and ammonification rates with P addition from soil of a saline-alkaline grassland in the Loess Plateau of northern China. They calculated mineralization rates from differences in soil NH_4^+ and NO_3^- concentrations by using the buried soil technique (Raison, Connell & Khanna 1987) during a 28 day in-situ incubation on permanent treatment plots. They suggested that there was a shift in the microbial community structure with the addition of P, indicated from a significant decrease in the microbial biomass carbon and microbial biomass nitrogen (MBC/MBN) ratio with P addition. However, in contrast, another study (Li *et al.* 2010) found no effect of P addition on MBC: MBN ratios and negligible effects on soil net N mineralization rates or microbial properties in a semi-arid, sandy grassland in Northeast china. They found that N addition alone (and not N and P treated together, or P alone), caused a reduction in microbial biomass, which is also true of nitrogen addition in temperate climates (Treseder 2008; Liu *et al.* 2014). Other supporting studies that imply N mineralization is linked to available P include Oorschot *et al.* (1997) where the available P pool was positively correlated with N mineralization using *in-situ* soil core incubations when comparing high nutrient and low nutrient slopes on two riverine wetlands in the UK. Similarly on Dutch heathlands, N-mineralization was positively correlated with the labile soil organic P-pool observed during incubation experiments of intact soil cores (Troelstra, Wagenaar & Boer 1990).

In order to simulate this test in the field, PRS-probes® were used to measure the nutrient supply rate to the vegetation, in place of extracting soil cores from the field which was too destructive. However, the results were inconclusive with supply rates of NO3-N increasing only in plots receiving N addition in 2011 and no significant differences or trends between treatment plots in 2011 or 2012. The PRS probe nutrient supply rate was extremely low in this experiment; ranging from 1.0-20 micrograms per 10 cm^2 over eight weeks. This is atypical for a clay soil where values would usually fall in the range of 1500-1700 micrograms, which is for an agricultural manured soil (Qian & Schoenau 2005). In this case, it is a possibility that drought affected the soil nitrogen N pool since the microbial processes that regulate soil N availability are sensitive to variations in soil moisture (Bloor & Bardgett 2012), and therefore the ability of probes to acquire nitrogen as efficiently as usual at this time was affected by soil moisture deficit. Size and fluxes of the soil N pool are largely determined by precipitation events, which can impact N cycling (Verburg *et al.* 2009). The probes reflected a 'snap-shot' of eight weeks in measuring the nitrogen supply rate to the plants which may have been severely limited by the drought at the time of burial, and in contrast were inundated by floodwater in 2012. The field nutrient addition experiment demonstrated that the plants were able to take up N and P, indicated by the significant differences in tissue N and P content between treatment groups, and the increase in N offtake by the addition of N and P, and the increase in P offtake by the addition of N.

Although below-ground cycling and interactions between N and P were not directly measured, these results could indicate co-limitation of N and P and interactions between multiple resources, which is the current thinking behind nutrient limitation (Harpole *et al.* 2011). For example, Marklein & Houlton (2012) investigated the interactions between N and P cycles of phosphatase enzyme activity by means of meta-analysis synthesizing results from 34 separate factorial N and P experiments. They found that N fertilization enhanced phosphatase activity, and P fertilization suppressed rates of phosphatase activity which implies that phosphatase enzymes are strongly affected by changes in local nutrient cycles. This is also in agreement with other studies that have shown increasing N deposition increases phosphatase activity (Chung *et al.* 2007) and also in sites that are limited by P or co-limited by N and P (Olander & Vitousek 2000).

Mechanisms of plant uptake of P and N, and the differences between treatment groups in the field experiment could be partially explained by the behaviour of the below-ground arbuscular mycorrhizal (AM) fungi, which supply limiting nutrients to plant roots which are otherwise inaccessible (van der Heijden, Bardgett & van Straalen 2008). In this experiment, P tissue content percentage and indeed P offtake were enhanced significantly by the P2 treatments in all years, however above-ground biomass was not increased significantly in these plots, therefore the AM fungi may have altered the distribution of nutrients amongst co-existing species, without altering total vegetation productivity; as supported by van der Heijden *et al.* (2006b). Multiple studies have shown that enhanced P uptake by plants is controlled by AM fungi, especially for certain species with high P requirements such as legumes, although the control of N uptake by AM fungi is less well reported with one study showing that N acquisition is indeed enhanced by AM fungi (Hodge, Campbell & Fitter 2001), and others reporting no effects (Reynolds *et al.* 2005; van der Heijden *et al.* 2006).

Clearly from these array of field results, it is not as straightforward as assigning the vegetation to being exclusively N limited. The addition of N was the only treatment to cause a significant biomass increase in the vegetation, however offtake P and N were both increased by the addition of N and P together, which suggests co-limitation by both nutrients or other resource limitation (Harpole *et al.* 2011). This agrees with recent papers on co-limitation where there are likely synergistic interactions between multiple limiting resources (Elser *et al.* 2007; Farrior *et al.* 2013). Harpole *et al.* (2011) found support for strict definitions of co-limitation in 28% of 641 studies which applied a factorial addition of N and P in freshwater, marine and terrestrial ecosystems. Furthermore N and P stoichiometry can alter the competitive response of different species resulting in a changing community depending on the ability of different species or functional groups to take up N or P under P limited or N limited conditions (Venterink & Güsewell 2010). The species and community response to the factorial addition of N and P is investigated in chapter 5.

4.4.5 Legacy effects

In this study, significant differences in above-ground biomass between treatment plots and controls still existed in 2013 despite the last dose of N and P being administered in May 2011. Although N % in the plant tissue content was not significant between plots in 2013, but P in plant tissue still showed a significant difference between treatments in 2013. Therefore in 2013 offtake N differences were driven by vegetation productivity increases, and offtake P differences were driven by vegetation P tissue content percentage. Explanation for this difference could be due to the difference in resource acquisition of both N and P, in response to the specific nutrient requirements of the vegetation. The available P (Olsen P) in the soil was also significantly different between treatment plots in 2013 despite the most recent application being in May 2011, demonstrating that P had not leached from the plots after 2 years, and after successive flood events and inundation. The lingering effect of N on the above-ground biomass in 2013, despite cessation of treatment additions and no increase in tissue N content % could be due to a legacy effect of N on the microbial community contributing to changes in below-ground N cycling. Microbial activity could potentially remain elevated despite cessation of N additions after a number of years, with microbial biomass showing no significant effect (Power *et al.* 2006). Similar results were found in another study on a prairie grassland where total plant N had declined to control levels, with above-ground biomass increasing, following cessation of N addition after 12 years with surface soil nitrate pool remaining higher than the controls, with higher rates of N mineralization detected, suggesting that the cycling of N was continuing long after the inputs of N ceased (Clark *et al.* 2009). Stevens *et al.* (2012) also found above ground biomass to be higher where N had been added, but with no significant effects on plant tissue chemistry, 15 years following the cessation of N addition on a peat grassland in the UK. This demonstrates that recovery from N addition can be very slow, with the effects of N addition seen for many years following its cessation. This long lasting effect of N is also reflected in the changing vegetation species composition of grasslands, and is investigated in chapter 5 of this study, where species redundancy analysis scores were correlated with Ellenberg N values.

4.5 Conclusion

From these results, the hypothesis in this investigation that the addition of P can significantly increase N uptake by plants in floodplain meadows can be partially accepted. Despite the addition of P exclusively, not stimulating the vegetation to increase uptake of N in the field experiment, the addition of P in combination with N produced a significant increase in N offtake. Supporting the hypothesis that increased P addition can lead to N mineralization was the laboratory experiment, where significantly increased levels of NH_4^+ were detected with increasing P concentration, which can be explained by elevated N mineralization. Addition of P in the field induced a non-significant increase in offtake N in 2013 which was the year of greatest precipitation in the factorial experiment and where the vegetation was not limited by moisture deficit. Therefore, the type of nutrient limitation may not be a static site characteristic, but may vary with changing environmental conditions (Venterink, Vliet & Wassen 2001), so the exact same experiment could produce different results in terms of above-ground productivity or nutrient offtake if carried out over a different time frame or at different sites.

The above-ground vegetation biomass was primarily limited by N alone, although from the tissue nutrient content and offtake results and from recent publications on co-limitation, the vegetation is unlikely to be limited by a single resource. The growing season in the first two years of the experiment (2010 and 2011), were uncommonly dry and therefore plant growth is likely to have been limited by water above any other resource. Significant changes in above-ground biomass were noted in 2013, demonstrating the persistence of nutrients within the system, however this was 2 years after the final nutrient addition. Similarly if 'average' rainfall had ensued in 2010 and 2011, above-ground biomass would have been very different and perhaps significant differences would have occurred between treatment groups. This study confirms links between P and N cycling which support multiple resource limitation theories in that changes in the cycling of one nutrient can affect the availability of another. These results imply that increased P content in floodplain soils can lead to increased uptake of N by the vegetation and thus enhancing the effects of N deposition. The results also support that of Fujita *et al.* (2010) and Phoenix *et al.* (2003) who found increased N supply stimulated phosphatase activity to potentially increase plant P uptake in a species-specific way. With this in mind N deposition could lead to changes in plant community structure not only by increasing productivity, but by favouring species that are able to persist under P limited conditions. My findings not only support this, but may also imply the reverse of this; that increased P supply could favour species that are more adapted to N limited conditions. Chapter 5 investigates the effect of N and P on vegetation composition.

Chapter 5

The effect of P and N addition on the botanical composition of floodplain meadows

5.1 Introduction

In the UK, much of the loss of unimproved grasslands and floodplain meadows has been attributed to the increased use of artificial fertilizers (Bakker & Society 1994; Kirkham & Tallowin 1995; Silvertown *et al.* 2006). This loss has also been associated with declines in many plant and animal species (Janssens *et al.* 1998; Tallowin *et al.* 1998; Stevens *et al.* 2004; Dupre *et al.* 2010).

Species richness and vegetation composition are often linked to nutrient levels in wetlands and grasslands. Variation in species richness and its relationship with productivity is described by Grime (1979) as a hump-shaped curve, with low species richness at low and high productivity. The hump-backed curve occurs because only a few species are adapted to nutrient poor conditions, and only a few dominant species are able to out-compete all others in nutrient rich environments. Janssens *et al.* (1998) investigated the relationship between soil chemical characteristics and plant diversity in West-Central European grasslands and demonstrated a hump-backed relationship between species richness and soil fertility. 281 sites were investigated and their soils sampled for pH, organic matter, total nitrogen, and exchangeable nutrients: phosphorus and potassium. They found that species number showed a hump-backed curve with soil extractable phosphorus and potassium, with maximum species achieved at only 4 mg/100 g of extractable P (acetate and EDTA extraction).

Species composition of plant communities can differ depending on whether nitrogen (N) or phosphorus (P) is the limiting nutrient, even if total productivity of the vegetation is the same (Venterink 2011). This has been demonstrated by long-term fertilization experiments, such as The Park Grass, where different species of grass dominate under N and P limitation, and legumes are more abundant under N limitation.

Further to this, plant-species richness is also influenced by the type of nutrient limitation, though both the addition of N and P can reduce species richness through higher biomass and taller growing graminoids competing for light, rather than a direct mechanism (Gough *et al.* 2000; Beltman, Willems & Güsewell 2007; Bobbink *et al.* 2010). It has been suggested that P limitation can favour a higher species richness than N limitation (Wassen *et al.* 2005; Ceulemans *et al.* 2011; Venterink 2011). Venterink (2011) explains this difference in species richness by four different mechanisms. Firstly, the number of forms in which P and N are present in the soil and the number of plants to acquire them; secondly the mechanisms and traits that control species competition and coexistence under N or P limitation; thirdly the sizes of regional species pools for N or P limited conditions; and lastly the interaction between the type of nutrient limitation and community productivity. Evidence is needed to support the above mechanisms in order to gain a better understanding of species competition under P limitation, and about the forms of N and P under different conditions and plant traits and mechanisms needed to acquire these nutrients.

Various papers have attempted to provide further evidence for differing levels of N and P application affecting species richness. In the Park Grass Experiment, as the amount of N applied increased, species richness decreased linearly with significant decreases in species richness between application rates of 0 and 50 kg N ha^{-1} y^{-1}, 100 kg N ha^{-1} y^{-1} and 150 kg N ha^{-1} y^{-1}, the greatest negative effects on species richness were when N and P were applied together (Crawley *et al.* 2005). Similar negative effects of N addition, even at low levels were found at Tadham Moor, Somerset, UK (Mountford, Lakhani & Kirkham 1993). Only 25 kg N ha^{-1} y^{-1} encouraged the spread of agriculturally productive grasses within two years, and at 50 kg N ha^{-1} y^{-1} species richness was significantly reduced within three years. In terms of soil P levels, species rich wet grasslands are associated with lower levels of soil P (Critchley *et al.* 2002). Further to this, Ceulemans, Stevens & Duchateau (2014) surveyed 105 lowland hay meadows and recorded species richness and soil phosphorus (using the Olsen-P extraction method) and found the highest species richness occurred at lower levels of soil P. Maximum observed species richness did not exceed 20 species per 4 m^2 beyond 80 mg P kg^{-1}, compared to more than 40 species below 40 mg P kg^{-1}.

Ceulemans *et al.* (2013) collected data from 132 semi-natural grasslands located along a gradient of nutrient availability and atmospheric N deposition, and then investigated the relation between soil nutrients, acidity, nutrient limitation and productivity against plant-species richness by use of linear mixed models. They found that soil P was significantly negatively related to total species number, forbs and endangered species, and soil N was only significantly negatively related to number of forbs and endangered species, but not species richness. They concluded that N and P driven species loss were independent to each other, but that P enrichment can present a greater threat to biodiversity than N enrichment in some terrestrial ecosystems. Blanck *et al.* (2011) studied shrub-land vegetation in northern Patagonia (Argentina) to ascertain whether there is a relationship between plant-species richness and plant-available N, P and water. They determined the leaf P and N content and the $\delta^{13}C$ of *Berberis buxifolia,* and the soil P and N content at each of the 20 sites. They found a negative correlation between species richness and foliar P concentration, and a positive correlation between plant species richness and foliar N:P ratios. Foliar N showed no correlation with species richness, despite the fact that N:P ratios indicated that N was limiting plant growth while soil extractable NH_4 showed a weak positive correlation with the number of shrub layer species. They concluded that low levels of P correlate with high plant-species richness, and available N did not show such a relationship despite N:P ratios indicating that N limited vegetation productivity.

In terms of the effect of P on specific plant communities, there have been many long-term experiments and numerous studies of nutrient addition on a range of grassland communities (Kirkham, Mountford & Wilkins 1996; Tallowin *et al.* 1998; Silvertown *et al.* 2006; Hejcman *et al.* 2010a). In the Park Grass Experiment legumes were favoured in plots receiving P alone (at rates of 35 kg P ha^{-1} y^{-1}) and no N, whereas grasses were found to dominate in plots receiving only N (Silvertown *et al.* 2006). Similarly legumes, and in particular *Trifolium pratense* were also found to increase with the application of P and K on a species rich meadow at Tadham Moor (Kirkham, Mountford & Wilkins 1996), and all legumes were found to be suppressed with high rates of N application (100 or 200 kg N ha^{-1} y^{-1}). High rates of P (application rate was 75 kg P ha^{-1} y^{-1}) were also found to increase biomass significantly and severely reducing species diversity leading to dominance of *Holcus lanatus*, *Rumex acetosa* and *Lolium perenne.* More recently published papers on another long-term experiment, the Rengen Grassland Experiment (Hejcman *et al.* 2007, 2010b; Chytry *et al.* 2009) found that the largest difference in vegetation structure and composition was between the treatments with and without P application (rates were 35 kg P ha^{-1} y^{-1}) . And among plots receiving P, plant species composition was similar with tall grasses such as *Alopecurus pratensis*, *Arrhenatherum elatius* and *Trisetum flavescens* dominating the sward. They also found that N application was not detrimental to species richness unless it was accompanied by another limiting nutrient such as P (Hejcman *et al.* 2007).

Clearly there is conflicting evidence between various studies as to which nutrient is most damaging to grasslands in terms of biodiversity. Consequently, there is still a need for further research into N and P limitation on floodplain meadows, and specifically MG4 vegetation as there is a lack of published work in relation to this habitat. In this chapter we address how plant community and species richness on a floodplain-meadow MG4 community is altered by the addition of N and P, alone and in combination. The following questions will be addressed in this chapter:

1. Does the addition of P change the species composition of MG4 vegetation? (Page 141-142)
2. Are there temporal changes in species composition and abundance with the addition of P? (Page 143-144)
3. What environmental variables are important in explaining species variation at Leaches Meadow? (Page 151-154)

5.2 Methods

Details of the experimental set up for the factorial experiment are given in chapter 4. Additions of N and P were administered to specific plots in 2010 and 2011 in three treatment doses. Plots that had been treated with N (N+P0 and N+P+ plots) were cut between 21st and 28th June in 2010 and 2011. Plots that had been treated with P (P2C1 and P2C2) were cut between 28th June and 4th July in 2010 and 2011. Controls for the N+ plots were labelled 'C6' and controls for the P2 plots were labelled 'C7'. The cut vegetation was split into functional groups, oven dried and weighed to establish the dry biomass for each treatment group. The vegetation of each factorial plot was surveyed before cutting, by estimating percentage ground cover of each species present in 2010, 2011 and 2013. Plots were not cut in 2013, so cover abundance of each functional group was used to replace dry weight in this year

5.2.1 Statistical analysis

For the years 2010 and 2011, above-ground dry biomass data were used to perform analysis on vegetation composition by functional group. For 2013 because cuts were not made, percentage cover data were used in the place of dry weight data and split into functional groups. These data were log transformed for statistical analysis to achieve homogeneity of variance. The relationship between above-ground dry biomass and percentage cover data was investigated using Pearson correlation. For each functional group there was a strong positive correlation between cover abundances and dry weight; the r^2 value for graminoids were 0.919, forbs were 0.92, and legumes were 0.772 (table 5.1 in results section). Significance between treatment groups and year for species richness, Shannon-Wiener diversity and functional group was assessed using General linear models (GLM) in SPSS 21, incorporating analysis of variance with Tukey *post-hoc* tests to detect any significant differences between treatment groups, with significance set at the $p < 0.05$ level. Block effects and hydrological variation were taken into account when building the model, by using block as a fixed factor and water-table depth as a covariate. Differences in percentage cover values between species, between treatments and between years were also assessed using General Linear Model repeated measures multivariate analysis in SPSS. Multivariate analysis of species data using ordination techniques was performed in Canoco 4.5.

The ordination techniques used in this chapter are based on the gradient lengths of the initial detrended correspondence analysis (DCA). As the length of the longest gradient was 2.359, the use of linear methods for the dataset were more appropriate. Principle components analysis (PCA) was used for investigating the variation in the dataset for species alone, and redundancy analysis (RDA) for investigating the variation in species which can be explained by the environmental variables. The significance of the environmental variables was tested in the constrained ordination by using Monte Carlo permutation tests. Variance partitioning was used to quantify the effects of groups of environmental variables.

5.3 Results

5.3.1 Species richness and diversity indices.

Species richness was significantly different between all years (p<0.001) but not between treatments (p=0.148) (figure 5.1). The Shannon-Wiener (SW) diversity index was significantly different between years (p=0.042) although *post-hoc* analysis showed differences between 2010 and 2011 only (*p*=0.032). Shannon-Wiener diversity was not significantly different between treatment groups (*p*=0.275) (figure 5.2). In terms of species number per quadrat, the most diverse year was 2010 with a mean species richness of 17.5, with 14.3 in 2011 and 15.9 in 2013. Species richness and SW diversity, declined across the whole site in 2011, and showed some recovery by 2013, which was significant for species richness, but not SW diversity. Despite no significant differences in species richness between treatments, all treatment plots declined in species richness from 2010 to 2011, but the greatest decline was seen in the P2 treatments, although this was not significant. Despite no significant difference between treatment groups, all treatment plots increased in species richness in 2013, except the N+P0 plots, which remained the same as 2011. Greatest recovery was in the N+P+ plots. This same trend was demonstrated by the SW index, except N+P0 plots declined again in 2013 (figure 5.2).

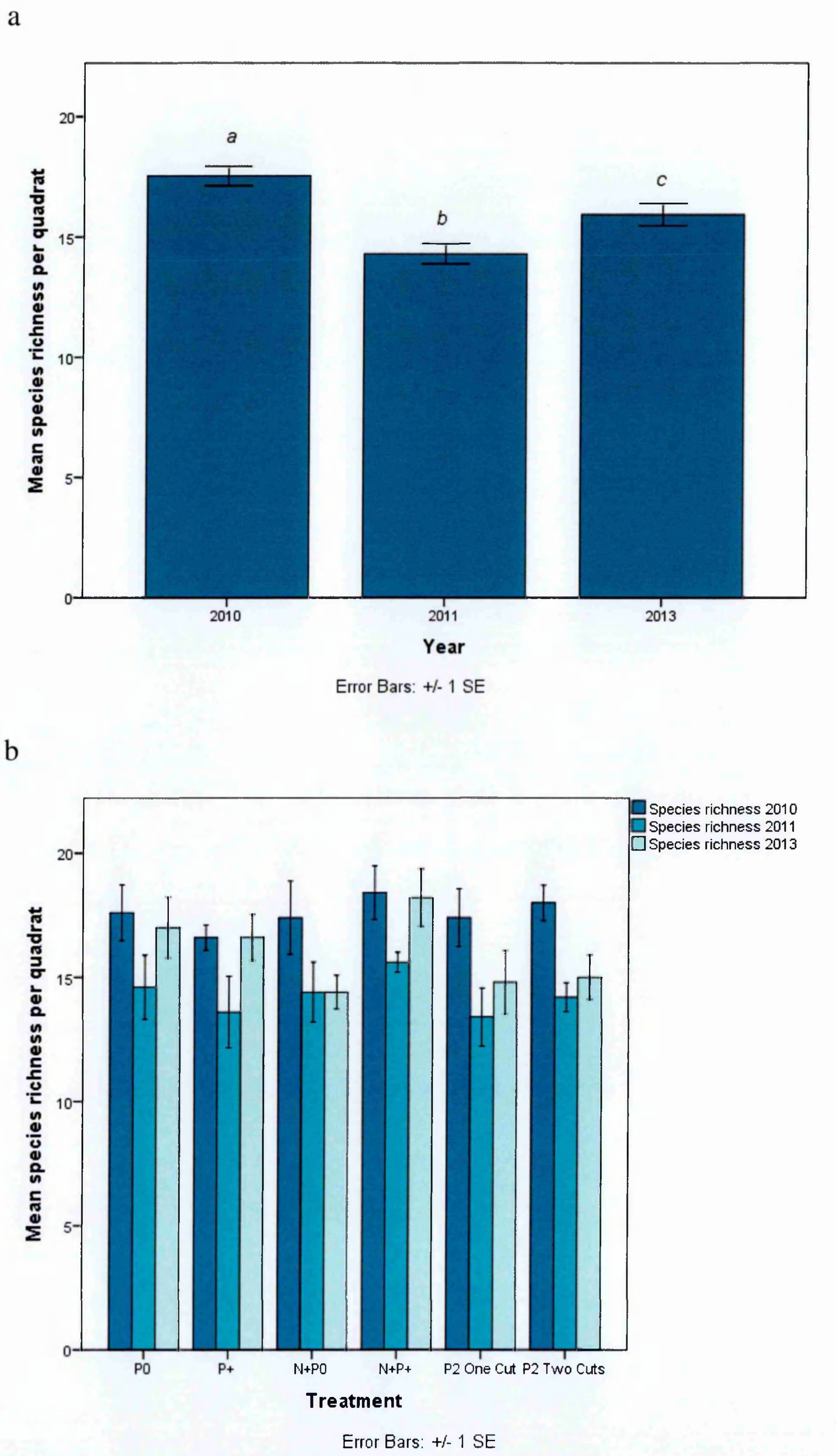


Figure 5.1 Mean and standard error for (a) species richness per quadrat (m^2) in each year and (b) species richness per quadrat for each treatment in each year

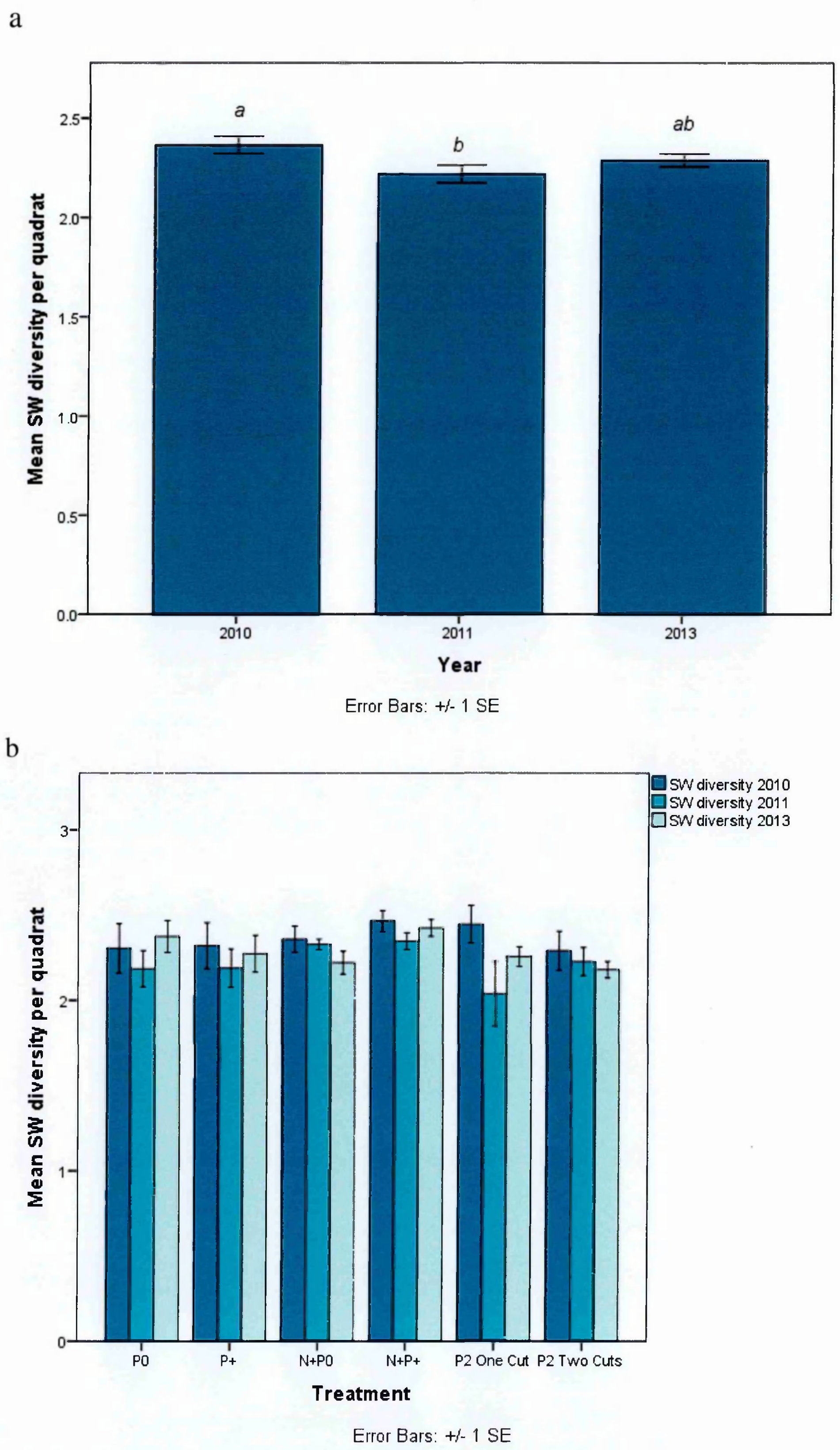
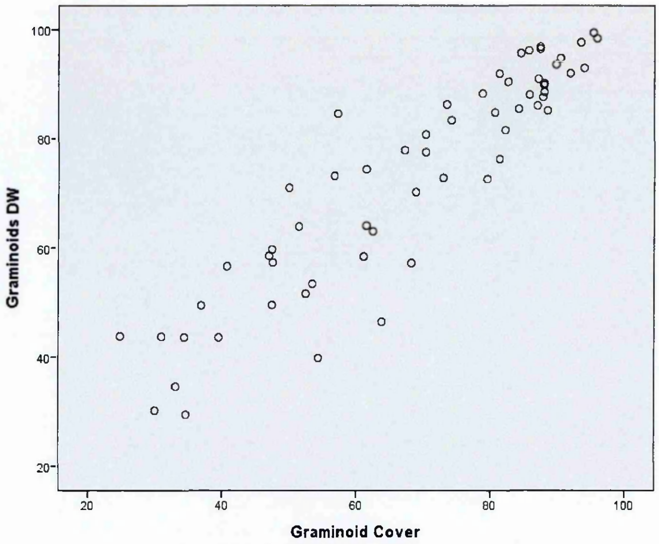


Figure 5.2 Mean and standard error for species richness per quadrat (m^2) for each treatment group in each year

5.3.2 Functional group

Cover percentage values and above-ground biomass dry weight for each functional group were correlated with each other in analysis including all factorial plots for 2010 and 2011 (table 5.1, figure 5.3). The correlation for each functional group was strongly significant ($p<0.01$ for all groups, r=0.772 for legumes, r= 0.919 for graminoids and r=0.920 for forbs) and therefore when comparing functional group between years, cover was used as a substitute for dry weight in 2013 (figure 5.4, 5.5), as factorial plots were not harvested in 2013. These values were log transformed to obtain homogeneity of variance before calculating statistical significance between treatments and years.



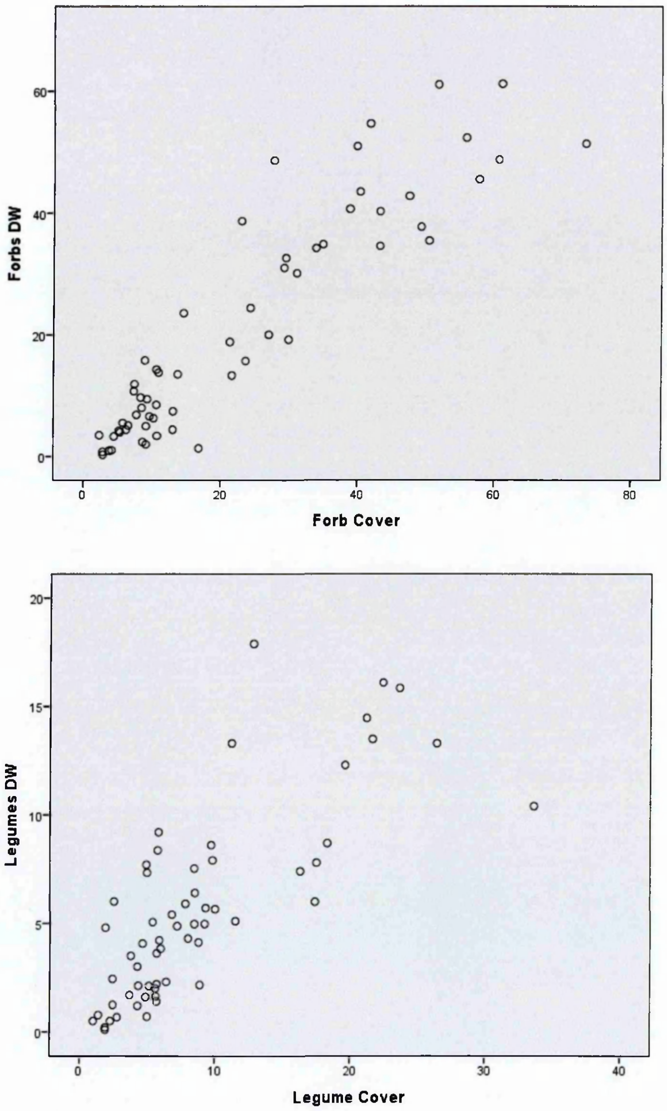


Figure 5.3 Relationship between percentage cover and dry weight for each quadrat in 2010 and 2011

	Pearson correlation between cover and dry weight	p value
Legumes	0.772	<0.01
Graminoids	0.919	<0.01
Forbs	0.92	<0.01

Table 5.1 Pearson correlation between cover percentage and dry weight for each quadrat in 2010 and 2011

Table 5.2 shows the results of the General Linear Model to test significance between species richness, SW diversity, and functional group between years and between treatment groups. Only graminoids and legumes were significant for year with post hoc differences displayed on figure 5.4.

Functional group was significantly different between years for graminoids and legumes ($p<0.01$), but not forbs ($p=0.266$) (table 5.2). *Post-hoc* tests showed that graminoids increased significantly between 2010 and 2013 ($p<0.05$), and 2011 and 2013 ($p<0.05$) but showed no significant difference between 2010 and 2011 ($p=0.946$). Legumes decreased significantly between 2010 and 2013 ($p<0.05$), and 2011 and 2013 ($p<0.05$), but also showed no significant difference between 2010 and 2011 ($p=0.09$). Forbs reduced in 2013 compared to other years, but this was not significant ($p=0.266$).

In terms of differences between treatment group within years, only legumes showed a difference in dry weight between treatments overall ($p<0.01$) with *post-hoc* differences significant for 2011. There were no other significant differences within years between treatments. In 2010 there was an increase in legume dry weight in P+ plots compared with P0 plots, which was also reflected in 2011 with a significant increase in legumes between controls and P+ plots ($p=0.047$). Legumes in 2013 showed no significant differences between treatment group, and the P+ and P2 treatments did not show any increase in legumes unlike the other years. Although not significant, there was an increase in graminoids in N+P0 plots in 2011. Trends in functional group that were apparent in 2010 and 2011 were diminished by 2013 with no significant differences in functional group between any treatments.

Species richness was also correlated with dry matter P percentage, dry matter N percentage and N:P ratio, which were not significant ($p>0.05$).

Factor	Variable	df	F	Sig.
Treatment	Species Richness	5	1.691	0.148
	SW diversity	5	1.297	0.275
	Graminoid %	5	1.333	0.26
	Forb %	5	0.973	0.44
	Legume %	5	4.636	p<0.01
Year	Species Richness	2	13.684	p<0.01
	SW diversity	2	3.308	P<0.05
	Graminoid %	2	7.511	p<0.01
	Forb %	2	1.347	0.266
	Legume %	2	29.674	p<0.01

Table 5.2 ANOVA table for species richness, SW diversity and functional group for each treatment group. Significance is shown for each variable.

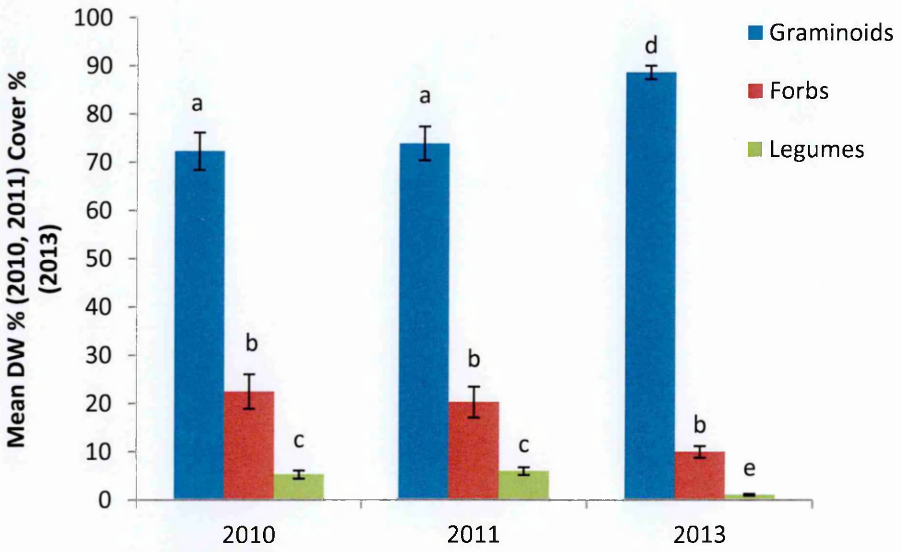
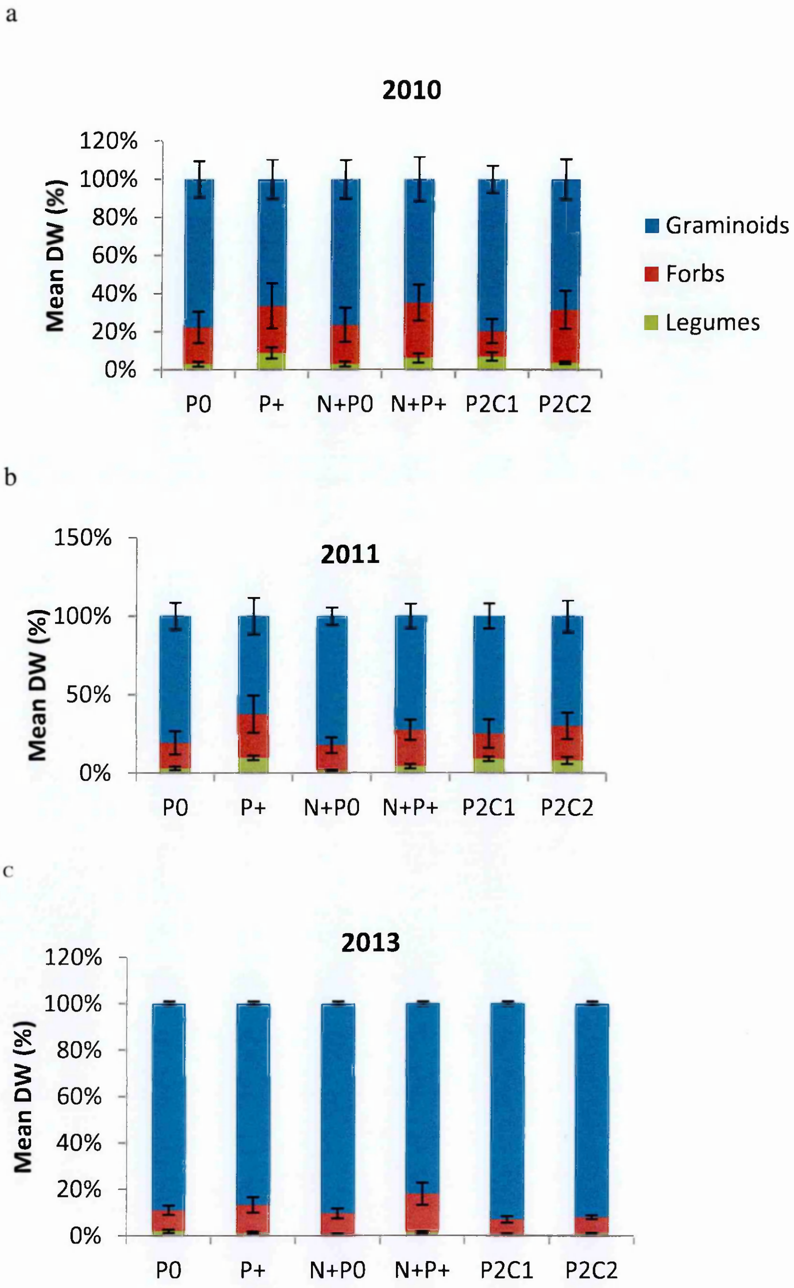


Figure 5.4 Mean and standard error for dry weight of vegetation for each functional group in 2010 and 2011 and cover % for each functional group in 2013


a
2010
Mean DW (%)
120%
100%
80%
60%
40%
20%
0%
P0
P+
N+P0
N+P+
P2C1
P2C2
■ Graminoids
■ Forbs
■ Legumes
b
2011
Mean DW (%)
150%
100%
50%
0%
P0
P+
N+P0
N+P+
P2C1
P2C2
c
2013
Mean DW (%)
120%
100%
80%
60%
40%
20%
0%
P0
P+
N+P0
N+P+
P2C1
P2C2

d

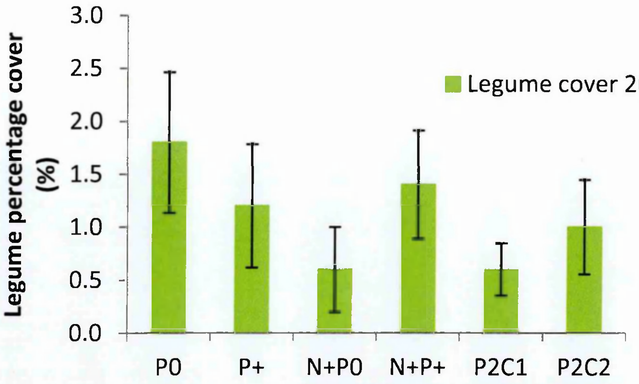
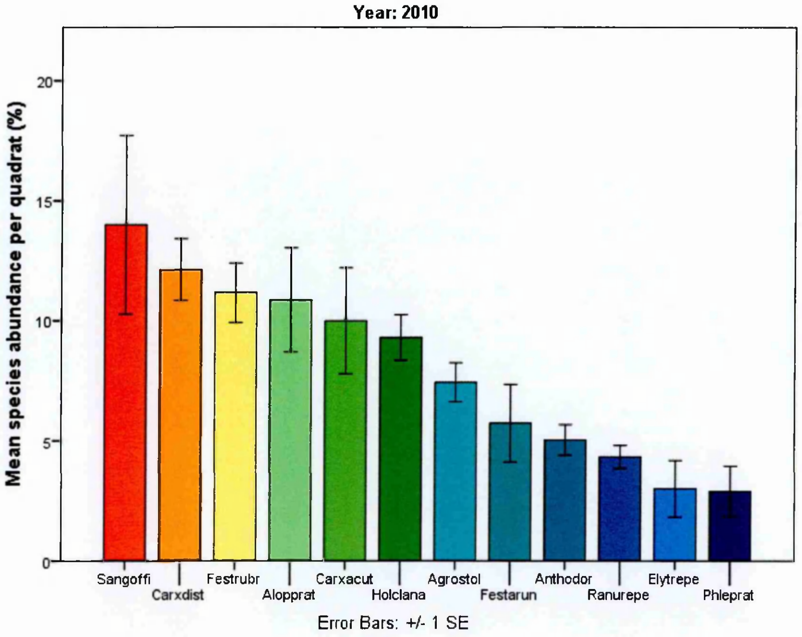


Figure 5.5 Mean and standard error for dry weight of vegetation for each functional group in (a) 2010 and (b) 2011 and (c) cover % for each functional group in 2013 and (d) cover % for legumes in 2013- separate graph for clarity (no significant differences between treatments)

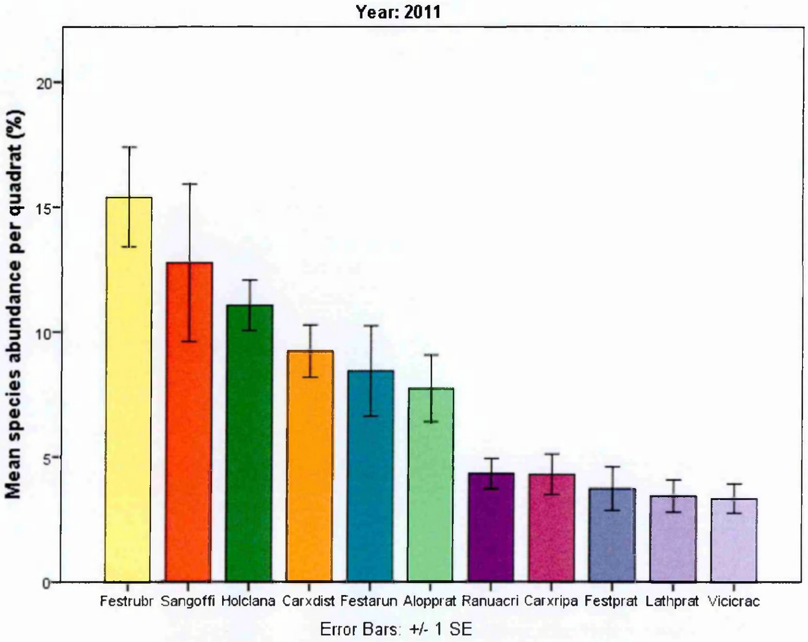
5.3.4 Species cover abundance

The most abundant species within the monitored treatment plots changed from 2010 to 2013 (figure 5.6). In 2010 the most abundant species was *Sanguisorba officinalis*, and most abundant graminoids were *Carex disticha, Festuca rubra,* and *Alopecurus pratensis.* In 2011 there were two species of legumes (*Lathyrus pratensis* and *Vicia cracca*) which were two of the most abundant species in the plots, but were not present in the top twelve in 2010. By 2013 *Sanguisorba officinalis* was much less abundant reducing from a mean of 14% per quadrat in 2010, to just 2.8% with the most abundant species being mostly graminoids and the appearance of *Carex riparia, Agrostis canina, Poa trivialis* and *Cynosurus cristatus* as the most dominant graminoids.

a


Year: 2010
Mean species abundance per quadrat (%)
0
5
10
15
20
Sangoffi
Carxdist
Festrubr
Alopprat
Carxacut
Holclana
Agrostol
Festarun
Anthodor
Ranurepe
Elytrepe
Phleprat
Error Bars: +/- 1 SE

b


Year: 2011
Mean species abundance per quadrat (%)
0
5
10
15
20
Festrubr
Sangoffi
Holclana
Carxdist
Festarun
Alopprat
Ranuacri
Carxripa
Festprat
Lathprat
Vicicrac
Error Bars: +/- 1 SE

c

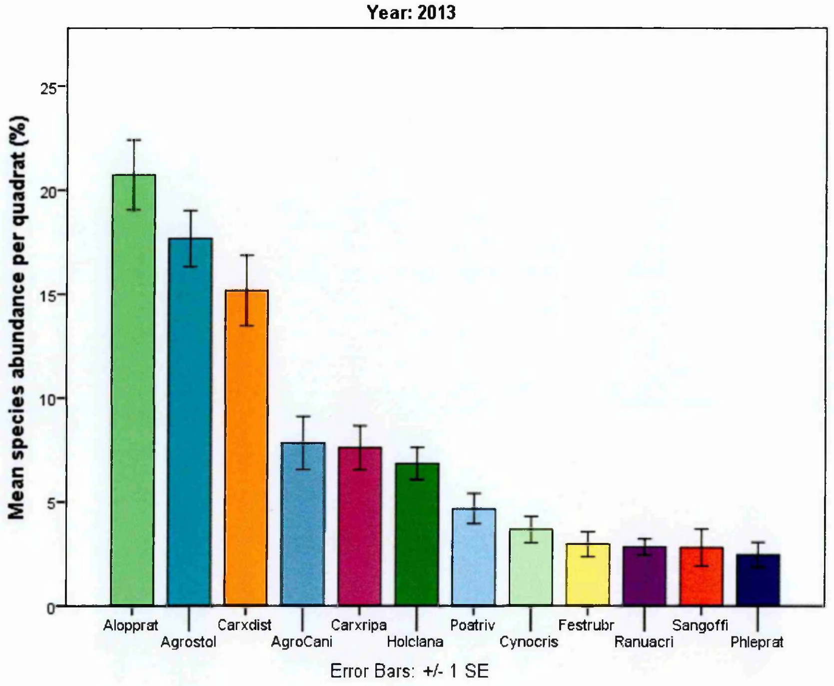


Figure 5.6 Mean and standard error cover abundance for top 10 species in a) 2010 b) 2011 and c) 2013

Multivariate analysis of the species data showed that there was a significant difference in species abundance between treatment group overall and between years (*p*<0.001). There was no interaction effect between treatment and year. Species that significantly increased or decreased between years are displayed on figure 5.7. The most pronounced differences were between 2013 and the other years where there was a significant increase in abundance of graminoids (*p*<0.05), such as *Agrostis stolonifera, Alopecurus pratensis. Poa trivialis, Carex riparia, Cynosurus cristatus* and *Carex disticha*. This was accompanied by a significant decrease in legumes *Lathyrus pratensis, Trifolium pratense* and *Vicia cracca* (*p*<0.05). *Sanguisorba officinalis, Anthoxanthum odoratum* and *Festuca rubra* all reduced significantly (*p*<0.05) between 2010, 2011 and 2013.

Species that were significant between treatments combining all years (2010, 2011 and 2013) were *Agrostis canina, Festuca rubra, Lathyrus pratensis, Lotus corniculatus and Sanguisorba officinalis* (p<0.05) (figure 5.8*). Lathyrus pratensis* was significantly increased in the P+ plots compared with the controls (p=0.021), and the N+P0 plots compared with the P+ plots (p=0.013). *Agrostis canina* was significantly decreased in the P+ plots compared with the controls (p=0.022), and in the N+P+ plots compared with the controls (p=0.022). *Festuca rubra* was significantly decreased between cutting frequencies, with an extra cut decreasing abundance (p=0.004). *Sanguisorba officinalis* was significantly increased in the P2C2 cuts compared with the controls and the P2C1 treatment plots (p=0.033, and p=0.015). The abundance of *Lotus corniculatus* was significantly increased in the N+P+ plots compared with N+P0 (p=0.043).

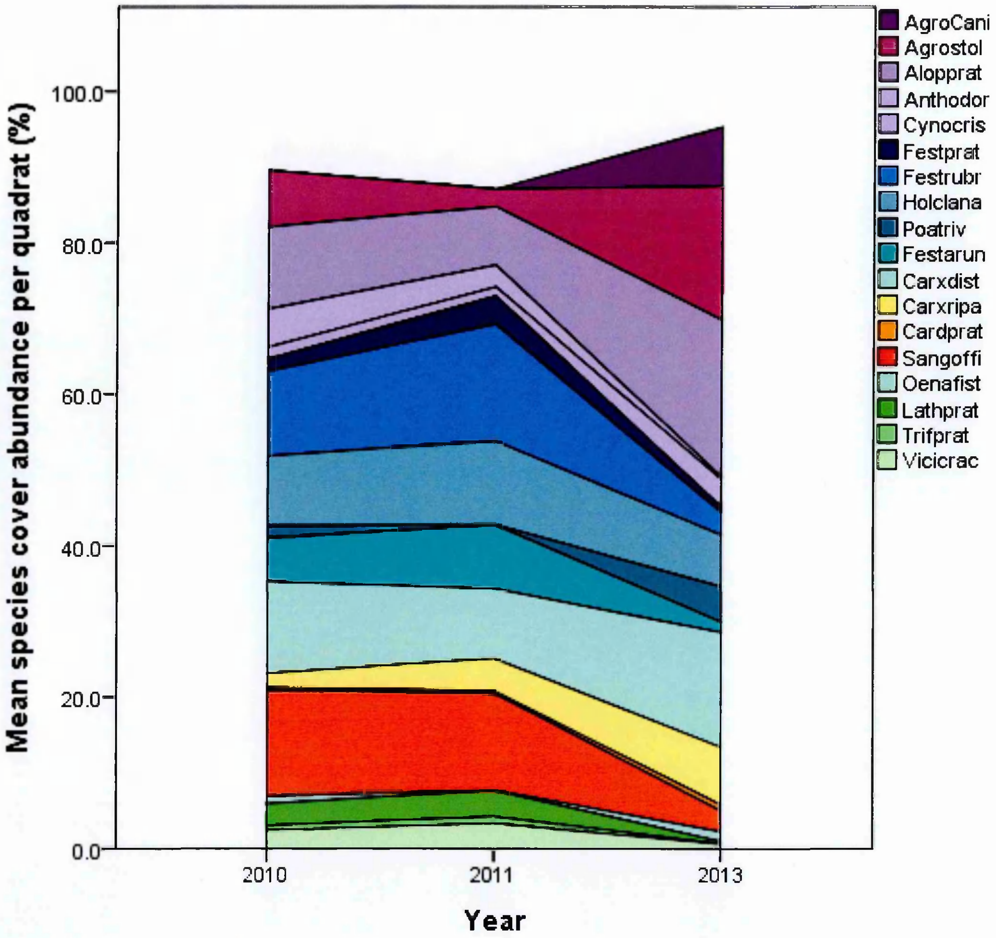


Figure 5.7 Mean species cover abundance for each year for species that showed a significant difference between years

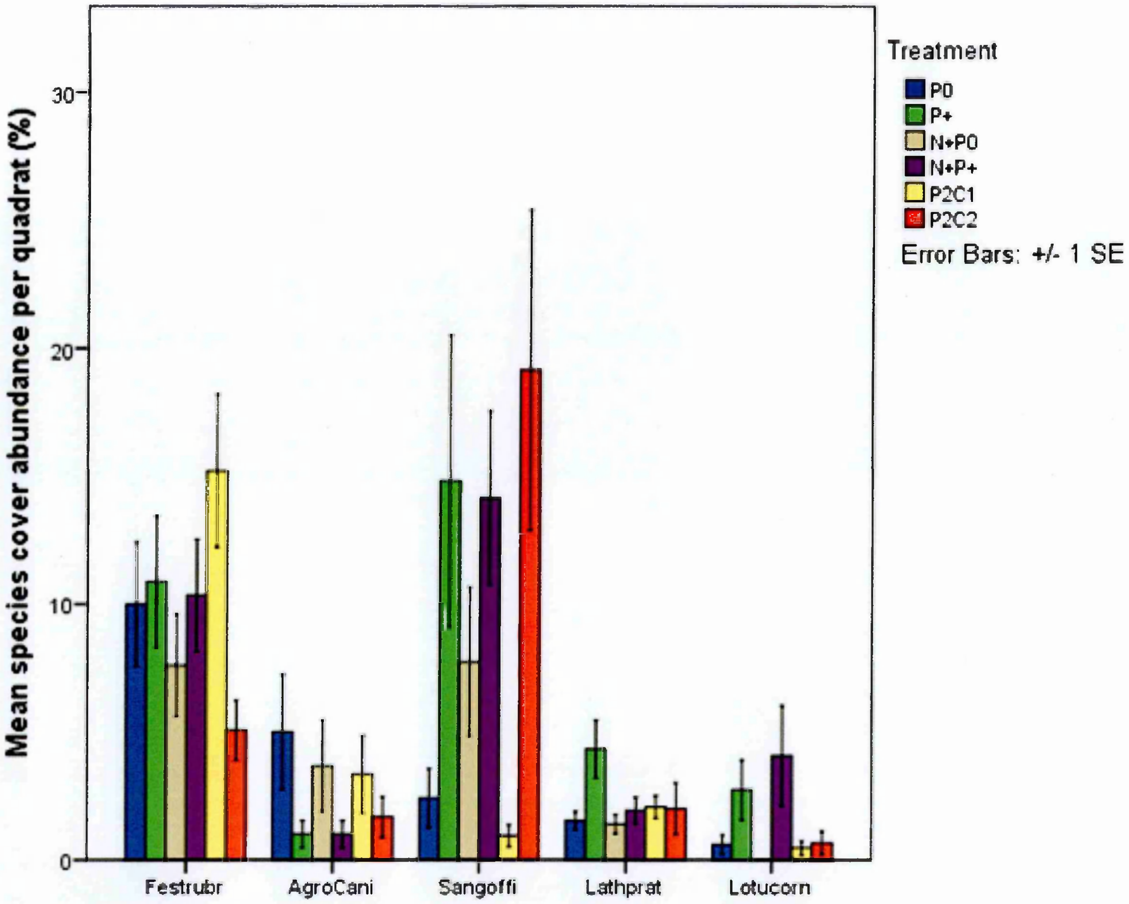
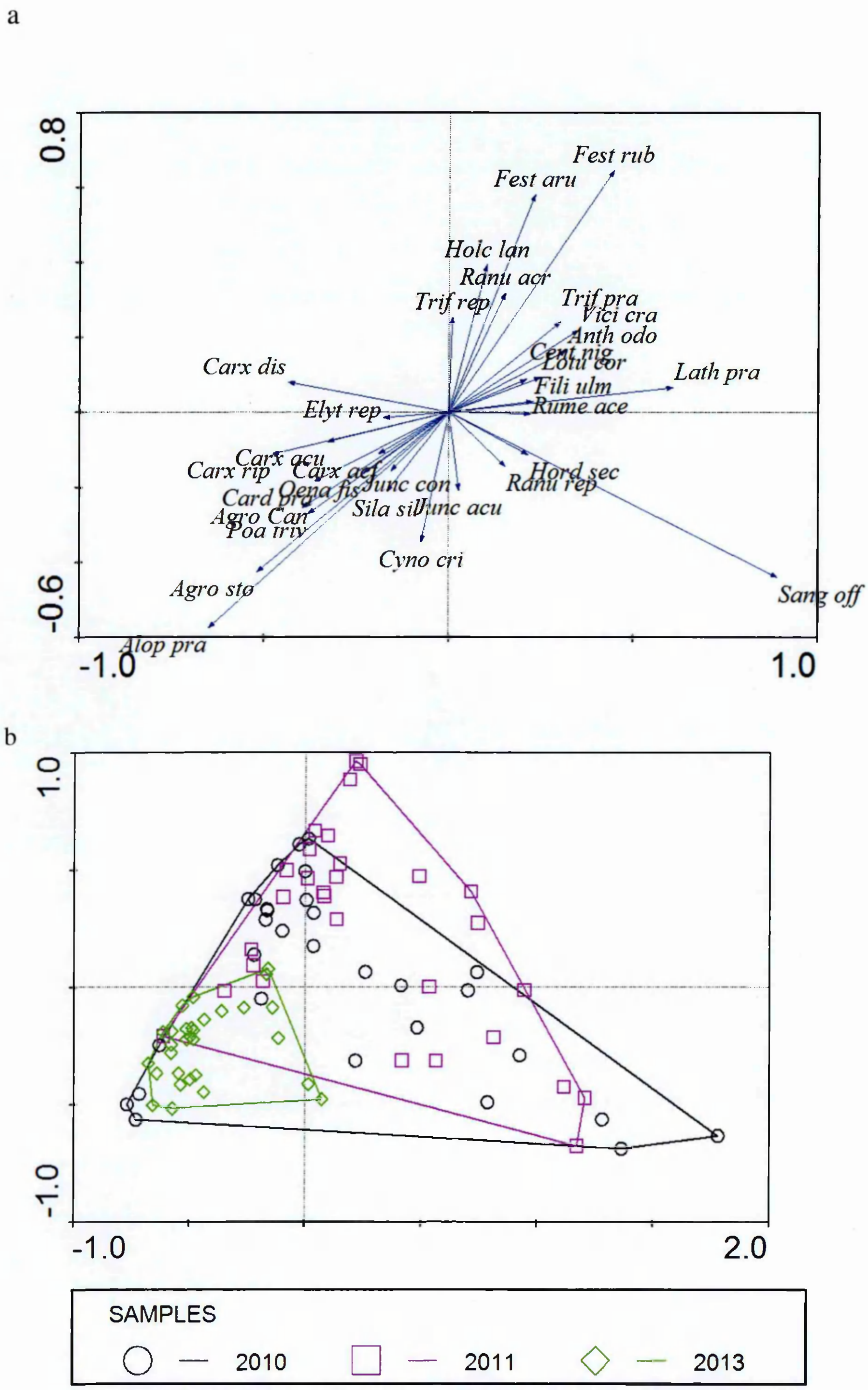


Figure 5.8 Mean and standard error cover abundance for species that were significantly different between treatments including all years

5.3.2 Ordinations

The PCA of species and samples for 2010, 2011 and 2013 show overlap of 2010 and 2011 species data, with 2013 displaying an obvious difference in species composition to the other two years (figure 5.9). Axis 1 on the PCA is correlated significantly with Ellenberg F values for moisture using Pearson correlation (r= 0.330, *p*=0.027). On the combined species and samples PCA diagram (figure 5.9c), species correlated with the year 2013 were *Carex riparia, Carex acuta, Agrostis stolonifera, Alopecurus pratensis* and *Cynosurus cristatus*. Whilst years 2010 and 2011 overlapped in terms of their species composition, Year 2010 was more highly associated with *Sanguisorba officinalis* and Year 2011 with *Festuca rubra* and *Festuca arundinacea*.



c

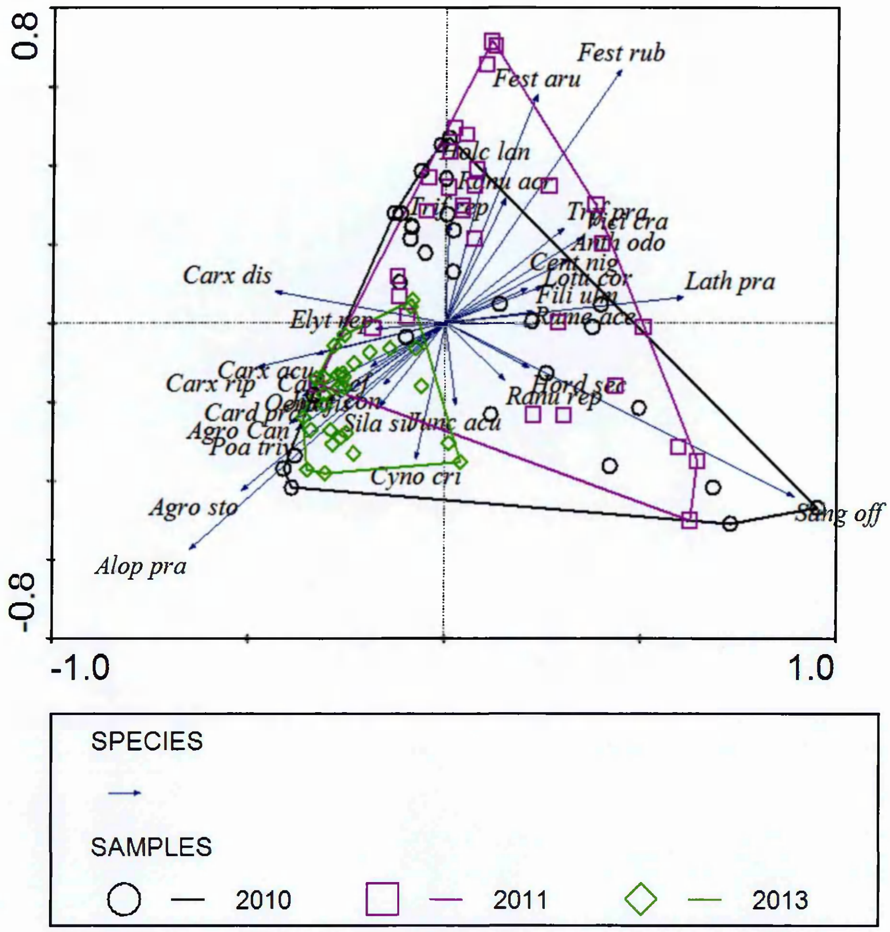


Figure 5.9 Principle components analysis ordination diagrams (PCA) of (a) species data (b) samples (quadrat) data, and (c) species and samples data for 2010, 2011 and 2013

The redundancy analysis (and Pearson correlation analysis) showed that water-table depth (WTD) was highly correlated with axis 1 on the ordination diagram (figure 5.10). 30.7 % of species variation can be explained by the environmental variables (elevation, water-table depth, calculated growing day degrees, N addition, P addition and cutting twice), with all variables showing significance ($p<0.05$) except for N addition and year. Variance partitioning showed that the variables explaining most of the variation in species data were elevation (explaining 6.3% of species variation), plots cut twice (explaining 3.8% of species variation) and P dose (explaining 2% of species variation). If water-table depth (WTD) and elevation were grouped together in analysis (due to being highly correlated), they explain 7.4% of the total variability in species data. Sample distribution shows 2010 and 2011 from -1.0 to 0 along axis 2, with 2013 from 0 to +1.0. The species associated with plots monitored in 2013 were *Carex* spp, *Juncus* spp. and certain grasses (*Alopecurus pratensis, Agrostis stolonifera, Poa trivialis*) indicating a shift to a community dominated by graminoids rather than forbs or legumes. This is also apparent in the functional group data analysis.

N dose was not significant after performing Monte Carlo permutation tests (table 5.3). Environmental variables that had a significant effect on species composition over the three years were calculated growing day degrees, water-table depth, elevation, P dose and cutting twice (p<0.05). Non-significant variables were year (p=0.206) and N dose (p=0.138). On the RDA (figure 5.10), species correlated with water-table depth were *Centaurea nigra, Lotus corniculatus*. Species correlated with elevation were *Sanguisorba officinalis*, and species correlated with cgdd were *Festuca rubra, Trifolium pratense, Holcus lanatus* and *Vicia cracca.*

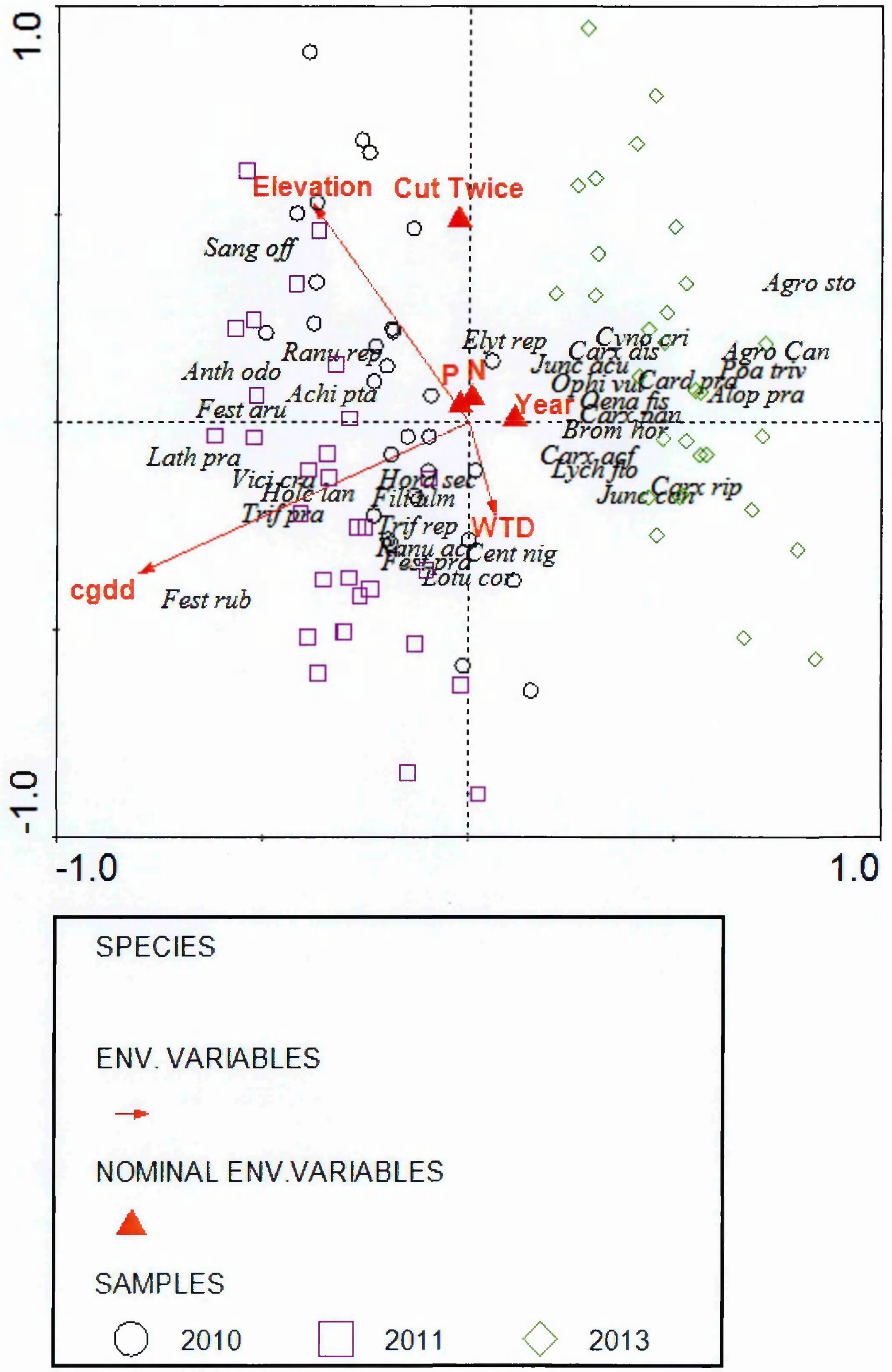


Figure 5.10 Redundancy analysis (RDA) ordination diagrams for environmental variables, species and samples data for 2010, 2011 and 2013. Sum of all canonical eigenvalues 0.307

Variable	p value	F ratio	% expl. 1st axis
cgdd	0.002	12.16	1.9
Elevation	0.002	6.06	6.3
WTD	0.002	6.06	1
Cut Twice	0.004	3.48	3.8
P dose	0.014	2.52	2
N dose	0.138	1.46	1.3
Year	0.206	1.25	1.1

Table 5.3 Summary of Monte Carlo permutation tests for RDA for environmental and species data for 2010, 2011 and 2013

In order to separate out the effect of treatment additions from time; an additional RDA was produced using year and the interaction between year and treatment as environmental variables in the analysis (figure 5.11). Variables that were significant in explaining the species variation were 2011*P2C1 (p=0.004), 2011*P+ (p=0.004), 2013*P2C2 (p=0.004), 2013*N+P+ (p=0.036) and 2011*N+P0 (p=0.004). Plots in 2010 and interactions between treatment group were not significant, and therefore not displayed on the diagram to avoid overcrowding. Plots in 2013 and interaction between N+P0, P+ and P2C1 were not significant but left on the diagram as there was correlation between these treatment groups and certain species. The ordination diagram indicated significant increase in *Sanguisorba officinalis*, *Lotus corniculatus* and *Vicia cracca* in 2011 in P2C2 plots. *Lathyrus pratensis, Trifolium pratense, Trifoium repens, Ranunculus acris* and *Festuca rubra* were all increased in the P2C1 plots and P+ plots, which was significant (p=0.004). Plots in 2013 had increased cover of *Cynosurus cristatus, Agrostis sp, Carex acuta, Alopecurus pratensis, Poa trivialis* and *Juncus acutifloris*, but only treatment N+P+ was significant.

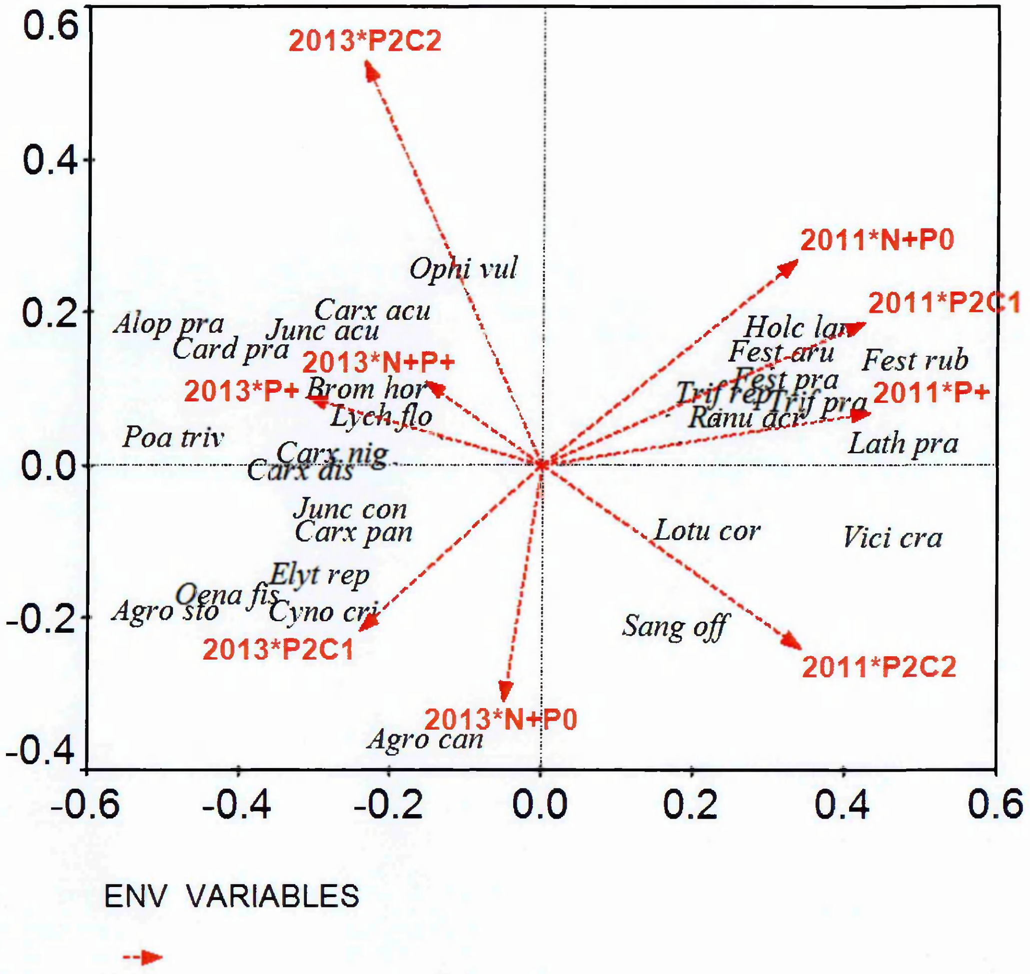


Figure 5.11 RDA of environmental variables as treatment and year, and species data. 18.8 % of species variability was explained by the 1st axis, with an F-ratio of 3.72, p=0.004 using 499 permutations under the Monte Carlo permutation test

5.2.4 Principal response curve

The principal response curve (PRC) displays the development of the vegetation community under the different treatments from 2010 to 2013 (figure 5.12), with the baseline at 0.0 representing the control group. The vertical scores of the PRC are based on the scores of the environmental variables (in this case, the treatment groups) where the sampling time indicators are used as covariables and the interactions between the treatment levels and sampling times stand as environmental variables (Šmilauer & Lepš

2003). Testing for the significance of the first principle curve (the first axis) in the analysis was not significant (*p*=0.628), the second, third and 4th axes were also not significant (*p*>0.05). However, there are some trends in species composition that can be observed. The dominant species shifted (such as *Sanguisorba officinalis* and *Filipendula ulmaria*) from 2010 to *Carex riparia, Agrostis stolonifera*, *Cynosurus cristatus* and *Carex acuta* in 2013. Changes in time had more of an effect than the treatments; although the greatest change in species composition was seen in the N+P+ and the P2C1 treatments. P2C1 shifting from *Lotus corniculatus* and *Achillea ptarmica* to *Carex disticha*, and N+P+ from *Sanguisorba officinalis* towards dominance by graminoids.

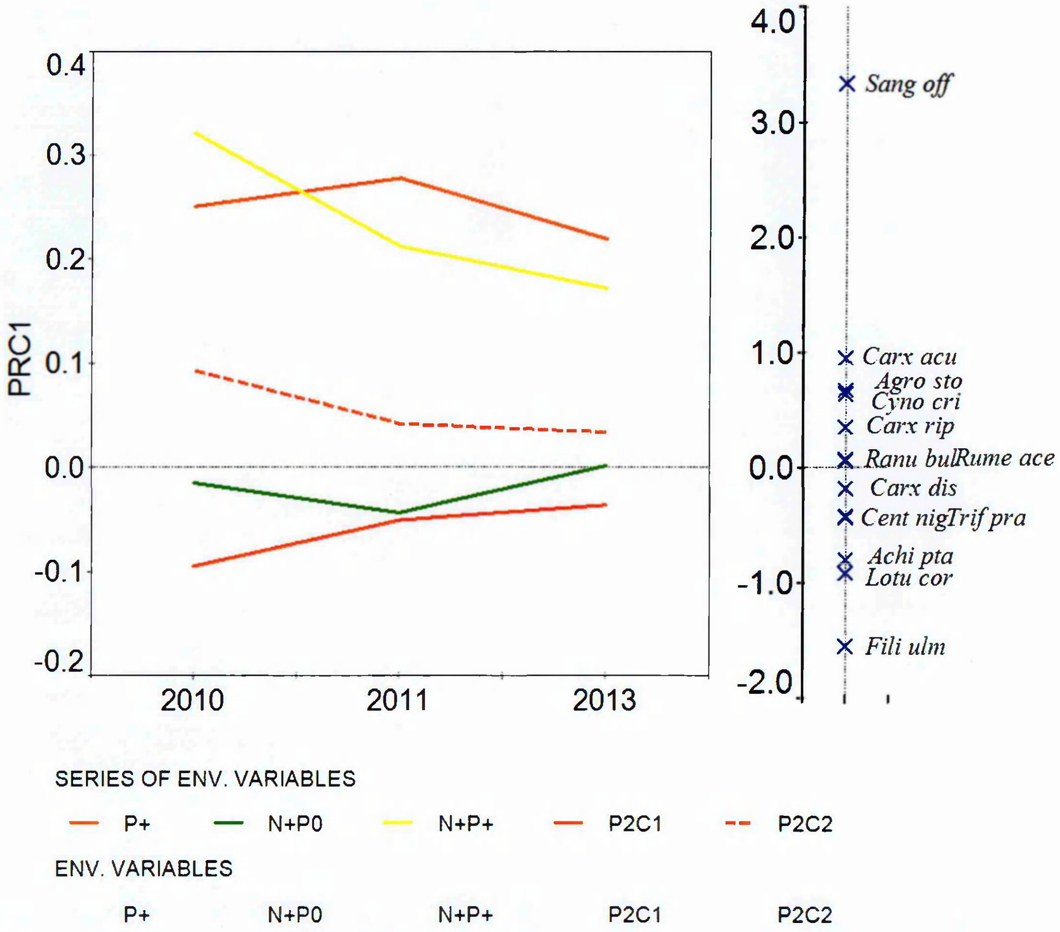


Figure 5.12 Principal response curve of species data and treatment effect from 2010 to 2013

5.4 Discussion

5.4.1 Species richness

The results for species richness and Shannon-Wiener diversity demonstrate no significant changes between treatment group, but significant changes over time (from 2010 to 2013). Declining from 2010 to 2011, and then showing some recovery in 2013, which is site specific rather than dependent on treatment additions, as the controls behaved in the same way as the treatment groups. The lack of treatment effect on species richness is contrary to the findings of the majority of studies, which show a negative effect of nutrient addition on species richness (Mountford, Lakhani & Kirkham 1993; Janssens *et al.* 1998; Ceulemans *et al.* 2011, 2013). Although the results were not significant in terms of mean species richness, the trend was for this variable to reduce in response to the addition of P compared to the controls in all years. Therefore if the P addition had been continued for longer than 2 years a significant detrimental effect on species richness may have occurred. Other studies found that application of N at relatively low levels (25 kg N ha^{-1} y^{-1}) encouraged the spread of agriculturally productive grasses within two years of application, although species richness was not significantly reduced until three years after application with 50 kg N ha^{-1} y^{-1} (Mountford, Lakhani & Kirkham 1993). Kirkham, Mountford & Wilkins (1996) found that N application caused a species decline more quickly than P application.

The treatment group with the greatest decline in species richness from 2010 to 2011 was the P2 treatments. The addition of N alone, and N and P together had no effect on mean species richness. This aligns with other studies that suggest that P could be more detrimental to species richness than N (Janssens *et al.* 1998; Wassen *et al.* 2005; Ceulemans *et al.* 2011). The reason for decline in species number between 2010 and 2011 could be due to drought from the exceptionally dry year in 2010 (Tilman & Elhaddi 1992; Van Peer *et al.* 2004). van Oorschot *et al.* (2000) found that summer drought on floodplain soils reduced nutrient availability, plant production and nutrient uptake after simulating effects of different flooding regimes on soil nutrient availability. Had there been more rainfall in the dry growing seasons of 2010 and 2011, there may have been more of an effect of the nutrient additions, as uptake would not be limited by moisture deficit (Araya, Gowing & Dise 2013).

The mean species decline from 2010 to 2011 was a reduction in three species per plot, and 4 species in the P2 plots. Species *Rhinanthus minor*, *Rumex acetosa*, *Oenanthe fistulosa*, *Carex nigra*, *Cerastium fontanum*, *Elytrigia repens* and *Poa trivialis* all declined in abundance from 2010 to 2011. *Rhinanthus minor* can be absent from habitats affected by drought, which may be the reason for its absence in 2011 compared with 2010, following very dry conditions in early 2011 which may have affected its germination (Grime, Hodgson & Hunt 1988). *Rhinathus minor* is also susceptible to competition by taller growing species and sensitive to high sward density and biomass production (Lindborg, Cousins & Eriksson 2005) and suffers from long-term N, NP and NPK application in grasslands (Hejcman, Schellberg & Pavlu 2011). At Leaches, the highest abundance of *Rhinanthus minor* was in the control plots in 2010, although this was not significant. In plots where abundance of *Sangusiorba officinalis* was high (>15%), *Rhinanthus minor* cover was low, therefore its disappearance may be associated with being outcompeted by taller growing forbs (Hejcman, Schellberg & Pavlu 2011). There was also notable absence of *Poa trivialis* in 2011, which could be explained by its very shallow root system and sensitivity to drought (Grime, Hodgson & Hunt 1988).

5.4.2 Functional group composition

The significant increase in legumes observed in 2011 with the addition of P, agrees with other studies and long-term experiments where legumes were more abundant under N limitation (Tilman 1982; Bobbink & Willems 1991; Kirkham & Wilkins 1994; Silvertown *et al.* 2006; Honsova, Hejcman & Klaudisova 2007). This is due to legumes being able to grow with a lower N supply than grasses, so when given sufficient N, grasses have the competitive advantage over legumes in competition for P thus grasses dominate under N-fertilized conditions, and legumes under P fertilization in the absence of N (Silvertown *et al.* 2006). This would explain the significant increase in legumes in the P fertilized plots in this experiment, and also the non-significant trend for an increase in graminoids in the N+P0 plots in 2010 and 2011. By 2013, trends showing an increase of legumes and decrease in graminoids in relation to treatment addition had diminished which could be due to the response of the vegetation community to summer flooding in 2012, which may have had more of an effect on the species composition than the treatment additions (Beltman, Willems & Güsewell 2007).

5.4.3 Species composition

The results of the PCA demonstrate that the vegetation community altered from overlapping in 2010 and 2011 to a narrower community dominated by graminoids in 2013; this is also in agreement with the functional group dry weight and cover percentage results where graminoids increased and legumes decreased significantly in 2013 compared to other years, which again was likely to be weather related than treatment dependent. Forbs decreased in 2013 relative to other years, but this was not significant. The significant correlation between axis 1 and Ellenberg F values (soil moisture status) indicate that the composition of the vegetation community was driven by changes in soil moisture. The significance of soil moisture was confirmed by the redundancy analysis in which water-table depth was highly significant in explaining 7.4% of the total variability in species data. Many studies have also confirmed that soil-water regime has a significant role in the composition of plant communities (van Oorschot *et al.* 2000; Leyer 2005; Toogood & Joyce 2009). On floodplains of the Elbe river in Germany, the response of 30 common grassland species to soil moisture and water level fluctuations was assessed, with the majority of species responding significantly to water level fluctuations (Leyer 2005). Similarly, Toogood & Joyce (2009) also found that increases in water levels can significantly alter plant community structure with the potential for rapid conversion to wetland vegetation following substantial increases in water levels. Increased productivity of graminoid species after prolonged flooding events has been shown by other studies (Insausti & Soriano 1987; Chaneton, Facelli & Leon 1988; Chaneton & Facelli 1991). For example, Insausti, Chaneton & Soriano (1999) demonstrated a remarkable increase in the abundance of graminoid species with increased flooding in a mesocosm experiment due to graminoids maintaining substantial below-ground growth during inundation. Most graminoids that increase with flooding possess anatomical adaptations such as root aerenchyma, hollowed shoots or the capacity to elongate shoots above the water surface to avoid submergence (Rubio, Casasola & Lavado 1995; Loreti & Oesterheld 1996; Insausti, Chaneton & Soriano 1999). At Leaches Meadow, species correlated with lower water tables were *Lotus corniculatus*, *Centaurea nigra, Ranunculus acris* and *Festuca pratensis* which are less tolerant of waterlogging (Grime, Hodgson & Hunt 1988). Species that were more abundant in 2013 than other years were not necessarily correlated with water-table depth on the RDA but were separated from other species by difference in calculated growing day degrees; these were *Carex riparia, Agrostis canina, Agrostis* *stolonifera, Alopecurus pratensis, Poa trivialis* which were all more abundant in 2013 and closely associated with the wetter end of the MG4 vegetation classification (Rodwell 1992). *Holcus lanatus* reduced in dominance in 2013 compared to 2010 or 2011, which is known to have lower productivity with higher water tables (Mountford, Lakhani & Kirkham 1993). Similar results are also shown by the principal response curves with the vegetation becoming dominated by graminoids over time, although this only took treatment addition into account and no other environmental variables. The implications of this are floodplain meadows becoming increasingly dominated by graminoids with wetter and warmer conditions, which can outcompete forbs and slower growing species. This could result in a rapid vegetation community shift from MG4 to species poor MG4 (with increase in *Poa trivialis* and *Agrostis stolonifera* higher in constancy than in the published floristic table for the community), to *Lolium perenne-Alopecurus pratensis-Festuca pratensis* flood pasture (MG7), which was observed in permanent quadrats in North Meadow NNR at Cricklade in response to heavy rainfall and floods experienced between 1999 and 2001 (Gowing *et al.* 2002).

In terms of treatment effect on species composition; the results of the Monte Carlo test and variance partitioning from the RDA demonstrate that P addition had a significant effect on species composition explaining 2% of total variation in species data. Although the effect of P was significant, it was not as pronounced as other environmental variables such as elevation, water-table depth, plots cut twice and calculated growing day degrees. The small amount of variation explained in the RDA provides an indication of why the lower P treatment does not correlate with any species in the ordination diagram. However, the significance of P in explaining species composition is a fundamental result of the investigation that in spite of fluctuations in water table and soil moisture due to extreme weather events, the community composition was still significantly changed by the addition of P. This demonstrates the importance of P in determining floodplain meadow composition and is a key outcome of this study.

The principal response curve demonstrates that the P2 treatments encouraged species such as *Lotus corniculatus* in 2010, *Trifolium pratense* and *Centaurea nigra* in 2011 and progressed to *Carex disticha* in 2013, which also agrees with the functional group data as the addition of P increased species of legumes, which has been well documented (Kirkham & Wilkins 1994; Silvertown *et al.* 2006; Honsova, Hejcman & Klaudisova 2007). N addition was not significant in explaining species variation, and indeed N+P0 plots did not differ too much from the controls on the PRC. N+P+ plots showed most change in species composition from 2010 to 2013 according to the PRC, mostly due to the reduction in abundance of *Sanguisorba officinalis* in these plots, however this was not significant. Negative effects of N addition and deposition has been widely reported in the literature, reducing species richness overall which is driven by loss of forbs rather than other functional groups (Stevens *et al.* 2004, 2010; Dupre *et al.* 2010; Maskell, Smart & Bullock 2010). N was not significant in explaining the species variation in this study, nor was it detrimental to species richness, however its effects cannot be compared like for like with P, as the design of the experiment was such to test the effects of P addition rather than N so explanatory power of the results relating to N are slightly diluted. Nevertheless, the effects of N on species composition were still non-significant. This was consistent with the findings of Hejcman *et al.* (2007) who found that N was only detrimental to species richness when applied in combination with P, with the lowest species richness occurring in those plots. Furthermore, Blanck *et al.* (2011) found that increasing levels of available P was one of the factors that reduced species on a local scale in a temperate shrub-land, despite N:P ratios indicating that plant growth was N limited. In this thesis there was no effect on species richness with either N or P addition, so parallels cannot be drawn from a biodiversity perspective. However, P was significant in explaining community composition, in spite of the productivity being N limited (chapter 4), and N:P ratios also confirming N limitation (Koerselman & Meuleman 1996; Verhoeven, Koerselman & Meuleman 1996), which is consistent with the findings of Blanck *et al.* (2011).

The P2 treatments that were cut twice were significant in the redundancy analysis, *Sanguisorba officinalis* significantly increased in these plots, and *Festuca rubra* significantly decreased. This is contrary to the findings of chapter 3 and other studies where cover of *Festuca rubra* was increased by cutting due to less competition for light (Huhta 2001; Hellstrom *et al.* 2006). However, in this case the reduction of *Festuca rubra* could be a direct response to the increase in *Sanguisorba* in these plots outcompeting species of smaller stature. Abundance of *Sanguisorba officinalis* was also increased significantly by the addition of P (apparent in 2010 and 2011), which could also explain the significant decrease of other species in these plots such as *Agrostis canina* due to shading from *Sanguisorba* preventing seed setting (Grime, Hodgson & Hunt 1988). These results are in agreement with a number of studies that demonstrate P to have differing effects on species composition to N (Honsova, Hejcman & Klaudisova 2007; Chytry *et al.* 2009). Hejcman *et al.* (2010a) found that the most powerful predictors of plant species composition in the Rengen Grassland experiment in Germany were soil P, K and Mg contents with soil P leading to a significant negative effect on species richness.

Species richness and Shannon-Wiener diversity remained unchanged by nutrient additions in the plots at Leaches, P dose was significant in explaining more variability in species composition than N (which was not significant) which supports the findings of other studies carried out in North-west Europe which demonstrated that most sites were more sensitive to the effects of P, than the effects of N in terms of species richness composition (Wassen *et al.* 2005; Hejcman *et al.* 2007; Ceulemans *et al.* 2011).

Redundancy analysis was used to separate out the effects of treatment and year, by using interactions between year and treatment as covariables, which demonstrated that treatment had a significant effect on vegetation community independent of year, and that year was significant in explaining variability in species community independent of treatment group. Year was not significant in the first redundancy analysis, probably because its effects were outweighed by the effect of growing day degrees and water table. In separating out year and treatment from the other environmental variables, species such as *Carex riparia, Carex disticha, Agrostis stolonifera,* and *Agrostis canina* were correlated with year, which supports the results from the species abundance graphs and original PCA analysis and are species that are able to withstand more waterlogged conditions (Kalusova *et al.* 2009). *Agrostis stolonifera* is able to exploit aquatic habitats and mires and has capacity to exploit pockets of nutrient enrichment and canopy gaps, and is most abundant where growth of tall dominant species has been restricted (Grime, Hodgson & Hunt 1988). *Sanguisoriba officinalis* decreased from 2010 to 2013, despite being tolerant of moist conditions, this could be due to this species occupying a distinct zone within a narrow range of water-table depths, thus the flooding in 2012 gave rise to waterlogged conditions that were too wet for the optimum growth of *Sanguisorba* (Araya, Gowing & Dise 2013). The overall site community change from a slightly more diverse sward containing more species of herbs to a site dominated by graminoids may have been driven by a series of flood events in 2012. Particularly long periods of inundation in the spring and summer can be more detrimental to species survival (van Eck *et al.* 2004), as floodplain meadow species are more sensitive to a change from dry to wet conditions, than the reverse. This was investigated in a study by Jung, Hoffmann & Muller (2009) who recreated two contrasting hydrological scenarios; a flooding scenario and a dry scenario. They found certain species increased their above-ground biomass significantly in the flooding scenario (*Holcus lanatus, Festuca arundinacea*, and *Alopecurus pratensis*) but were not significantly affected by the drying scenario. However at Leaches, *Alopecurus pratensis* increased in abundance in 2013, but *Festuca arundinacea* and *Holcus lanatus* both reduced, suggesting different species were affected by inundation in this case. *Festuca arundinacea* has a well-developed root system and is more tolerant of drought than waterlogging (Perlikowski *et al.* 2014), which may be the reason for not increasing in abundance in 2013, following the floods of 2012. Although *Holcus lanatus* is more tolerant of waterlogging and occurs in high percentage occurrence in hydrology class A (Grime, Hodgson & Hunt 1988) so its decrease in 2013 could be due to increase of other species rather than an actual response to waterlogging. Species of frequently flooded areas can show many characteristics of a competitive strategy such as erect tall stature and high growth rate and hence are stress-tolerant competitors (Grime 1979). Such species (*Alopecuris pratensis, Agrostis stolonifera, Carex disticha* and *Carex riparia*) became more abundant at Leaches following the extremely wet year of 2012 which resulted in an overall site specific vegetation community shift to more flood tolerant species. This supports evidence from a study by Araya, Gowing & Dise (2013) who found that graminoids dominate meadow communities in areas with wetter regimes or higher N availability; they were able to determine the point at which a switch between forb to graminoid dominance occurs below a mean spring water-table depth of 55 cm where soil N availability decreases substantially. A poorly drained MG4 vegetation community can shift to MG7C (*Lolium perenne-Alopecurus pratensis-Festuca pratensis)* flood pasture (Rodwell 1992), which has been observed over a relatively short time frame of under two years at Cricklade in the UK, in response to heavy rainfall and floods after the winters of 2000/2001 (Gowing *et al.* 2002) which supports the reason for rapid community change at Leaches being due to a wetter water regime adopted following wet conditions in 2012.

5.5 Conclusion

Overall, no treatment combination in this experiment reduced species richness of this MG4 habitat. This is perhaps due to treatments needing longer to elicit a response on the vegetation; most treatment addition studies have applied treatments in excess of three years (Bobbink & Willems 1991; Mountford, Lakhani & Kirkham 1993; Silvertown *et al.* 1994; Honsova, Hejcman & Klaudisova 2007). However, the most likely reason is the impact of the extreme weather conditions; firstly the droughts in the spring and summer of 2010 and 2011 leading to reduced nutrient uptake and alteration of soil nutrient dynamics (Verburg *et al.* 2009). And secondly the impact of the major flood events in the spring and summer of 2012, leading to a shift in the vegetation community from drier vegetation, to a vegetation community more tolerant of waterlogging, despite previous fertilizer treatments. However, there was a small effect of treatment on the vegetation community regardless of time; the addition of P was significant in increasing the proportion of legumes in 2011. Furthermore, the addition of P increased individual species such as *Sanguisorba officinalis* and was significant in explaining some of the variation in species data at Leaches. This is particularly significant as extreme weather conditions between 2010 and 2012 had a considerable effect on the species composition, but in spite of this P was still significant. N was not significant in explaining species variation or changing the species composition at the site, however this cannot be attributed to the greater effect of P than N overall, due to the design of the experiment and the role of other environmental variables in explaining the species composition. Longer term treatment additions at multiple MG4 sites would be needed to confirm the significance of P addition in relation to N addition in determining the species composition of floodplain meadows.

Nevertheless this is a crucial outcome of this thesis, that despite water table fluctuations throughout the duration of the experiment and extreme weather events from 2010 to 2012, the community composition still responded to P addition. This demonstrates the importance of P in determining floodplain meadow composition, in conjunction with and regardless of water regime. Furthermore, chapter 4 indicated that above-ground production was N limited (or co-limited) which highlights the relevance of P despite not limiting vegetation productivity. This is the only solid evidence of this nature reported on this vegetation type in the UK, and dispels earlier theories about N:P ratios able to determine the type of nutrient limitation on floodplains (Verhoeven, Koerselman & Meuleman 1996).

Chapter 6

Discussion

6.1 Introduction

The primary aim of this thesis was to investigate the role of phosphorus availability in determining the plant community composition of floodplain meadows. The effect of P addition on MG4 vegetation was studied in terms of above-ground biomass production, nutrient offtake and nitrogen mineralization. This was achieved by conducting two field experiments; a factorial design nutrient addition and sequential cutting date experiment; and a laboratory experiment investigating the influence of P addition on soil-nitrogen mineralization. A secondary aim was to determine the type of nutrient limitation on floodplain meadows. This was incorporated into the factorial design element of the experiment.

The community composition was assessed by a field survey, and by dividing dry weight samples of each plot into functional groups. Results of this work are relevant for the management and conservation of floodplain meadows in terms of identifying when to cut a meadow, and the implications of nutrient inputs for community composition.

6.2 The impact of P addition on N uptake

Contrary to the initial hypothesis that the rate of N-mineralization in floodplain soils may be limited by the availability of P, the results of the field experiment did not demonstrate that P addition necessarily increases plant N uptake. However the addition of P in combination with N did produce a significant increase in N offtake suggesting a role for phosphorus in nitrogen uptake. The laboratory incubation experiment was also used to test this hypothesis, and its findings demonstrated that phosphorus availability was significant in increasing ammonium and nitrate ions in the soil, indicating increased N mineralization (section 4.3.7). Other studies have found that an increase in the P loading rate can lead to increased organic N mineralization in wetlands (White & Reddy 2000; Changhui *et al.* 2014), leading to an increased availability of N which could have detrimental effects to floodplain meadows in terms of altering species composition and potentially reducing species richness.

N:P ratios in plant tissues suggested N limitation (section 4.3.4). This supports the initial view on N:P ratios when this thesis was proposed; that they can be used to determine the type of nutrient limitation on grasslands (Koerselman & Meuleman 1996; Verhoeven, Koerselman & Meuleman 1996; Güsewell, Koerselman & Verhoeven 2003). However the results of this thesis also refute this, showing that community composition and nutrient offtake was responsive to phosphorus addition. Therefore N:P ratios cannot be used exclusively to assign the type of nutrient limitation on grasslands or floodplains. Studies published since this thesis commenced also support this view (Ceulemans *et al.* 2011, 2013), which also agrees with evidence that phosphorus is important even when it is not limiting productivity (Wassen *et al.* 2005; Venterink 2011).

The results observed in this study suggest that co-limitation of N and P is more likely on floodplain meadows than single resource limitation, as the addition of N and P together gave rise to the greatest offtake N from the vegetation. The results support synergistic interactions between multiple limiting resources (Elser *et al.* 2007). Patterns of nutrient limitation in floodplain meadows need to be considered in management, especially the interactions between N and P. The use of N:P ratios for defining nutrient limitation on floodplain meadows therefore has restricted uses; due to the results demonstrating productivity was limited by N, but the vegetation community and N offtake was responsive to P addition. Hence P is important despite not necessarily regulating above-ground production. This supports recent work by Ceulemans, Stevens & Duchateau (2014), who found plant species richness to be consistently negatively related to soil phosphorus. They also highlight the importance of including phosphorus free fertilization as part of agro-environmental schemes.

The effects of P addition are potentially two fold with direct effects of P loading, and indirect effects with increased N availability. Coupled with the increasing rates of nitrogen deposition, addition of phosphorus could be substantially damaging to grassland communities and result in further species loss and habitat decline, as the negative impacts of nitrogen on grasslands have been widely reported with overall reduction in species richness (Stevens *et al.* 2004, 2010; Cleland & Harpole 2010). This is especially relevant in view of recent climate change predictions of wetter, milder winters in the UK (Hulme *et al.* 2002), which may increase flood events leading to enhanced P deposition on rural floodplains by increased sediment inputs rich in P.

6.3 Management in response to increased nutrients

To avoid further species loss and habitat decline of these important floodplain ecosystems, they must be carefully managed (Hansson & Fogelfors 2000; Mountford, Roy & Cooper 2006; Gowing & Wallace 2010). Management must consider the balance of its impact on species richness, above-ground productivity and the nutrient status of the ecosystem, as mowing and hay removal can directly influence productivity and nutrient content by removing biomass, leading to a reduction in nutrient status of the soil and vegetation. In this study a cutting trial was implemented to investigate if the timing of the hay cut was important in terms of maximum nutrient removal, and the subsequent impact on species composition. The nutrient tissue content of the vegetation (both N and P percentages) declined from May to July in the growing season, and combined with patterns of the vegetation productivity to generate values for nutrient removal in terms of N and P offtake. The optimum time to cut this type of vegetation community was around the middle of June for unfertilized plots, and circa the middle of July for P fertilized plots (section 2.3.3). P addition in this experiment delayed the peak standing biomass of the vegetation within the growing season. Other studies have reported ceiling biomass yield to occur later in the season for unfertilized grasslands than for agriculturally improved grasslands, which is due to species-rich unfertilized grasslands having a slower growth in the spring and early summer (Robson 1981; Tallowin & Jefferson 1999). This is the reverse of the findings of chapter 2 where the addition of P was found to delay the peak standing biomass, however other factors may have driven the nutrient uptake and productivity of the vegetation. For example, summer drought has been found to reduce nutrient uptake by plants and limit vegetation productivity of floodplain soils (van Oorschot *et al.* 2000; Venterink, Vliet & Wassen 2001).

In this experiment the results of the cutting trial were markedly influenced by the exceptionally dry weather conditions experienced in the spring and summer of 2010 and 2011. Soil-moisture deficit is a key factor influencing the variability of yield between years (Dodd *et al.* 1994), which agrees with the findings of Smith (1960) that there is a close relationship between hay yield and actual transpiration, which in turn is related to soil-moisture deficit during the growing season. The relationship between hay yield and transpiration was tested in this experiment (section 2.3.2) and also found to be in agreement with Smith (1960) resulting in above-ground biomass being limited by soil-moisture deficit rather than nutrient availability in 2010 and 2011. Coupled with the results of chapter 3 (section 3.3.4), cutting date was significant in explaining the species composition of the vegetation, which reinforces current management practices in floodplain meadows, for maintaining typical species assemblages (Baker 1937; Gowing *et al.* 2002).

Based on these results, it is important that cutting dates for floodplain meadows remain flexible for example on sites that are nutrient deficient, a later cut might be beneficial in removing less N and P from the system, whilst a cut in advance of peak biomass might remove excess nutrients from comparatively P and N rich sites. Cutting frequency can also be used to manage undesirable species such as coarse species of sedge (Newman 2013), although cutting twice was not significant in explaining the species composition in this experiment, and did not decrease the availability of P (Olsen extractant method) in these plots over three years of cutting management or significantly increase the offtake N and P in the above-ground biomass. Individual species can also be managed to increase or decrease in abundance by changing cutting dates (section 3.3.3); for example, *Festuca rubra* was increased in abundance by cutting earlier, possibly due to the removal of other more competitive species and the reduction in canopy height of those plots. Some species thrived in plots cut later including *Phleum pratense, Anthoxanthum odoratum, Centaurea nigra* and *Oenanthe fistulosa.* This is likely to be due to a combination of individual species phenology such as late season growth in the case of *Anthoxanthum odoratum* (Lambrechtsen 1968) and competitive exclusion where taller growing forbs gain advantage over as the vegetation sward height increases later into the season (Hellstrom *et al.* 2006).

6.4 Water-regime influences

Species richness was not affected by the timing or frequency of the hay cut (chapter 3) or by the addition of N and P (chapter 5). Whereas P was significant in explaining some of the species variation at Leaches Meadow, the main determinant of species composition from the measured variables at Leaches Meadow from 2010 to 2013 was water-table depth. This was significant in explaining species variation on the RDA ordination, but was also reflected in the species assemblages at the site where the community changed from species tolerant of drier conditions to those tolerant of waterlogging over time (section 5.3.2). This was demonstrated by increased dominance of graminoids in 2013 compared with 2010 and 2011, which was also identified in other studies with increased waterlogging (Antheunisse & Verhoeven 2008; Araya, Gowing & Dise 2013). These results from chapter 5 support the results of chapter 2 where the relationship between above-ground biomass and relations between transpiration and rainfall, was significantly correlated by using the 'Smith calculation' (Smith 1960).

Although water regime was not included in the hypotheses of this thesis and was not an experimental variable here, it was the main driver of plant community change at the site and may have been more important in limiting vegetation biomass and nutrient uptake than any other resource in this instance. Weather conditions from 2010-2013 were extreme events compared to 'average' conditions. Drought conditions developed in the first half of 2010, and 2011 was the warmest year on record for the UK since records began in 1766 (Met Office). Some parts of England recorded their lowest 18-month rainfall in at least 100 years (figure 6.1). In huge contrast to this, April 2012 was the wettest on record for 230 years, and the period from April to July had the highest rainfall in the England and Wales series which extends back to 1766 (figure 6.1). As a consequence, soil conditions changed from that of drought stress to waterlogging during the timeframe of the experiment. Furthermore, saturated soil conditions resulted in restricted harvesting opportunities due to restricted access to the experimental plots (Marsh *et al.* 2013).

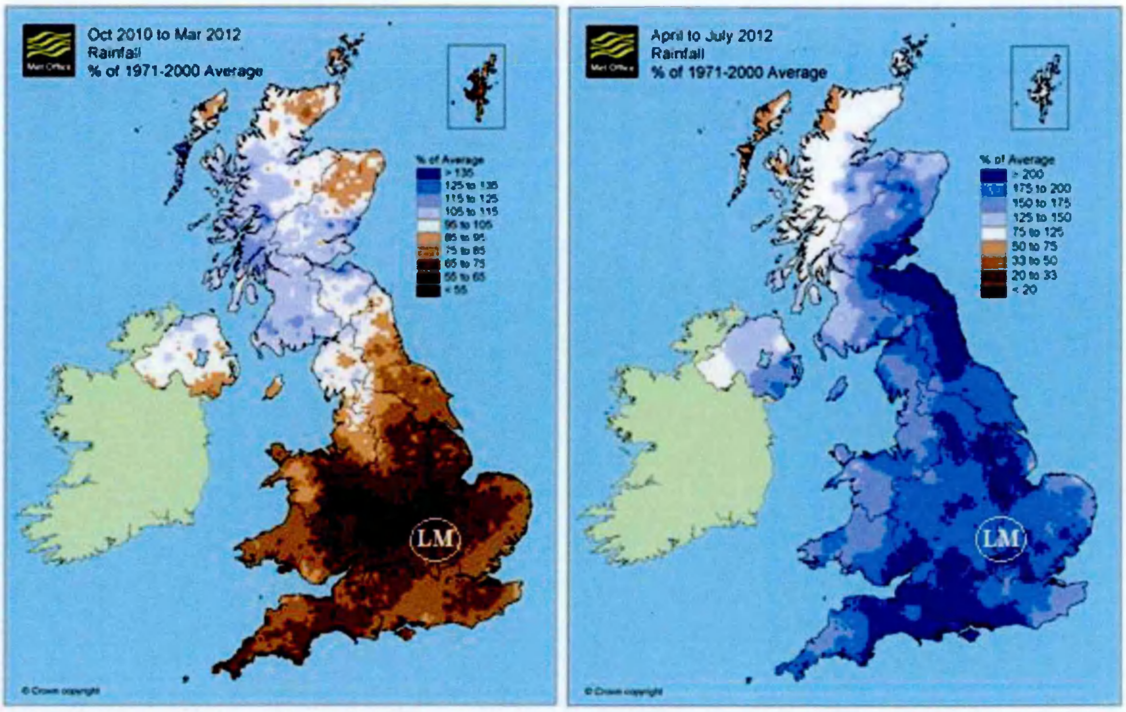


Figure 6.1 October 2010 to March 2012, and April-July 2012 rainfall as a percentage of the 1971-2000 average (Marsh *et al.* 2013) LM indicates location of Leaches Meadow.

This dramatic variation in hydrological conditions, would be likely to have had a pronounced effect on the nutrient dynamics of Leaches meadow and thus productivity and community composition independent of the nutrient-addition experiment as effects of fertilization can be overruled by flood events (Beltman, Willems & Güsewell 2007). In this thesis, separating out the effects of the treatment additions was possible, but changing weather patterns made year a significant variable, therefore making it difficult to identify consistent trends across the whole experimental period.

Many studies have investigated the relationship between plant N limitation and water availability in arid ecosystems to attempt to ascertain which resource is most limiting under dry conditions with the majority of findings indicating that plants are co-limited by multiple resources (Hooper & Johnson 1999; Harpole, Potts & Suding 2007; Elser *et al.* 2007; Bai, Wu & Xing 2008). There is not necessarily a shift of primary limitation from water to N, across a geographic water availability gradient. However, this is the opposite of the findings of Xia & Wan (2008) who found water availability to be more important than N availability in regulating plant growth in regions with low annual precipitation and positive responses of plant growth to N addition are likely to be suppressed by water limitation. Both concepts were found in this thesis; where plant biomass was limited by N (chapter 4, section 4.3.1), N offtake was limited by a combination of N and P (chapter 4, section 4.3.2) and that above ground production was restricted by soil moisture deficit (chapter 2) present in the drought years of 2010 and 2011. At the other end of the spectrum following extensive flooding in 2012, the effects of the treatment additions were possibly superseded by the effects of water regime, which was also observed by Beltman, Willems & Güsewell (2007) who found the effects of fertilization on the vegetation response to be eradicated by extreme winter floods and spring floods between 1998 and 2001. Nevertheless, they also found that the effects of nutrient additions reappeared after these years. Reasons behind the disappearance of treatment effect could be due to soil moisture controlling the availability of nutrients to plants in wet meadows, with soil N mineralization rates declining as soil becomes more saturated (Araya, Gowing & Dise 2013).

In terms of species composition, a number of studies have found the effect of summer flooding to be of primary importance in determining plant survival and biomass response (Van de Steeg & Blom 1998; Vervuren, Blom & de Kroon 2003; van Eck *et al.* 2004), with flood tolerant species occurring at higher elevations and ability to recover biomass more quickly than flood sensitive species. Floodplain meadow species also differ in their short-term susceptibility to hydrological scenarios, and are more likely to be affected by a change from dry to wet conditions than the reverse (Jung, Hoffmann & Muller 2009) from the results of experimental manipulation of two contrasting hydrological scenarios after just five weeks.

In Leaches Meadow, functional group composition was significantly different between all years, with an increase in graminoids in 2013 relative to legumes (which significantly decreased) and forbs (section 5.3.2). Species dominance changed from those more tolerant of drying to those more tolerant of waterlogging, which was reflected in the species dynamics and the key driver of plant community change (section 5.3.4). *Sanguisorba officinalis*, *Anthoxanthum odoratum* and *Festuca rubra* all declined in 2013 compared with previous years and are known to be intolerant of waterlogging, whereas *Agrostis stolonifera*, *Carex disticha* and *Carex riparia* all increased and are much more tolerant of waterlogging (Gowing *et al.* 1997). *Alopecurus pratensis* was the dominant species in 2013 and generally has moderate soil moisture requirements, but can adapt to waterlogged conditions due to the possession of large lysigenous lacunae which maintain maximum oxygenation of the cortex under conditions of waterlogging (Soper 1959), and therefore was able to thrive over other species in 2013 after inundation experienced in the previous year.

6.5 Further work

The results of this thesis are based on research and data from four-year field and laboratory experiments. Extension of the experimental period in excess of five years would provide more substantial evidence and give a clearer indication of the effects of cutting and P addition to the species composition and nutrient dynamics within a floodplain meadow. This P addition experiment would also be relevant on different habitats to continue to explore the effect of P on N cycling in the soil, as current literature is scarce. There is also a risk that effects of P on terrestrial ecosystems could be overlooked as most recent work has been focussed on the effects of N pollution. However, as we have shown that P addition can contribute to plant N uptake and enhance N mineralization in the soil; effects of P addition could potentially be two-fold with the direct effects of P pollution, and indirect effects on N availability which could enhance N pollution. Further work on multiple terrestrial ecosystems in addition to more detailed research on floodplains and dry grasslands would provide further evidence for this, specifically influences of water regime together with P availability and the subsequent effects on the microbial community of floodplain soils.

6.5.1 Hydrological modelling

Altering water-table depth, in conjunction with P application under controlled conditions by means of mesocosm experiments would enable further testing of hypotheses relating to increases in N mineralization with P addition on floodplain soils. This would remove the uncontrolled variability of water-table depth in the field, along with speculation about the effects of waterlogging on nutrient dynamics. Further water-table modelling using diver data gathered in the field, could also be used to interpret water-table depths using the sun exceedance values (SEV) concept for aeration and drying stress (Gowing *et al.* 1998).

Given the influence of water-table depth and variation in weather conditions between years on the experiment, the next logical step would be to investigate the interrelationships between soil water regime, N mineralization and P availability. This would involve repeating the P addition incubation experiment, under a range of different water tensions, which cover the range that N mineralization is particularly sensitive. This technique was used by Araya, Gowing & Dise (2013) who demonstrated that soil moisture content and soil aeration controlled the availability of N. Including the influence of P availability in this hypothesis would contribute further to understanding nutrient dynamics and water regime in floodplains and would add to the results of this study.

6.5.2 Microbial indices

After finding that the addition of P significantly increased the concentration in extraction of ammonium and nitrate ions, alluding to increased N mineralization in the soil (section 4.3.7), the next step would be to measure this *in-situ* in the field (Raison, Connell & Khanna 1987). This would indicate if the same microbial processes were occurring as in the laboratory. Further to this, microbial biomass C and N could be measured to indicate if P addition enhances microbial activity or decreases the total microbial biomass of floodplain soils, which has been shown for upland grasslands (Bardgett, Frankland & Whittaker 1993; Lovell, Jarvis & Bardgett 1995), but has received very little attention for floodplain soils. In addition to microbial biomass, the effect of P addition on fungal:bacterial biomass ratios (measured by phospholipid fatty acid analysis; PLFA) would be useful to assess if fungal biomass will decrease in plots receiving P as has been reported for N (Bardgett & McAlister 1999; Bardgett *et al.* 1999). PLFA could be used as an indicator of change among the microbial community of the soil between floodplain meadow sites to determine associations between the prevalence of certain plant species and soil microbial groups (Smith *et al.* 2003). A study focussing on P effects of the microbial community of floodplain soils would enhance the results of this thesis.

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Appendix 1 Diver data output from Leaches Meadow