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Climate change implications for grassland ecosystems: a biodiversity approach

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ABSTRACT

Grassland species assemblages are vulnerable to changes in rainfall patterns, with consequences of change including less species-rich communities, and changes in carbon, nitrogen and hydrological cycles. Research has indicated that increasing species diversity can lead to better resource and water use efficiency, and experiments are now targeted at identifying species characteristics that can modify ecosystem responses to climate change. This thesis aimed to evaluate the extent plant functional diversity can modify the effect of climate change in a field experiment on acid grassland in South-East England. Other climate scenarios, namely spring and summer drought, and highly variable rainfall were tested concurrently, and a modelling technique was developed to predict ecosystem functions from abiotic and plant trait-based variables.

Climate change treatments generally decreased rates of ecosystem processes such as mineralisation and ecosystem respiration. Plots dominated by perennial plant species exhibited lower rates of processes such as net ecosystem CO_2 exchange and soil respiration under climate stress. Results suggest annual plants are adapted to take advantage of very small rainfall input, and are less affected by climate change, thus generally maintaining overall ecosystem function through times of drought. A spring and summer drought regime was associated with slowing of ecosystem processes. This treatment was more deleterious to ecosystem function than increasing rainfall variability, where process rates did not differ discernibly from ambient, although plants suffered a higher level of dieback in general. While climate change could have detrimental effects on all aspects of ecosystem function, using information regarding species traits which are more resistant to climate change may aid grassland management in order to preserve more vulnerable species. Experiments such as these are vital to further understanding of the links between plant community composition and ecosystem function in order to target management schemes and policy to reduce the effects of climate change.

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To my family, with their rather endearing bafflement at my work but constant support despite it, thank you for believing in me, and for being so so proud.

And finally to CT, always.

DECLARATION

I confirm that all of the written work within this thesis is my own, although Chapter 2 has been subject to extensive revisions to prepare it for publication. Some of the trait data used in Chapter 2 was collected by Alexander Kendall and Annie Pagan, and much of the data from the 2009 season used in Chapter 3 was collected by Alex Hurst, Martin Rimmler, Dave Allen, Katelyn Faulkner and Ana Margaridas. The rest of the data collection is my own work.

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CHAPTER 1: INTRODUCTION

Grassland ecosystems are likely to be detrimentally affected by climate change through the coming century, but there is increasing evidence that some characteristics of grassland species may have a role in mitigating the effects (Suttle *et al.* 2007; Kardol *et al.* 2010; Van Ruijven & Berendse 2010; Wardle *et al.* 2011). Altering patterns of frequency and intensity of rainfall have measurably different effects on grassland communities in terms of how they use and cycle nutrient and water resources (Knapp *et al.* 2002; Jentsch *et al.* 2011), and so more work is needed on both responses and effects of species assemblages when exposed to rainfall changes. Global climate models are more accurate than ever before, and the forthcoming Intergovernmental Panel for Climate Change (hereafter IPCC) 5th Assessment Report will warn of the possibility of more unpredictable or extreme events than previously assumed (www.ipcc.ch). Therefore the likelihood of grassland ecosystem function responding differently to various rainfall scenarios is high, and this will have profound implications for ecosystem services such as provision of fodder, clean air and water.

Altering functional diversity of natural plant species assemblages under climate change, as well as exploration of the effect of different rainfall regimes, are the main focuses of this thesis. First, a method of categorising species into groups, based purely on potential functional effects, is described. This is then demonstrated in a large scale field experiment investigating ecosystem processes, combining the functional groups with rainfall manipulation. In tandem with this, two more extreme rainfall regimes without diversity manipulations are evaluated. Finally, all data collected is used in linear models to predict ecosystem function using a combination of abiotic and biotic variables.

1.1 BACKGROUND

1.1.1 CLIMATE

Climate change is becoming an increasing concern; its effects are already being felt (and quantified) around the world (IPCC 2007). Natural disease vectors and crop failures are increasing, putting unprecedented pressure upon the poorest of the world's population (Millennium Ecosystem Assessment 2005). With the added problem of widespread land use change, many of the most vulnerable natural ecosystems, and the services they provide, are becoming increasingly degraded or lost. While efforts continue to slow these climate changes and reduce atmospheric greenhouse gas concentrations, an equally pressing issue is how to preserve sufficient natural ecosystem services for mankind to sustain itself in future centuries. To date, these efforts have been focussed on agriculture and food security (Godfray *et al.* 2010; Scheffran & Battaglini 2010). In recent years research on natural systems has begun to move from more theoretical ecological questions towards quantifying ecosystem functioning under stressful conditions. This is necessary to understand the complex responses of the more vulnerable systems to climate change (Hooper *et al.* 2005; Williams *et al.* 2008; Mooney *et al.* 2009).

1.1.2 CLIMATE MODELS

While it is accepted that global temperatures will increase through this century as a result of increasing greenhouse gas 'forcing', and that precipitation patterns will change, there is still contention about the exact climate model outputs used by the world's leading climate authority, the IPCC. IPCC climate projections are based on a number of general circulation models (GCMs) that are rigorously evaluated and constantly compared using multimodel comparisons. Each one is strongly anchored to physical principles and laws (energy flow *etc*.), and calibrated using present and past weather patterns (IPCC-TGICA 2007).

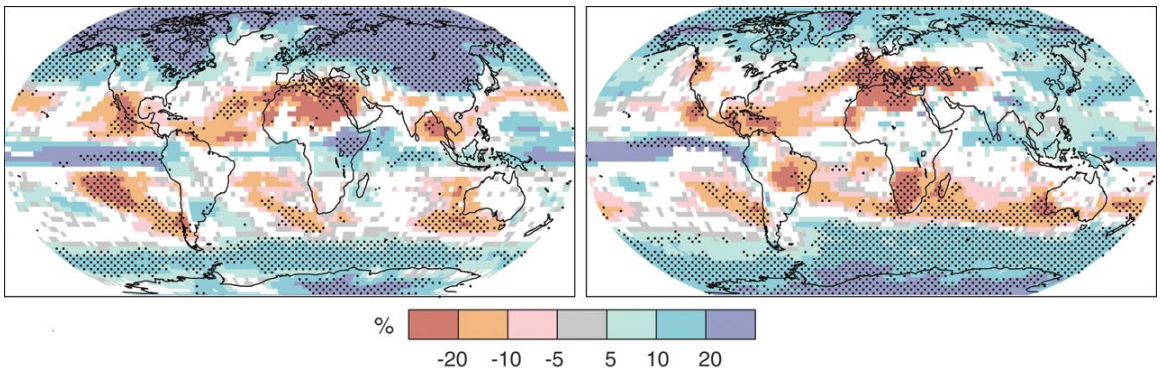

Figure 1.1: Relative changes in rainfall (%) between 2090-2099. The images are a synthesis of model outputs (left: winter DJF, right: summer JJA). Stippled areas reflect 90% model agreement, while white areas should be disregarded due to <66% model agreement (IPCC 2007).

There are many different GCMs; these differ in their spatial resolutions and temporal scale (seasonal, monthly or daily means). They use the same fundamental equations for calculating the model parameters, the likelihood of increased CO_2 forcing and their consideration of temperature, precipitation and pressure changes (IPCC 2010). These models all predict rising temperatures throughout this century in the order of 0.5-4°C global annual average, and general alterations in precipitation. In temperate areas these changes are projected to increase the duration of summer drought and number of heavy rainfall events, with a net reduction in volume (JJA ~25-30%), and an increase in volume of winter rainfall (DJF ~12.5-15%) projected for the UK in 2090-2099 (Fig. 1.1 and Fig. 1.2, IPCC 2007).

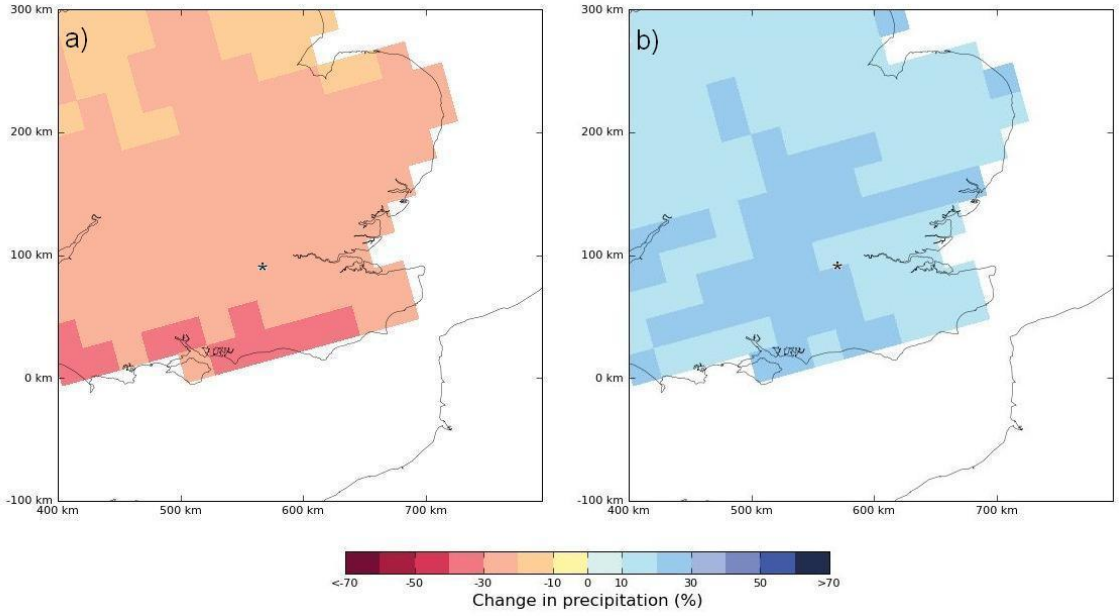

Figure 1.2: Projections from UKCIP09 of 2080-2099 rainfall in South-East UK as a function of 2009 in a) summer JJA and b) winter DJF (Murphy *et al.* 2009). The study site for this experiment is marked with a star.

There are three main areas of uncertainty within these GCMs, and they result in very different projections of future climate (IPCC-TGICA 2007). The first relates to socioeconomic factors, where future emissions are described in six potential SRES scenarios (Nakićenović & Swart 2000). The highest change in climate is A1F1, where fossil fuel use continues unabated and atmospheric CO_2 concentrations continue to rise through the century, while the lowest is B1, which includes a global approach to finding more efficient technologies. The second uncertainty comes from differences in the way emissions profiles are interpreted when used to quantify the carbon cycle and atmospheric chemistry, which leads to differences in global climate sensitivity, i.e. some models predict greater global warming per unit of greenhouse gas. The third area of uncertainty is unpredictability of small scale processes and how they are modelled by different GCMs. These are determined by parameterisation, a technique to average the known properties of difficult-to-measure factors such as cloud cover across a region (IPCC-TGICA 2007). Consequently, there is little agreement of models regarding frequency and intensity changes of extreme events, such as storms, although precipitation models are more congruent (Fowler & Hennessey 1995; Kattenberg 1996).

A more general area of uncertainty is the coarse resolution of GCMs (250–600km horizontal resolution), which cannot take into account small scale processes. This results in large grid squares (Fig. 1.2; Murphy *et al.* 2009). These uncertainties create problems with predicting stochastic events such as storms, but they are suitable for general estimates of seasonal rainfall frequencies and volumes, which can then be used to generate simple rainfall regimes for use in field experiments.

1.1.3 CLIMATE EFFECTS ON GRASSLANDS

Biological experiments that investigate climate change treatments have mostly concentrated on one key aspect of global change, such as precipitation changes or CO_2 enrichment. There are two ways of designing a rainfall regime. The first is to add a predetermined volume, or alter the frequency of rainfall inputs to the plots as a function of the ambient mean for the study site (Chimner & Welker 2005; Yahdjian & Sala 2002, 2006; Fiala *et al.* 2009), or to simulate an anomalous year (e.g. 2003- Joos *et al.* 2010). This has been favoured in the past, partly because lagged timing of effects, and general resilience of grasslands tend to mean

results are sometimes unclear or non-significant, and so more stressful treatments are implemented to ensure a result (Laporte *et al.* 2002; Yahdjian & Sala 2006). The second option is to base studies on altering ambient rainfall. Variability, rather than volume, could be more important in affecting ecosystem function (Knapp *et al.* 2002), and so more 'natural' rainfall patterns should be applied.

The inherent uncertainties in GCMs and the increasing occurrence of naturally occurring extreme climate events have led to calls for the design of biodiversity-ecosystem function (BDEF) experiments to shift to extreme weather events and multiple scenarios (Jentsch *et al.* 2007; Smith 2011). Experimenters are beginning to include as many different global change factors into their designs as possible and so many experiments have sought to account for interactions between two or more aspects of global change such as elevated CO_2 and nitrogen deposition (Reich *et al.* 2001; Volk *et al.* 2011) or elevated CO_2 and rainfall changes (Kardol *et al.* 2007; Larsen *et al.* 2011). Global change factors come in many forms, but some argue that increasing experimental complexity leads to difficulties with mechanistic understanding of responses. Additionally, three-way interactions (e.g. CO_2, drought and N) have reduced effect sizes in empirical experiments, so the magnitude of responses to combined stresses could be lower than previously feared (Leuzinger *et al*. 2011).

Experiments that seek to determine the effect of a given climate change upon an ecosystem typically measure different aspects of ecosystem function, or compositional change. The working definition of ecosystem function used in this thesis is that of Pacala & Kinzig (2001), who define ecosystem function as a general term for processes which allow a biological community to support itself through soil, geography and climate processes. It can be quantified through biogeochemical or biotic parameters such as rates of N-mineralisation, amounts of standing carbon (stocks or pools) or resilience of processes or composition to disturbance (Pacala & Kinzig 2001). Ecosystem functions can be used as proxies to investigate overarching themes including resistance to perturbation (Schwartz *et al*. 2000), water cycling (Chapin *et al*. 2000), nutrient cycling and retention (Manning *et al*. 2006), and the ability of a system to support microbial communities (Wardle 2002) and higher fauna (Morehouse *et al*. 2008). These factors determine the provision of ecosystem services and the benefit that ecosystems give to people, including clean air and water, food and medicines (IPCC 2007).

Most rainfall change studies that consider effects on ecosystem function are based on unmanipulated semi-natural systems (Table 1.1), although exceptions include Fay *et al*. (2008) and St Clair *et al.* (2009) who used a mesocosm approach. Naturally, soil moisture content (SMC) is reduced under precipitation shortages fairly proportionally with the severity of the drought enforced (Laporte *et al.* 2002). This usually leads to plant tissue dieback, and decreases in annual net primary productivity (ANPP) under drought conditions (Knapp *et al.* 2002), although Kreyling *et al.* (2008) reported stable ANPP under a regime with once-in-a-century drought and downpour events in lowland grassland. Belowground processes are less simple to monitor in open field experiments, so there is little information on these. However, the consensus is that there are few effects of extreme rainfall on these in lowland grassland, but other ecosystem types may be more sensitive (Fiala *et al.*2009; Jentsch *et al.* 2011). Generally, carbon fluxes from plants and soil are more sensitive to drought than decomposition and mineralisation processes over the course of a growing season (Yahdjian & Sala 2002, 2006). Net ecosystem exchange (NEE) is very sensitive to both SMC and rainfall changes (Knapp *et al.* 2002; St Clair *et al.* 2009). However, it is far less widely researched than ecosystem respiration (R_{eco}), so more work is needed. R_{eco} was decreased under drought in an experiment on fallow fields, by up to 80% in the driest treatments (Laporte *et al.* 2002); this was mirrored in a mesocosm study which had long periods of very low SMC (St Clair *et al.* 2009). These effects were alleviated in unmanaged grasslands by adding extra winter snowfall (Chimner & Welker 2005) and maintaining high plant diversity (Aanderud *et al.* 2011).

The experiments described in Table 1.1 have enhanced our understanding of grassland ecosystem function by using real systems and taking place, for the most part, over a few growing seasons. This is important because natural succession and soil organic matter status change over time, and affect ecosystem processing, but are both very difficult to emulate in an artificial experiment (although St Clair *et al.* (2009) successfully retained accumulated biomass throughout their mesocosm experiment). In general, a single year of study is undesirable because of potential unexpected weather patterns and the problem of using only one period of succession; these do not give a true representation of an ecosystem's potential response to climate change (Thompson *et al*. 2005; Leuzinger *et al.* 2011). Studies in this review will not include experiments from Mediterranean biome regions such as California, as their predictions are for more rainfall in spring rather than less, and they include plants with

survival and regeneration strategies not found in temperate grasslands, such as highly sclerophyllous leaves and formation of lignotubers (Specht 1969).

Rainfall manipulation experiments would also benefit from basing their rainfall regimes upon real climate change scenarios, giving more realistic estimates of process and compositional responses to climate change. Currently, many experiments that report proportional changes of functions under artificial climate change regimes are not based on real projections, and many only focus on one or a few functions, and do not offer a holistic view of ecosystem response with strong links to biomass and traits. With the wealth of literature that suggests that plant community composition, weighted by abundance, could be a key driver of almost every aspect of ecosystem function (Chapin *et al.* 2000; Dìaz & Cabido 2001; Lavorel & Garnier 2002; Garnier *et al.* 2004; Dìaz *et al.* 2007a), there is a gap in the literature for a detailed, methodical experiment that measures a number of ecosystem functions for a number of growing seasons.

Table 1.1: Key experiments testing effects of altered precipitation under rain shelters. Experiments not included here consist of experiments from arid, Mediterranean, arctic and alpine environments. See key at the bottom for abbreviations.

Author	Location	Habitat	Grassland type	Duration	Rainfall regime	Response
Knapp *et al.* 2002,Fay *et al.* 2000, 2003, Harper *et al.* 2005	Kansas, USA	Mixed grass prairie	C_4 perennials	4 years	Increase interval by 50%, apply all rain.	SMC down 11.6%, 27% more variability. p^{syn} reduced ~20%.ANPP reduced ~10%. R_{eco} reduced 16%. Increased sp richness and turnover.
Yahdjian & Sala 2002, 2006	Argentina	Semi-arid Patagonian steppe	Tussock grasses and shrubs	3 years	30%, 55% and 80% reduction from ambient.	ANPP increased with increased rain. Min. and NH_4, unaffected. NO_3 reduced with increased rain. Decomposition varies with rainfall, mostly negative with reduced rain.
Jentsch *et al.* 2004, 2011 Kreyling *et al.* 2008,Mirzaei *et al.* 2008, Walter *et al.* 2010	Bayreuth, Germany	Temperate lowland grassland	Arrhenatherion grassland	10 years	Extreme events based upon 100 year trends-prolonged drought, heavy downpours.	No decrease of soil processes with extreme events. ANPP stable, though more tissue dieback with extreme climate. With increased diversity, less dieback. No effect of legumes.
Chimner & Welker 2005	Wyoming , USA	Mixed grass prairie	C_3 and C_4 grass mixture	1 year	±50% precipitation amounts. Winter snow addition	R_{eco} decreased under drought, but in winter snow addition plots it increased. Increased plant growth more of a driver of R_{eco} than decomposition.
Laporte *et al.* 2002	Ontario, Canada	Temperate grassland	Fallow field. Mostly grasses and *Trifolium*	1 year	6 regimes of absolute volume and frequency based upon the June average	R_{eco} reduced by 80% in the mot droughted plots.SMC reduced by 42%. AGB was decreased under droughted plots.

Table 1.1 (cont.): Key experiments testing effects of altered precipitation on grassland under rain shelters. Experiments not included here consist of experiments from arid, Mediterranean, arctic and alpine environments.

Author	Location	Habitat	Grassland type	Duration	Rainfall regime	Direction
Aanderud *et al.* 2011	Michigan, USA	Perennial mesic grassland	Agricultural land reverted to successional grassland	One growing season	Increased or decreased variability: 3 20mm events every 7 days, and 2.3mm every day.	Increased manipulated diversity mitigates the effect of drought on R_{eco}. More rainfall, higher R_{eco}.
Fiala *et al.* 2009	Czech Republic	Mountain, highland and lowland	Highland: wet grassland, lowland: acid grassland	2 years	Three treatments: 50%, 100% and 150% of ambient	No difference between rainfall at the lowland site but positive root growth with moisture in mountains.
Kahmen *et al.* 2005	Germany	Mountain plateau (temperate conditions)	Semi-natural grassland, *Lolium* dominated	2 years	100% exclusion mid-April to mid-June	$\delta\ ^{13}C$ can be explained by soil cation and nutrient variables, but responses to drought with increasing species richness are variable.
Joos *et al.* 2010	Switzerland	Temperate grassland	Intensively managed perennial grassland	1 year	30% exclusion like 2003	Drought reduced soil R_{eco} by 59% and litter R_{eco} by 81%.

SMC = Soil Moisture Content p^{syn} *= Net photosynthetic rate* R_{eco} *= Ecosystem respiration, ANPP= Annual net primary productivity Min= Mineralisation rate AGB= Aboveground biomass BGB= Belowground biomass* $\delta\ ^{13}C$ *= Leaf labelled with* ^{13}C *as a correlate with stomatal conductance.*

1.2 BIODIVERSITY

Most plant species are adapted to a relatively small range of environmental conditions which both influence and are influenced by community level interactions and composition (Elton 1927; Silvertown 2004). Plant communities are being exposed to unprecedented levels of stress. This is particularly important given changing global climate and widespread land use change. These stresses are drastically altering ecosystems and understanding the relationships between plants and their individual responses is crucial in directing conservation and remedial efforts in a changing climate. When conditions are extremely inhospitable or competition is exceptionally strong, resulting extinctions are very rarely random because of differing adaptations and tolerances of plants (Schläpfer *et al.* 2005; Cross & Harte 2007).

A long tradition of experiments set out to ascertain whether there is a relationship between species richness and ecosystem function; this search for general principles precluded species and functional identity, merely focussing on species number (Loreau 2001). This led to a proliferation of experiments that selected randomly from a predetermined species pool and usually grew grassland plants in assemblages of 1, 2, 4, 8 *etc*. species. These experiments provided some interesting insights into various long-standing ecological questions, such as how plant interactions can positively or negatively affect one another, but there were also many confounding results caused by sampling effect and complementarity not accounted for in the design (Mulder *et al.* 2001). Sampling effect postulates that in a given species pool, higher species diversity is likely to include more productive species, or those that have a disproportionate effect on their surroundings (Huston 1997; Bengtsson 1998; Wardle *et al.* 1999; Schwartz *et al.* 2000; Loreau & Hector 2001). It is then difficult to attribute effects to species number alone. Complementarity refers to a mechanism that allows resource use to be partitioned by different species, allowing them to exploit different spaces in the soil, so they may coexist while still fulfilling their resource requirements (Hooper 1998). Classic species diversity experiments did not allow for complementarity, rather, assuming ecosystem function would change in an additive fashion as species were added. Realistically they are more likely to be synergistic or antagonistic, as interactions are inevitable. These experiments have been superseded by designs that grouped species with regard to their perceived ‘functional effect’ on the environment (Hooper & Vitousek 1997, 1998; Roscher *et al*. 2004).

Idiosyncratic differences between species' impacts on their environment led to the realisation that their characteristics needed to be accounted for when quantifying ecosystem function (Tilman & Downing 1994; Hooper & Vitousek 1997; Hooper *et al.* 2005). For example, when legumes are added to a mixture, their nitrogen-rich exudates and litter allow increased resource uptake by their non-nitrogen fixing neighbours (Spehn *et al.* 2002; van Ruijven & Berendse 2003). There are few simple links between increasing biodiversity and most ecosystem functions, and this is partly because function has been shown to be depressed at both extremes of diversity (Schwartz *et al.* 2000). In order to investigate the effects of species in a system, grouping species into sets with similar effects upon their environment is a simple approach and has become widely used. Efforts to categorise plant species have gone through many permutations, beginning with the grass, legume, forb classification (GFL), then with some modifications (annual and perennial or deep and shallow rooted forbs, or C_3 and C_4 grasses, He *et al.* 2002; Reich *et al.* 2004). However, there was no consistent link made with functions other than primary productivity, and no difference in the functions of aboveground and belowground biomass and soil N concentration could be discerned when species from the GFL classification were reshuffled and reanalysed (Wright *et al.* 2006).

Most of these studies focussed on relationships between aboveground biomass and increasing diversity, usually finding a positive relationship between the two (Tilman *et al.* 1996; Hector *et al.* 1999; Pfisterer & Schmid 2002; Fridley 2003). It has been found, however, that species identity is more important than richness alone (Hooper & Vitousek 1997; Symstad *et al.* 1998; He *et al.* 2002). There is some evidence for a relationship between higher diversity correlating with stability of ecosystem function, where a number of studies have reported increased resistance of productivity to drought with more diverse species mixtures (Frank & McNaughton 1991; Tilman & Downing 1994; Pfisterer & Schmid 2002), and Ewel (1991) also described more fertile soils under mixed groups than monocultures on a volcanic substrate. However, others noted that complementarity of resource use between species were associated with depleted nutrient pools in the soil in diverse mixtures and led to much lower leaching losses (Tilman *et al.* 1996; Knops *et al.* 2001; De Deyn *et al.* 2009).

The main experiments looking for an association of plant biomass with litter decomposition have had mixed results which could reflect the still somewhat unknown relationship of plant diversity with microbial biomass. Knops *et al.* (2001) report that foliar N concentration in increasingly diverse mixtures did not correlate with decomposition rate, as averaging foliar N across a community caused no net change. Knops' result was an example

of how mean trait values alone can be poorly correlated with function, although Bardgett & Shine (1999) found a positive relationship between decomposition rate and adding species to the mixture with high foliar N content. Wardle *et al.* (1997) also showed that longevity of a species has a strongly negative effect on decomposition which is directly linked to leaf toughness. These results, and more direct measures of microbial biomass, have led to a conflicting view of how plant diversity affects the microbial community. De Deyn *et al.* (2009) showed an increase in microbial biomass with increasing functional diversity, while Mulder *et al.* (2002) and Hedlund (2003) found no difference. This could be due to a problem with assigning species according to function, which is still highly disputed. Therefore clearer partitioning of species effects is needed (Bardgett & Wardle 2010). Plant species are influential of microbial diversity, mainly via litter inputs though to some extent through root exudates, but the lack of consistency of findings suggests that abiotic factors have much stronger effects than plant species.

More recently, groups were based on functional effects traits, for which a wealth of literature had already been amassed (Lavorel & Garnier 2002; Cornelissen *et al.* 2003; Hooper *et al.* 2005; McGill *et al.* 2006; Kattge *et al.* 2011). Functional effects traits are defined as measurable characteristics of plants that affect ecosystem functioning (Hooper *et al.* 2005). The literature had shown links between a given functional effect trait of a species, and the assumed effect it would have on its surroundings, measured per unit biomass (Violle *et al.* 2007). Therefore, effects traits are carefully chosen and species grouped according to these, and so ecosystem functioning should be discrete between groups. Grouping traits using a simple clustering or ordination analysis has been implemented for many years and across many organism types (Jaksic & Medel 1990; Roscher *et al.* 2004; Al Haj Khaled *et al.* 2005), although agreement on exact techniques remains elusive, and there is some evidence that more continuous trait-based measures of function, such as community weighted means, may yet be more appropriate (Dìaz *et al.* 2007a).

It is difficult to ascertain why experiments using bespoke species grouping designs are rare. In this thesis the functional trait group design has come from previously mooted techniques by Lavorel *et al.* (1997) and Petchey & Gaston (2002, 2006). Nevertheless, most researchers use the GFL classification, possibly because creating a customised grouping to fit the requirements of individual experiments is difficult and time consuming There is a need for some consistency in approach to functional grouping, only then can results be compared across systems and true insight obtained into species effects on ecosystem function. Past

work has given glimpses of the power of functional diversity to mitigate the effects of stress (Wardle & Nicholson 1996), and more than ever we need to understand this in order to make informed decisions about the future of our ecosystems and appropriate ways of targeting resources for conservation.

So far, few studies have combined climate changes and manipulated functional diversity in a long term, large-scale field experiment on grasslands (though note ongoing work by the EVENT team; Jentsch *et al.* 2007; Mirzaei *et al.* 2008; Walter *et al.* 2010). Duration of experiments is important as some functional responses could result from transient 'bedding in' effects and accumulation of plant necromass. This thesis offers a new approach using substantiated statistical methods in order to group species according to their functional effects. It proceeds to apply the classification to a long term field experiment and follows through the whole process to the prediction of ecosystem function under certain conditions. This is necessary because there have been many different ideas offered in the past, few of which have been applied to empirical tests or followed through from species grouping to field application, to predictive modelling.

1.3 AIMS AND OBJECTIVES

This thesis aims to:

- Design and test a method to create plant functional groups that are tightly linked to ecosystem function, manipulation of which will have discrete and measurable effects.
- Implement a large-scale grassland field experiment with a climate and a functional diversity manipulation, to evaluate the response of a set of ecosystem processes describing carbon, nitrogen, phosphate and water use.
- Design contrasting climate projections to create extreme climate regimes, to evaluate the varying responses of a set of ecosystem processes.
- Create a generalised linear model method to use the data collected throughout the experiment to predict ecosystem processes using a range of biotic and abiotic variables.

1.4 THESIS OUTLINE

Chapter 2 presents a method of assigning species to functional groups for use in diversity experiments by clustering plant species according to their functional effects traits. This approach is intended to be used as a general method, applicable to a wide range of species, and possibly for a number of trophic levels. I discuss choosing the most appropriate traits according to the functions to be measured, and the reasons for choosing divisive hierarchical cluster analysis for categorising species. I then recommend two simple methods of validating the groups chosen, in order to confirm that the groups are functionally discrete. Finally, I present a worked example based upon species native to British lowland grassland.

Chapter 3 describes a large-scale field experiment, which comprised a rainfall manipulation and a functional diversity treatment, using the groups described in Chapter 2 to mesotrophic grassland in South-East England. Through the last two years of the experiment, many measures relating to biogeochemistry were taken, and results suggest that there are marked differences in response to climate alterations between the functional groups. Notably, functions under perennial species seem more vulnerable to severe summer drought, while functions under systems containing annuals are less strongly affected.

Chapter 4 used the framework of the climate change experiment in Chapter 3, and considered the response of ecosystem functions to two differing (more extreme) precipitation regimes; drought in spring *and* summer, and highly variable summer rainfall frequencies and volumes. There was a strong difference in the response to the two treatments; spring/summer drought seemed to experience strongly reduced microbial-driven processes such as N-mineralisation and ecosystem respiration, while the more variable treatment rarely responded differently from ambient, except with regard to plant species cover and richness, which were both much reduced.

Chapter 5 used the data from Chapter 3 to create generalised linear models describing the ecosystem functions measured. These models consisted of four components that were

intended to predict the main drivers of ecosystem functions throughout the seasons. I offer an examination of these drivers, highlighting key patterns and trends across seasons and groups of ecosystem functions.

Chapter 6 discusses the key outcomes of this experiment with regard to its position alongside similar global change studies, and suggests areas where further work might be useful.

Figure 2: Progress of experiment through summer 2008.

CHAPTER 2: TRAIT BASED CLASSIFICATION AND MANIPULATION OF FUNCTIONAL GROUPS IN DIVERSITY EXPERIMENTS

2.1 ABSTRACT

Concerns over the consequences of species loss for ecosystem functions and the services they underpin have led to a proliferation of experiments in which functional group identity is manipulated. The norm in these experiments has been to assemble communities from arbitrary groups. However, there is evidence to suggest that such groups are unable to adequately predict ecosystem function because the mean trait values per group are not sufficiently different. More meaningful functional groupings can be developed by classifying organisms by their functional effects traits, thus making the link between the biota and the functions of interest easier to identify. Here I describe a process for establishing functional groups based upon the identification of traits that affect the function of interest by using divisive hierarchical cluster analysis. This approach can be applied to a wide range of ecosystems to assess the impact of functional diversity upon ecosystem processes, particularly when functions to be measured are relevant predetermined. I suggest two rigorous procedures for validating the groups, and present an example of a large-scale field experiment where this approach has been implemented. This method is applicable to a wide range of communities and trophic levels. I intend that it should be used to contribute to a more standardised approach to grouping species, which in turn will aid predictive power of ecosystem models.

2.2 INTRODUCTION

Concerns over the implications of global biodiversity decline for ecosystem functioning and associated services has led to a proliferation of biodiversity and ecosystem function (BDEF) experiments (Hooper *et al.* 2005; Cardinale *et al.* 2006; Hillebrand & Matthiessen 2009). These have generally found that the diversity of species and functional groups positively influences function but our mechanistic understanding of this relationship is incomplete, with the species traits that influence function often remaining unidentified.

It is impractical to establish experiments containing all possible combinations of species from a given species pool, and there are advantages to simplifying diversity by reducing it to functional groupings based upon morphological, physiological and/or phenological traits to study ecosystem functions (Dìaz & Cabido 1997; Lavorel & Garnier 2002; Petchey 2004). This is more comprehensive and defendable than choosing taxonomic species combinations, which some experiments have done in the past, because the aim is to create groups where trait variation is lower within groups than between (Tilman *et al.* 1996; Fridley 2003). The assumption is that if the traits chosen are closely linked to the functions to be studied, the groups will exert discrete effects on their surroundings. This approach also allows researchers to evaluate influential trait combinations, which can then be compared across experiments and possibly lead to generalisation across systems.

At present there is no standardised measure of functional diversity in a given assemblage, and there have been many calls for a universal protocol for both trait measurement and the classification of functional groups (Lavorel *et al.* 1997; Tilman 1999; Lavorel & Garnier, 2002; Cornelissen *et al*. 2003; Naeem & Wright 2003; Harrington *et al.* 2010). Many field experiments, which have commonly been performed in grasslands, have used the traditional grass/forb/legume (GFL) classification, or slightly more mechanistic groupings using rooting depth and the seasonality of annuals, to investigate links with decomposition and nitrogen cycling (Hooper 1998; Wright *et al*. 2006). These classifications lack a sound mechanistic basis and make no concession to the high likelihood of substantial within-group variation in other functionally important traits, or the possibility of strong trait overlap between groups. A multi-site analysis by Wright *et al.* (2006) re-sorted species from GFL groups into random combinations from a number of experiments and related the new groupings to function data. Their conclusion was that that the GFL classification has no

greater explanatory power than randomly allocated groups. There is a vast amount of literature that describes potential methods of grouping, but a good starting point for linking effect to response is to use a hierarchical technique based upon measuring plant functional traits, and choosing to use traits that have the best support in the literature for correlation with function (Lavorel *et al.* 1997).

Here I describe a systematic process for the formulation of *a-priori* functional effects groupings for use in BDEF experiments, illustrated with a worked example. It builds upon the work of Roscher *et al.* (2004) and the use of functional diversity measures (e.g. Petchey & Gaston 2002) to produce a scheme that allows for both rigorous hypothesis testing and continuous trait based approaches to explain function (e.g. Dìaz et al. 2007b). In this method the functions of interest and the species pool are defined, appropriate species traits are selected for measurement and a dendrogram of species is constructed, segregated by cluster analysis. The species groups returned by the dendrogram should show similar within-group relationships to the chosen functions, thus allowing for clear and testable predictions for the effect that they have on ecosystem function. A forerunner to our approach is that employed in the Jena experiment, a long-term field study that used customised functional groups to predict the role of functional diversity in a grassland with respect to a wide range of ecosystem functions, properties and services (Roscher, 2004). Their clustering method for various reasons, (including the double weighting of the nitrogen fixation trait), produced groupings that closely align with the GFL classification. Such approaches have also often used morphological traits, such as plant height and leaf size, but lack a clear hypothesised link to most ecosystem functions.

My approach to grouping species has been refined from clustering strategies that originally sought to describe plant life history (Raunkiaer 1934), habitat preferences (Ellenberg *et al.* 1991) and life history strategies (Grime 1988). I offer this process as a successor to these studies, aiming to group species into the most functionally homogeneous clusters possible, disregarding taxonomic, life history and morphological associations. In doing so I acknowledge that this approach is more suitable for explaining biogeochemical processes than functions based upon co-evolved species interactions (e.g. pollination) or interactions with ecosystem physiognomy (e.g. those involving habitat selection). I illustrate our approach with an example of a field experiment investigating the modification of plant functional diversity on the nitrogen and water cycles of a grassland ecosystem affected by climate change. My example focuses on plant traits and terrestrial ecosystems, but the

process should be applicable to a wide range of systems. I also discuss the pitfalls and advances that such an approach offers, with consideration given to potential problems, such as a lack of empirical evidence for trait-function linkages.

2.3 METHODS

2.3.1 CHOOSING TRAITS

The first step in generating tailored functional effects groups is to define the functions of interest and select a group of functional traits that drive or determine them (Hillebrand & Matthiessen 2009). Generally, if there is a theoretical or empirical link between a trait and the function to be measured, then it should be included. For example, traits relating to leaf nutrient concentration or toughness can be directly linked to decomposability (Garnier *et al.* 2004; McLaren & Turkington 2010). Correlation between many traits may be unavoidable, but *direct* correlation should be avoided, for example specific leaf area (SLA) and leaf dry matter content (LDMC) describe almost identical characteristics, so only one should be included. Other correlations are frequent, such as between leaf nitrogen content (LNC), SLA and photosynthetic rate, but even though their links with different processes are likely to overlap, between them they will describe a large range of different processes, and do not directly measure the same aspect of function (Table 2.1).

Table 2.1: Correlations of traits using Pearson's Product Moment Coefficient. AGB = Aboveground biomass, BGB = Belowground biomass, SLA = Specific leaf area, LNC = leaf nitrogen content, Gs = stomatal conductance, p^{syn}= photosynthetic rate

	BGB	AGB	SLA	LNC	Gs
AGB	0.42				
SLA	0.41	0.14			
LNC	0.24	0.13	0.29		
Gs	0.11	0.09	0.03	0.07	
P^{syn}	0.03	0.4	0.05	0.12	0.56

While I recommend keeping the number of traits as low as possible, Hector and Bacchi (2007) present evidence that the more functions to be explained, the more species (and by extension, functional diversity) are needed. If traits are given equal weighting in cluster analysis (see below), this will generate bias towards the correlated traits, so I recommend testing for correlations before commencing the cluster analysis; an *r* value of over 0.7 is generally considered to indicate excessive correlation. Trait choice will depend upon community type and the functions in question, but I suggest that they are practical to measure, with strong support in the literature for their ability to describe the relevant functions (Westoby 1998; Wright *et al.* 2006; Kraft 2008). Mixtures of physiological and morphological traits have been recommended in the literature in order to represent a full spectrum of growth forms and resource capture strategies, which is useful as long as they are strongly supported by evidence (Dìaz *et al.* 1999a; 2004). Traits used can be ordinal or continuous. If a discontinuous trait variable cannot be assigned a logical rank order (e.g. from annual to perennial- 1-3), I recommend avoiding these data, as they are not well suited to cluster analysis (see below). However, if they are instrumental in describing the functions required, Jongman *et al.* (1995) suggest treating such traits as nominal data.

There are several published examples of systematic evaluations of trait and process linkages, which are good starting points for trait selection (Klumpp & Soussana, 2009; de Bello *et al.* 2010). Failure to identify groups closely linked to function is likely to result in a weak capacity to explain ecosystem processes. The functional diversity (FD) work presented by Petchey and Gaston (2002) indicates that if processes are measured with no consideration of the trait variation of species present, they are often poorly explained, and the authors question whether a single functional classification scheme can describe a wide range of functions. Creating many different functional effects classifications to describe different responses is likely to be unfeasible in a field experiment (although not in model simulations), so using a set of the most comprehensive traits possible is likely to be the most practical alternative.

2.3.2 DEFINING THE SPECIES POOL

The next step in the process is to delimit the species pool from which the groups are drawn. Deciding which species to include can be problematic, particularly if the study system

is open to invasion. It is further complicated by the fact that the majority of field experiments using these methods will undergo successional changes. The simplest and most cost-effective method is to begin with all the species present in the field site at the beginning of the study, and to screen and add invaders to the groups as and when they appear, on a similarity basis. In cases where communities are artificially assembled it may be sensible to use species that associate frequently and that are typical of the sites' environmental conditions e.g. the plant species found in a class of the UK's National Vegetation Classification (NVC) (Rodwell 1992).

2.3.3 OBTAINING A TRAITS DATABASE

Trait data can be obtained from database or literature sources (Fitter & Peat 1994; Kleyer *et al.* 2008; Royal Botanic Gardens, Kew 2008; Kattge *et al.* 2010; USDA 2010b), or measured directly. Database trait values can be very useful as they are drawn from several studies, and save a lot of time and expense (Lavorel *et al.* 2008). However, there are many limitations with using such values, particularly the lack of standardisation. A number of standardised protocol suggestions have been offered, with the aim of making published trait data largely comparable (Grime *et al.* 1997; Cornelissen *et al.* 2003). Standardisation is still generally lacking in plant trait measurement studies, which differ in growth conditions and substrate, season of measurement, seed source and trait measurement protocols. In addition not all species are represented in the databases. A second problem is that a single trait value does not represent the full intra-specific range of a species, or its expression in field conditions (Albert *et al.* 2010). These problems can be addressed by creating as many replicates of each species as possible and averaging between them. Using local seeds and soil, and keeping greenhouse conditions as similar to the site as possible could go some way to alleviate criticism; if using one set of trait data taken *ex situ*, trait-based field experiments should be confined to one site to reduce intra-specific trait variation, by the same token, attempting to capture the whole range of a species' trait distribution in a greenhouse could lead to 'noisy' data and lack of fit to functions in the field Dìaz *et al.* (1999b).

2.3.4 USING DIVISIVE HIERARCHICAL CLUSTER ANALYSIS TO CREATE FUNCTIONAL EFFECTS GROUPS

Once a list of trait values for all species has been established, the next step is classification into functional groups, to create groups of species that have similar effects on the functions of interest. An efficient means of doing this is to use cluster analysis (Jongman 1995; Shaw 2003), which allows clusters of similar values to be identified within multivariate datasets. Of the various techniques available I recommend divisive hierarchical cluster analysis as this allows for a pre-determined number of groupings to be derived. This is desirable for many experiments, as an unrestricted number of groupings could result in uninformative (if low) or unmanageable (if high) numbers of functional groups. Cluster analysis can create a dendrogram (Fig. 1), which provides the added benefit of visualising the relationships between species. If the assumptions about the relationship between traits and function are correct, and trait values are representative of those expressed by species at the study site, the groups derived from the analysis should have more discrete and predictable effects on ecosystem function than random species assemblages.

In order to create groups, trait means for each species should be calculated and the cluster analysis carried out using S-PLUS 6.0 (Insightful, Gothenburg, Sweden) or the freeware R2.12.0 (R Core Development Team 2009). Categorical variables should be treated as ranked factors. Weighting of traits is somewhat contentious, and I do not recommend it unless there is strong justification for it. For example, if responses concerned with nitrogen cycling are the focus of the experiment, traits such as leaf nitrogen content or nitrogen fixation capacity could be double weighted (Petchey & Gaston 2002; Roscher *et al.* 2004), although this could lead to heavily biased groups, particularly since legumes do not always fix nitrogen, for example in immature communities or water-stressed communities (Serraj *et al.* 1999). As clustering is based upon dissimilarity matrices, an appropriate distance measure must be chosen. The simplest (and default in R) is Euclidean distance, which calculates the distance between every trait combination in Cartesian space (n-species dimensions), and creates a matrix of these distances (McCune & Mefford, 1999). Euclidean distance is commonly used, although it emphasises outliers so the data must be standardised with a mean of 0 and variance of 1. Most other distance measures have fairly rigid requirements, and compute data in less intuitive ways. For example, Sørenson (Bray-Curtis) dissimilarity gives less weight to

outliers, but it is recommended for ecological community data, as it gives proportions based on overlap of two communities, so is better for community turnover.

When carrying out this analysis in S-PLUS, it requires the user to set the number of groups required before clustering begins. In R, the grouping occurs in a post-hoc fashion. The number of groups to be included has a large impact on the type of study to be carried out, and the model system.

2.3.5 ESTABLISHING THE GROUPS IN THE FIELD

The optimal method for generating an experimental functional diversity gradient from the groups identified will depend upon the type of species in question, and the functions to be studied i.e. whether species are removed from an existing community or whether an artificially assembled community is established. If disturbance strongly affects the measured functions (e.g. intensive weeding can dry and warm the soil, altering microbial activity), then it may be best to assemble species. Clearly, it is not feasible to assemble late-successional ecosystems comprised of species with long generation times, so here species removals or simulations (e.g. Bunker *et al.* 2005) are the only options. Weeding to establish functional groups should cause a directional change in community level trait values and their distribution, although shifts in trait distribution can only be expected for traits that show significant differences between groups. In other cases niche space may be made available by the removal or exclusion of a functional group, and the remaining species that are most similar to those removed are likely to utilise this space and increase disproportionately. Where this occurs, the observed change in trait values following functional group exclusion may be less than expected. When trait data are combined with a survey of species abundances in experimental units, a number of metrics, including functional diversity (Petchey & Gaston 2002), community weighted means (Garnier *et al.* 2004; Lavorel *et al.* 2008) and dissimilarity measures (Hillebrand & Matthiessen 2009) can be calculated and used to estimate the impact of the functional group manipulation on community level functional properties. These can also be used as explanatory variables in statistical models describing function.

2.3.6 ASSIGNING NEW SPECIES TO EXISTING FUNCTIONAL GROUPS

In open ecosystems new species may colonise or emerge from stasis, (e.g. seedbank). These may not have been considered when originally delimiting the species pool of the site, and their absence from the functional dendrogram means that their functional group assignment is unknown. There are two options in this situation, the first of which is to remove the colonising species. This is simple and may be desired to avoid the emergence of entirely new functional properties (e.g. the entry of an N fixer into a system that did not contain them), but risks reducing ecological realism; the species may be a potential new dominant, for example. The other option is to obtain trait data for the new species and allocate it to the most appropriate of the existing functional groups. This *a posteriori* integration into groups can be achieved by using dissimilarity indices to add the new species to the dendrogram. Dissimilarity values follow the same principles as a cluster analysis; again Euclidean distance measures are recommended to arrange the data in multidimensional space.

To assign new species to functional groups, calculate a mean trait value for each of the three functional groups, to compare to the mean trait values of the new species. This method requires a sequential addition of species, and because the mean trait value of the groups would be altered with each addition, it is important to add new species in order of their abundance in the field, in case the new species have outlying traits which would alter group means. Dissimilarity indices can be calculated using R2.12.0, choosing Euclidean distances, and standardising the data as before. Categorical variables should be converted to a continuous format by averaging values across species, and labelling them as numeric, not factor values. The new species is then assigned to the group with the lowest dissimilarity value, and the new trait means calculated. The mean trait value for the functional group must be adjusted with each new species.

2.3.7 VALIDATION OF THE FUNCTIONAL EFFECTS GROUPS

An important criticism of hierarchical cluster analyses is that there is no measure of whether the groups identified are the most effective combination to explain function, or whether they are statistically different from one another. For this I recommend using linear discriminant analysis (LDA) in the MASS library of R2.12.0. LDA is an *a posteriori* method of verifying

that species have been allocated to the most appropriate group. LDA tests the within-group covariance matrix of standardised traits, and generates a probability of each species being in the most appropriate group, i.e. it generates a percentage comparing the similarity of the species to each of the groups. The highest probability generated is taken to be the group the species belongs in. If the analysis finds that almost every species is appropriately categorised, this is strong justification for the groupings. High percentages of correctly allocated species in the LDA confirm that the functional groups are as discrete as possible. If there are some species that the LDA suggests are misclassified, close inspection is needed. It is possible that the species was classified on the basis of a single trait. It is for the user's discretion to decide whether this trait is particularly important to their functions of interest. If not, the analysis could be repeated with the trait removed and results re-evaluated.

Before hypotheses about the effects of functional group removal can be formulated it is important to check that there are quantifiable differences between the trait means of the groups. If there are no clear differences between the groups, this suggests that there is too much functional divergence, or there are too many groups, and the outcome of manipulating these groups in a system could be confounding or inconclusive. Simple analyses such as one-way ANOVAs and post-hoc Tukey's HSD tests allow evaluation of differences in trait means across functional groupings.

2.3.8 GENERATING HYPOTHESES ABOUT FUNCTIONAL GROUP EXCLUSION

Identification of functional group differences, coupled with hypothesised trait-function relationships, enables predictions about the consequences of functional group removal. Despite these hypotheses, it is possible that the removal of a functional group will not change functional properties. For example, if the group removed was rare, or if trait expression in the remaining species shifted to encompass the trait identity of the lost group (Walker *et al.* 1999). To measure the trait distribution and means across treatments in the field, I suggest using community weighted mean (CWM) and functional divergence measures (FDvar), (Mason *et al.* 2003; Garnier *et al.* 2004). These both weight traits by abundances in the field, and give a weighted mean trait value and trait variation value, respectively. Once intergroup variability has been established, these metrics can then be applied to ecosystem process measures. FDvar is particularly useful as it hypothesises that increasing functional diversity

would lead to higher FDvar. This could then imply niche complementarity if correlated with higher levels of function.

2.4 EXAMPLE: THE DIRECT EXPERIMENT

The DIRECT experiment (DIversity, Rainfall and Elemental Cycling in a Terrestrial ecosystem) was set up in 2008 on mesotrophic grassland in South-East England. I created tailored functional groupings to assess the effect that altering functional diversity would have upon ecosystem response to climate stress, focussing on the cycling of carbon, nitrogen and water, and their temporal stability as the ecosystem functions of interest (after Pacala & Kinzig, 2001). I used an existing species list from Silwood Park, Berkshire, UK (Crawley 2005), where our field site is located (for details of growing conditions and trait protocols, see Appendix 1).

I selected eight traits with established links to C, N and water cycling and the temporal dynamics of these processes. Temperate grassland species have been extensively studied, particularly with regard to effects traits, with the result that there are comprehensive databases, standardised protocols and informed evaluations of their links to function (Grime *et al.* 1997; Cornelissen *et al.* 2003; Kleyer *et al*. 2008; Kattge *et al.* 2010). I used specific leaf area (SLA), leaf nitrogen content (LNC), nitrogen fixation ability (as a binary factor), leaf stomatal conductance rates (Gs), above- and belowground biomass (AGB and BGB), leaf photosynthetic rate (p^{syn}) and perennation. SLA is a proxy for leaf thickness, and is associated with decomposability, relative growth rate and leaf construction costs (Reich *et al*. 1998b; Keddy *et al.* 2002), thus potentially explaining many other processes (Wilson *et al.* 1999). LNC is closely linked to litter quality, and therefore decomposability and soil nutrient turnover (Cheng *et al*. 2010). Nitrogen fixation plays a key role in ecosystem nitrogen input and availability (Hartwig 1998). Gs is a potential proxy for ecosystem evapotranspiration rate and water use efficiency (WUE) which may indicate the capacity to preserve water under soil moisture stress (Hsiao & Acevedo 1974). AGB and BGB describe potential carbon allocation and are associated with plant nutrient and water uptake (McConnaughey & Bazzaz 1991; Dìaz *et al.* 2004; Fornara & Tilman 2008; de Bello *et al.* 2010). P^{syn} can be considered an indirect measure of C fixation (Gilmanov *et al.* 2009). Perennation is likely to correspond to the temporal dynamics of the processes outlined above.

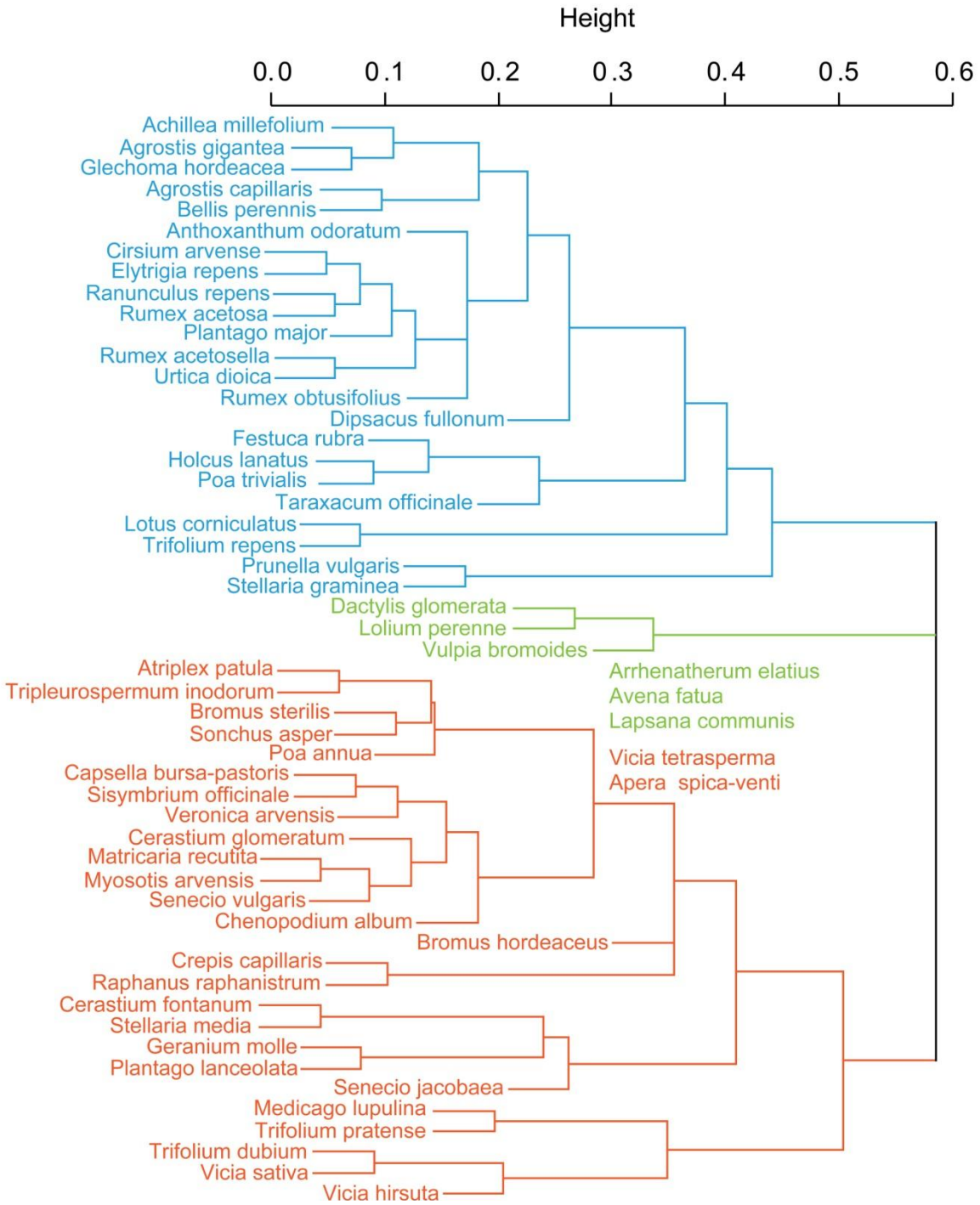

Figure 2.1: Functional dendrogram of species found in Silwood Park, Berkshire, UK, created using functional effects traits in a divisive hierarchical cluster analysis. Species added later using dissimilarity indices are placed next to their group. The red group will hereafter be known as group one, while two is in green and three is in red.

Table 2.2: Functional group validation using linear discriminant analysis to test the robustness of the species allocation by divisive hierarchical cluster analysis. Percentages describe the proportion of species that have been allocated to the 'correct' group by the discriminant analysis. The removal of each trait individually determines whether any trait has a disproportionate influence on the cluster analysis and subsequent groupings. AGB = Aboveground biomass, BGB = Belowground biomass, SLA = Specific leaf area, LNC = leaf nitrogen content, Gs = stomatal conductance, p^{syn}= photosynthetic rate

Trait group removed	Group 1	Group 2	Group 3
All traits present	92%	78%	89%
AGB (g)	80%	66%	89%
BGB (g)	92%	67%	82%
SLA (mm^2mg^{-1})	84%	78%	82%
LNC (mg/kg)	84%	67%	82%
Gs ($molm^{-2}s^{-1}$)	80%	67%	86%
Photosynthetic rate ($\mu mol\ m^{-2}\ s^{-1}$)	84%	100%	82%
Perennation	72%	67%	93%

I tested for correlations between traits using Pearson's Product-Moment Correlation Coefficient (Table 2.1), and then carried out a cluster analysis as described above using S-PLUS 6.0. The groups returned consisted of perennial grasses, forbs and legumes (group 1), caespitose (bunch) grasses and tall forbs (group 2) and annual forbs, grasses and legumes (group 3), (Fig. 2.1). The species were closely clustered on LDA ordination axes (Fig. 2), indicating that there is strong similarity within groups, particularly groups one and three. Only ~10% of species had a <50% agreement by the LDA, and so appeared misclassified. The removal of each trait in turn did not create a change in accuracy of group allocation (Table 2), suggesting that no single trait had undue weight in the analysis.

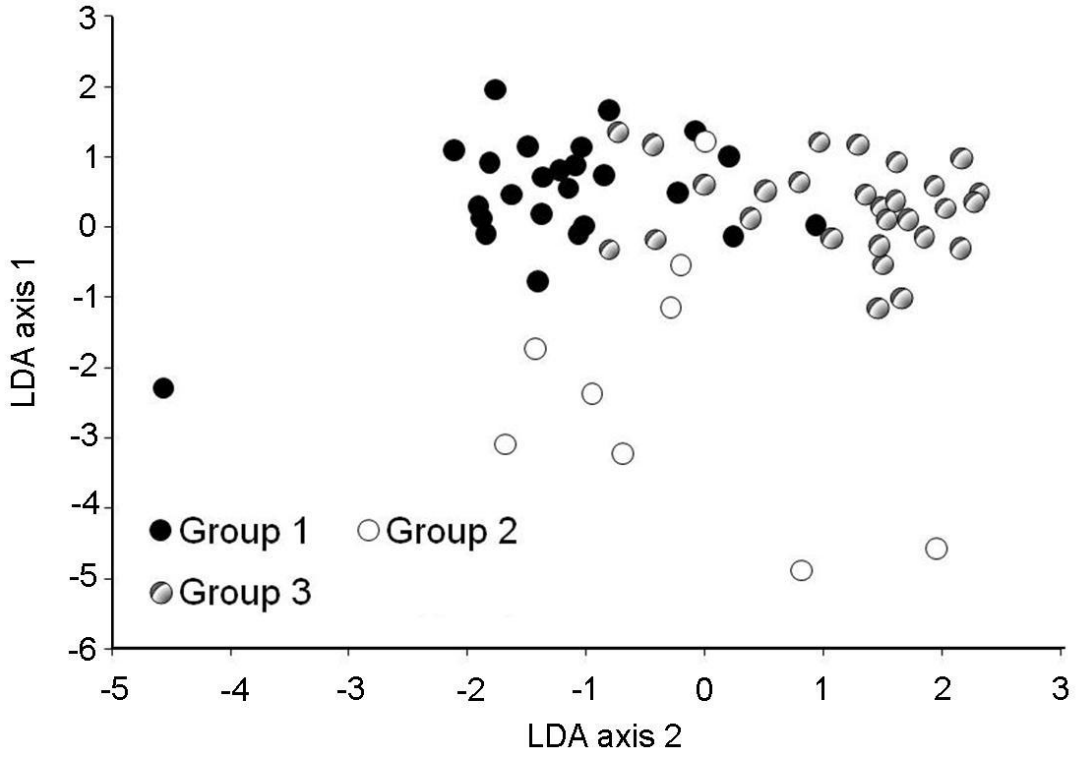

Figure 2.2: Ordination plot showing delineation of the three functional effects groups using linear discriminant analysis (LDA). Filled circles denote group 1, empty circles denote group 2, and grey circles denote group 3.

One-way ANOVAs revealed significant differences between the mean trait values of all three groups (Fig. 2.3). For example, groups one and three had similar AGB and BGB, but differed in their values for perennation and SLA. The ANOVAs justified the group two classification which appeared rather dispersed on the LDA ordination (Fig. 2.2), by illustrating that high AGB and BGB, and low LNC distinguish this group from the others (Fig. 2.3). Group one had the highest average SLA, so the loss of this group would be expected to result in lower community light capture and potentially slower decomposition rates due to the loss of thin leaves with little structural tissue content. It is also the group with the most perennial species, and so biomass stocks would be expected to be variable where it was present. Whilst most species in the second group are caespitose (bunch) grasses, a tall herb *Lapsana communis* L. is also included. Species from this group have a high biomass both above and belowground and lower LNCs than the other two groups, so its elimination would be expected to decrease nutrient pools at the community level and reduce decomposition and N mineralisation rates. The third group was mainly comprised of annual forbs, but also includes numerous legumes and three annual grass species (*Bromus sterilis* L.,

Bromus hordeaceus L. and *Poa annua* L.). This group had the fewest perennial species and so communities containing them may see a high seasonal variability in standing biomass.

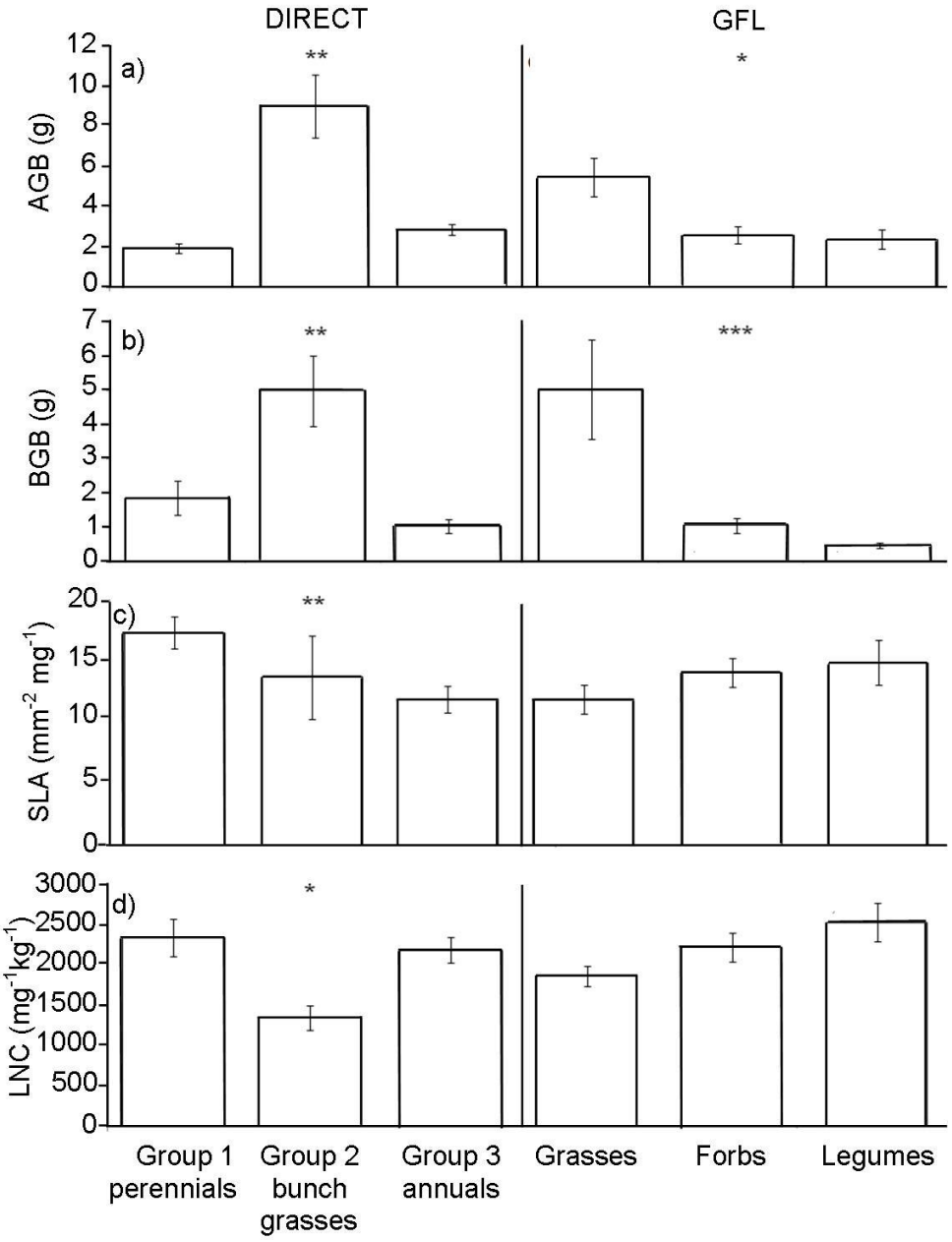

Figure 2.3: Trait differences between functional groups comparing the DIRECT with the GFL classification. a) aboveground biomass, b) belowground biomass, c) specific leaf area, d) leaf nitrogen content. Asterisks above bars indicate a significant difference (*p<0.05, **p<0.01, ***p<0.001), assessed using Tukey's HSD test. Error bars signify standard error of the mean.

I carried out an analysis to test whether our classification created stronger functional trait differences between groups than the GFL classification. This consisted of allocating our species into grass, forb and legume groups and carrying out one-way ANOVAs to test for

significant trait differences (Fig. 2.3). By including different traits in this way the differences between groups are stronger and could describe more functions than GFL, as recommended by Petchey & Gaston (2002). In the GFL classification the only discernible difference between traits was regarding biomass, which was higher in the grass group both above and belowground. There were no significant differences between groups for SLA or LNC, which are both implicated in a large number of ecosystem processes. Additionally, at no point were the forb and legume groups different in terms of traits, so it is unlikely that they would cause

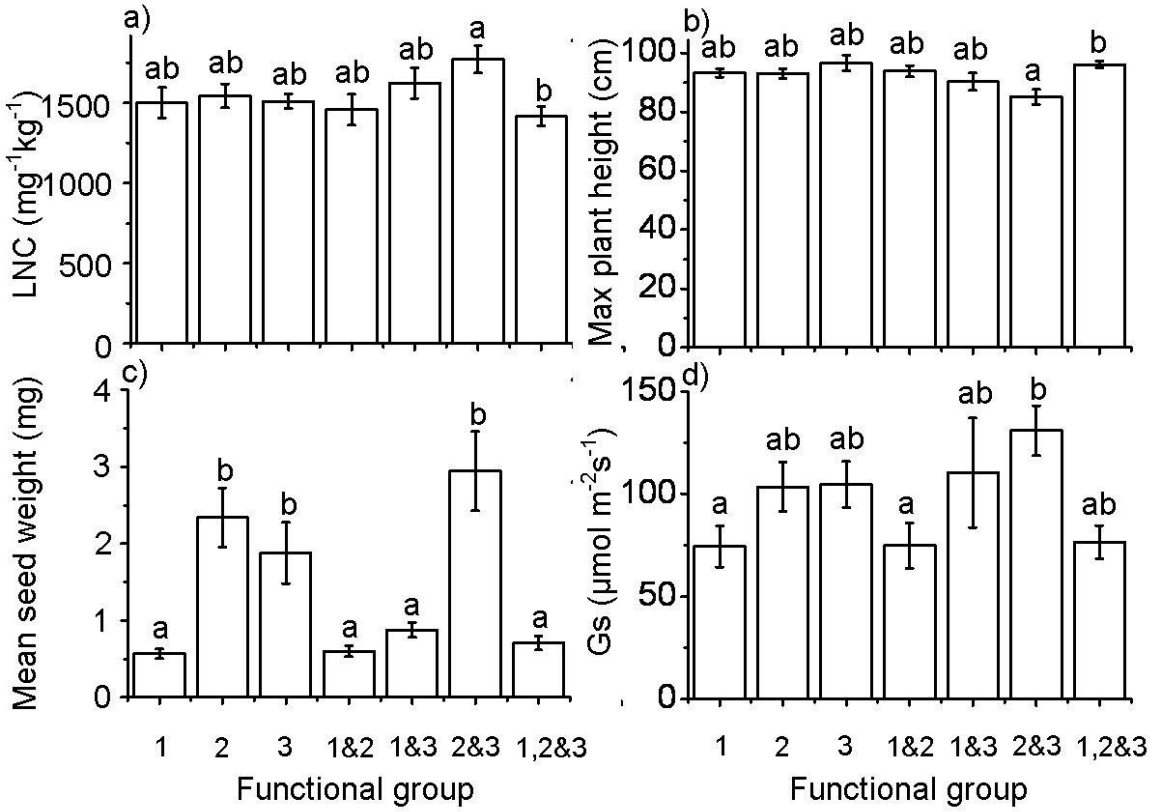

Figure 2.4: Community weighted trait means measured in October 2008, evaluated using one-way ANOVA. Letters refer to differences at the $p<0.05$ level. a) leaf N content, b) maximum plant height, c) seed weight, d) stomatal conductance. Error bars signify standard error of the mean.

measurable differences in function.

I established an experiment where every permutation of the three diversity treatments was represented, to test the relationship between the functional groups and the ecosystem process(es) in question. I established functional group treatments by removing young plants from a recently ploughed field undergoing secondary succession. This kept community assemblage as natural as possible, while minimising disturbance effects. After functional

groups were created, in October 2008, I tested whether trait differences were sustained in the field. Relative abundance of each species was measured in each plot and used to calculate CWMs for each of the measured traits in each plot, for trait values at the community level (Garnier *et al.* 2004). I carried out one-way ANOVAs on each trait used in the original groupings, as well as leaf dry matter content (LDMC) and typical maximum plant height (a proxy for shading and canopy cover) obtained from the Leda Traitbase (Kleyer *et al.* 2008) to test whether the trait differences occurred in the field when weighted by species abundance. I also used photosynthetic surface area (PSA) from our own data, and seed weight from Kew's Seed Information Database (SID, RBG Kew 2008) to investigate whether the groups showed demonstrable differences between traits that had not been used to cluster the groups. In general there was a significant difference between CWM traits when all groups were present compared to when group 1 was absent (Fig. 2.4). These differences support the assumption that differences in function will occur during the experiment.

2.5 DISCUSSION

The demarcation of groups of species with similar trait characteristics means that if the chosen traits are linked closely to function then the removal of groups will have different effects upon ecosystem function (Hillebrand & Matthiessen 2009). In presenting our new approach, I hope to show that field experiments based upon rigorously tested trait groupings offer substantial benefits over arbitrary groupings with regard to predictive capacity of ecosystem function. I feel that hierarchical cluster analysis improves upon these by allocating species to groups in a way that is unbiased towards any one trait and is applicable to many ecosystems. It offers a method of grouping that result in clear, significant differences between traits, which can be theoretically linked to function. Follow up with *post hoc* tests such as LDA provides confirmation of the appropriateness of allocations.

A limitation of the trait grouping approach is that it does not account for the functional consequences of species interactions. It is very difficult to predict, particularly when species are allowed to join the system organically, what adaptations of form and function will occur due to competition and various stresses (Petchey 2004; Maestre *et al.* 2010). In our study, traits were chosen carefully, using a wealth of knowledge about each, but nevertheless there remain doubts of the efficacy of the trait-based approach to explain

compositional controls of ecosystem function (Lavorel & Garnier 2002; Petchey 2004). These doubts are well grounded; the few large-scale BDEF studies that have used this approach indicate that traits are not silver bullets that fully explain how plants influence their environment (Dormann & Woodin 2002; Wright *et al*. 2006; Dìaz *et al*. 2007b). The optimal way of defining compositional effects on function has yet to be established, but future approaches may incorporate careful screenings of potentially important species interactions and responses to abiotic factors, which will then be pared back to the most effective set of descriptors. Careful consideration also needs to be afforded to the traits themselves. I chose to include root biomass as a trait because of the importance of belowground processes in determining ecosystem function. However, root biomass correlates weakly with many belowground functions, and aboveground traits often have better explanatory power over soil moisture and nutritional status, so there is a pressing need for higher quality belowground trait measures (Wright *et al.* 2006).

In the DIRECT experiment I aimed to compile the most comprehensive traits database with the resources and time available, using so-called 'soft traits', because often the most useful and accurate traits are too costly, time consuming or otherwise impractical to easily measure (Hodgson *et al.* 1999). A case in point would be key root traits (e.g. root exudation rates), many of which are difficult to quantify (Hishi 2007). Soft traits such as root length and root biomass are used as proxies for these traits (Poorter & de Jong 1999), and there is a large body of support for them.

Using a standardised protocol for grouping species, experimental results from a range of biomes will be comparable, making both generalisation and mechanistic insight more likely. Results gained in this way can be extrapolated to describe interactions between different trophic levels such as insect herbivore interactions with plant functional groups. Our system of deriving functional trait groupings is extremely flexible with regard to traits, ecosystems, functions, and trophic levels, making it potentially applicable to a wide range of both terrestrial and aquatic systems.

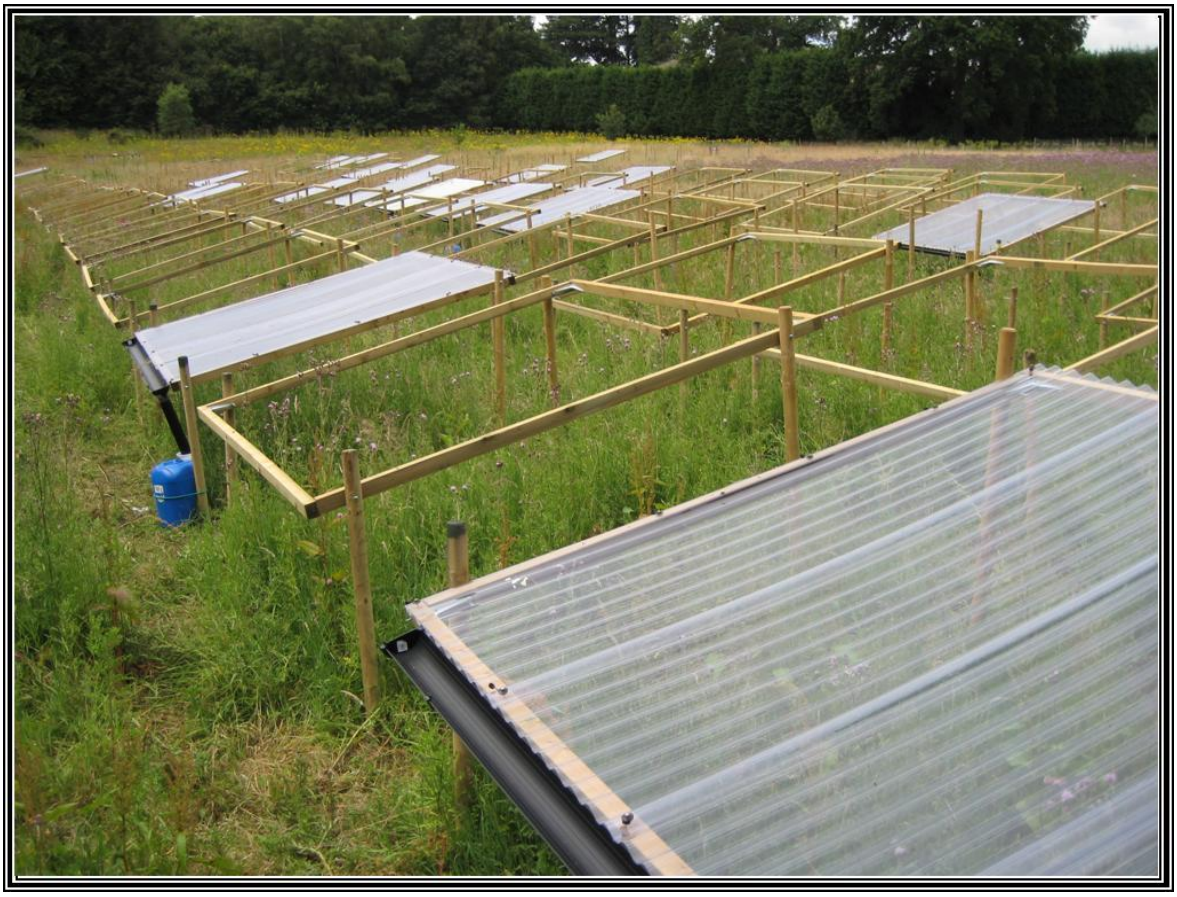

Figure 3: Roofing of the initial 56 plots, summer 2008.

CHAPTER 3: THE MITIGATION OF CLIMATE CHANGE BY FUNCTIONAL DIVERSITY AND THEIR COMBINED EFFECTS ON ECOSYSTEM FUNCTION

3.1 ABSTRACT

Temperate grassland ecosystems are increasingly threatened by climate change and plant diversity loss. Changes in rainfall regime, with increased summer drought and winter flooding are expected to negatively affect ecosystem functions and possibly result in grasslands becoming carbon sources through reduced photosynthesis. However, there is evidence that maintaining plant communities with a variety of traits may mitigate these climate effects. To investigate the complex effects of climate change and diversity loss on grassland, a field experiment was set up in southern England which consisted of a winter rainfall addition phase and a summer drought where summer rainout shelters were built over plots. Throughout the experiment the climate treatment had significant and long lasting effects upon soil moisture. There were interactions between the climate and the functional diversity treatment, indicating that perennial forbs and grasses are key groups and instrumental in the functioning of ecosystems. This is partly because of the cool humid microclimate they contribute to and the habitat they create, but they were more vulnerable to climate change than annual forbs and legumes. Both sets of species are necessary because they seem to be temporally separated, maintaining ecosystem functions at different times of year and under different conditions. This is likely to be attributable to differing rooting patterns and the characteristics that are inherent in being annual or perennial, such as frequency of senescence and nutrient uptake. Therefore retaining species with these characteristics may become more crucial to ecosystem functioning in future climate scenarios.

3.2 INTRODUCTION

Projected changes in climate patterns are likely to affect ecosystem functioning across grassland biomes in the coming century, potentially causing a deterioration of ecosystem services including clean air and provision of animal fodder (Schröter *et al.* 2005; Lee *et al.* 2010). These services are sustained by processes related to the cycling and retention of nitrogen, carbon and water (e.g. the uptake and release of CO_2). Temperate grasslands are crucial carbon sinks (~200PgC in the top 3m, Fischlin *et al.* 2007), but their vulnerability to intra-annual rainfall variability means that stored carbon may be subject to unpredictable, non-linear changes (Fischlin *et al.* 2007; St. Clair *et al*. 2009). In Europe, summer rainfall is projected to decrease in volume and occur in more extreme downpours, with more severe interim drought by 2100 (Murphy *et al.* 2009); this could change grassland ecosystems from carbon sinks to sources, and have far-reaching impacts upon soil fertility and atmospheric carbon levels.

Climate change experiments typically use rainout shelters to create drought regimes in order to investigate associated impacts on ecosystem functions (Chimner & Walker 2005; Yahdjian & Sala 2006; Signarbieux & Feller 2011). The focus for these studies has been on primary productivity and carbon fluxes, with less emphasis on impacts on species compositional change and nutrient cycling. They suggest that climate change has long-term effects upon function, even after stress has been alleviated. Root growth and carbon and mineral exudation are constrained by drought, so less soluble carbon is passed to the soil for uptake by microorganisms (Gorissen *et al.* 2004); these combined processes could initiate an overall decline in soil C storage, with potentially severe consequences for global climate (Börken & Matzner 2009; St Clair *et al.* 2009). Nitrogen cycling could also be affected by reduced microbial activity under droughted conditions, potentially limiting processes that provide nitrogen in a usable form to plants. Combined with root dieback suffered by many species, soil fertility could decline through immobilisation of nutrients by microbes and leaching losses, although uncertainties still exist regarding the exact impact that microbial adaptation to drought would have on these process rates (Börken & Matzner 2009). Longer term studies of the effects of drought on ecosystem function are few, and so short-term responses are much better understood (St Clair *et al.* 2009).

Biodiversity loss is another pressure exerted upon grasslands; estimates suggest ~50% was lost globally between 1950 and 1990, and over 35% has been converted to cropland (Millennium Ecosystem Assessment 2005). Losses are also caused by climate change and excessive nutrient loading which have already modified species assemblages and lowered diversity (Morecroft *et al.* 2004; Thuiller 2007). This in turn has led to fewer resource capture strategies (Fornara & Tilman 2008), shifts in function (Hooper & Vitousek 1998) and increased invasibility (Symstad, 2000; Pfisterer *et al.* 2004). The effect of altering a community assemblage, such as changes in relative abundance of species or species losses, has been much more comprehensively studied with regard to ecosystem function than has climate. A positive link between species diversity and productivity is well established (Hector 1999; Fridley 2003; Cross & Harte 2007), and the same pattern occurs with ecosystem functions such as resource uptake efficiency (Weigelt *et al.* 2005), mineralisation rates (Fornara *et al.* 2009), and nutrient retention (Scherer-Lorenzen *et al.* 2003), although many remain unclear (decomposition, leaching losses, *etc*.). While general themes of using increasing species number to explain community responses to disturbance have been explored, work on identifying the traits needed for effective ecosystem functioning, and grouping species according to these is still in its infancy; this is a crucial step because of the disproportionate effects of some species, such as nitrogen fixation by legumes.

Characterising plant's effects on ecosystem functioning has been focussed more on 'functional diversity', which considers plant species in terms of the effects they have upon their environment, thus enabling them to be categorised in terms of 'functional effects groups' (FG). Numerous studies have sought to prove that these groups are more functionally descriptive than species richness or the grass, forb, legume- GFL classification (Petchey & Gaston 2002; Wright *et al.* 2006), and consequently studies such as the Jena experiment aim to improve understanding with bespoke groupings (Roscher *et al.* 2004). Recognising the need for tailored functional groupings is the first step toward gaining a mechanistic understanding of the response of ecosystems to stresses instigated by global change (Petchey & Gaston 2006).

While experiments have increased knowledge about grassland responses to individual stresses, few have specifically directed their approach to the interaction between climate change and plant functional diversity loss. In recent years, large scale and long-term experiments have been set up in order to test these interactions, beginning with classical ecological questions such as whether species richness increases resilience to drought (Cedar

Creek: Tilman 1994), and moving on to more detailed ecosystem processes and interactions with CO_2, rainfall and warming (Niklaus *et al.* 2001; Jentsch *et al.* 2007; De Boeck *et al.* 2007). Temperate grasslands are an ideal system to use for these studies, as they are easily manipulated and have a vast body of literature describing many aspects of their processes. They are also one of the least protected biomes, and biodiversity trends are less well known than in forest biomes (Millennium Ecosystem Assessment 2005). It is therefore critical that a more complete understanding of the effects of climate and functional diversity, alone and in tandem, is achieved.

My overall hypothesis is that by reducing functional diversity, a grassland ecosystem is less resistant and resilient to changes in rainfall regimes. The first hypothesis is that summer drought will cause lower photosynthetic and evaporative rates and induce water stress in plants. This stress will result in lowered capability to access soil nutrients, so they will be lost through leaching. The second is that removing functional groups will substantially impact ecosystem processes, leading to reduced water, N and C retention. The third is that climate change and plant functional diversity interact to alter ecosystem functioning, i.e. that increased plant functional diversity, or certain characteristics of plant functional groups, reduces the impact of climate change upon the system.

I present an investigation that combined plant functional groupings within a precipitation manipulation experiment in a factorial design to examine the complex relationships between functional diversity and climate change. This experiment began in 2008, and concentrates on the first three years of the long-term experiment. Over the course of the study I measured ecosystem functions encompassing a range of biogeochemical processes, including CO_2 flux rates, mineralisation rates, availability of soil nutrients and leachate nutrient concentrations.

3.3 METHODS

3.3.1 STUDY SITE

The experiment is located in South-East England, in Silwood Park, Berkshire, (OS: SU946686), on a lowland mesotrophic grassland (UK national vegetation classification

(NVC) MG6, Rodwell 1991) on loamy sand where the dominant species are *Holcus mollis*, *Agrostis capillaris* and *Cirsium arvense* (Appendix 3.2). The field has a rabbit-proof fence, although there is some roe deer (*Capreolus capreolus* L.) grazing and mole (*Talpa europaea* L.) activity. It is shaded on two sides (south and west) by mixed deciduous woodland dominated by *Quercus robur* and *Acer pseudoplatanus* (NVC W10, Rodwell 1992), and has a main road running close to the southern perimeter. The climate is wet (averaging 833mm yr^{-1} between 2000 and 2010, Appendix 1 Table 1A), and relatively warm (January mean temperature 4.8˚C, July mean temperature 17.2˚C, 2000-2010, Met Office 2010). Some soil characteristics vary across the site due to a shallow incline from east to west (Appendix 3.2). The field was ploughed in October 2007, and standing biomass removed.

3.3.2 EXPERIMENTAL DESIGN

The experiment was a fully factorial design consisting of two levels of climate (climate change and ambient climate) and seven levels of functional diversity based upon every combination of three functional groups. The gradient of conditions across the site necessitated a randomised block design. Four blocks were arranged in a row from east to west. Each block had one representative of each treatment combination (climate (n=2) and functional diversity (n=7), resulting in a total of 56 plots).

Each plot was 2.4m x 2.4m, with a 70cm buffer zone on all four sides to account for lateral drift of rain, and a 1m x 1m focal area for plant measurement in the centre. The plots were positioned in grids with a 1m walkway between, this was narrow due to space constraints, but was justified by the free-draining soil.

3.3.3 CLIMATE TREATMENT

The climate treatment was a rainfall regime based upon IPCC projections of the climate in 2080-2099 (Fischlin *et al.* 2007). This was to be compared with unmanipulated ambient rainfall. Individual rainout shelters were built (Appendix 3.4) which were placed over all plots including ambient for the summer months (JJA).

The climate treatment was based on several sets of modelled A2 scenario projections. The IPCC 4th Assessment Report (Fischlin *et al.* 2007) amalgamated model outputs from 21 institutes worldwide and returned climate projections at a coarse scale. The UKCP09 Report (Murphy *et al.* 2009) used HadCM3 models to make projections of UK climate in 2099. South-East England in the years 2080-2099 is projected to undergo a reduction of ~30% rainfall volume overall during the summer months (JJA), with these rainfall events becoming less frequent than at present, and concentrated into more intense downpours. Conversely, in the winter (DJF) a 10-15% volume increase is projected, with frequency and intensity remaining approximately the same as present day (Appendix 3.3, Fig. 3C Murphy *et al.* 2009).

To create a practical climate change regime that reduced the overall volume of rainfall to ~70% of ambient and accounted for the different intensities, a regime was designed based upon rainfall data from the previous four years at the site, depending upon the rainfall volume in a 24 hour period. All the rain removed from the climate change plots was collected in individual water butts. If less than 20mm fell in 24 hours, 50% of the water was reapplied using watering cans, and the rest discarded. If more than 20mm fell, the full amount was reapplied. We predicted that approximately 30% of the water would be removed using this system. In reality, it resulted in a reduction of 50% in 2008 (no events over 20mm), 44% in 2009 and 25% in 2010 (Fig. 3.1). In spring (MAM) and autumn (SON) the plots were left unroofed.

Light transmission through the plastic was measured using a photosynthetically active radiation (PAR) meter (Skye Instruments, Wales); there was no difference in light intensity between the climate and ambient treatments despite the holes in the ambient treatment ($F_{1,62}$=0.2275, $p>0.05$, mean: 27.6% omitted). A linear relationship illustrated that with increased light intensity, the proportion of light omitted increased (Appendix 3.5, Fig. 3E). The extent of moisture encroachment into one climate change and one ambient plot was measured using a ThetaProbe Soil Moisture Meter HH2 with ML2x probe (Delta-T, Cambridge, UK) in August 2008 after heavy rain, and confirmed using contour plots in S-PLUS 6.0 (Insightful Corps, Canada) that the buffer zone was effective in preventing lateral flow of soil water into the experimental zone.

Winter (DJF) changes were applied (an extra 10-15%) by placing weather-resistant water butts adjacent to each climate change plot, with surface area of 15% of plot size (approx 8640cm^2). The water collected was reapplied to the plots after every rainfall event.

3.3.4 FUNCTIONAL DIVERSITY TREATMENT

Three plant functional groups were derived using a divisive hierarchical cluster analysis using trait data obtained from growing all species present at the site to maturity in a glasshouse (see Chapter 2 for more details). The groups clustered as follows:

1. Perennial grasses, forbs and legumes (hereafter FG1),

2. Bunch grasses and tall forbs (FG2),

3. Annual forbs, grasses and legumes (FG3).

These were then combined into every possible combination (three individual groups, three combinations of two, one combination of three). This treatment was implemented by weeding out unwanted species from naturally occurring secondary succession grassland. A slight gradient, accounted for by blocking, caused different species compositions across the site. Weeding took place in August 2008, June 2009 and May 2010.

There were concerns about under-representation of species in FG2 leading to plots of >90% *H. mollis*, as well as some species in other groups. The decision was taken to add seeds to encourage their appearance in their allotted plots. This was achieved by using the October 2008 vegetation survey to determine the proportion of each species in each functional group occurring in the plots, and seeding in underrepresented species from each group in May 2008 (Appendix 3.7, eq.3.1).

3.3.5 FIELD MEASURES

3.3.5.1 MICROCLIMATE OF PLOTS

Silwood Weather Station data was used (unpublished data) to make a dataset of rainfall for the duration of the experiment, and a rain gauge to dictate the implementation of the rainfall treatment from day to day. The average soil moisture content of each plot was measured on a weekly basis, year round, using a ThetaProbe at a distance of 1m from the edge on all four sides.

Dataloggers were installed (iButtons DS1923, Homechip, Milton Keynes) at the vegetation height specific to each plot, which logged temperature and relative humidity (%RH) every thirty minutes between January 2009 and September 2010. They were calibrated using a Weatherlink 5.8.0 (Davis Instruments, Brighton, UK) station at Silwood Park, fitting a correction factor to both datasets (Appendix 3.6, Fig. 3F, 3G).

3.3.5.2 VEGETATION SURVEYS

Vegetation surveys of all plots were completed at the beginning and end of the summer seasons. An additional survey was carried out in July 2010 because severe drought had caused vegetation dieback. A $1m^2$ quadrat with $25cm^2$ subdivisions was placed in the centre of each plot and percentage cover was visually estimated for each species (http://www.itis.gov). Percentage cover of necromass and bare earth were also assessed.

3.3.5.3 CO_2 FLUX

To measure ecosystem CO_2 flux rates, PVC ring collars (20cm diameter, 10cm long) were inserted into the soil to a depth of 5cm on each plot to create a seal with the soil. A transparent Perspex chamber (area $299cm^2$, volume, including average collar volume

8959cm^3) was then attached to a CIRAS-2 infra-red gas analyser (IRGA) in 2009 and a CIRAS-1 IRGA in 2010 (PP Systems, Hitchin, UK), which was clipped onto the collars to create a sealed area over the plants. The CIRAS measured CO_2 and water flux for four minutes (CIRAS-2) and two minutes (CIRAS-1). In light conditions, the returned values were net ecosystem exchange (NEE) (μmol CO_2 $m^{-2}s^{-1}$) and evapotranspiration (ET), (mmol $m^{-2}s^{-1}$). This was repeated with an opaque cover to simulate night-time ecosystem respiration (R_{eco}). Soil moisture, PAR and soil temperature (Hanna HI 98501, Bedfordshire, UK) were measured as covariates, and a set of values from the iButton data was derived, which consisted of the temperature and humidity of each plot on the specific day it was measured, averaged between 9:30am and 5pm. These measures were taken monthly during the summer and in alternate months through the winter.

3.3.5.4 DECOMPOSITION

Decomposition rate measures of the individual plots began in December 2008. 2g of dried, cut samples of the most dominant species in FG1 (*Holcus mollis*) and FG2 (*Arrhenatherum elatius*) were placed separately in mesh bags with a 1mm aperture (Normesh, Oldham, UK) and secured to the soil in each plot using staples. A representative for FG3 was not used due to lack of biomass at the time of the experiment. Four bags for each species were placed in each plot and one bag per species was removed at three month intervals (March, June and September 2009). All new biomass growing through the mesh was removed and the remaining material dried at 80˚C for 24 hours, before being weighed, and the mass lost from the bags calculated.

3.3.5.5 MINERALISATION

Mineralisation of nitrogen was ascertained quarterly between December 2008 and September 2010. The net mineralisation rate in each plot was determined using 4cm diameter by 10cm depth *in situ* soil cores, which were sealed with tape to prevent leaching, and incubated in the soil for three months (time= t_0). Four initial samples of soil were taken and homogenised to

create an initial composite sample for each plot. Nitrogen was extracted from the samples using Allen (1989)'s method (Appendix 3.9), and analysed colourimetrically using a Skalar SAN^{++} Continuous Flow Analyser (CFA, York, UK) (Appendix 3.10, Table 3A).

After three months had elapsed, the incubated cores were removed (t_{0-1}) and the soil available nitrogen content analysed in the same manner, along with a new composite sample (t_1). The cores were then replaced in the plots with fresh soil. The mineralisation rate of the plot was calculated by summing the NH_4^+ and NO_3^-/NO_2^- of each sample to give total extractable nitrogen before subtracting the t_0 value from the t_{0-1} value and multiplying the total by the bulk density. This gave a value of nitrogen accumulation over three months. These calculations were repeated with NO_3^-/NO_2^- only to gain a quarterly estimate of nitrification.

3.3.5.6 TOTAL NITROGEN AND PHOSPHORUS

Total soil nutrient concentrations were determined in October 2008, September 2009 and September 2010. Soil samples were collected and homogenised from four areas of each plot to create composite samples and dried at 80˚C for 24 hours, before being partially digested using a modified Kjeldahl method (Kjeldahl 1883, modified using a selenium catalyst, Appendix 3.8). These were stored at 5˚C overnight, then analysed using a Skalar SAN^{++} CFA.

3.3.5.7 C:N RATIO

Soil was taken from the May 2009, September 2009 and September 2010 samples, dried at 80˚C for 24 hours, finely ground and weighed into tin cups (15-20mg- exact weight recorded). Carbon and nitrogen content were then determined using a CNS total combustion analyser (FLASH EA 1112 Series, CE Instruments, Wigan, UK). The machine was calibrated using three aspartic acid standards, which were verified by measuring three further standards

as “unknown” and comparing to the known values. Two blanks were also included in each run (empty cups). The soil C:N ratio was then calculated for each plot.

3.3.5.8 CATIONS

Composite soil samples were collected for cation analysis in July 2009, which were homogenised, sieved and dried at 80ºC overnight. Cation concentration was measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES, Agilent 7500c Series, Wokingham, UK). Edgell (1988)’s method was followed to determine the concentrations of the cations ^{27}Al, ^{24}Mg, ^{44}Ca, ^{39}K, ^{55}Mn and ^{47}Ti in the plots (Appendix 3.11, Tables 3B-3D).

3.3.5.9 EXTRACTABLE NITROGEN AND PHOSPHATE

Four samples were collected from each plot every three months from December 2008, with a preliminary baseline test in October 2008, and monthly between May and September inclusively in 2009 and 2010. The soil was homogenised and sieved, then plant available nitrogen and phosphate were extracted (NH_4^+, NO_3^-/NO_2^-, PO_4^+) using Allen (1989)’s KCl and Truogs methods (Appendix 3.9). Both extracts were analysed using a Skalar SAN^{++} CFA.

3.3.5.10 LEACHING LOSSES

Leaching losses of soil nitrogen were measured by installing suction cup lysimeters (SCLs) in every plot in January 2009. The SCLs consisted of a ceramic cup attached to a 3cm diameter pipe installed 50cm deep into the soil, which draws soil water up a tube into a syringe when placed under a vacuum. Soil water was collected monthly from March 2009 to June 2010 and determined the NH_4^+ and NO_3^-/NO_2^- content using a Skalar SAN^{++} CFA. A sample of rainwater was also analysed at each timepoint. In the summer periods there was insufficient leachate for measurement.

3.3.5.11 STATISTICAL ANALYSIS

The effect of the climate treatment on soil moisture content was tested using a one-way analysis of variance (ANOVA) on averaged plot data at each time point. Monthly means of temperature and %RH were calculated from iButton data and used in a two-way ANOVA testing climate and functional diversity interactions with block as a factor to find whether the microclimate was affected by the treatments.

When variables were measured on more than five occasions, a repeated measures ANOVA (RMANOVA) was carried out to test for effects of time and interactions of time with treatment. Block was treated as a factor, with plot number (1-56) included as an error term. Month, climate and functional diversity were modelled with a three way interaction that was retained as the maximal model. Data transformations were carried out as before to meet the requirements of ANOVA.

For each ecosystem function response variable an ANOVA was carried out using the generalised linear model function in R2.12.0 (R Core Development Team 2009), at each timepoint measured. Each variable was modelled with block as a factor, the appropriate covariates as described in table 3.1, and the treatments climate and functional diversity, with interaction terms for the treatments and the covariates.

Models were simplified to remove non-significant covariates, and also block where non-significant using likelihood ratio deletion tests to derive the minimum adequate model. The main effects and interaction of climate change and functional diversity were retained (Manning *et al.* 2004). Likelihood ratio tests compared the more complex model with the simplified one to test for an unacceptable increase in unexplained deviance.

Table 3.1 Covariates used in ANOVAs. Omitted variables were modelled without covariates.

Response variable	Covariate			
	PAR	**Soil moisture**	**Soil temperature**	**Air temperature**
NEE	x	x		
R_{eco}		x	x	
ET	x	x		
Decomposition		x		x
Mineralisation		x		x
Extractable Nutrients		x		x
Leachate		x		

Outliers were removed if they were not readily explainable biologically, created a 5% or more decrease in unexplained variance when removed, or substantially altered the model on removal. Percentage cover of vegetation and mass loss percentages allowing estimation of decomposition rates were arcsine transformed to remove the constraints of bounded data. Any other variables that displayed a non-normal distribution were log or square root-transformed to meet the assumptions of ANOVA.

ANOVA tables were generated for each analysis in order to obtain F-statistics of each variable and interaction, and Tukey's Honest Significant Difference was used to generate post-hoc comparisons between factor levels, although caution was required when applying this to interactions.

3.4 RESULTS

Soil moisture followed a similar pattern to rainfall, after a short lag time. The winter and summer phases of the rainfall treatment had clear measureable effects upon soil moisture, also in a lagged fashion (Fig. 3.1). The first winter of the experiment (1st December 2008 – 31st March 2009) received 206.1mm of rain, while the climate change plots received 15% more (233.3mm). In the first summer (1st June – 31st August 2009), the climate change treatment received a total of 93.2mm of rainfall, while the ambient treatment received

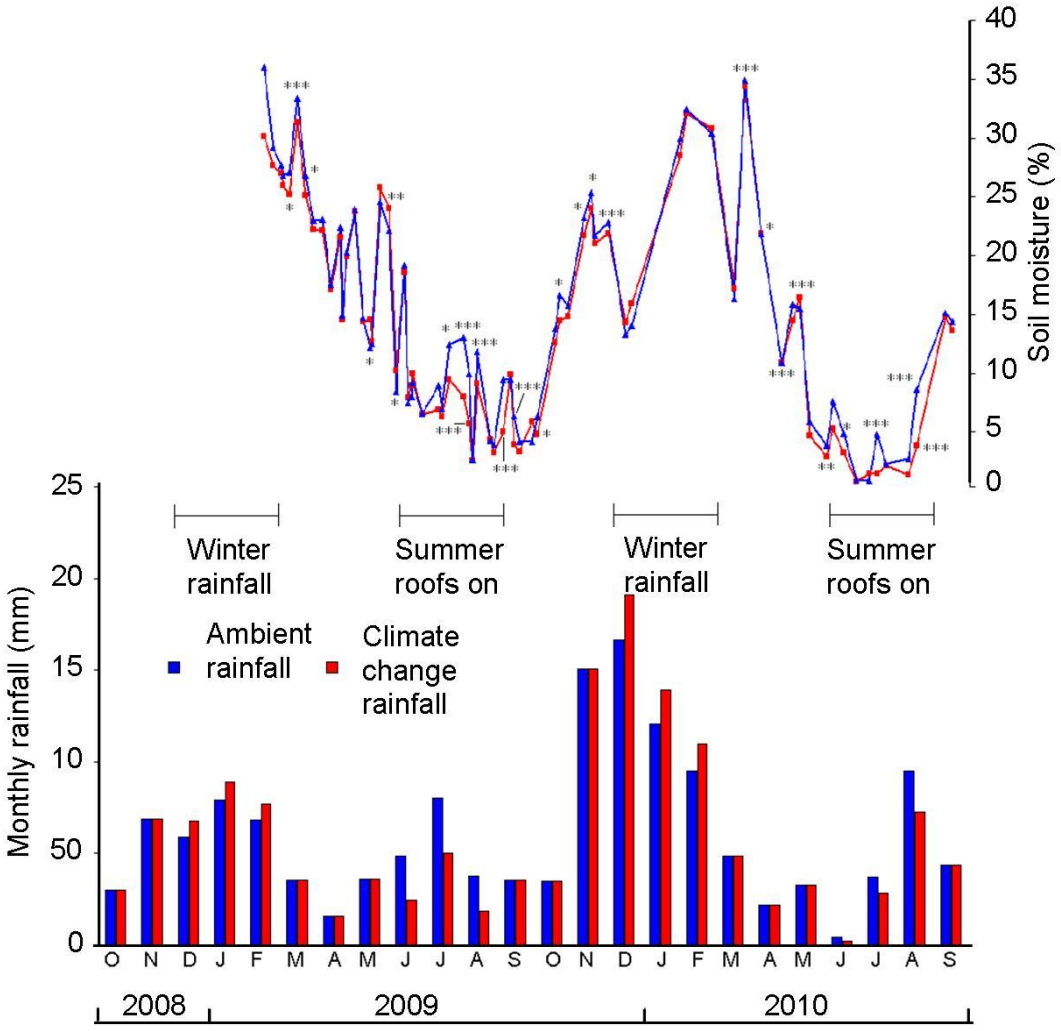

Figure 3.1: Rainfall applied to the treatments for the duration of the experiment. Significant differences in soil moisture between climate treatments are represented by asterisks *= p<0.05, **= p<0.01, ***=p<0.001.

166.3mm, representing a 44% decrease. The second winter (1st December 2009 – 28th February 2010) was exceptionally cold and wet (Prior & Kendon 2011), with the climate

change treatment receiving 439.59mm compared to 382.25mm in the ambient plots (a 15% increase). The final summer (1st June – 31st August 2010) was drier than the first one but had three heavy rain events rather than two, so a higher proportion overall was applied to the climate change plots (75% of 136.30mm= 103.15mm).

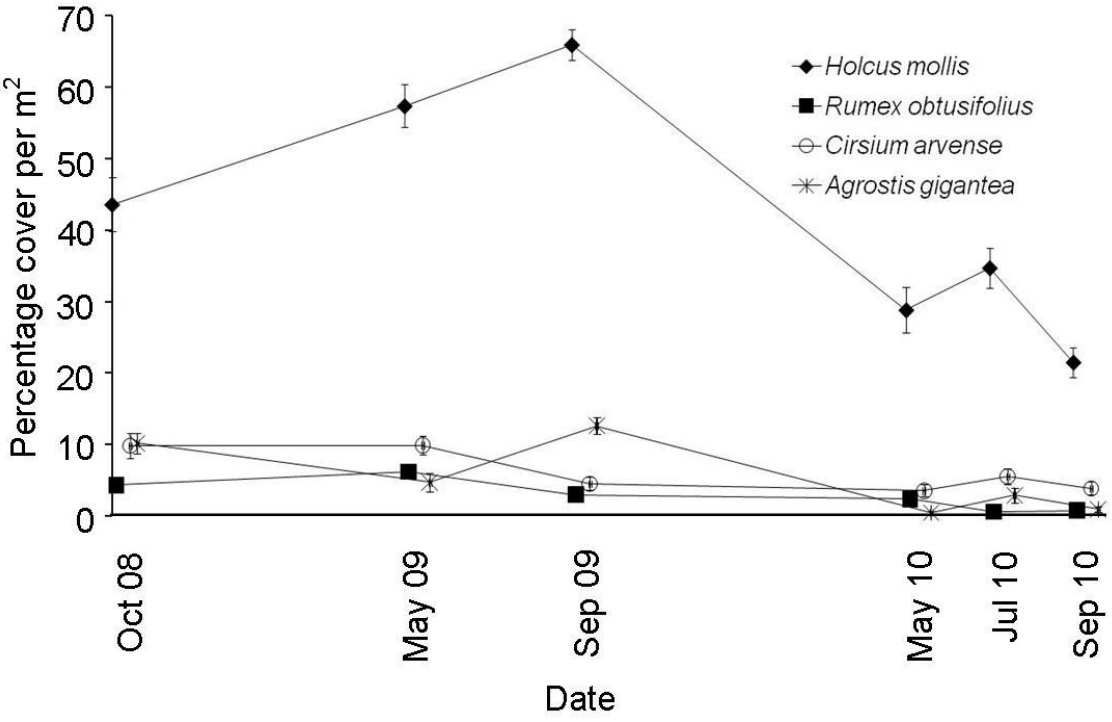

Figure 3.2: Average percentage cover of the four most dominant species in the DIRECT experiment over time. Climate effects were not significant so are not shown here. Error bars depict ±1 SEM.

The experiment was performed against a backdrop of seasonal and successional change, where all process rates including net ecosystem exchange (NEE, Fig. 3.3) and mineralisation were highest in the summer and lowest in the winter (Appendix 3.12.3, table 3J). These trends were followed closely by nutrient concentrations and soil leaching rates, which showed similar patterns to soil moisture, i.e. with a slight time delay. All CO_2 and water flux processes were highest in spring 2009 (NEE, ecosystem respiration- R_{eco} and evapotranspiration- ET, Fig. 3.3-3.5 respectively), as well as mineralisation rates, and correspondingly extractable nitrogen and phosphate were fairly high in August and September 2009, although some plant uptake would have occurred (Fig. 3.8 & 3.9). This is likely to have been mostly nitrate, as its pattern of availability did not match the seasonal fluctuation of ammonium. The cover of all dominant species was highest in May 2009 (12%±2% bare earth) and subsequently declined, with the canopy opening and bare gaps

appearing through 2010 after the severe drought in June (From 15%±2% bare earth in May to 53%±5% bare earth in July). Functional diversity was associated with bare earth appearance in the plot across all time points (Fig. 3.11, RMANOVA: Month*climate: $F_{5,210}$=36.32, p<0.001). This was mostly because in October 2008 there was a significant difference between functional groups ($F_{6,42}$=14.44, p<0.001, R^2=0.68) where FG2 and FG3, and the two combined, were significantly barer than the other combinations of groups. Later in the experiment climate change was a key factor where the climate change treatments had significantly more bare patches than the ambient (Fig. 3.11, RMANOVA Month*diversity= $F_{30,210}$=3.78, p<0.001). The dieback of biomass in the plots in this period corresponded with lower process rates (Fig. 3.3-3.5), an accumulation of nutrients in the soil and higher nutrient availability (Fig. 3.6-3.8) in summer 2010 than 2009.

Concentrations of leached ammonium were generally lower than those of nitrate (Fig. 3.9), except in December 2009, which saw the heaviest rainfall of the entire period (see Fig. 3.1- rain graph). January 2010 saw heavy and sustained snowfall, with much of the snowmelt happening in February, resulting in substantial nitrate leaching. In 2010 the severe natural drought in June led to a slowing of microbial driven processes in July (Fig. 3.1), as evidenced by the low R_{eco} which led to a high NEE rate when the balance between uptake and release of CO_2 shifted. In summer 2010 lack of plant cover and low R_{eco} was accompanied by lower mineralisation rates than those seen in 2009, and higher concentrations of extractable nutrients. The slight increase of plant cover following a wet July (Fig 3.2) corresponded with a decrease in extractable soil nutrient concentrations, although more fixed nutrients, i.e. nitrogen and phosphate including plant unavailable forms generally increased in the soil throughout the experiment.

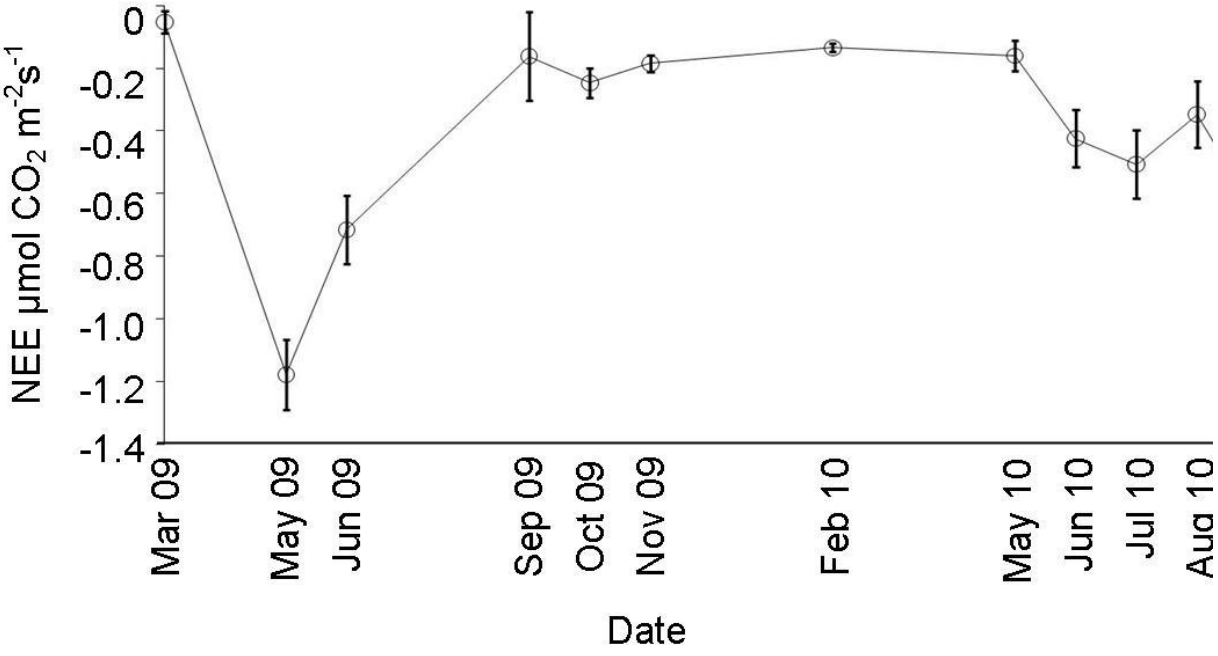

Figure 3.3: Net ecosystem exchange (NEE), which changes significantly over time (RMANOVA: 2009: $F_{6,252}$=2.52, p<0.05, 2010: $F_{6,252}$=4.23, p<0.01). The graphs are arranged as 2009 and 2010 due to different equipment being used. (±1 SEM).

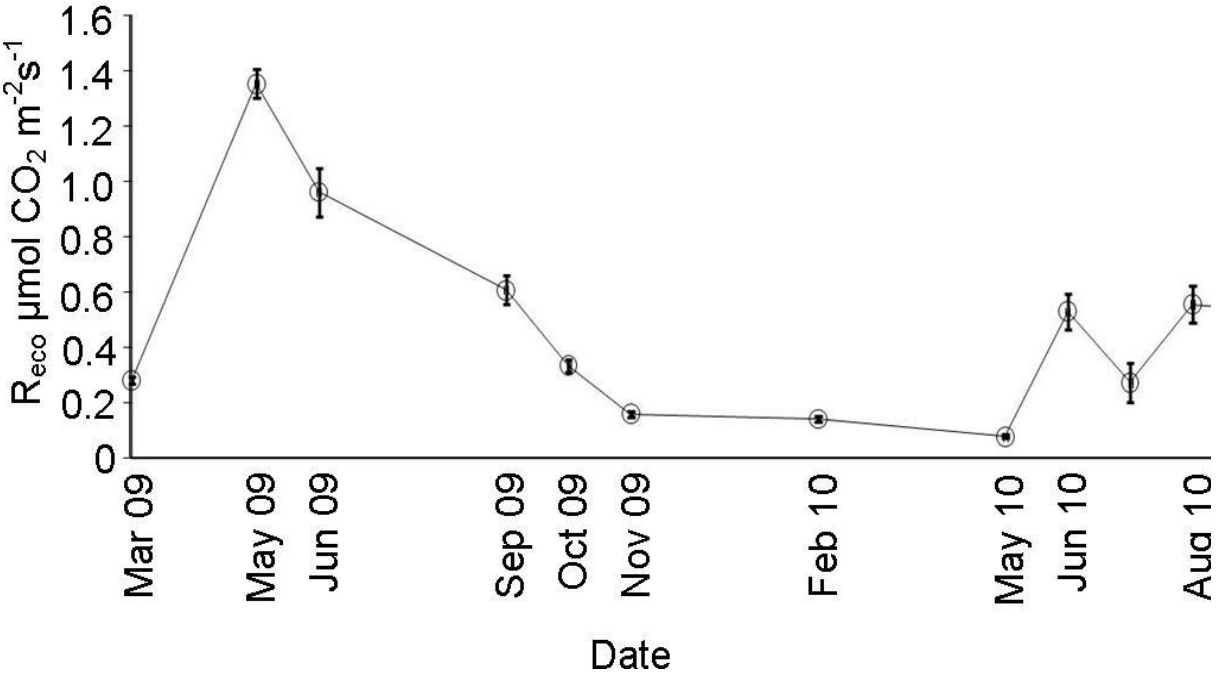

Figure 3.4: Change over time of soil respiration rate (R_{eco}) had a significant time effect in both 2009 (RMANOVA: $F_{6,252}$=106.94, p<0.001) and 2010 ($F_{6,252}$=12.90, p<0.001) (±1 SEM).

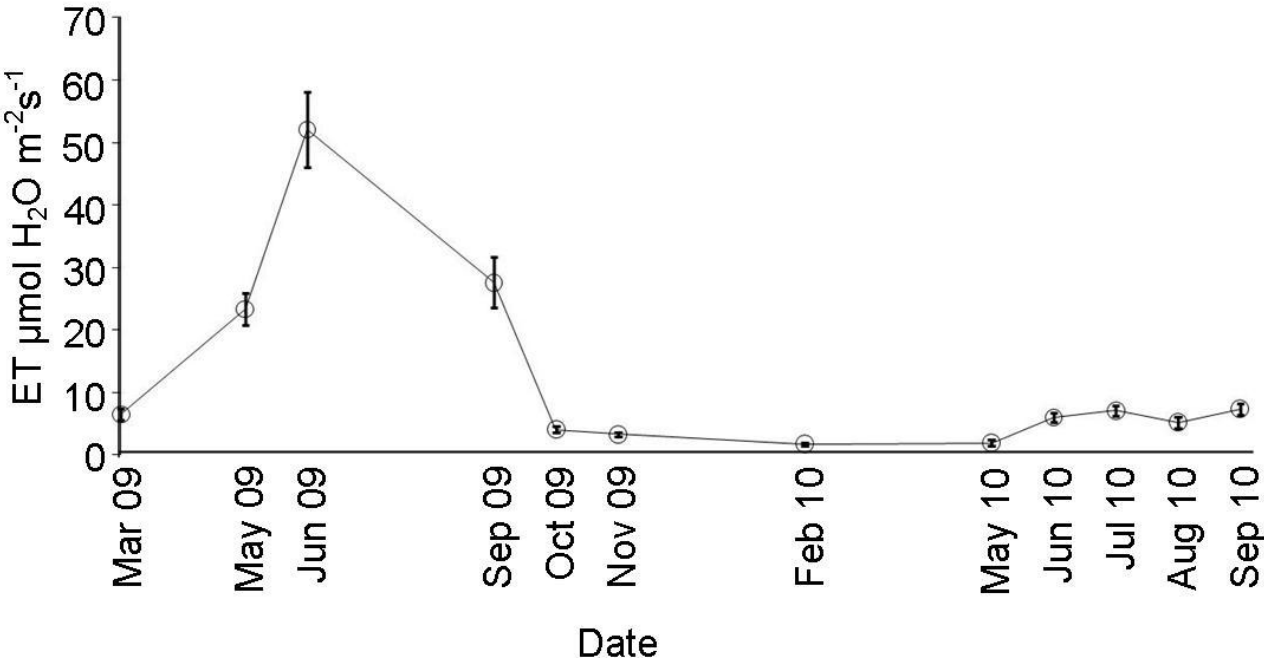

Figure 3.5: Trend of evapotranspiration (under light conditions) over time. Due to the lack of significant interactions with time, no treatment effects are shown here (appendix 3.12.1, table G). Error bars depict ±1 SEM. (RMANOVA: 2009: $F_{6,252}$=29.45, p<0.001, 2010: $F_{4,148}$=30.00, p<0.001).

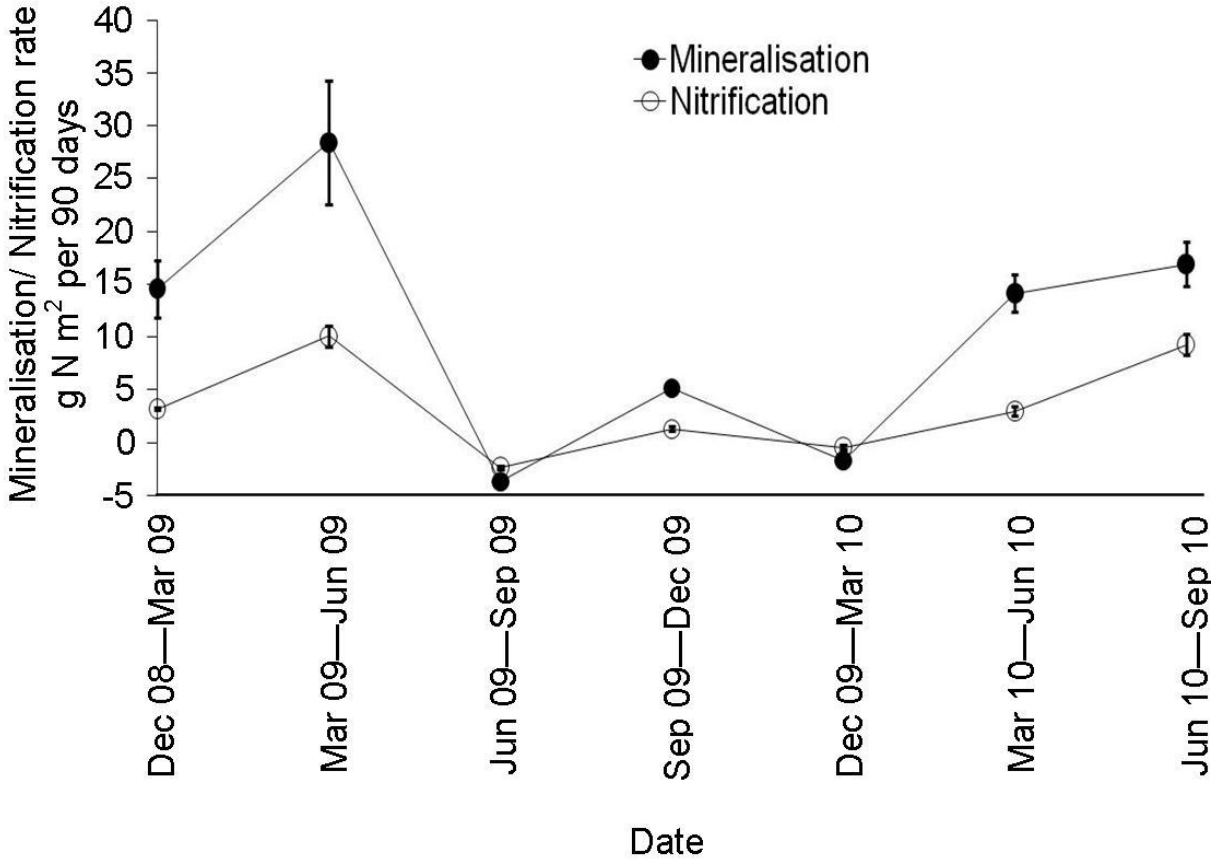

Figure 3.6: Time series analysis of mineralisation rate (accumulation of both ammonium [NH_4^+] and nitrate/nitrite [NO_3^-/NO_2^-]) and nitrification rate (accumulation of nitrate [NO_3^-] alone) (±1 SEM). Both change significantly over time (RMANOVA: $F_{6,252}$=17.82 p<0.001, $F_{6,252}$=68.09 p<0.001 respectively). The June-Sept 09 period is likely to be anomalous and should be disregarded.

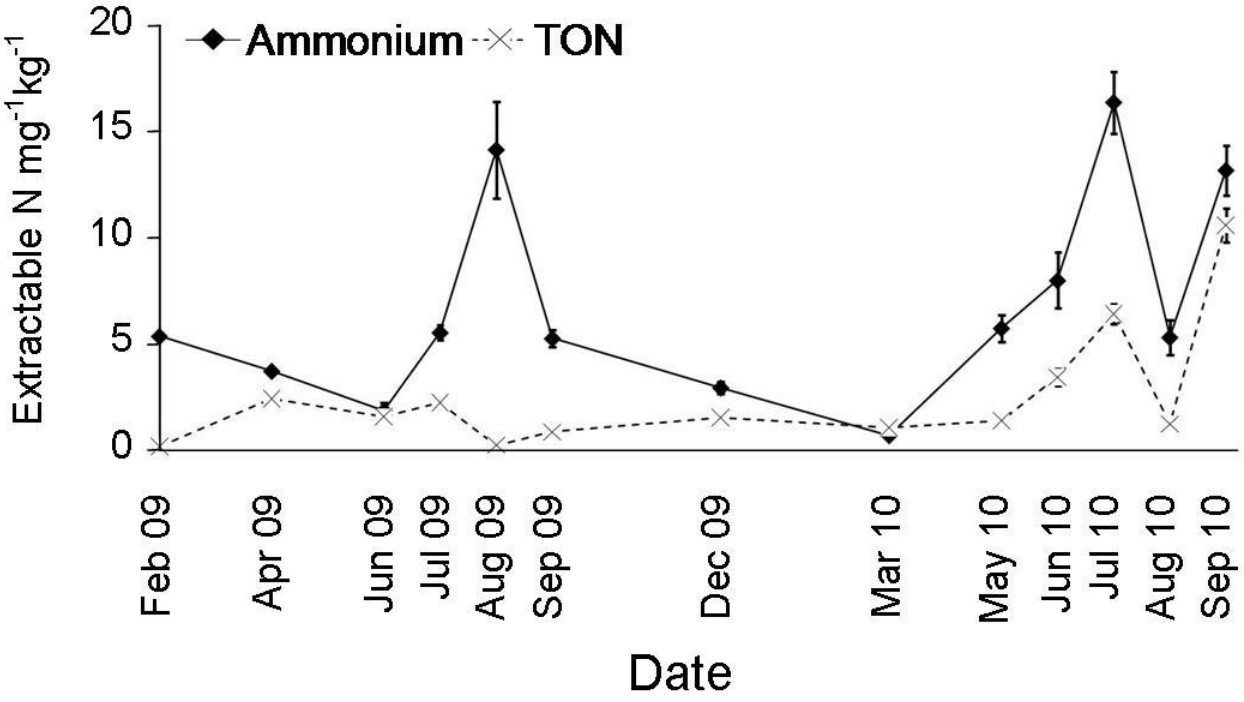

Figure 3.7: Time series patterns of extractable nitrogen in the soil (±1 SEM). Overall treatment effects are non-significant, so have not been presented here. (RMANOVA: NH_4= $F_{12,504}$=147.87, p<0.001, $NO_{2/3}$= $F_{12,504}$= 158.52, p<0.001).

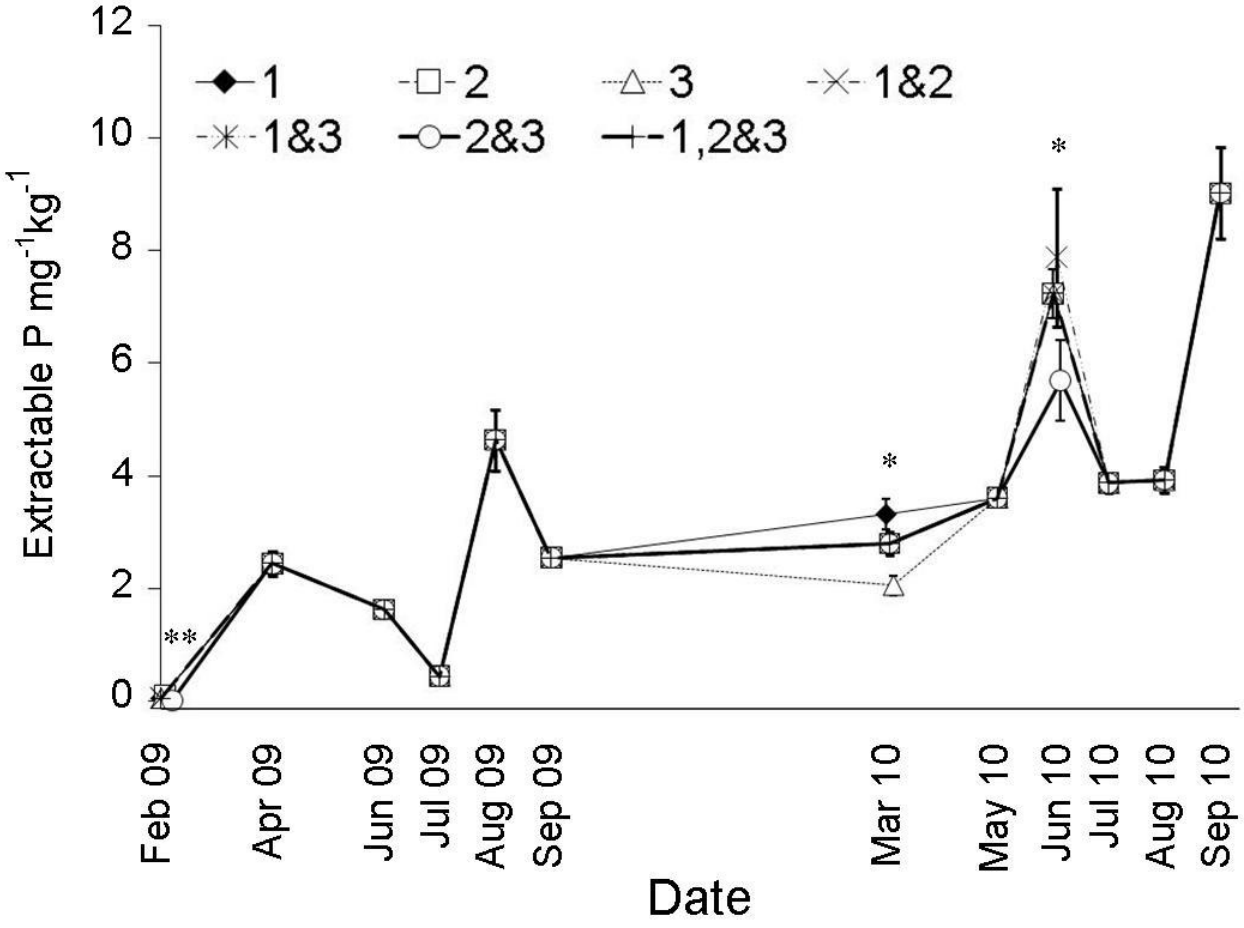

Figure 3.8: Extractable phosphate varies over time and according to climate change treatment and plant functional group combination (±1 SEM). Asterisks denote *p<0.05, **p<0.01, ***p<0.001. (RMANOVA: $F_{66,462}$=1.55, p<0.01).

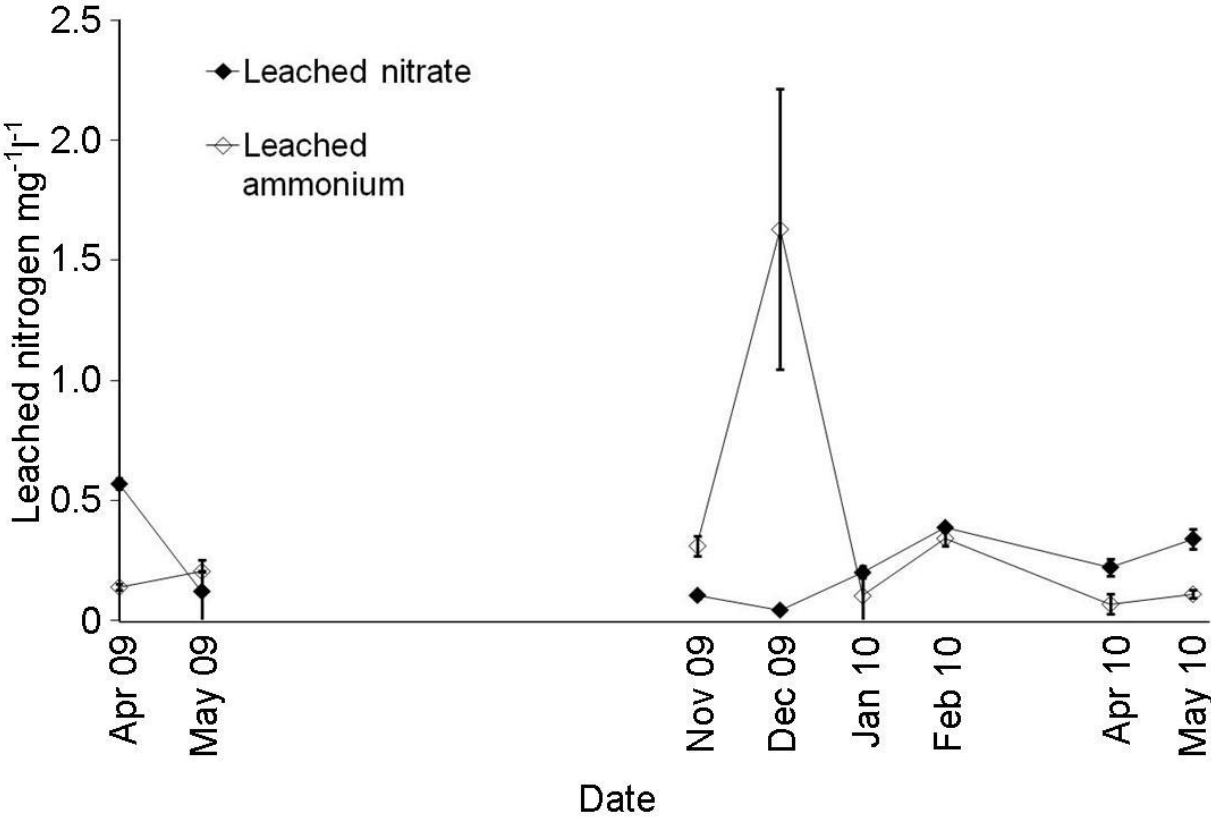

Figure 3.9: Leached plant available nitrogen over time; initially ammonium and nitrate are leached in different concentrations; latterly they follow one another. (RMANOVA: NH_4= $F_{6,252}$=6.34, p<0.001, $NO_{2/3}$= $F_{6,252}$=10.00, p<0.001)

3.4.1 CLIMATE

In general climate the climate change treatment suppressed function and caused plant dieback, leading to slower process rates, an accumulation of decomposable material and more nutrient rich soils. The climate change treatment highly significantly reduced soil moisture in the summer periods of the experiment, and increased it in the winter when rainfall addition was occurring (Fig. 3.1). A slight lag time in this change resulted in effects on function that were later than the key rainfall regime periods, and so most effects were noted in spring and autumn (Fig. 3.11a-d). The climate treatment did not significantly alter microclimate of the plots until the final month of the experiment (September 2010) when the climate change

treatment was 0.3°C warmer on average (Appendix 3.6, Fig 3G), having been much barer than the ambient throughout the summer (Fig. 3.10, 84.5% bare under climate change compared with 20.9% under ambient in July).

In May 2010 NEE was almost at equilibrium under climate change, so it is likely that the winter rainfall addition resulted in higher ecosystem respiration outweighing the effect of photosynthesis, since plant growth seemed largely unaffected (Fig. 3.11a $F_{1,34}$=9.78, p<0.01). Between March and June 2010 nitrification was faster in the wetter soils of ambient climate plots, while the climate treatment, which had received extra rainfall through the winter, was unresponsive to soil moisture changes (Appendix 3.12.3, Table 3K, $F_{1,31}$=8.76, p<0.01). The increased provision of nitrate in the soil can be inferred to have resulted from heightened microbial activity. This trend of higher microbial driven processes under wetter conditions also occurred when R_{eco} was significantly reduced in the climate change plots at the end of the first summer season of rainfall manipulation (Fig. 3.11b, $F_{1,34}$=8.37, p<0.01, R^2=0.46). The resulting increase in nitrate in ambient plots over the summer was subsequently lost as leachate more than in the drier climate change plots (Fig. 3.11d, $F_{6,39}$=49.13 p<0.001).

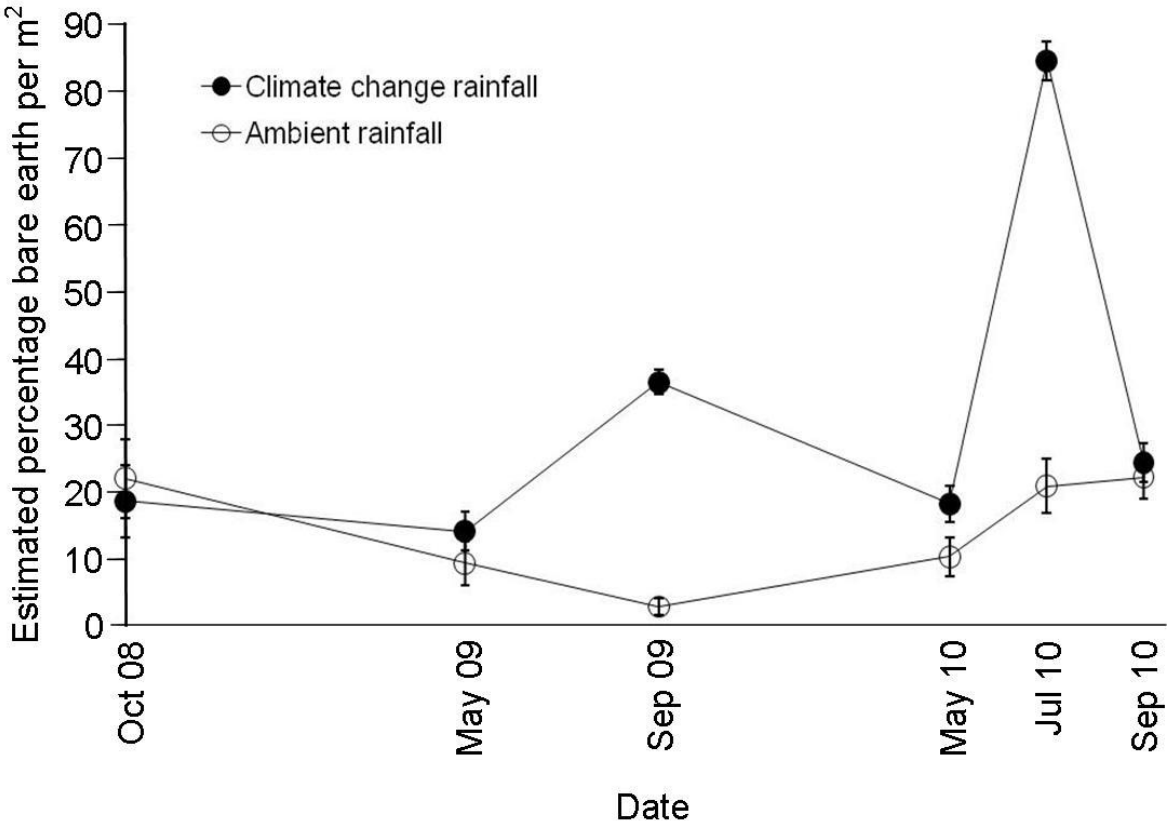

Figure 3.10: The climate change regime significantly increases the amount of bare ground in the summer periods (RMANOVA: $F_{30,210}$=36.32, p<0.001). Error bars denote ±1 SEM.

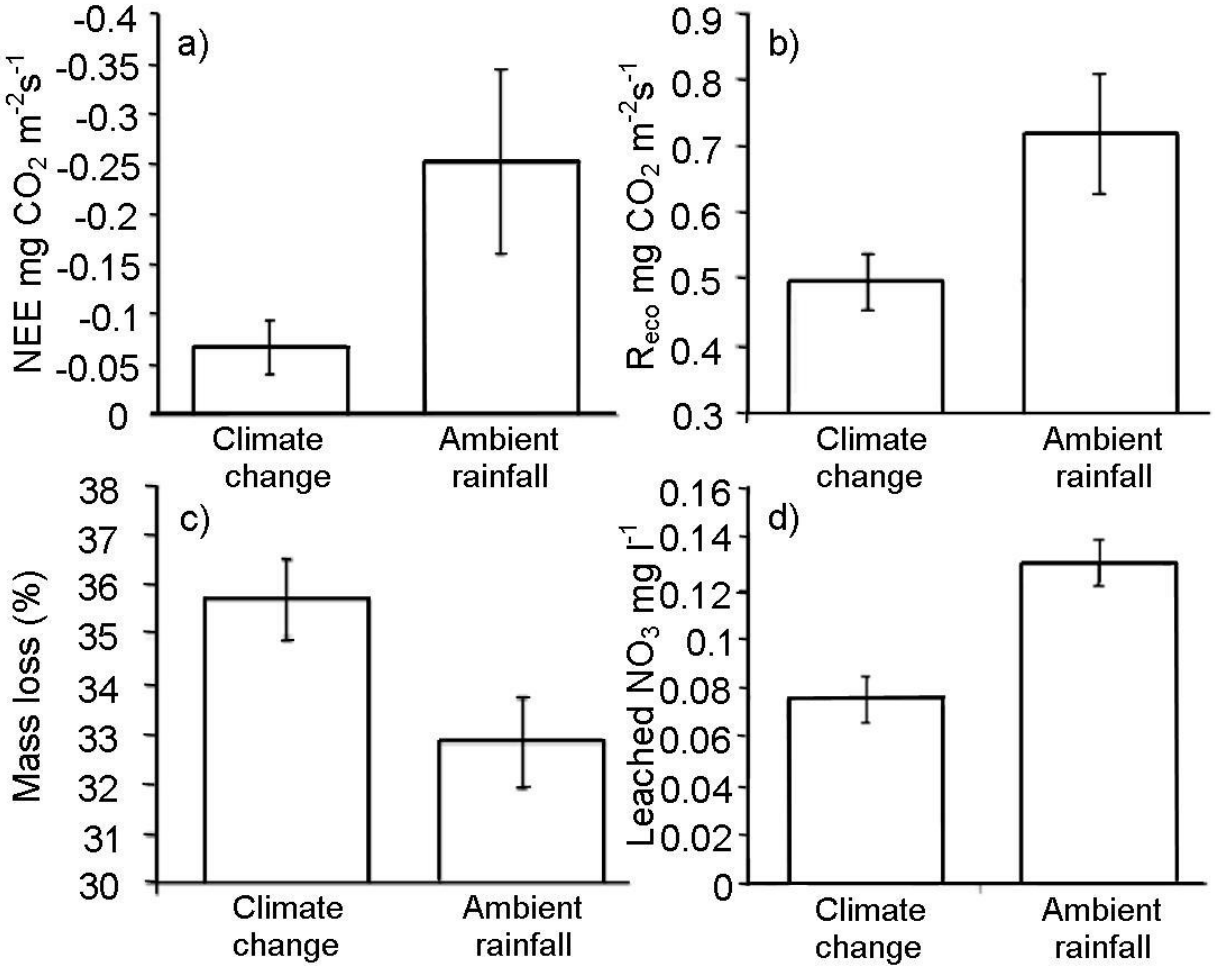

Figure 3.11: a) In May 2010, soil respiration balances photosynthesis more than in the ambient treatment ($F_{1,34}$=9.78, p<0.01) (±1 SEM). The negative values imply photosynthesis is occurring (net CO_2 reduction). b) In September 2009 at the end of the first summer climate change phase, there is a significant reduction in soil respiration in climate change manipulated plots ($F_{1,34}$=8.37, p<0.01, R^2=0.46). Error bars correspond to ±1 SEM. c) The climate change regime affects decomposition of *Arrhenatherum elatius* between December 08 – June 09 ($F_{1,39}$=7.47, p<0.001). Error bars describe ±1 SEM. d) In November 2009, nitrate was lost through leaching in different amounts according to climate regime ($F_{6,39}$=49.13 p<0.001). Error bars represent ±1 SEM.

3.4.2 DIVERSITY

There were many significant effects of composition that were independent of climate manipulations. The main difference between the functional effects treatments was between plots containing FG1 and those containing FG2 and FG3. This was particularly marked with regard to decomposition of *H. mollis* and *A. elatius*, which lost significantly more litter mass under plots where FG1 was present (Fig. 2.14 & 2.15, Table 3.2). The same effect occurred in

all seasons, and it is likely to be due to higher microbial activity under the denser canopy under FG1-dominated plots. This is supported by higher R_{eco} rates recorded under plots where FG1 was present in early summer (June 2009), and consequently lower NEE values, as the high R_{eco} counteracted the effect of photosynthetic CO_2 intake (Fig. 3.12; $F_{6,39}=2.60$, $p<0.05$). In the same month ET was lowest when FG1 and 3 were absent, their absence causing much barer plots (Fig. 3.13; $F_{6,34}=2.97$, $p<0.05$).

Soil nutrient concentrations were highest throughout the year in plots where FG1 was present, and no discernible effect of increasing functional group diversity was observed, particularly with regard to extractable phosphate (Fig. 3.18). This was particularly evident in February 2009 for extractable nitrogen (Fig. 3.17; ammonium: $F_{6,39}=7.11$, $p<0.001$, nitrate: $F_{6,39}=3.03$, $p<0.05$) and September 2009 (Fig. 3.16; total phosphate, $F_{6,38}=2.75$, $p<0.05$). In line with these findings leachate was therefore higher in plots containing FG1, which was significantly higher than plots containing FG3 in November 2009 (Fig. 3.19 $F_{6,39}=4.50$ $p<0.05$).

Table 3.2: Significant mass loss of *Holcus mollis* litter over three, six and nine month periods. Non- significant interactions have been removed. (*p<0.05, **p<0.01, ***p<0.001)

Factor	d.f	**Dec 08 - Mar 09**		**Dec 08 - Jun 09**		**Dec 08 – Sept 09**	
		F	*p*	*F*	*p*	*F*	*p*
Model	16,39	**3.97**	***	**3.82**	***	**2.34**	*
Block	3	0.69	NS	2.76	NS	-	-
Climate change	1	0.35	NS	2.33	NS	0.11	NS
Functional diversity	6	**9.46**	***	**7.97**	***	**4.33**	***
Climate x diversity	6	0.73	NS	0.69	NS	0.73	NS

Table 3.3: Significant mass loss of *Arrhenatherum elatius* litter over three, six and nine month periods. Non-significant interactions have been removed. (*p<0.05, **p<0.01, ***p<0.001)

Factor	d.f	**Dec 08 - Mar 09**		**Dec 08 - Jun 09**		**Dec 08 – Sept 09**	
		F	*p*	*F*	*p*	*F*	*p*
Model	16,39	**6.28**	***	**3.69**	***	1.35	NS
Block	3	**4.84**	*	**5.32**	**	**3.36**	*
Climate change	1	0.18	NS	**7.47**	**	0.28	NS
Functional diversity	6	**17.41**	***	**4.86**	***	0.95	NS
Climate x diversity	6	**4.12**	**	0.26	NS	0.92	NS

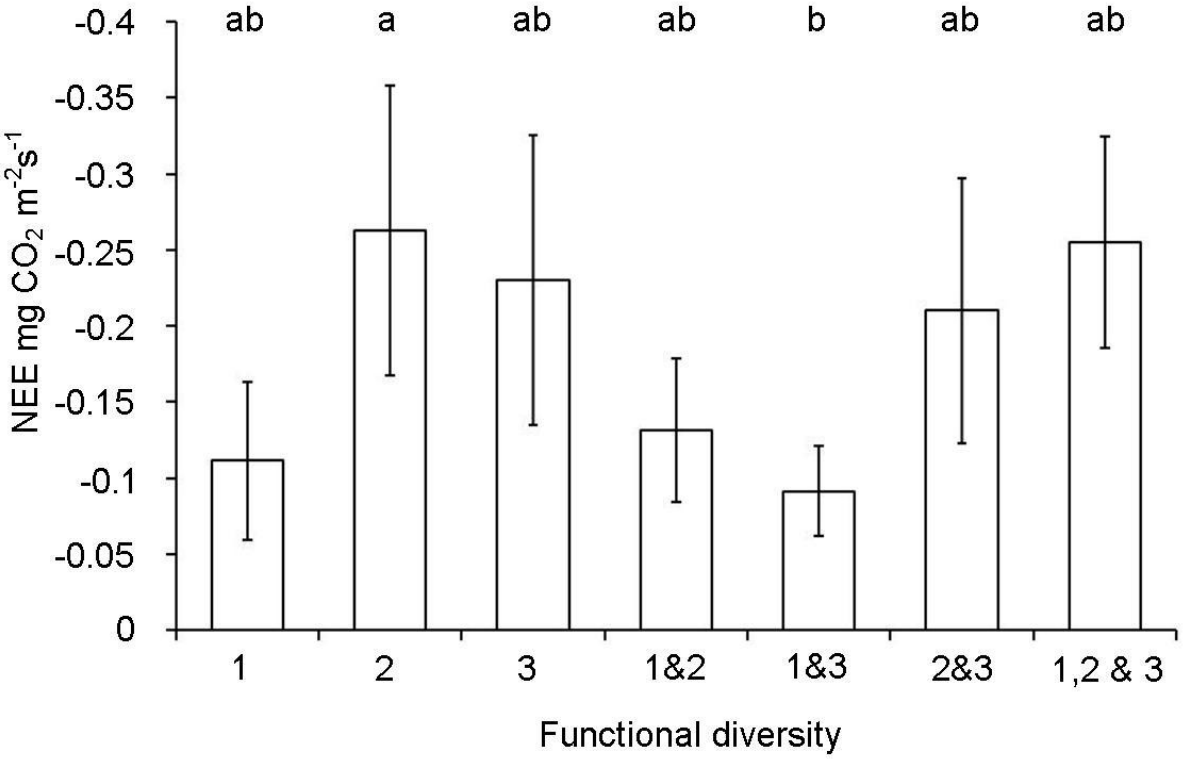

Figure 3.12: In June 2009 functional diversity affected net ecosystem exchange ($F_{6,39}$=2.60, $p<0.05$). The closer to zero, the more respiration is balancing photosynthesis.

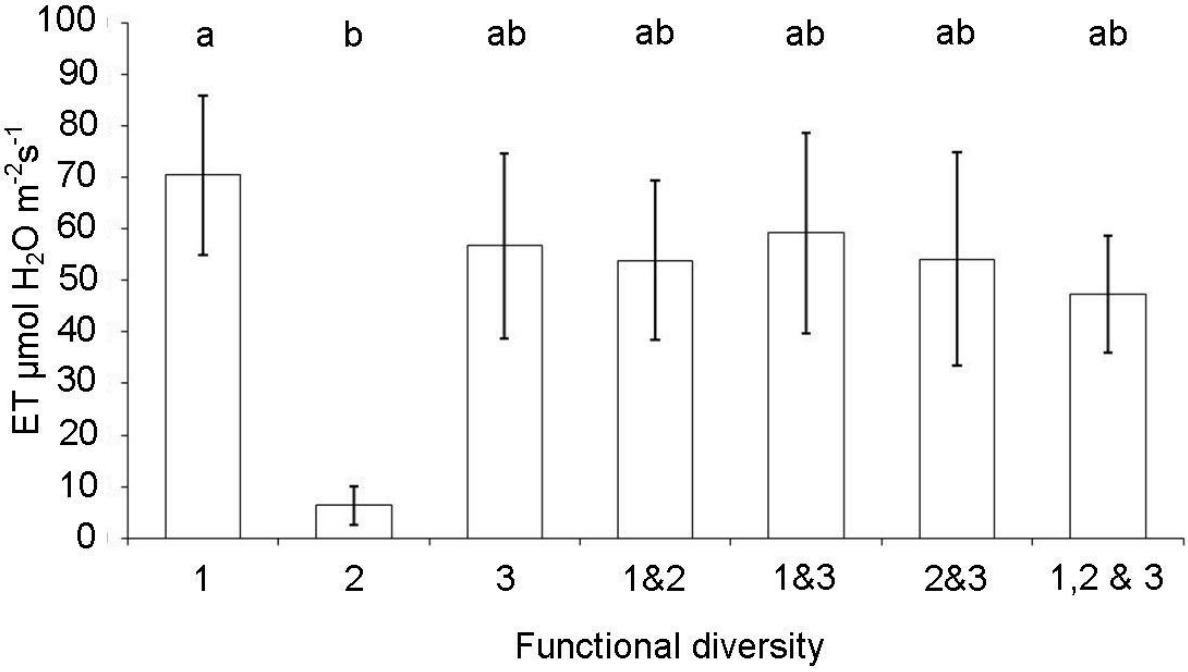

Figure 3.13: Evapotranspiration (ET) in June 2009. There is a significant diversity effect which shows a lower ET rate when fg2 is present, compared to fg1 ($F_{6,34}$=2.97, $p<0.05$). Bars describe ±1 SEM.

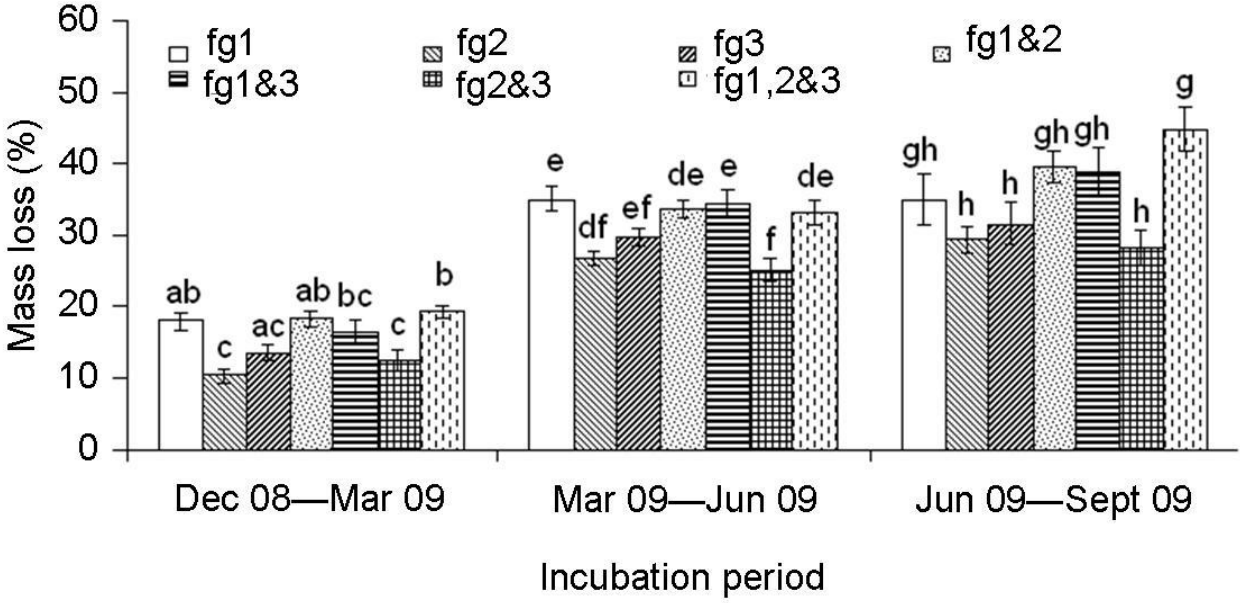

Figure 3.14: Decomposition of *H. mollis* litter over three time periods (±SEM). Groups containing group one (perennials) caused significantly faster mass loss than those with two or three alone (Dec-Mar $F_{6,39}$=9.46, p<0.001, R^2=0.63, Mar-Jun $F_{6,39}$=7.97, p<0.001, R^2=0.65, Jun-Sept $F_{6,39}$=4.33, p<0.01).

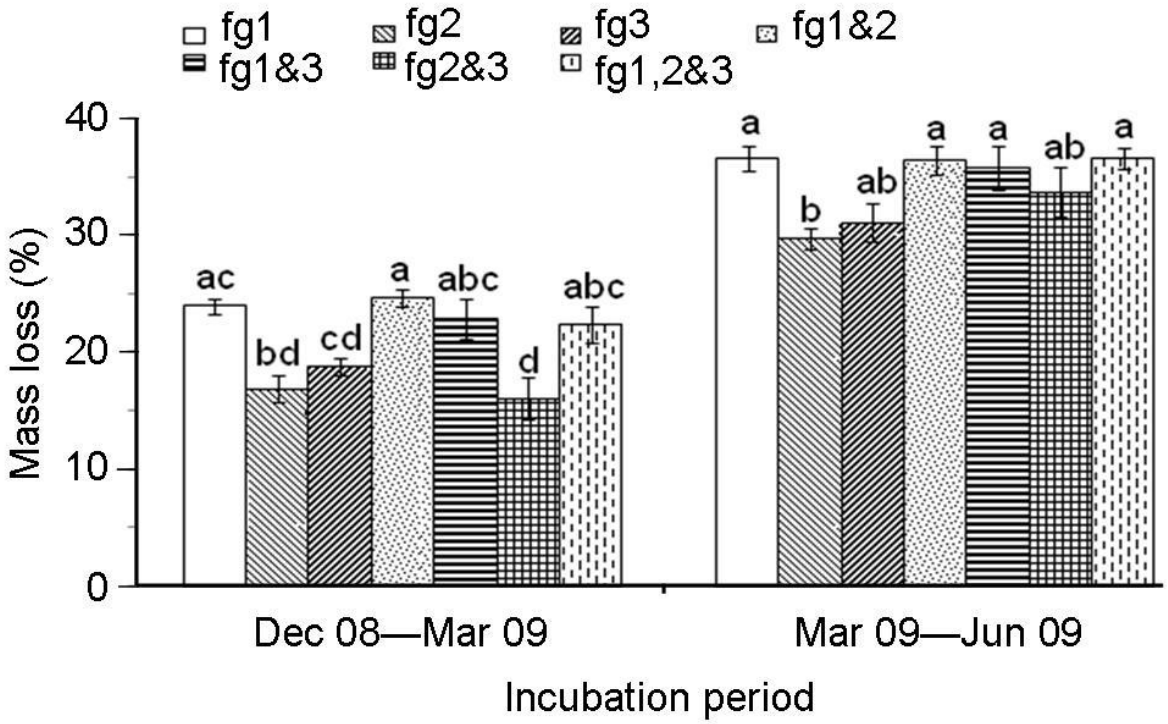

Figure 3.15: Treatment effects of mass loss of *Arrhenatherum elatius* litter (±1 SEM). December 08 -March 09, diversity effect ($F_{6,39}$=17.41 p<0.001). December 08– June 09, diversity effect ($F_{6,39}$=4.86, p<0.001).

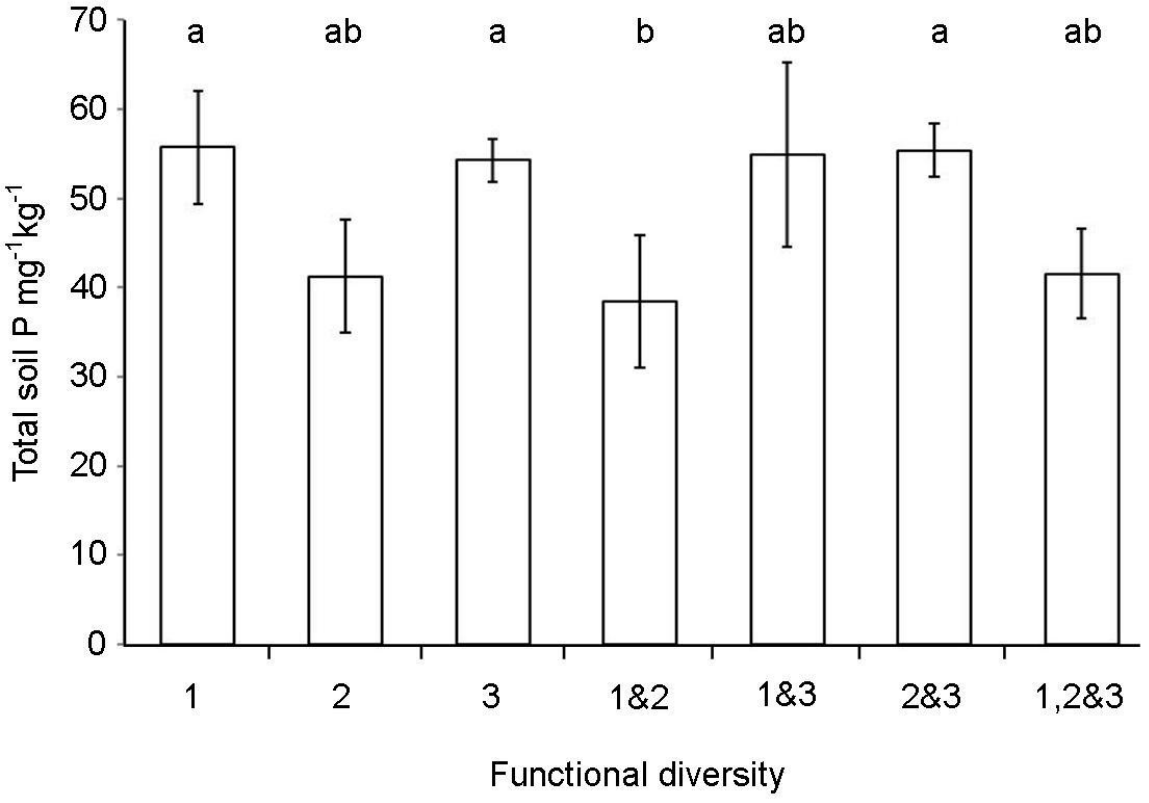

Figure 3.16: Functional diversity effects on total soil phosphorus content in September 2009, at the end of the second summer rainfall regime ($F_{6,38}$=2.75, p<0.05).

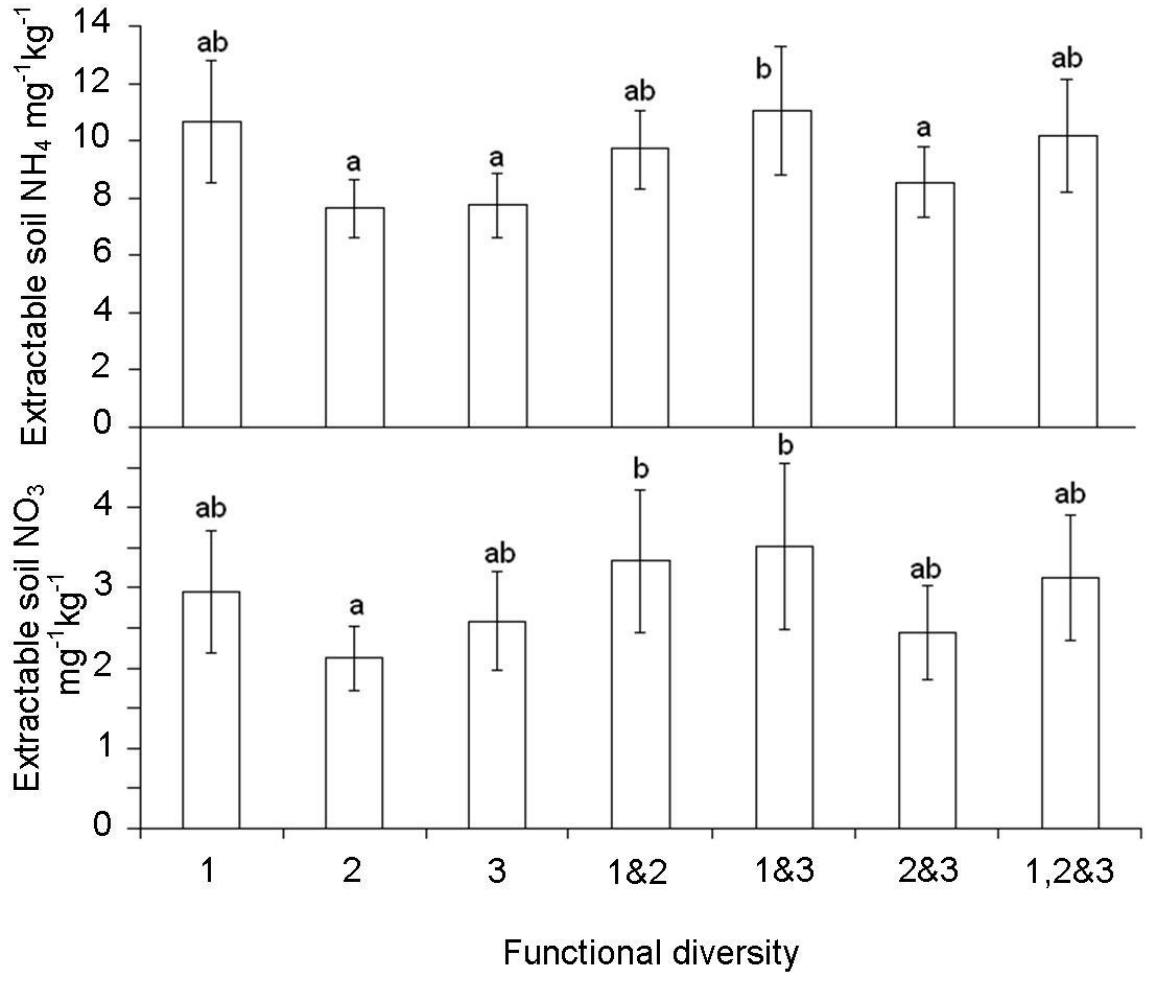

Figure 3.17: Diversity effects on pool sizes of a) NH_4 and b) NO_3 in February 2009 (±1 SEM). Ammonium: $F_{6,39}$=7.11, p<0.001, nitrate: $F_{6,39}$=3.03, p<0.05.

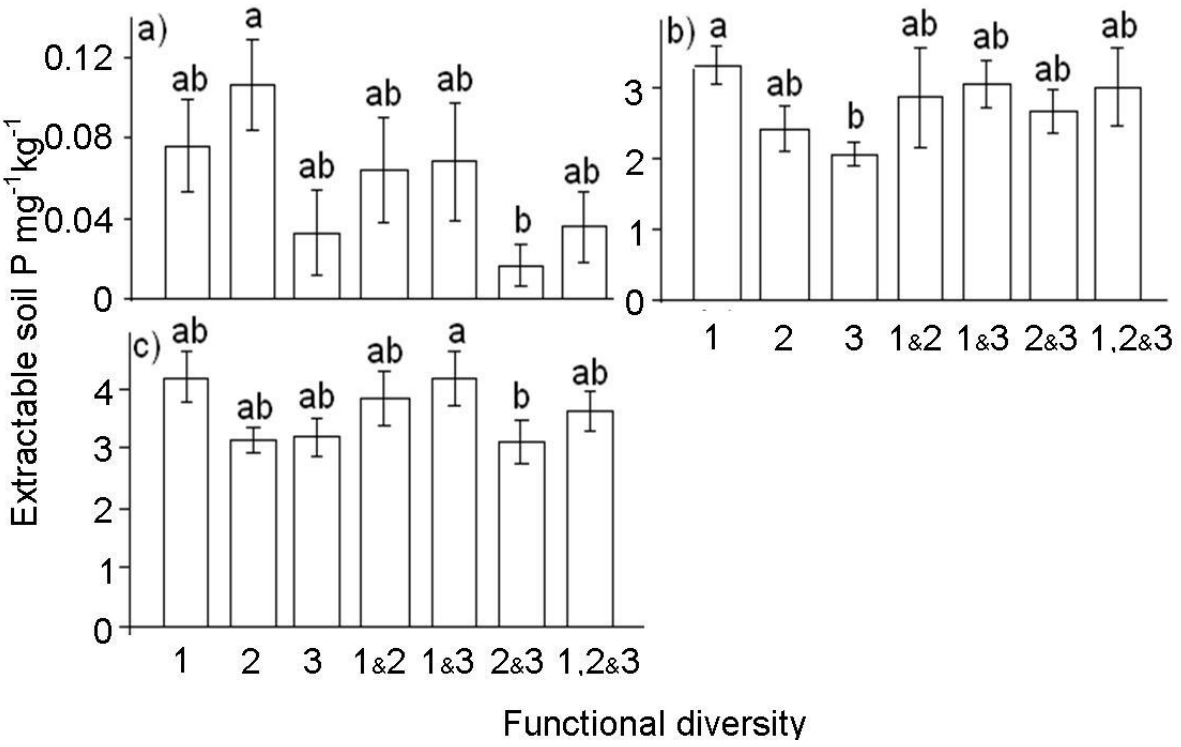

Figure 3.18: Diversity effects upon availability of phosphate in the soil (±SEM). Significant effects were only seen in spring and early summer, and they are presented here. a) February 2009 ($F_{6,39}$=3.80, p<0.01), b) March 2010 ($F_{6,39}$=2.39, p<0.05), c) May 2010 ($F_{6,39}$=2.38, p<0.05).

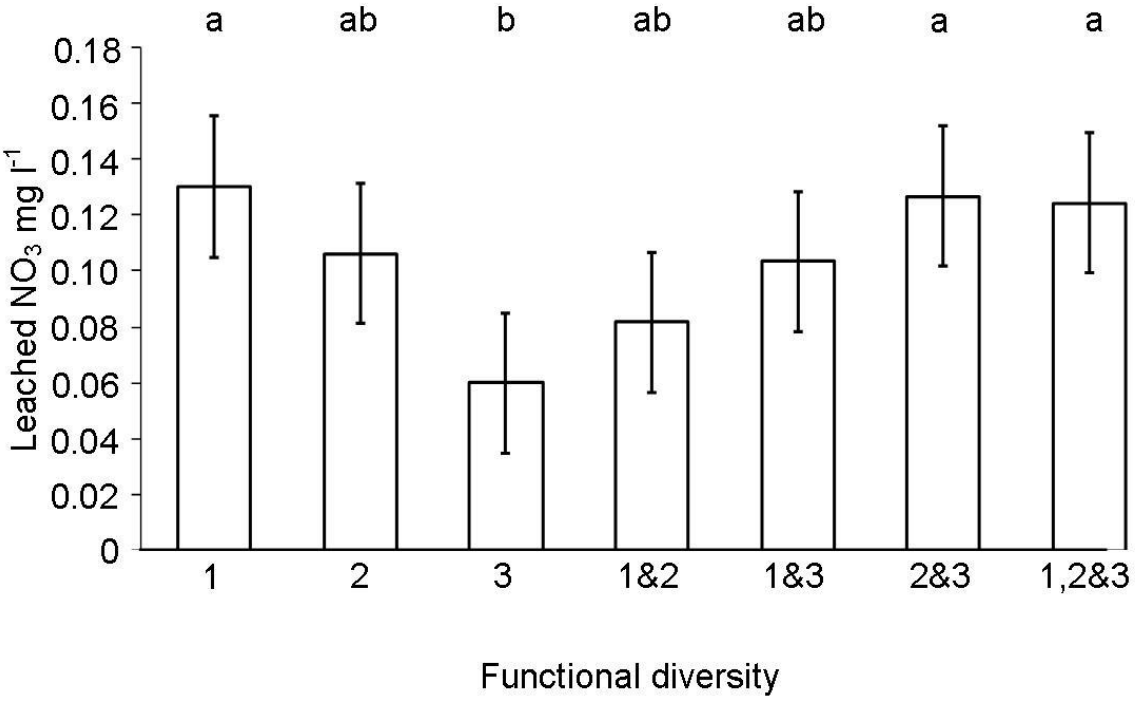

Figure 3.19: In November 2009, nitrate was lost through leaching in different amounts according to functional diversity ($F_{6,39}$=4.50 p<0.05). Error bars represent ±1 SEM.

3.4.3 CLIMATE AND FUNCTIONAL DIVERSITY INTERACTIONS

Interactions between the climate and functional diversity treatments began to appear towards the end of the experiment, with most appearing after the second summer rainfall phase in 2009. The interactions characterised a differing response to climate change between plots with FG1 present, and FG2 and FG3. In general, FG1 had faster process rates and therefore higher stocks of nutrients, but this seemed to be dependent on constantly high soil moisture. Plots where FG1 was absent seemed much less responsive to climate change and performed equally well under either regime.

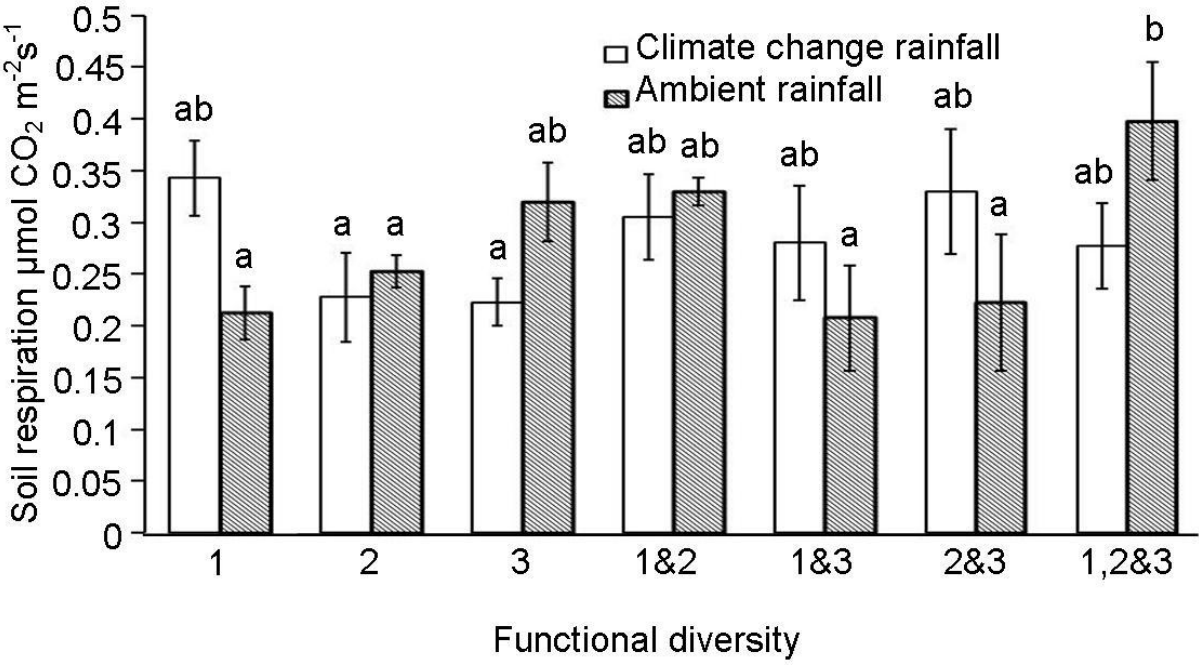

Figure 3.20: Treatment effects in March 2009 there is a significant climate and diversity interaction on soil respiration ($F_{6,34}$=5.35, p<0.001, R^2=0.66). Error bars correspond to ±1 SEM.

March 2009 saw the lowest R_{eco} in ambient plots with individual functional groups. R_{eco} was higher in the wetter climate change plots with FG1 alone, and most plots with two functional groups, and highest in ambient plots with all three functional groups present (Fig. 3.20, $F_{6,34}$=5.35, p<0.001). In May 2009 R_{eco} was higher in plots with FG1 present under climate change, and FG2 and 3 saw higher R_{eco} where the ambient part of the climate

treatment was implemented (Appendix 3.12.1, Table 3F, $F_{6,34}$=4.96, p<0.01). This pattern was very similar to that of March, but there were smaller effect sizes between treatments in May when climate manipulations had temporarily ceased. The positive effect of soil moisture on FG1 plots was explicitly demonstrated in a study showing that R_{eco} increased with soil moisture under FG1, and decreased under wetter conditions under FG2 and FG3 ($F_{6,34}$=3.03, p<0.05). This continued throughout the summer phase of the rainfall treatment, where FG1 plots responded better under the wetter ambient conditions. This is illustrated in June 2010, the plots which had the highest R_{eco} after the wet winter treatment, were now the lowest during the summer drought (Appendix 3.12.1, Table 3F). This particularly refers to FG1 under climate change and FG1,2&3 under ambient conditions ($F_{6,34}$=10.94, p<0.01).

In general, plots where FG1 was absent had a significantly warmer average air temperature due to less or taller plant cover, which correlated with lower mineralisation rates between September and December 2009 (Appendix 3.12.3, Table 3J: $F_{6,31}$=6.35, p<0.001). A warmer, more open canopy also saw higher extractable soil P under plots with FG1 present in August 2009, and lower P under climate change plots where only FG2 or FG3 were present (Appendix 3.12.5, Table 3O: $F_{6,39}$=8.07, p<0.001). Nitrification rates were highest in these barer plots where FG1 and FG3 were absent and drier conditions prevailed; therefore between December 08-March 09, FG2 plots under ambient conditions had the highest nitrification rates, and FG1,2&3 had the lowest under climate change (Appendix 3.12.3, Table 3K, $F_{,31}$=5.48, p<0.001). Between June-September 2010, the FG2 plots under climate change exhibited the highest rate of nitrification, so overall the barest plots under dry conditions were the most nitrate rich (Fig. 3.23, $F_{6,31}$=3.59, p<0.01). In December 2009 ammonium was leached in a more straightforward manner, where more functional groups in the plot caused less ammonium to be leached. A caveat to this was that under drier ambient conditions more ammonium was lost when two functional groups were present, than under climate change ($F_{6,8}$=4.5, p<0.01).

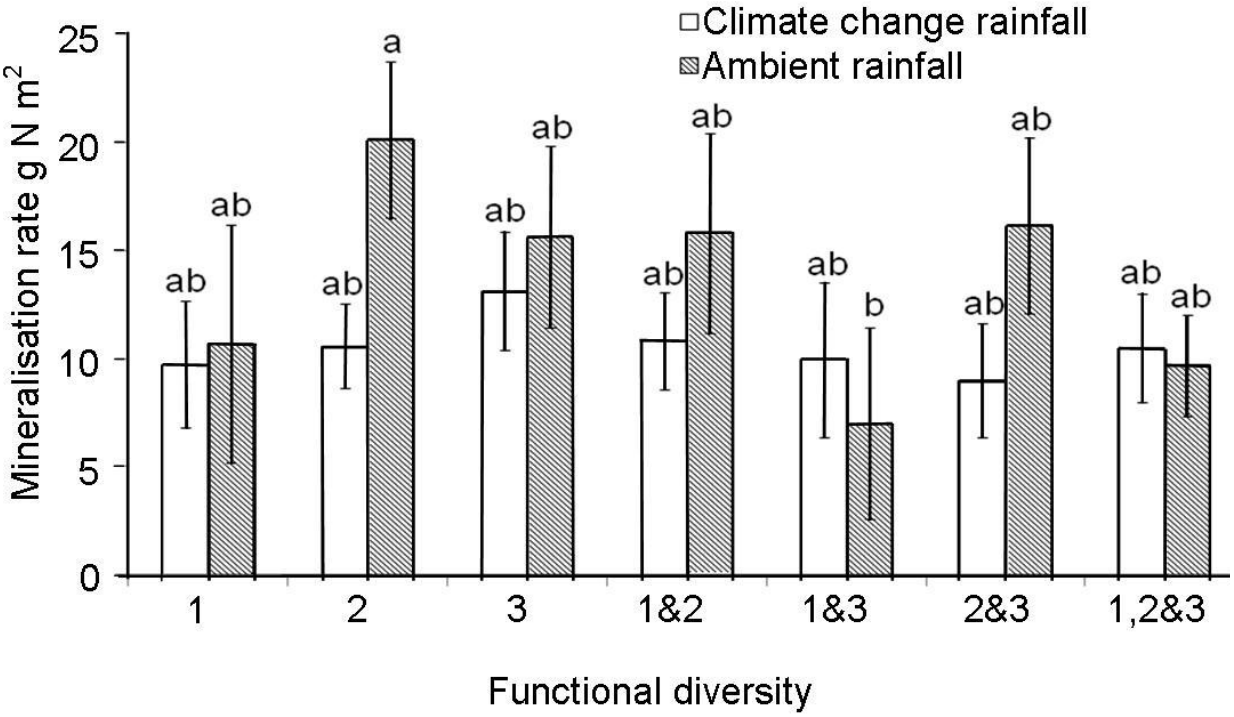

Figure 3.21: Climate and diversity interaction effects on mineralisation rate (±1 SEM). March-June 2010 ($F_{6,31}$=3.45, p<0.05).

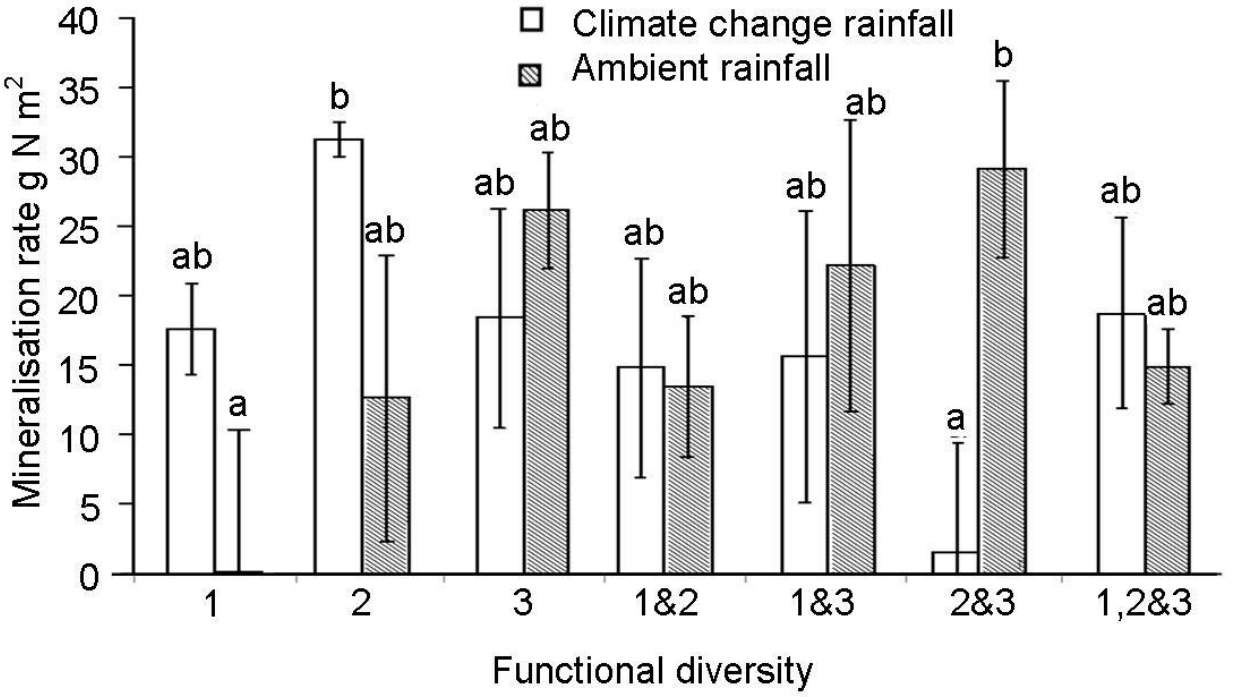

Figure 3.22: Climate and diversity interaction effects on mineralisation rate (±1 SEM). June-September 2010 ($F_{6,31}$=2.33, p<0.05).

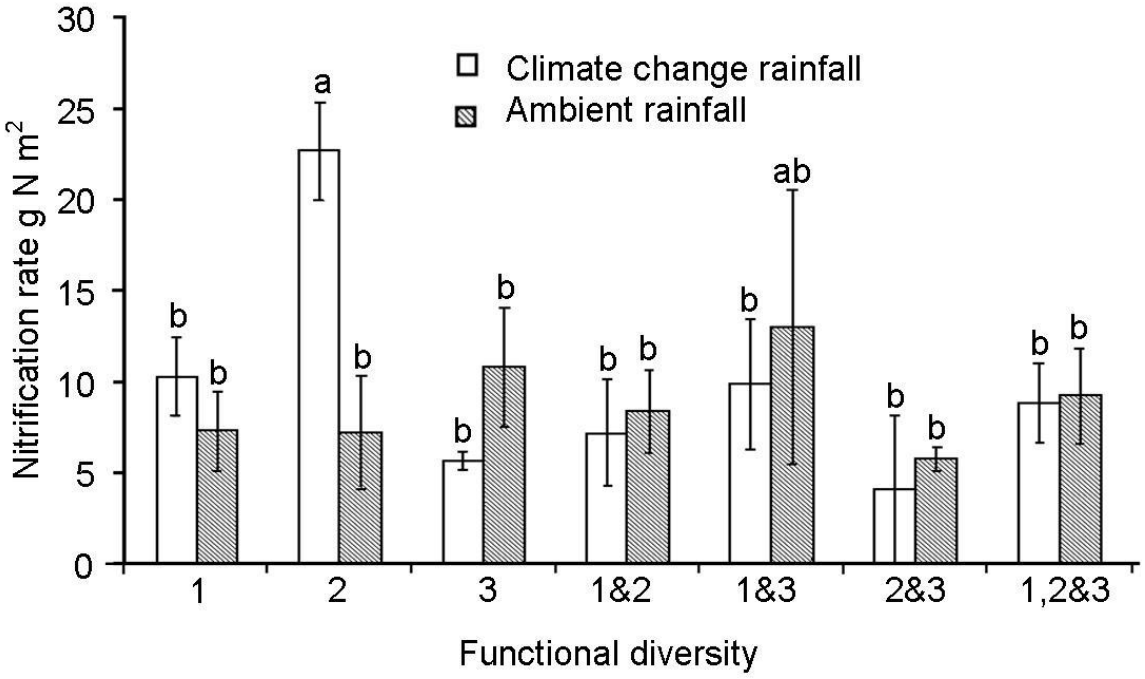

Figure 3.23: Climate and diversity interaction nitrification in the June 2010 – September 2010 period (±1 SEM). Nitrification rates were generally lower under climate change, although fg2 was associated with a very high level of nitrification under droughted conditions ($F_{6,31}$=3.59, p<0.01).

3.5 DISCUSSION

The results of this study indicate that certain ecosystem processes are altered by changes in rainfall, and the magnitude and direction of these changes can be modified by functional diversity and are subject to seasonal and successional influences. In other processes, functional diversity supersedes the importance of climate as a determinant of process rates. It is evident that the implementation of functionally discrete species groups has successfully differentiated responses to climate change.

3.5.1 SEASONALITY

All ecosystem processes measured in the experiment experienced winter periods where process rates were slow, and much faster rates in summer periods of high biological activity. This was despite an anomalously wet winter in 2009-2010, and an extremely dry spring and early summer in 2010 (Prior & Kendon 2011). In spring 2009 onwards, the responses to climate change under the perennial group were different from the annual group. The perennial and annual species present in this study had high relative growth rates (Grime & Hunt 1975), but were driven by differing resource acquisition strategies (conservative versus exploitative, respectively), with the result that in spring, function rates including mineralisation and uptake of extractable nitrogen were lower when annuals were absent.

Net ecosystem exchange of CO_2 (NEE) is the sum of ecosystem respiration (R_{eco}) and photosynthesis, and it can be inferred the two processes balance at a NEE value of zero, with more negative NEE implying photosynthesis is outweighing R_{eco} (Wu *et al.* 2011). NEE and evapotranspiration (ET) followed a clear seasonal pattern where they were higher during the summer, despite very high corresponding R_{eco}, and closer to zero throughout the winter. The grassland is therefore a carbon sink in the summer, and carbon neutral in winter (Baldocci *et al.* 2001). In the drier year of 2010 both R_{eco} and NEE were lower than 2009, which suggests that this site is sensitive to water deficiency, although still an overall carbon sink. This is common to most temperate and alpine grasslands, although more waterlogged grasslands display very little response to drought (Kato *et al.* 2004; Jaksic *et al.* 2006). R_{eco} was lower in late summer 2010 under climate change compared to ambient, while NEE remained more constant, suggesting that root and microbial activity were strongly suppressed by the long period of dry weather, while leaf level processes were affected to a lesser degree. This suggests that a drought avoidance strategy will confer an advantage in future years where deeper root systems could access water sources and prevent wilting, because in the drier top layers, activity of both roots and microbes was suppressed. The exact relationship between soil microbial respiration and abiotic factors including moisture is not understood due to complex variables involving soil organic matter quantity and quality (Bardgett *et al.* 2008).

The seasonal fluctuations of R_{eco} are associated with fluxes of microbial activity in the soil; these dictate processes such as decomposition and mineralisation rates. These were higher in late spring and summer, which is likely to be due to warm damp conditions and an abundance of decomposable substrate (summer 2009 showed an anomalously low

mineralisation result which is likely to be due to operator error and should be disregarded), and lower in winter when microbial activity was at its lowest (Börken & Matzner 2009; Wu *et al.* 2011). Consequently, soil ammonium and phosphate concentrations were very high in late summer in both years due to a large accumulation of biomass available for decomposition, and probable nutrient excretion and reduced N immobilisation by microbes (Bardgett 2002; Börken & Matzner 2009).

In recent years, interest in leachate from grasslands appears to have declined, which may be due to the inconsistency of data obtained from lysimeters (Zotarelli *et al.* 2007). In this experiment, leaching losses were tiny, even during the wettest period in December 2009, so nutrient retention in the system was high. Ammonium and nitrate leaching followed one another through 2010, which suggests that they are similarly poorly bound to the sandy soil. This changed in December 2009 when a large influx of rainfall led to disruption of soil aggregates and loss of ammonium (Bronick & Lal 2005; Riaz *et al.* 2010).

3.5.2 CLIMATE CHANGE

The climate change treatment began to show strong effects on function in late 2009. Significant effects of climate manipulations upon measured ecosystem processes occurred after a lag time of up to three months after the roofs were removed, chiefly because of the delayed response of soil moisture to these treatments. The summer drought phase was associated with reduced NEE and lower living plant cover, and R_{eco} was also reduced in the drier plots. There have been numerous conflicting studies regarding whether photosynthesis or R_{eco} is more sensitive to precipitation change, though a recent meta-analysis by Wu *et al.* (2011) found the overall magnitude of response to rainfall manipulations (both increased and decreased) were almost identical. The sudden increase in R_{eco} in May 2009 coincided with a sharp increase in temperature, and the soil moisture content lowered accordingly. In field conditions, air temperature is often a stronger driver of R_{eco} than soil moisture (a 10˚C increase in air temperature can double respiration rates across most biomes, Davidson *et al.* 2000), although moisture was the main driver overall found in my experiment (Yuste 2003). However, this is very difficult to uncouple from the inevitable soil drying which results from warming (Lloyd & Taylor 1994). After the summer climate phase of the experiment in 2009, R_{eco} was still suppressed in the climate change treatment as late as one month after roofs were

removed, suggesting that a substantial proportion of the microbial biomass was less active due to slow recovery of soil moisture levels. Therefore, R_{eco} can be driven by both current and historical conditions (Xu *et al.* 2004).

The sudden increase in microbial activity in May 2009 is likely to have contributed to the increased mass loss of *Arrhenatherum elatius* litter under the climate change treatment in the December-June 2009 period, a difference not seen in the three month period prior to this. This climate effect can be attributed to the preference of microbes for warm, moist conditions which would have occurred in these plots in May 2009 after the winter rainfall addition phase (Bardgett 2005). The lack of many climate effects on decomposition is likely to be because microorganisms that control decomposition are usually Gram positive bacteria and fungi, neither of which are overly sensitive to drought (Schimel *et al.* 2007). While some climate effects occurred with regard to decomposition, these effects were not continued through the process of nutrient provision; there was no effect seen on mineralisation rates or nutrient stocks. This could be due to very fast plant uptake in these plots; in November 2009 it was the wetter ambient plots that lost the most nitrate through leaching, which suggests that any senesced material in the climate change plots was either still in an organic form or had been strongly bound by microbes in climate change plots.

In May 2010 climate change depressed both NEE and R_{eco} significantly, attributable to the prolonged snow and subsequent natural drought in late spring. This early summer drought was particularly deleterious to ET, which continued to be low through the summer due to lack of plant cover and low canopy height. ET is often positively correlated with higher vapour pressure deficit and plant biomass (St Clair *et al.* 2009). Some evidence of a relationship between vegetation type and ET has been demonstrated elsewhere in the literature, but this mostly describes rooting depth (which can only be loosely aligned with the groups in this experiment) and has mostly been tested in arid environments (Schenk & Jackson 2002, although see Tilman & Wedin (1991) for a temperate example).

3.5.3 FUNCTIONAL DIVERSITY

In June 2009, NEE was lower in plots containing the perennial group than others, which may be due to a combination of high R_{eco} from increased litter inputs, and drier conditions (Suyker

& Verma 2001). The density of the foliage could have caused light competition, which was less of a factor with the sparser annual and bunch grass groups (Reekie & Bazzaz 1989). There has seldom been a direct link made between microbial activity and functional plant diversity, mainly because a clearer link has already been made to microbial influences on soil and substrate properties (Tufekcioglu *et al.* 2001), though Ryan (1991, 1995) has suggested that there could be an impact on R_{eco} based upon species-specific tissue chemistry. However, the mean foliar N content of FG1 and FG3 are not significantly different, so there is no evidence for this here (Chapter 2).

R_{eco} is largely driven by processes associated with decomposition (up to 50% is attributable to this, Ryan & Law 2005). Plots under perennial dominated groups saw consistently higher decomposition rates compared with plots where they were absent, and this difference was consistent through the study. It can be inferred that decomposition is affected by microclimate, so the denser canopies provided by perennial species can be assumed to favour microbial activity (Bardgett & Shine 1999; Hector *et al.* 2000; Zak *et al.* 2003). The theory of perennial species creating a warmer, more humid microclimate during hot dry summers is broadly supported by the less negative NEE in June under perennial plants, which is likely to be due to increased microbial activity and consequent R_{eco} (Raich &Tufekcioglu 2000). This is supported by Wardle *et al.* (1997) who reported stronger linkages of litter decomposition rates with living plant functional diversity than litter quality (species composition); although litter (independent from root) respiration was not strongly driven by functional group.

When annuals and bunch grasses appeared alone or together, there were low rates of mineralisation, which is directly linked to the lower decomposition rates in these treatments. Accumulation of plant available nitrogen from the microbial breakdown of litter is therefore subject to the same requirements for warm, moist conditions, and this link continues through all processes. There are few studies which directly highlight the connection between mineralisation rate and functional diversity, with the majority of the literature focussing more upon effects on microbial biomass, with effects on mineralisation rates inferred (Wardle & Nicholson 1996, Spehn *et al.* 2000). The consensus from the literature is that increasing functional or species diversity allows more niches for microbes, and therefore faster soil processes, with no direct plant species or functional effect (Zak *et al.* 2003); however, in this experiment functional group identity had larger effect sizes than functional group diversity. February 2009 had the most striking difference in extractable nitrogen pool size associated

with functional grouping; in the case of both ammonium and nitrate, bunch grasses and annuals had the lowest soil concentration. According to Grime's CSR classification (Grime 1985, 1988), these species are mostly ruderals or stress tolerators, with bunch grasses having low relative growth rates and annuals being high. In February it is unlikely that the temperature would have been high enough for perennials to be growing, whereas germination of annuals may have been beginning. Extractable nitrogen is transient in the soil and is easily taken up (especially NH_4 which has a lower energetic cost to the plant, Lumme 1994), therefore it is likely that under plots with annual plant groups, extractable nitrogen was either being used in early spring or lost to leachate from deeper soil layers.

Diversity treatment effects of leaching were seen mostly in the autumn, when plots with a high abundance of annuals lost less nitrogen after a summer with lower nitrogen availability in the soil (Börken & Matzner 2009, Riaz *et al.* 2010). Most studies using leachate to consider the role of plant species identity or diversity, have concluded that increasing species richness leads to decreased leaching losses (Tilman *et al.* 1996, Scherer-Lorenzen 2003). This is readily explainable in terms of more numerous resource use strategies (Hooper & Vitousek 1998), more habitat heterogeneity for microbes (Bardgett 2005) and increased litter (Riaz *et al.* 2010). While Tilman *et al.* (1996) surmised that more species result in less leaching, Hooper & Vitousek (1998) considered the problem more analytically with the aid of the grass, forb, legume (GFL) functional diversity classification, and found that while this is often indeed the case, there is a marked seasonal complementarity with regard to functional groups' resource retention. In this experiment, species richness itself was not measured, but increased functional diversity caused lower leaching in December 2009. It is possible that in other months too little was lost to draw accurate conclusions about the role of functional diversity in the current study.

3.5.4 CLIMATE AND DIVERSITY INTERACTIONS

In spring 2009 the winter phase of the climate treatment resulted in increased respiration under the perennial group, compared to the annuals and bunch grasses. Other studies have corroborated this by demonstrating increased growth and abundance of perennials under wetter conditions, while ruderals are competitive in drought (Morecroft *et al.* 2004). Using groups closely linked to function is important in this case because failure to do this leads to

confounding effects, such as with St Clair *et al.* (2009), who created functional groups consisting of a pre-selected species mixture, and compared it with two monocultures. Processes under the perennial group in my study favoured wetter conditions all year round, illustrated by lower R_{eco} than under the annual groups in the dry summer climate change phase, and higher R_{eco} under the perennial groups than annual in the winter phase. This was likely to be due to a combination of microbial flushes of activity caused by rainfall pulses (Xu *et al.* 2004), and rapid plant root metabolism of annual plants (Ryan and Law 2005). Annual plants, with their shallow roots, are ideally situated to take advantage of small rainfall pulses (less than 3mm), which would otherwise be quickly re-evaporated (Schwinning & Sala 2004). Perennials require either larger or longer pulses, or rises of the water table left over from a wet winter (Chimner & Welker 2005). This study indicates that carbon sequestration in an acid grassland community is sensitive to drought in the long-term, so short term drought effects are less likely to be noticed. However, there is some evidence that in the summer perennials may be more adversely affected by drought, and annual plants can compensate for this.

Mineralisation was lower when perennials and bunch grasses were absent under climate change in the summer, so the loss of the closed canopy under drought conditions led to reduced nitrogen availability. Many studies demonstrate less plant available nitrogen under drought, which often does not recover even after wetting (Voroney *et al.* 2007; Börken & Matzner 2009). This is likely to be a combination of less plant litter availability and lower microbial activity under climate change (Wu *et al.* 2011).

This experiment has demonstrated that ecosystem function in temperate grasslands is intricately linked to water availability and the functional diversity of the plant species present. In essence, while climate will have a detrimental effect upon nutrient cycling processes in grasslands, functional diversity of the plants present have a role in mitigating this, and functional group identity is more important than species diversity. These results indicate that grassland systems can endure a certain amount of climate variability, because functional groups respond differently to climate conditions and in different seasons. This has important connotations for managers of grazed and managed grasslands, where species are often lost through nutrient enrichment or selective sowing. For all these processes to work together and grassland ecosystem services to be sustainable through the 21st century, a selection of perennial grasses and forbs, and annual forbs and legumes should be recommended initiatives

looking to optimise ecosystem function in the future. This lends support to the theory of insurance species, an idea that has displaced the redundancy hypothesis (Tilman 1994).

The group comprising bunch grasses and tall forbs were comparatively rare in the plots throughout the experiment, and they did not have a significant impact upon ecosystem function, supporting the biomass ratio hypothesis (Grime 1998). There are signs that by the third year, this group was beginning to behave differently (for example, mineralisation became more similar to perennial than annual), so it is likely that the group will have a more unique identity in future years.

Climate change will alter ecosystem processes in years to come, but functional diversity is crucial in buffering this effect in grassland ecosystems. Each functional type has a different role through the seasons, all contributing to optimal resource use and functioning. Plant functional groups show different temporal and stress response patterns which can be manipulated to protect grasslands from the strongest effects of climate change.

Figure 4: Roofing continues.

CHAPTER 4: CHANGES TO GRASSLAND BIODIVERSITY AND ECOSYSTEM FUNCTION UNDER A RANGE OF PRECIPITATION CHANGE SCENARIOS.

4.1 ABSTRACT

There is some uncertainty regarding the exact climate projections general circulation models provide, with a number of possible climate scenarios through the 21st century depending on socioeconomic and political trends, response of ecosystems to greenhouse gas emissions and temporal and spatial model scales. Accordingly, experimental designs should consider a range of climate scenarios in order to make reasonable predictions about plant ecosystem responses to climate change. In this experiment I applied two rainfall regimes to lowland grassland in South-East UK; 1) spring and summer drought treatment, and 2) variable summer rainfall treatment, with an ambient control. I measured various aspects of ecosystem functioning, encompassing CO_2 and water fluxes, and soil nutrient stocks, in order to understand how prolonged drought or variable rainfall inputs affect the system. The spring/summer rainfall treatment was affected in terms of measures driven by microbial activity, most notably mineralisation rate, reducing nutrient turnover. Many processes were less obviously affected under the variable treatment and there were few differences from the ambient treatment, but plant species diversity and cover were much lower after the summer drought, and did not recover as fast as other treatments. Shortages of water are detrimental to plant communities in complex ways, and much more study is needed to be able to predict future effects on grassland systems, despite climate model uncertainties.

4.2 INTRODUCTION

Grasslands are important biomes which provide a wide range of ecosystem services, and concern is increasing because of widespread degradation and conversion to cropland (Millennium Ecosystem Assessment, 2005). In 2005 it was estimated over 800 million people were directly dependent on grasslands. They comprise many fast-growing, productive and grazing-resilient species which are used for food, fodder and pollinator refuges (Reynolds *et al.* 2005), and support about 4000 vertebrate species worldwide (Millennium Ecosystem Assessment, 2005, grasslands including shrublands). Grasslands are finely balanced systems, which can turn from carbon sinks to sources under climate stress (Cao & Woodward 1998; Murphy *et al.* 2009). Since they cover 25% of the terrestrial surface, and are estimated to account for as much as 10% of global C stocks, it is becoming increasingly apparent that safeguarding these systems is a global priority (Hui & Jackson 2006; Sitch *et al.* 2008).

There have been recent calls for experimental studies on 'extremes' of climate, including droughts, floods and heatwaves (Smith 2011b). Evidence that 'extreme' or anomalous climate events are already occurring is increasing, yet there is little evidence of studies on extremes proliferating. For example, the anomalously hot and dry summer of 2003 in Europe cost $12.3 billion in lost crops (Schär & Jendritsky 2004), and extreme weather events in China have been increasing in frequency since the 1970s (Piao *et al.* 2010). This gap in the literature needs to be urgently addressed (Planton *et al.* 2008).

It is likely that precipitation changes will cause stronger impacts on grassland ecosystem function in the coming century than CO_2 or warming (Blankinship *et al.* 2011). Many drought experiments have been undertaken in recent years, for the most part considering a single climate scenario, and most have noted almost immediate effects of limited rainfall on every aspect of ecosystem function (Knapp *et al.* 2002; Xu *et al.* 2004; St Clair *et al.* 2009). Recently there have been attempts to consider a range of factors, such as the three factor CLIMAITE experiment on Danish grassland and heath, which showed a much stronger effect of drought on nitrogen cycling than CO_2 enrichment or temperature after two years (Mikkelsen *et al.* 2008; Larsen *et al.* 2011). As well as direct effects on productivity, changes in precipitation have been consistently found to cause larger effect sizes upon soil biota across terrestrial ecosystems than CO_2 or warming, and the longer the climate stress continues, the stronger the effects on ecosystem processes become (Heimann &

Reichstein 2008; Blankinship *et al.* 2011). However, given the lack of literature on different potential rainfall regimes, and their likely effects, this could be a more logical starting point than beginning with extremely complex interactions (Leuzinger *et al.* 2011).

While most rainfall models agree that spring and autumn will not see consistent proportional changes like the other seasons, even minor or infrequent changes in spring precipitation are likely to have a marked effect on ecosystem functioning in grasslands (Kim *et al.* 1992; Gorissen *et al.* 2004). Drought extended over two seasons is likely to have stronger direct effects upon primary productivity (Fay *et al.* 2003); seed germination (Stampfli & Zeiter 2008) and net ecosystem CO_2 exchange rates (Kim *et al.* 1992) than drought in summer alone. It could also highlight differing sensitivities to drought across plant groups such as perennial grasses and annual forbs (Morecroft *et al.* 2004; Fay & Schultz 2009). Seedlings are the most vulnerable phase of a plant's development, so spring drought could cause some species to be lost (Lloret *et al.* 2004), although some evidence suggests that species with strong seed dormancy patterns will be less affected (Fay & Schultz, 2009). This could be a contributing factor to changes in community assemblages and more rapid species turnover (Knapp *et al.* 2002). Studies on spring and summer drought are few, but some suggest that when directly compared, a dry spring and wet summer could reduce productivity and net ecosystem exchange more than a dry summer and wet spring (Heitschmidt & Vermeire 2006). The overall lack of literature on spring drought needs to be addressed, as given the projected phenological advance of spring over recent decades (2.5 days per decade^{-1}) spring conditions could be far more important than previously assumed (Menzel *et al.* 2006).

Some studies have begun to investigate the effect of heavy rainfall events juxtaposed with severe drought phases in temperate grasslands, such as EVENT at Bayreuth, Germany (Jentsch *et al.* 2007; Mirzaei *et al.* 2008). These studies were implemented after many climate models predicted increasingly infrequent summer rainfall events in the coming century, with occasional flash flooding in temperate areas (Fischlin *et al.* 2007; Murphy *et al.* 2009). Between the second and third IPCC Assessment Report (SAR, 1995 and TAR, 2001 respectively), evidence of increased extreme events was noted. In AR5, due in 2013/2014, the IPCC plans to give more emphasis to 'rare' and extreme events, so systems biology must incorporate these into designs (IPCC 2011, www.ipcc.ch). Other studies are also beginning to incorporate more complex and realistic rainfall models, although the idea of creating extreme climate regimes is still underrepresented in the literature (Laporte *et al.* 2002; Smith 2011b)

There are increasing indications that altering rainfall frequency and intensity could have unpredictable and profound consequences for water, carbon and nutrient processes in grasslands. The EVENT experiment used a temperate grassland system with rainout shelters to create a series of severe rainfall events including drought, heavy rain and freeze-thaw cycles (Mirzaei *et al.* 2008). While they reported net carbon uptake after drought due to increased productivity and stable respiration rates, Laporte *et al.* (2002) and Knapp *et al.* (2002) demonstrated a decrease in CO_2 efflux under their most severe drought regimes. Many ecosystem processes are driven by microbial activity, which are also strongly affected by water availability, but are often quickly reactivated when a pulse of water is applied (Lee *et al.* 2004; St Clair *et al.* 2009). Estimates vary for how long this reactivation can take, but in general processes such as ecosystem respiration do not regain the level of the ambient treatment for a long time after stress is alleviated (Högberg *et al.* 2001). However, with the build up of decomposable organic matter that often occurs during periods of severe drought, subsequent rainfall pulses correlate with a sudden increase in decomposition causing a peak of mineralisation that often overshoots that of ambient conditions (Sponseller 2007; Börken & Matzner 2009).

The increasing frequency of extreme events and the uncertainties inherent in general circulation models (GCMs) means that future climate experiments should incorporate as many different scenarios as possible. In this experiment I implemented two different climate regimes in lowland grassland in South-East England. A spring and summer drought treatment (with additional winter rainfall) and a summer drought of variable duration with intermittent downpour events and winter rainfall addition, were contrasted with an ambient control. I then measured a set of ecosystem processes and soil nutrient concentrations in order to monitor climate effects upon ecosystem functioning.

I hypothesised that:

1. Implementing rainfall stress in spring and summer, as well as increasing winter precipitation, will reduce nitrogen and water retention, lower rates of net ecosystem exchange of CO_2 and measurably alter plant composition.
2. Grasslands are not only sensitive to the amount of rainfall received, but also the frequency, and ecosystem function will be affected in a measurable way. It is likely that sporadic pulses of rainfall will lead to bursts of microbial action, which is likely to

result in lower CO_2 and water cycling, and accumulations of nutrients that are not taken up by plants.

4.3 METHODS

4.3.1 STUDY SITE

The experiment described here was carried out on plots embedded within the framework of a larger experiment which had been running from October 2008. This experiment (the DIRECT experiment: DIversity, Rainfall and Elemental Cycling in a Terrestrial ecosystem) combined a rainfall manipulation treatment (summer drought, winter rainfall addition, IPCC 2007), with altered plant functional diversity treatments in a lowland grassland in South-East England. For more details of the study site, including dominant species and abiotic conditions, see Chapter 3. The rainfall regime was achieved by building rain shelters using transparent corrugated plastic.

The DIRECT experiment was a randomised blocked design where four blocks of plots were arranged east-west to accommodate a slight incline across the site. Three plots in each block (n=12) were used in the experiment described here. There were two rainfall regimes and one ambient control, with one plot assigned to each treatment in each block, making four replicates of each treatment. These were implemented between March 2009 and September 2010. The plots had not been sheltered or manipulated prior to the beginning of this study.

4.3.2 CLIMATE CHANGE REGIMES

The first climate change scenario comprised a spring and summer drought, the second a variable rainfall treatment. The rain shelters were put up on March 1st 2009 and 2010, and removed on August 31st. The spring/summer rainfall scenario administered 50% of rainfall when less than 20mm of rain fell in 24 hours. When more than 20mm fell, it was all reapplied. This regime was supported by model simulations from the GLIMCLIM program (Chandler 2002).

For the variable rainfall regime, between March and June 1st, the plots were roofed and all rainfall was reapplied so that the microclimate for all treatments was comparable. During the summer period (JJA) all rainfall was stored, and a complete drought imposed until a rainfall event occurred with more than 20mm in 24 hours, whereupon all stored rainfall was reapplied to emulate a flash flood. For the winter rainfall regime in both climate change treatments, 15% extra rainfall was reapplied after every precipitation event. These two treatments were compared to an ambient treatment which received all rain through drilled holes in the plastic rain shelters, while maintaining the microclimate of the plots (see Chapter 3). This regime was also supported by model simulations from the GLIMCLIM program (see appendix 4.2 for simulation methods and output).

In 2009 253 mm of rain fell between 1st March and 31st August; 166 mm of this in the period between June and August. In 2010 239 mm fell between March and August, of which 136mm fell between June and August. The volume of rainfall to be reapplied in the spring/summer climate change treatment was intended to be 70% of ambient between March and August. In fact the amount reapplied was 63% in 2009 and 65% in 2010 due to the infrequency of heavy rainfall events.

The design of the variable treatment was intended to reapply 100% of ambient. In 2009 the plots received 100% of ambient rainfall, but in 2010 due to the lack of heavy downpours of over 20mm in 24 hours, some rainfall was discarded at the end of the summer season and so 92% was applied (Fig. 4.1). Heavy rainfall events were more common in 2009 than 2010, with more rainfall being stored up for reapplication in the variable treatment. In 2010, the rainfall reapplication occurred much later in the season, which created strong water stress in the early summer.

Soil moisture was measured weekly using a ThetaProbe Soil Moisture Meter HH2 with ML2x probe (Delta-T, Cambridge, UK), four measures in each plot were taken and averaged, and then a monthly mean was generated for use as a covariate with other measures.

4.3.3 VEGETATION SURVEYS

The vegetation in the plots was surveyed twice a year in May/June and September in 2009 and 2010, with an additional survey in July 2010 to capture the effects of the natural drought that occurred in early summer. The survey consisted of placing a $1m^2$ quadrat in the centre of the plots, and estimating percentage cover of each vascular plant species (nomenclature follows ITIS, 2010).

4.3.4 CO_2 AND WATER FLUX

Ecosystem CO_2 and water exchange were measured in February 2010 then at monthly intervals between May and September using a Ciras-1 Infra-Red Gas Analyser (IRGA, PP Systems, Hitchin, UK) with attached perspex chamber cuvette ($299cm^2$ area, $8959cm^3$ volume). The cuvette was placed over a collar in the soil to create a seal, and measurements of net ecosystem exchange (NEE) and evapotranspiration were taken over two minute periods. This was carried out in full sunlight between 10am and 3pm in February, and 9am and 5pm between May and September. An opaque cover was placed over the cuvette after the initial measure, and a new measure taken after 60 seconds equilibration time. This allowed measures of ecosystem respiration (R_{eco}). Simultaneous measures of soil moisture, photosynthetically active radiation (PAR, Skye Instruments, Wales) and soil temperature (Hanna HI 98501, Bedfordshire, UK) were taken to act as covariates.

4.3.5 MINERALISATION RATE

A composite sample of four soil cores was taken from each plot every month in the spring and summer and every three months during autumn and winter. These were homogenised, put through a 2mm aperture sieve and stored for no more than 24 hours at 5ºC. Extractable nitrogen content of wet soil (NH_4^+-N and NO_3^--N) was extracted using Allen (1989)'s potassium chloride (KCl) extraction technique and analysed colourimetrically using a Skalar SAN^{++} continuous flow analyser (CFA, Skalar, York, UK). To determine N mineralisation rate in the soil, cores were placed in the soil in December 2009 (t_0), covered and incubated

for three months (t_{inc}). At the same time (t_0), extractable N concentration was analysed in a composite soil sample as a baseline point. In March 2010 the t_{inc} core was removed, the nitrogen extracted and analysed, and the core was replaced with fresh soil. Another composite sample was taken at the same time (t_1). The soil nitrogen concentration for t_0 was subtracted from that of t_{inc} and the result was multiplied by the bulk density to give a value of mineralisation rate in each plot. This process was repeated every three months until September 2010, and results are presented as g N m^2 per 90 days.

4.3.6 SOIL NUTRIENT CONCENTRATION

Total nitrogen and phosphorus content were measured in dried soil using a modified Kjeldahl method (Kjeldahl, 1883), and analysed using a Skalar SAN^{++} CFA. This was carried out annually after the summer season.

Extractable nutrients were measured using Allen's KCl and Truogs methods (Allen, 1989) for nitrogen and phosphate respectively and analysed using the CFA. Soil moisture was determined by weighing soil before and after drying and determining the proportion of moisture lost relative to soil dry weight. Thus extractable nutrients are presented in mg/kg of dry soil.

4.3.7 LEACHING LOSSES

Ammonium and nitrate losses through leaching were determined monthly using suction cup lysimeters installed in the plots in January 2010. Because these require one month for the soil to settle, the first month sampled was March 2010, and the final month was May 2010. The very dry weather through the summer meant that insufficient leachate was collected, so these were not included in the analysis. The samples were analysed immediately using a CFA, with distilled water as the matrix.

4.3.8 STATISTICAL ANALYSIS

Data were analysed using ANOVAs in R2.12.0 (R Development Core Team, 2009) at individual time points, which looked for climate treatment effects, and interactions between these and covariates specific to the variable in question. For NEE and ET, mean PAR, soil temperature and soil moisture taken simultaneously with the measure were used. For R_{eco}, only soil temperature and soil moisture were used. For mineralisation and nitrification rates, and all available nutrients, averaged monthly soil moisture for each plot was used. All variables were log-transformed where assumptions of normality were not met, and outliers were removed if they significantly altered the model or increased unexplained deviance to an unacceptable level, i.e. more than 5% in this case. The effects of block and covariates were sequentially omitted during model simplification where non-significant, to leave the optimal model in each case. Post-hoc Tukey's Honest Significant Difference tests were used to determine which climate treatments differed, where climate effects were significant.

Where there were more than five sampling times of a given dataset, a repeated measured ANOVA (RMANOVA) was carried out. This identified whether the overall trend of a response differed between climate treatments. Data were log-transformed where assumptions of normality were not met, and each plot was assigned a treatment number, which was treated as a factor.

4.4 RESULTS

4.4.1 CLIMATE AND SOIL MOISTURE

In 2009 the rainfall pattern was similar to long term trends, with a dry warm summer and autumn. However, the main measures of this experiment took place during an exceptionally wet winter period (166mm rain in December compared with a 10-year average of 63mm) followed by a very dry month in June (4.4mm compared with ten year average of 51mm). The soil moisture pattern follows that of the rainfall, with a slight lag time. Despite the differing regimes the spring/summer climate change and variable regimes never significantly differed from one another (Fig. 4.1). The effect of the spring phase of the spring/summer

climate change regime had little discernible effect on soil moisture, possibly because the differences in absolute rainfall volume were small (in 2010).

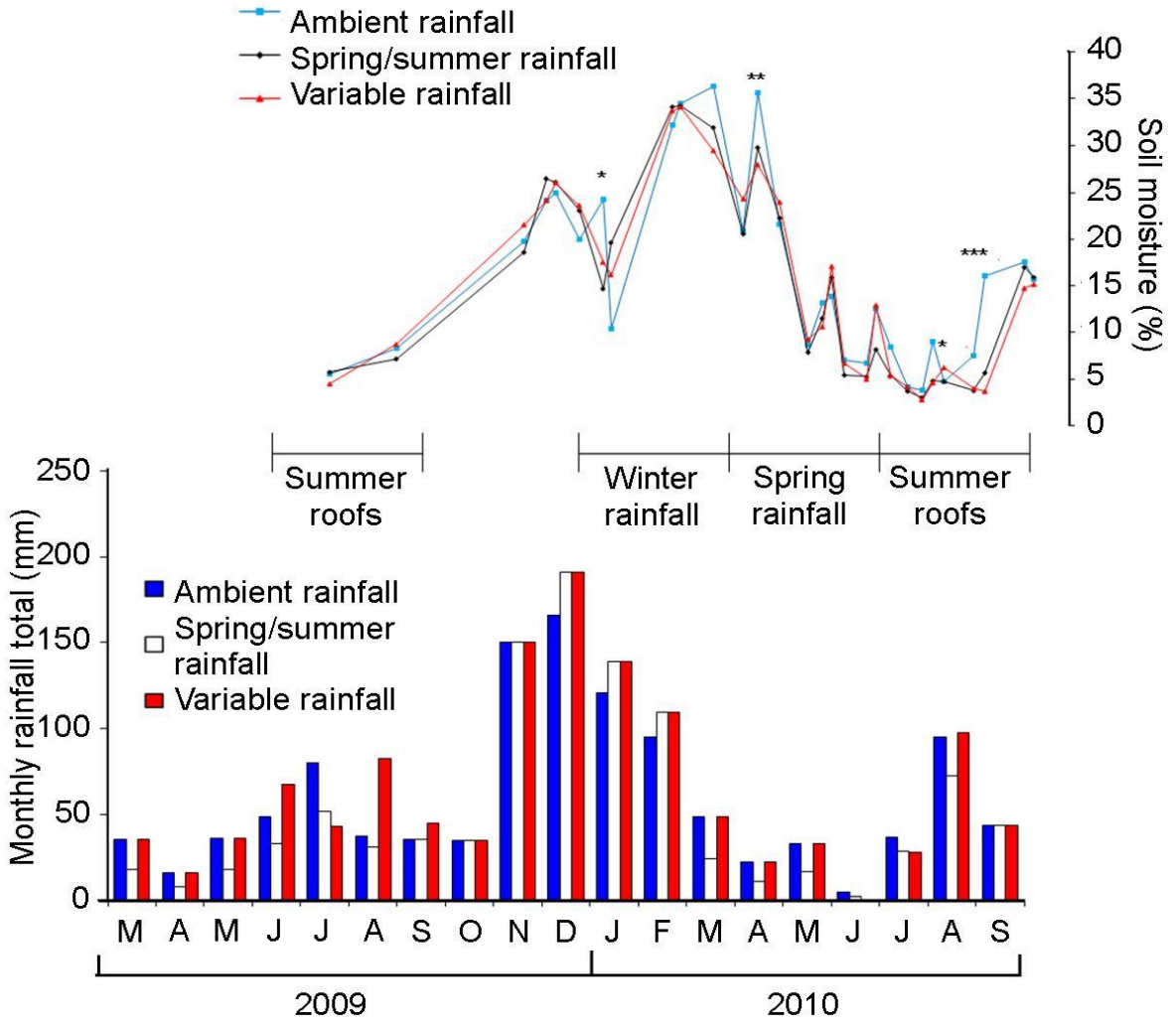

Figure 4.1: Soil moisture compared with applied rainfall over the course of the experiment. Asterisks represent differences in soil moisture between rainfall treatments (*p<0.05, **p<0.01, ***p<0.001). Error bars on the soil moisture lines are omitted for clarity.

4.4.2 VEGETATION SURVEYS

Species richness per m^2 was quite low across all climate regimes including ambient (2-6 spp) at all survey times. The spring/summer climate change regime had very constant species numbers throughout the experiment compared to the variable and the ambient treatments, which were more adversely affected by the drought in early spring 2010. The most dramatic of these was the variable treatment, which was most diverse in May 2010 (ten species), then lost an average of six species by September including *Arrhenatherum elatius, Holcus mollis, Rumex obtusifolius* and *Vicia* species. The most abundant species was *H. mollis* in all treatments and at all time points, except May 2010 when *H. lanatus* briefly superseded it in the spring/summer climate plots. The other common species throughout the experiment were *Cirsium arvense, Agrostis capillaris* and *Lotus corniculatus*, although these were more subject to fluctuation. RMANOVAs showed no effect of climate treatment on species richness per m^2, but there was a very highly significant change over time ($F_{4,36}$=5.93, $p<0.001$, Fig. 4.2a). Percentage of bare earth was closely linked to soil moisture, being highest during the drier seasons, although the sustained drought in June 2010 did not lead to more bare earth than in June 2009 (Fig. 4.2b). There was a significant month by climate interaction, where the variable treatment plots had the least bare earth through the drought period, but had the most dramatic dieback by September despite the increase in soil moisture (RMANOVA: $F_{8,36}$=11,72, $p<0.001$).

Grass percentage cover changed over time in all treatments, though most markedly in the variable treatment (RMANOVA: $F_{4,36}$=11.72, $p<0.01$, Fig. 4.3a). This mirrors the loss of species seen in Fig. 4.2a. The cover of forbs did not change significantly across treatment or time (RMANOVA: $F_{4,36}$=1.63, $p>0.05$ Fig. 4.3b). Grass cover (Fig. 4.2a) increased up to May 2010 then declined due to a combination of natural and manipulated drought. The variable treatment continued to decrease in grass cover to September after three months of prolonged droughts and heavy rainfall pulses, while the other two treatments showed signs of recovery. Forb cover was apparently unrelated to the amount of bare earth in the plots and appeared to be resilient to climate change. The spring/summer climate change treatment plots had less bare ground than the ambient (Fig. 4.3b).

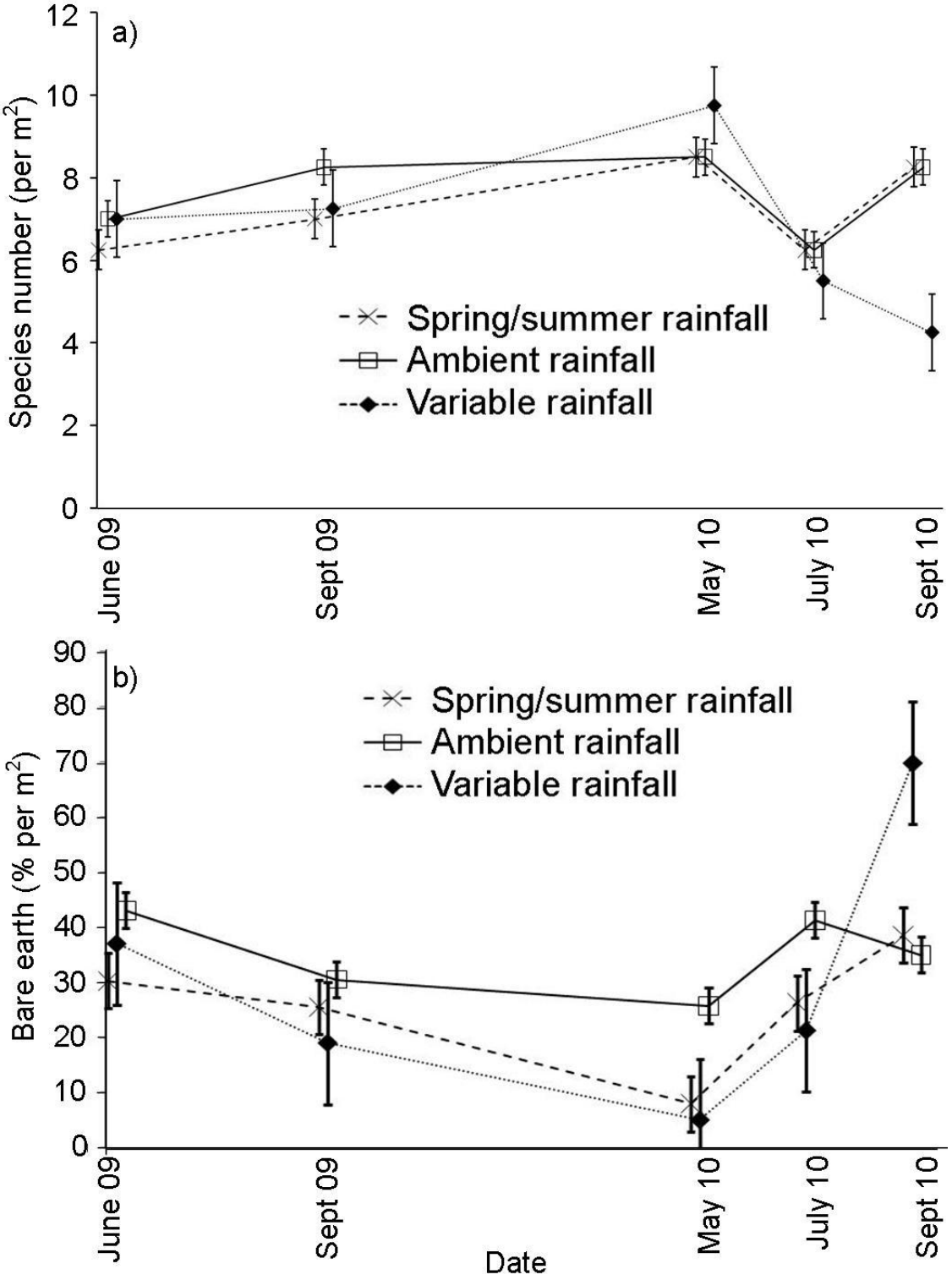

Figure 4.2: a) Average species number in the climate treatments (RMANOVA Month: $F_{4,36}$=5.93, p<0.001). b) Average percentage of bare earth in the plots in different climate treatments (RMANOVA Month*climate interaction: $F_{8,36}$=11,72, p<0.001). Error bars depict ±1 SEM in both cases, and are staggered to illustrate differences between treatments.

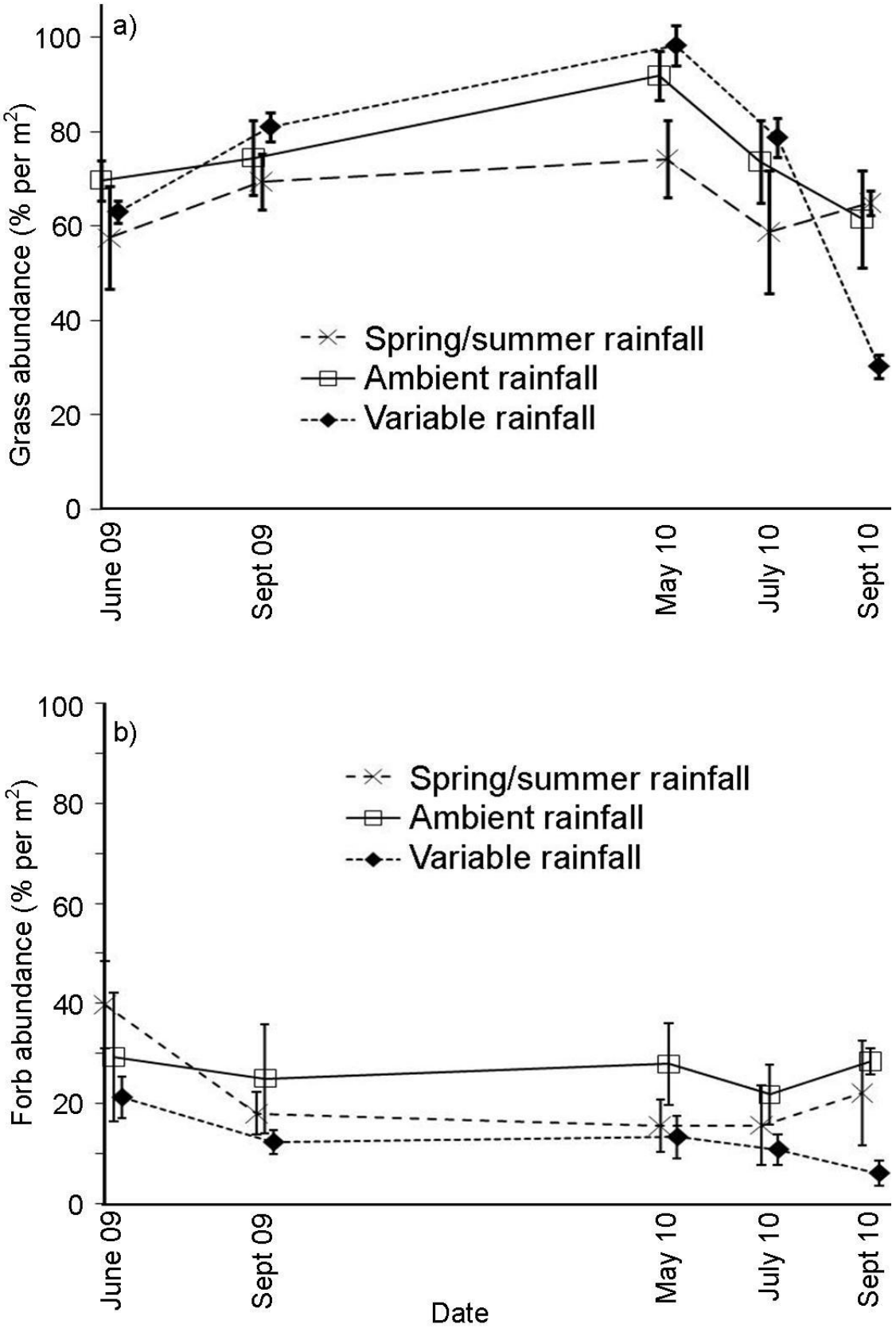

Figure 4.3: Grass and forb percentage cover over time. a) Grass cover changes over time, but not treatment (RMANOVA Month: $F_{4,36}=11.72$, $p<0.01$) b) Forb cover does not significantly change over time or treatment (RMANOVA Month x Climate: $F_{4,36}=1.63$, $p>0.05$). Error bars are staggered in order to illustrate differences between treatments and represent ±1 SEM.

4.4.3 CO_2 AND WATER FLUX

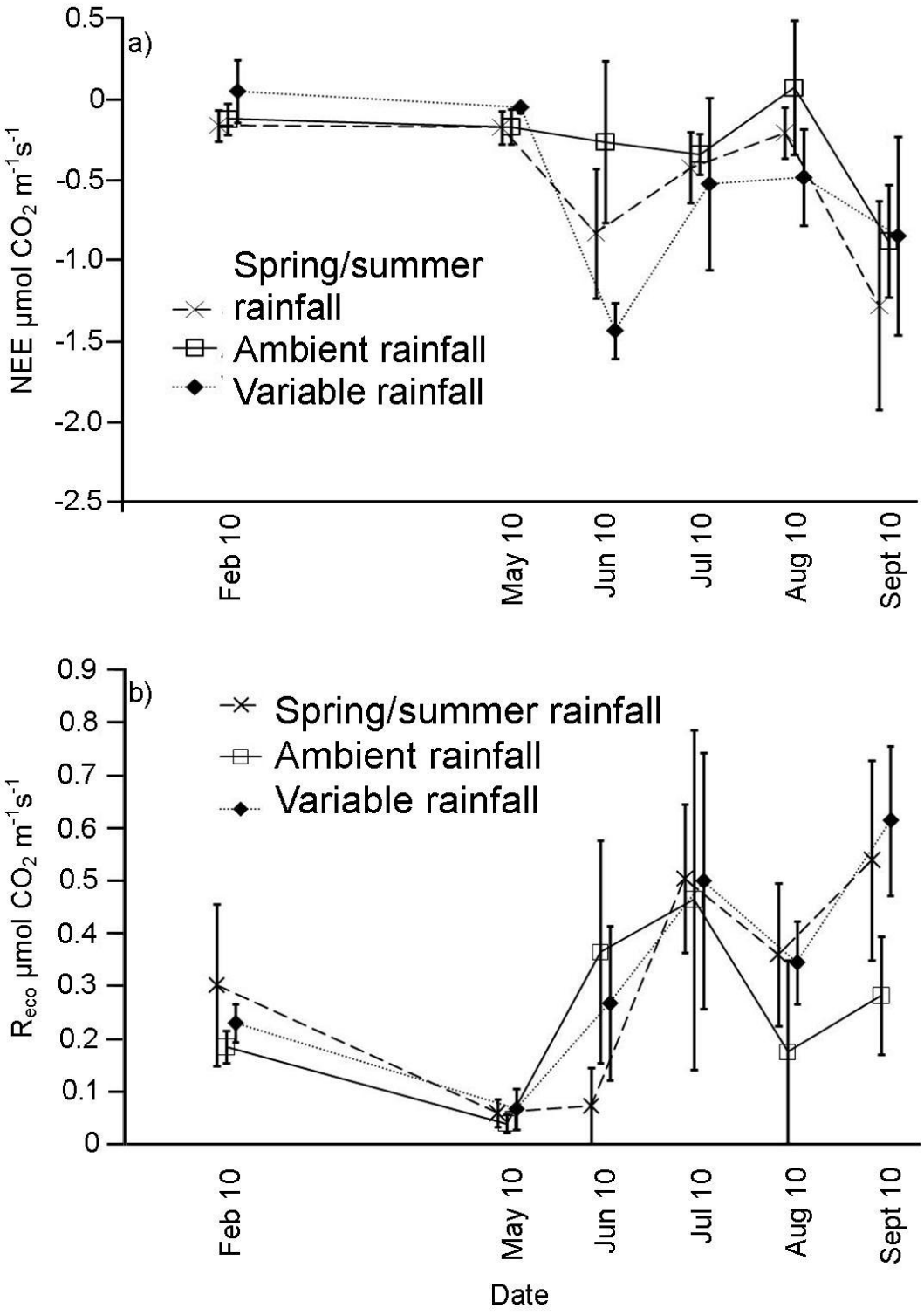

Figure 4.4: a) Net ecosystem exchange showed no difference between climate change treatments (RMANOVA: $F_{2,45}$=1.37, p>0.05) or over time (RMANOVA: $F_{10,45}$=0.32, p>0.05). Note that the x axis becomes more negative; this is to show greater reductions of CO_2 in the chamber. b) Ecosystem respiration changed over time (RMANOVA: $F_{5,45}$=5.11, p<0.001), but there was no effect of treatment (RMANOVA: $F_{2,45}$=0.77, p>0.05) or an interaction between them (RMANOVA: $F_{10,45}$=1.40, p>0.05). Error bars represent ±1 SEM, error bars are staggered to illustrate differences between treatments.

Net ecosystem exchange (NEE) is the balance between photosynthesis and ecosystem respiration (R_{eco}); it was closer to zero (equilibrium) in winter and more negative in summer which corresponds to with peak plant growth, becoming closer to equilibrium in June 10 when natural drought and mass plant dieback occurred (Fig. 4.4a). This change of NEE towards zero suggests photosynthesis was reduced, as the change was not reflected in R_{eco} rates (Fig. 4.4b). In February and May the key abiotic driver was photosynthetically active radiation, which had a strongly positive relationship with NEE (February: $F_{1,3}=21.71$, $p<0.05$, May: $F_{1,9}=12.64$, $p<0.01$). In July and August 2010 soil temperature interacted with NEE, as it became warmer the R_{eco} component of NEE increased and brought it closer to equilibrium (July: $F_{1,9}=9.13$, $p<0.05$, August: $F_{1,9}=8.51$, $p<0.05$).

R_{eco} was not significantly affected by climate change manipulations (Fig. 4.4b); the main driver appeared to be soil temperature, which was negatively correlated with R_{eco} in May ($F_{1,9}=10.94$, $p<0.05$) and June ($F_{1,9}=14.96$, $p<0.01$). It was also negatively correlated with soil moisture in May, so warmer (and drier) soils had lower rates of R_{eco} ($F_{1,9}=6.13$, $p<0.05$).

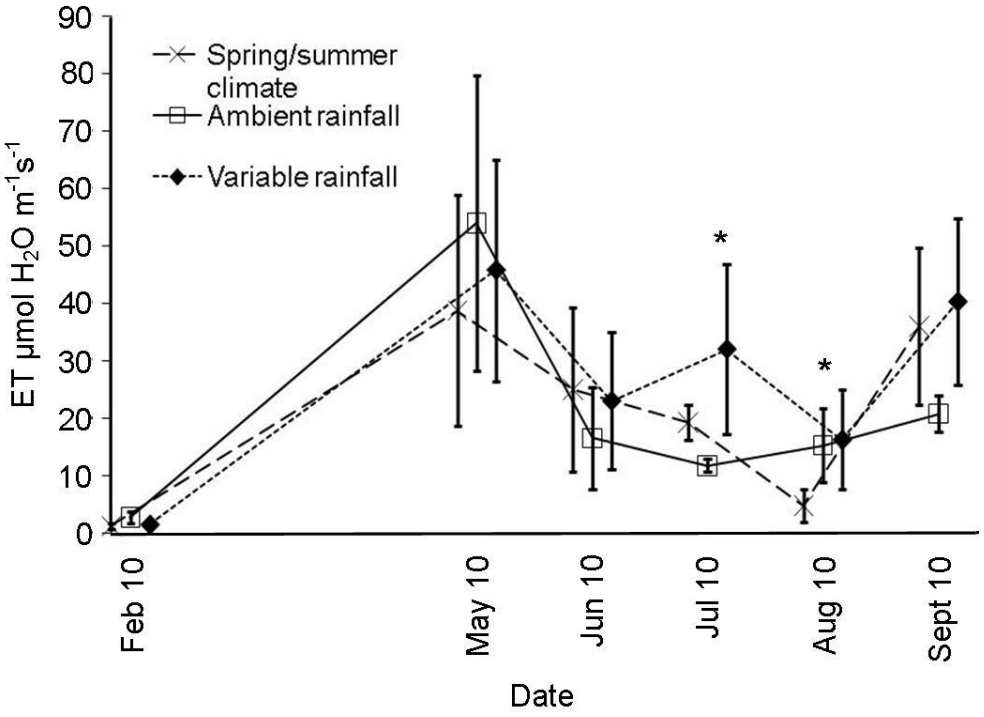

Figure 4.5: Evapotranspiration change over time with treatment effects (RMANOVA: $F_{5,45}=10.74$, $p<0.001$). There are significant effects of climate change in July (one-way ANOVA $F_{2,7}=7.20$, $p<0.05$) and August (one-way ANOVA $F_{2,7}=11.87$, $p<0.05$). Error bars are staggered to elucidate treatment effects. Significance stars denote *$p<0.05$, **$p<0.01$, ***$p<0.001$.

The pattern of evapotranspiration (ET) was most likely to reflect the pattern of growth and natural water stress upon the system throughout the summer (Fig. 4.5). ET was strongly reduced by increasing soil temperature (closely linked to plant species cover Fig. 4.2b) in May and August 2010, ($F_{1,9}$=85.31, p<0.001 and $F_{1,9}$=8.64, p<0.05 respectively). In July, the spring/summer treatment was significantly lower than the ambient ($F_{2,8}$=7.2, p<0.05). In August ambient had lower ET rates than spring/summer ($F_{2,8}$=11.87, p<0.05).

4.4.4 MINERALISATION RATES OF N

Mineralisation rate of nitrogen was affected by soil moisture in the December-March period ($F_{1,7}$=7.38, p<0.05), but not in warmer periods through the spring and summer. Mineralisation rates increased through the year, concurrent with increasing warmth and microbial activity (Fig. 4.6). In the June-September period, the six months of artificial drought led to lower mineralisation rates for the spring/summer regime ($F_{2,8}$=4.11, p<0.05).

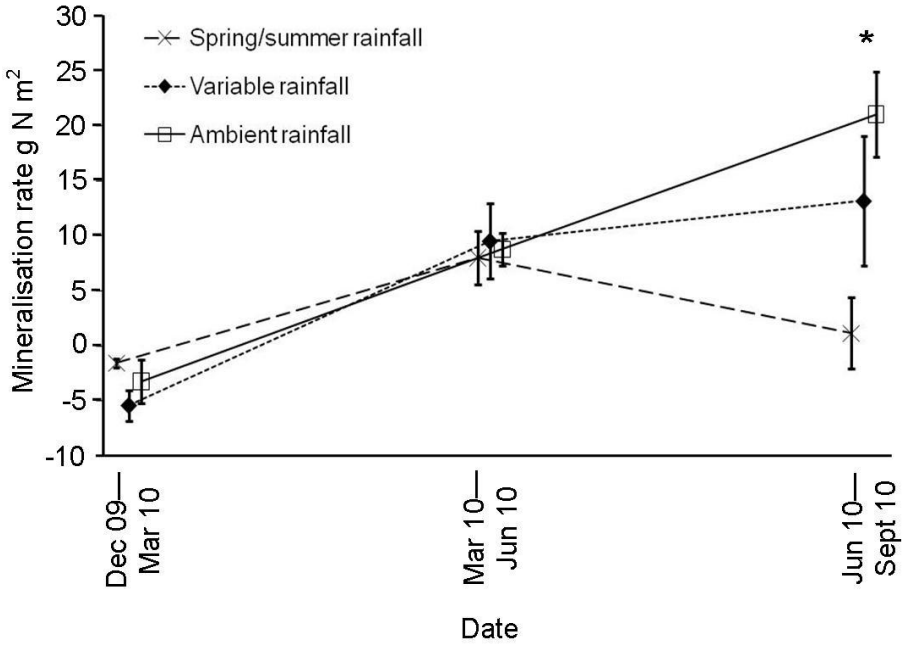

Figure 4.6: Mineralisation rate over the growing season of 2010. Climate change was significant ($F_{2,8}$=4.11, p<0.05). Asterisks signify *p<0.05, **p<0.01, ***p<0.001.

4.4.5 NITRIFICATION

Nitrification rate increased over the course of the growing season in a similar manner to mineralisation rates, although the spring/summer treatment increased very little over the course of the study. There was a significant interaction between nitrification and soil moisture in the period June-September 2010 where spring/summer and ambient plots showed no effect of soil moisture, while nitrification was generally higher in variable plots, with a negative relationship with soil moisture (Fig. 4.7, $F_{2,7}$=11.45, $p<0.05$).

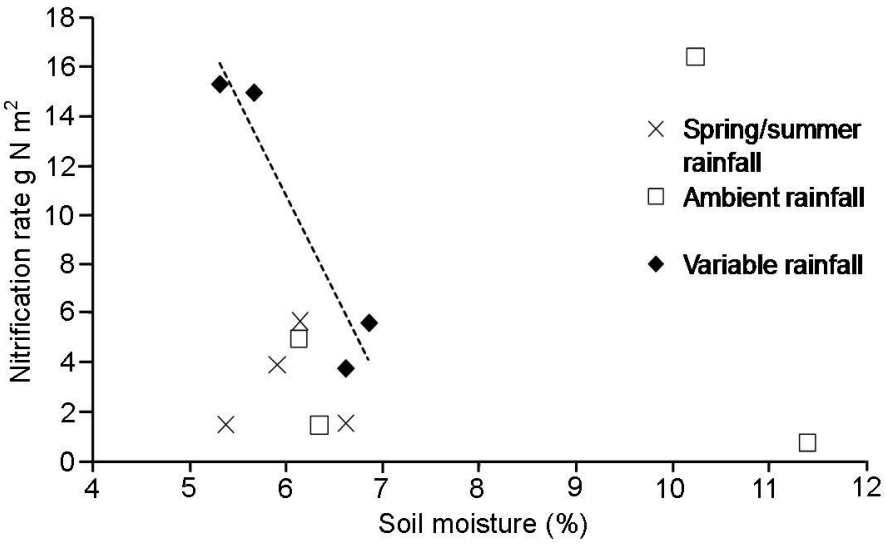

Figure 4.7: A significant interaction between soil moisture and nitrification in June-September 2010. Trend lines are presented where relevant ($F_{2,7}$=11.45, $p<0.05$).

4.4.6 SOIL NUTRIENT CONTENTS

After the first season of climate change treatment, no significant climate effects on soil nutrient concentrations were evident. In September 2010 soil N concentration was lower under the spring/summer climate regime after the low rates of mineralisation and drier conditions than the other treatments (Fig. 4.8, $F_{2,6}$=4.44, $p<0.05$).

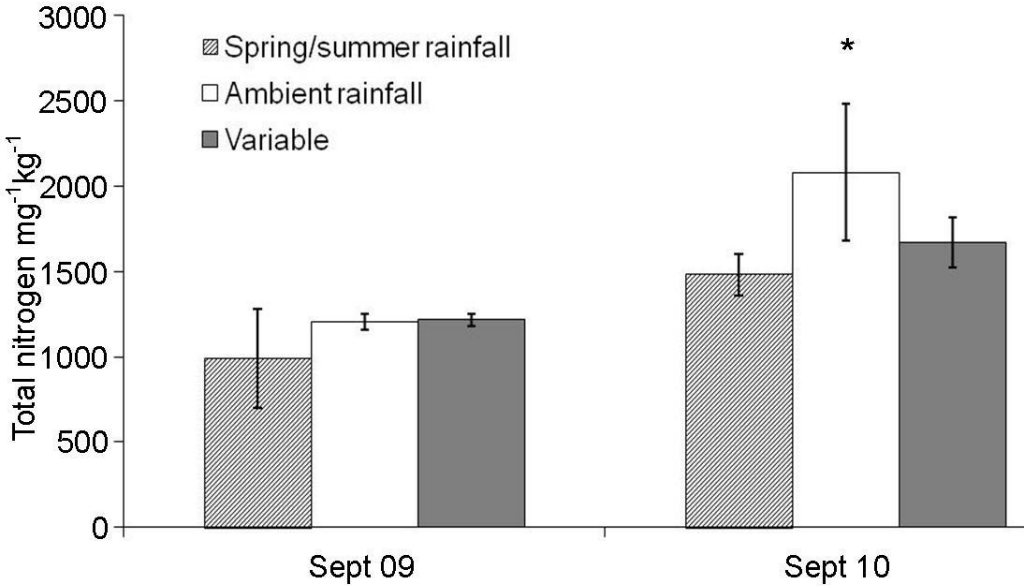

Figure 4.8: Effect of climate change regimes upon nitrogen concentrations in the soil in September 2009 and 2010 (2009: $F_{2,6}$=0.56, p>0.05, 2010: $F_{2,6}$=4.44, p<0.05)

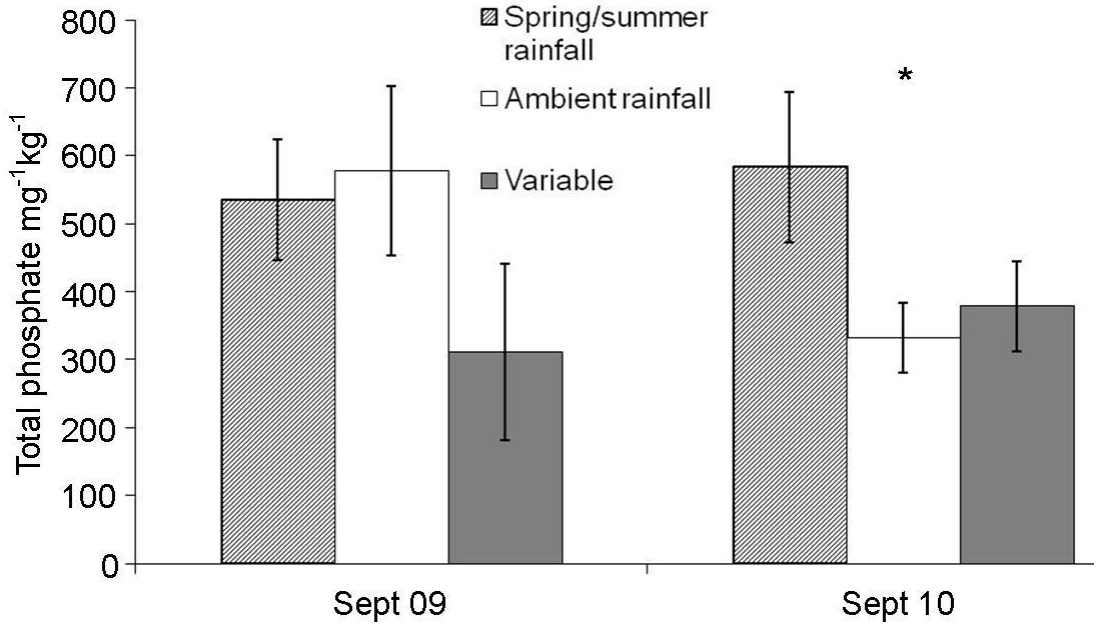

Figure 4.9: Effect of climate change regimes upon phosphorus concentrations in the soil in September 2009 and 2010 (2009: $F_{2,6}$=0.97, p>0.05, 2010: $F_{2,6}$=5.82, p<0.05). Significance stars signify *p<0.05.

Soil P content was also not significantly affected by climate in the first year, but showing strong effect of climate in the second year. Contrary to the pattern seen for nitrogen, the spring/summer rainfall treatment was significantly more P rich than the other treatments in 2010 (Fig. 4.9, $F_{2,6}$=5.82, p<0.05), and the ambient treatment was substantially lower in P in 2010.

4.4.7 EXTRACTABLE NUTRIENTS

There was a significant time effect on extractable ammonium, where very wet conditions in winter led to lower concentrations, and high concentrations occurred in summer after high mineralisation rates and plant dieback (RMANOVA: $F_{8,72}$=17.95, p<0.001), although interactions between climate and time were not significant ($F_{16,72}$=0.87, p>0.05). Ammonium decreased to very low levels through autumn and winter 2009, before increasing to ~25mg^{-1} kg^{-1} in July 2010.

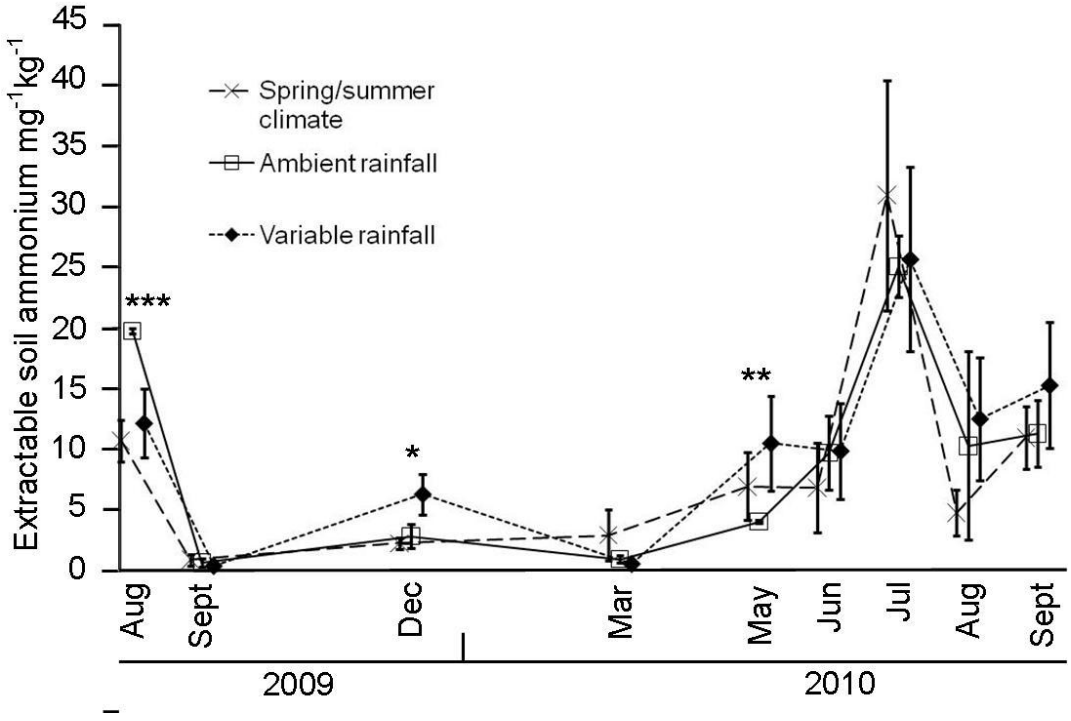

Figure 4.10: Change in concentration of extractable ammonium in the soil over the course of the experiment (RMANOVA Month: $F_{8,72}$=17.95, p<0.001). Error bars are standard error of the mean, and are staggered to highlight differences in treatment, not time. (*p<0.05, **p<0.01, ***p<0.001).

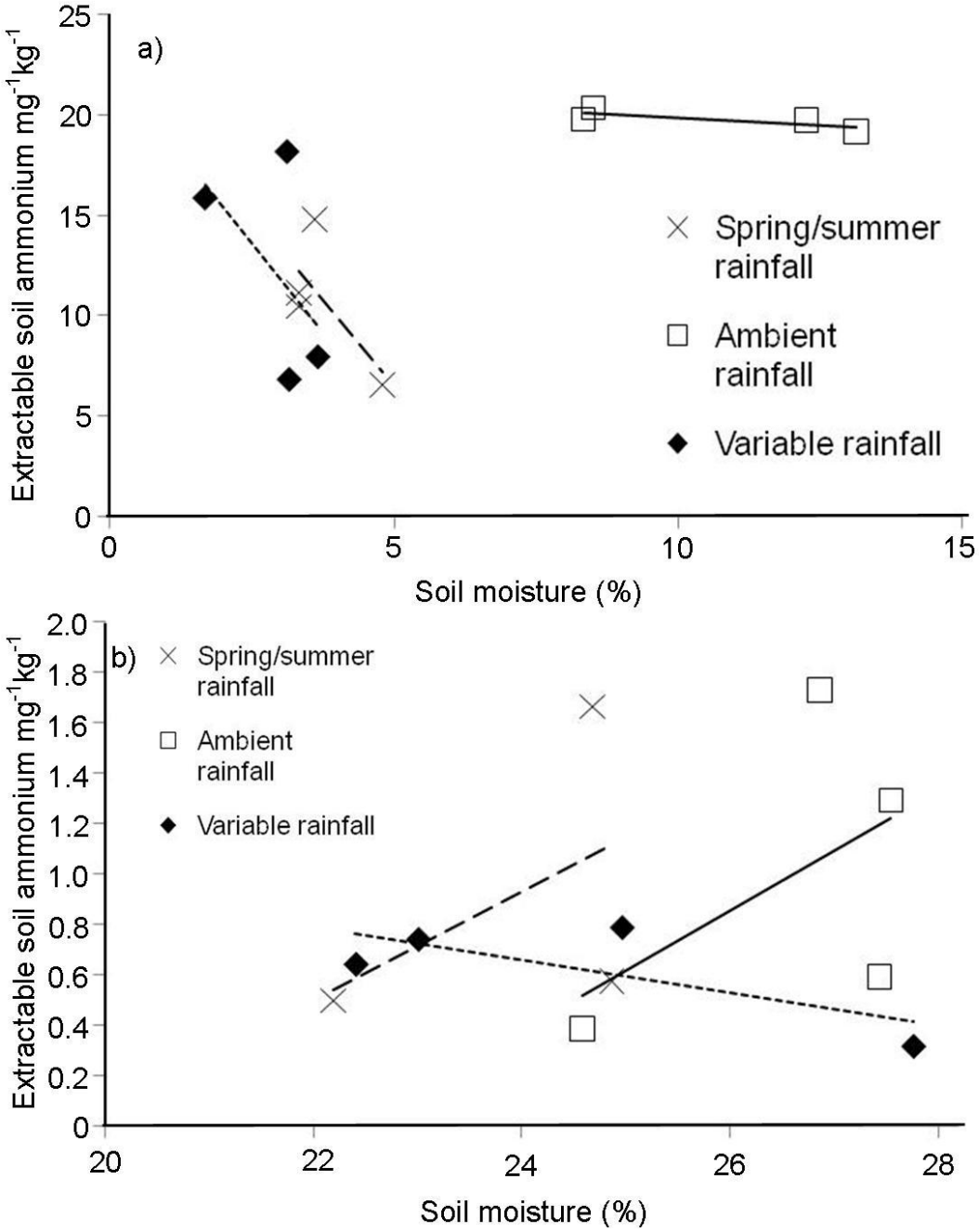

Figure 4.11: a) Effect of soil moisture upon concentration of extractable ammonium in August 2009 ($F_{2,6}$=321.59, p<0.01). b) Effect of soil moisture upon extractable ammonium concentration in March 2010 ($F_{2,6}$=77.79, p<0.01).

For some individual months there were significant climate effects (Fig. 4.10). August 2009 saw much lower ammonium levels in both climate change treatments than ambient during the dry summer conditions and high plant dieback ($F_{2,6}$=1920.68, p<0.001). Over the winter ammonium concentrations were low, although concentrations increased in the climate change plots in December 2009 during the winter addition treatment (variable treatment

higher than the others, $F_{2,6}$=10.1, p<0.05) and March 2010 (ambient treatment higher than the others, $F_{2,6}$=54.99, p<0.01). Soil moisture was associated with much lower soil ammonium in climate change treatments in August 2009 ($F_{2,6}$=321.59, p<0.01, Fig. 4.11a). In March 2010 there was a positive relationship between ammonium and soil moisture, with the drier ambient plots having a higher ammonium concentration ($F_{2,6}$=77.79, p<0.01, Fig. 4.11b).

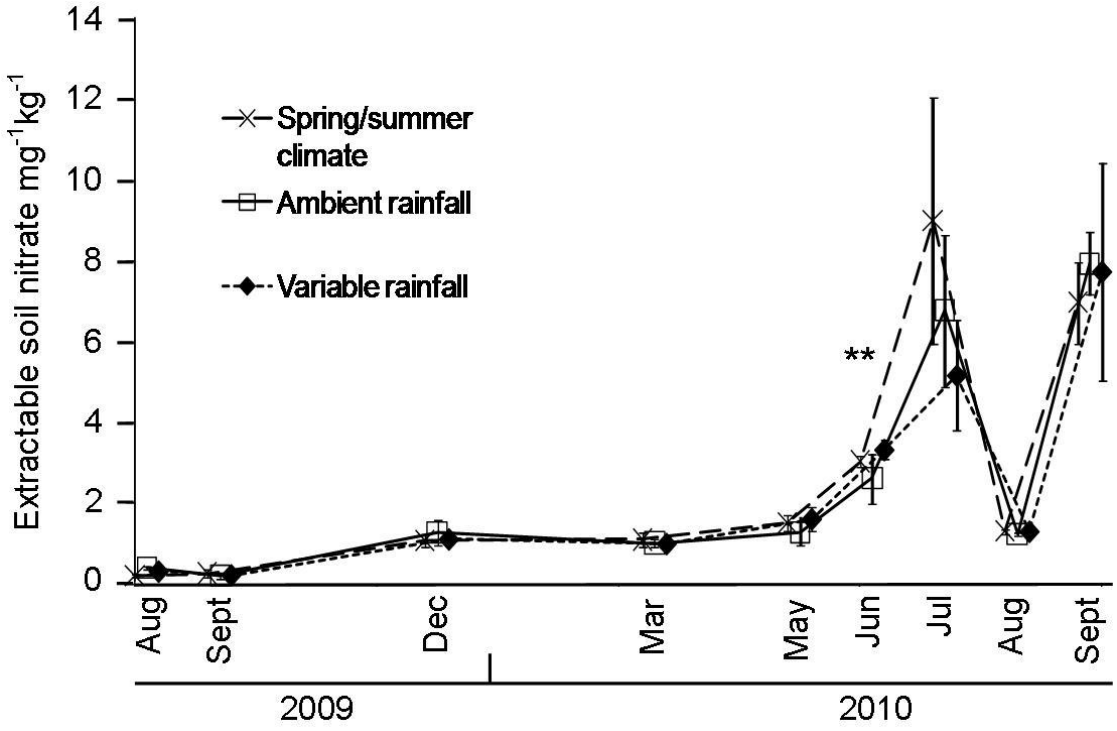

Figure 4.12: Change in concentration of extractable nitrate in the soil over the course of the experiment (RMANOVA: $F_{8,72}$=83.94, p<0.001). Error bars are ±1 SEM, and are staggered to highlight differences in treatment (*p<0.05, **p<0.01, ***p<0.001).

Extractable nitrate concentrations were very low through the summer and winter of 2009 when water was freely available, and increased throughout the dry summer of 2010 (Fig. 4.12; RMANOVA: $F_{8,72}$=83.94, p<0.001). In June 2010 the spring/summer climate change plots had significantly higher nitrate concentrations than ambient and variable treatments after undergoing three months of drought ($F_{2,6}$=11.14, p<0.01). In late summer, there was a small peak in July and a large increase of nitrate in September during the period of plant senescence.

Phosphate was at a low concentration throughout the experiment, with significant increases in September 2010 in all treatments except for the spring/summer climate change (RMANOVA $F_{8,72}$=14.64, p<0.001). Phosphate availability followed a similar pattern to nitrate, with an earlier peak in June 2010 (Fig. 4.13). The climate treatments only had significant effects in September 2010 after the shelters had been removed ($F_{2,6}$=9.3, p<0.05), where the ambient treatment has a significantly higher phosphate concentration in the soil than the spring/summer climate change treatment. There was no effect of soil moisture.

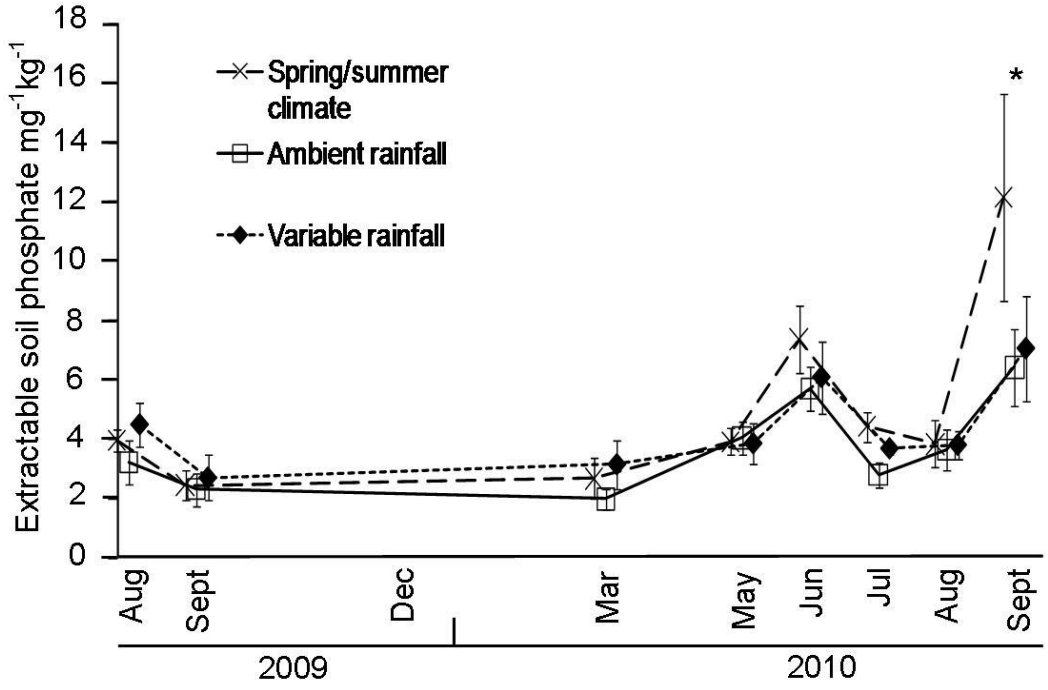

Figure 4.13: Change in concentration of extractable phosphate in the soil over the course of the experiment (RMANOVA Month x Climate: $F_{6,63}$=14.64, p<0.001). Error bars are standard error of the mean, and are staggered to highlight differences in treatment, not time. (*p<0.05, **p<0.01, ***p<0.001).

4.4.7 LEACHING LOSSES

Leaching losses in spring 2010 were not affected by climate treatment. Concentrations of leached ammonium were consistently low over the spring period, when mineralisation rates

and availability of nutrients were also low, while the temporal pattern of nitrate mimics the rainfall pattern over these three months (Fig. 4.1; Appendix 4.4, Fig. 4E).

4.5 DISCUSSION

The application of a drought treatment to temperate grassland was very effective, but in this case the lack of ambient rainfall between April and July 2010 were associated with stronger effects than the artificial ones imposed. The results suggest that a combined spring and summer drought have a stronger suppressive effect upon ecosystem functioning than long periods of summer drought interspersed with heavy downpours. This effect can be simply summarised by noting that in general the spring/summer treatment was associated with a net decrease in processes driven by microbial activity, while the variable rainfall treatment had a more marked impact upon plant species presence and cover. The stronger effect of changing spring rainfall rather than the interval between summer rainfall events contrasts with other experiments which found very detrimental effects of altering rainfall frequency, although it is possible a longer experimental period was needed (Hall & Scurlock 1991; Easterling 2000; Knapp *et al.* 2002; Suttle *et al.* 2007). Overall, the Silwood grassland system appears fairly resilient to periods of severe stress, as effects of drought on ecosystem function occurred shortly after treatments began, but most were transient and disappeared after the rainfall manipulations ended in September 2010.

The natural drought in June 2010 led to a build-up of soil nutrients and total lack of leaching throughout the growing season, indicating that while mineralisation of nutrients was still occurring at a low level, plants unable to make use of them. Other processes, including net ecosystem CO_2 exchange (NEE) and ecosystem respiration (R_{eco}), responded more to the natural lack of spring rainfall than the two climate scenarios imposed despite the mass dieback of grasses in the variable treatment. In the case of leachate before May 2010, nitrate followed the same pattern as rainfall because anions are very readily washed out of soil, which is likely to have superseded the effect of the climate treatments (Christensen & Christensen 1991). Other studies attribute similar lack of large-scale responses to climate change to small-scale soil effects and plant community reorganisation (Fridley *et al.* 2011), although these links were not apparent here. It is likely that the increase of rainfall after June 2010 caused a number of changes in the soil, including microbial cell lysis due to sudden

osmotic changes, comminution of soil aggregates and releases of nutrients through renewed decomposition, which would have made available formerly inaccessible sources of nutrients (Börken & Matzner 2009). These changes would have contributed to the renewal of microbial driven processes such as R_{eco} and mineralisation rates.

4.5.1 SPRING/SUMMER TREATMENT

The decline of forbs under the spring/summer treatment at the beginning of 2010 was exacerbated by the prolonged natural drought in May and June, and they never recovered to the same abundance of ambient or variable plots. This is likely to be caused by seedling mortality when the treatment changed from rainfall addition to reduction between February and March, because seedlings are the most stress-sensitive life stage (Padilla & Pugnaire 2007). Seedling mortality under drought is non-species specific (Chesson *et al.* 2004; Lloret *et al.* 2009; Walck *et al.* 2011), so in future years there could be a shift in dominant species due to the decline of the seed bank from 2010 and weedy species quickly adapting.

R_{eco} was suppressed through the growing season in the spring/summer treatment, although when the rainfall stress was alleviated at the end of the season it was very quick to recover to the level of the ambient plots. While this could be due to recovery of microbial populations and decomposition of accumulated plant necromass, mineralised nitrogen was significantly lower in this treatment than the ambient by the end of the summer. A likely explanation for this anomaly is that this increase in R_{eco} was mainly from a surge in root respiration, supported by the recovery of grass species in September, and that microbial biomass was metabolically inactive, or was reduced through either cell death because of prolonged drought, or cell lysis following osmotic stress when precipitation was no longer limiting (Börken & Matzner 2009). Microbial activity was seemingly more affected by reduced rainfall throughout the entire growing season (spring/summer treatment), than by prolonged drought and heavy rainfall events (variable). Many workers have described similar trends in the study of mineralisation and microbial activity, but microbial responses to rain pulses are poorly understood due to indirect confounding effects of plant diversity (and thatch), and differing methodologies for measuring microbial biomass and activity (Xu *et al.* 2004; Castro *et al.* 2010; Kardol *et al.* 2010; Maestre *et al.* 2010). Börken and Matzner (2009) however, state that during drought a lot of soil nitrogen is immobilised by microbes

which would not be readily released after a rainfall pulse, which would explain the higher concentration of total soil nitrogen in ambient plots where mineralisation by microbes was not constrained.

In the summer of 2009 the ambient rainfall was much higher than in 2010, and the spring/summer treatment had higher extractable ammonium levels than the other treatments in 2010. This is likely to be due to lower plant uptake after a very dry spring. Soil moisture appears to have a reverse parabolic relationship with ammonium accumulation in the soil; when soils were very wet, ammonium was low, probably through leaching and waterlogging of soil, but when it was dry ammonium was also low due to immobilisation or low rates of mineralisation, which could have serious soil fertility consequences under future climate changes (Fay *et al.* 2003; Emmett *et al.* 2004; Kardol *et al.* 2010).

4.5.2 VARIABLE TREATMENT

The variable scenario saw a marked loss of species and decreased plant cover compared with the ambient and spring/summer treatments, although very few ecosystem processes differed significantly from the ambient plots. The greater decline of grass cover than forbs through the growing season and more dieback in September under the variable treatment was consistent with Morecroft *et al.* (2004) and Stampfli & Zeiter (2008), who showed that grass seedlings are more susceptible than forb seedlings to long periods between rainfall inputs. Fay & Schultz (2009) reported the opposite effect, but their species pool consisted of only two grasses. Facilitation of forbs may occur where grasses create a closed canopy in spring and thus more humid conditions for under-storey plants, then later a thick thatch of decomposing litter and labile carbon inputs (Greenlee & Callaway 1996; Tewksbury & Lloyd 2001). The shallow roots of these small annual plants are then ideally placed to take advantage of short term small rainfall pulses (Padilla & Pugnaire 2007).

Microbial (and root) based activities were not as affected by pulses of water and short term drought as described in other studies, and it was long-term (spring/summer) drought that was linked to an appreciable decrease in activity, with a net decrease in CO_2 efflux for the season (Lee *et al.* 2004; Börken & Matzner 2009). R_{eco} was fairly unresponsive to soil drying in this study and many others (Fierer *et al.* 2003; Maestre *et al.* 2010, though see Knapp *et al.*

2002). St Clair *et al.*'s (2009) mesocosm experiment only saw significant drought treatment effects after six seasons, which they postulated was due to a need for sufficient biomass accumulation. The lack of effect of variable rainfall upon R_{eco} and mineralisation suggests that the microbial community is more resilient to rainfall pulses than prolonged drought. There was no difference in availability of nutrients between the variable and ambient plots. Given the loss of much living tissue in the variable treatment, plant uptake was unlikely to be occurring at the same rate as the ambient rainfall control. This then suggests that microbial immobilisation was occurring in the variable treatment, resulting in similar nutrient pool sizes between the variable and ambient treatments. The net effect of ecosystem function with variable rainfall pulses appears to be more centred on plant community composition than nutrient and water based functions, suggesting that effects on microbial and plant driven processes are compensated for in some way, potentially after rainfall pulses.

While more time is needed for this experiment to test for effects on the seed bank, and a closer investigation into microbial biomass and activity, the results reported in this study show how important monitoring different precipitation scenarios will be in the coming century. By illustrating the different impacts prolonged drought and variable rainfall exert on a grassland system, it is apparent that more consideration needs to be afforded to testing many different scenarios within a field experiment framework to ensure a more accurate picture of the effect complex climate changes will have.

Figure 5: Roofing completed, autumn 2008

CHAPTER 5: STATISTICAL MODELLING OF ECOSYSTEM FUNCTIONS IN A GRASSLAND EXPERIMENT USING TRAIT BASED MEASURES

5.1 ABSTRACT

Grassland ecosystems are being altered by a number of simultaneous stresses, and much effort has focussed on describing the effect plant assemblages have on ecosystem processes. The idea of using functional effects traits to characterise ecosystem processes in order to predict responses to global change is gathering support. A common approach to doing this has been to weight traits by abundance or biomass and explain function in terms of these measures, and those of the abiotic environment. In this study this approach was used to describe a set of ecosystem function measures taken throughout the year in a mesotrophic grassland in South-East England, which had been subjected to climate and diversity manipulations. I used a model comparison and selection approach to build general linear models starting with abiotic variables, followed by biomass measures, community weighted mean traits and trait divergence measures. The measures used here include ecosystem respiration, net ecosystem CO_2 exchange, evapotranspiration and extractable soil nutrients. The selected models showed that abiotic variables generally explained more variation than plant traits, especially outside the growing season. In the autumn, biomass measures explained variation in ecosystem respiration and evapotranspiration, suggesting that physical and chemical characteristics of senesced material have stronger effects on these processes than traits of the vegetation. Extractable soil phosphorus was explained by aboveground biomass and assemblages dominated by annual plants throughout the year, possibly indicating the high demand of this nutrient by legumes, which were abundant in this group. In the summer, leaf and root traits including specific leaf area and root to shoot ratio explained a significant proportion of the variance in functions including net ecosystem exchange. Important factors remained constant across seasons, lending support for the method. This technique could potentially be scaled up across grasslands, as the strong seasonality of key descriptors allows for predictions of the most important variables. A good next step would be to apply this method to a range of different grasslands to assess its applicability.

5.2 INTRODUCTION

Natural ecosystems are facing increasing pressure from global change factors including climate change, nutrient loading and land use change (Millennium Ecosystem Assessment, 2005). In order to preserve these systems to maintain vital ecosystem services, research is needed to gain a mechanistic understanding of their processes. Ecosystem functions such as water and nutrient cycling are modified by a number of factors including abiotic variables such as soil moisture and pH, and characteristics of the plant community present. There have been recent calls to find general principles to quantifying plant species effects upon a system (McGill 2006) by using "functional effects traits", these are plant characteristics that have an effect on their surroundings, (e.g. aboveground biomass, specific leaf area and leaf nitrogen content, Lavorel & Garnier 2002). By linking traits to functions, we may be able to make general predictions of how ecosystems will respond to global change, based upon the range of traits present in the system (Dìaz & Cabido 1997; Chapin *et al.* 2000). Accordingly, studies and debates have proliferated over the last two decades, mainly discussing effects traits (Lavorel *et al.* 1997; Dìaz & Cabido 2001; Lavorel & Garnier 2002; Cornelissen *et al.* 2003; Hooper *et al.* 2005), but some considering the theoretical links between response and effect (Gross *et al.* 2008; Klumpp & Soussana 2009). Traits have been used to demonstrate functional relationships in many areas of the literature, although it is unclear whether the direction of the relationship would be the same under particular stresses, at different times of year or across extensive environmental gradients (Shipley 2006).

Effects traits are simple to measure and many have considerable support in the literature for their close links to processes, such as specific leaf area (SLA) being a descriptor of resource use strategy (low SLA: conservative, high SLA: exploitative) and leaf toughness, which affects decomposition rates (Corneliessen & Thompson 2008) and photosynthetic rates (Reich *et al.* 1998). There is also some theoretical evidence that it contributes to leaf nitrogen and leaf gas exchange (Meziane & Shipley 2001). Many of these traits can be scaled up from leaf and community to ecosystem, i.e. they can be extrapolated arithmetically to larger scales (Dìaz & Cabido 1997). Net photosynthetic rate is one example of this. However, many other traits, for instance those modified by species interactions, are difficult to scale up without the aid of very complex equations such as Lotka-Volterra models, and as such are almost impossible to apply general principles to (McGill *et al.* 2006). Therefore, it is more appropriate to carry out simple models at the plot level.

Trait diversity in an assemblage can be linked to respective species biomass in a system in order to quantify species effect per unit biomass. For example, a nitrogen fixer would fix more nitrogen in monoculture in the absence of competition with many other species. Therefore the traits of each species must be weighted by their abundance in order to calculate a true trait average for the site (Grime 1998; Lavorel & Garnier 2002; Garnier *et al.* 2007). Defining species' impacts upon a system using biomass has long been recognised as a means of describing observed variables, beginning in rocky intertidal pools (Batzli 1969) before being used to describe response to climatic gradients of different grasses photosynthetic pathways (Boutton *et al.*1980). Linking legume biomass to biogeochemical cycling (particularly nitrogen) is also a long-running idea (Woodmansee & Duncan 1980).

The theory of mass of a species within a community being a key predictor of function was crystallised into a "biomass ratio" hypothesis by Grime (1998). The theory states that the effect of each species present in an ecosystem on its surroundings will be dependent upon its proportion of the total biomass of that system. Each species' traits should be weighted by its total biomass, then all species trait values summed across the site. Therefore, ecosystem processes should be predictable using carefully selected aggregate trait measures (Wardle *et al.* 1999; Garnier *et al.* 2007; Quetier *et al*. 2007; Vile *et al.* 2006). The biomass ratio hypothesis, however, has not been wholly supported because some studies have reported that weighting community traits by abundance have stronger results than biomass (Quested & Eriksson 2006). To counteract this, we suggest that plot-level trait means and biomass are separate variables and both should be built into models. This approach distinguishes whether the function can be predicted by some characteristic of composite biomass, so a total biomass value is all that is required, or the finer detail of mean trait values is more descriptive. Some workers have suggested that since the most dominant species are likely to have the strongest impacts upon a system, only two or three of the species making up the majority of the biomass should be included (Garnier *et al.* 2004). However, it is likely that rare or invasive species might comprise unusual traits, forming a functional type that would need to be included. Most studies have focussed upon leaf level responses to factors such as grazing (Klumpp & Soussana 2009; Zheng *et al.* 2010), or their effects on decomposability (Dìaz *et al.* 2007; Fortunel *et al.* 2009), and have confirmed that biomass is an important component during model fitting, but there is a lack of information on its relationship with nutrient, carbon and water cycling.

In order to link biomass, ecosystem trait distributions and ecosystem processes, a number of approaches have been proposed. RLQ analysis consists of a three-table ordination method combining traits, abundance and environmental variables (R, L and Q refer to each table, Doledec *et al.* 1996). While these components are indeed useful, a number of perceived problems with the models (chiefly that presence-absence data could only be entered in binary form), meant it has not been widely adopted (Dray & Legendre 2008). In the meantime, many metrics have been proposed to describe trait distributions in communities, although they have not been used extensively to predict function. There is wide variation in these metrics and their applications; some combine all traits to produce single values of ecosystems such as functional attribute diversity (FAD), which is the number of different trait combinations in a community (Walker *et al.* 1999), while another uses these data to create a "convex hull", where the traits are plotted in multidimensional space to create the smallest shape possible to compare functional richness between ecosystems (Cornwell 2006). These do not account for abundance however, and are fairly abstract in design and execution, so it is difficult to visualise species relationships. Other metrics that do incorporate both are much more widely used, and the recent release of a program called *f-diversity*, means that this approach is likely to become very popular in the future (Casanoves *et al.* 2011). The two metrics that have been most widely used to correlate with function are community weighted means (CWM, Garnier *et al.* 2004, 2007; Dìaz *et al.* 2007; Lavorel *et al.* 2008) and functional divergence (FDvar, Mason 2003), which describe individual traits. When CWMs are closely linked to ecosystem processes, they provide strong support for the biomass ratio hypothesis, as the weighted trait mean is a descriptor of the mean trait value per unit mass (Dìaz *et al.* 2007). FDvar, conversely, describes the variation in trait values within a plot, and is a proxy for complementarity of resource use (Klumpp & Soussana 2009). They are both simple to calculate and to incorporate into generalised linear models which explain ecosystem processes. This approach is more flexible than other methods such as principal components analysis (PCA) and other ordination procedures; the raw trait data can be of almost any type and linear parameters are easier to interpret than clusters of variables. In general, there have been few attempts to apply a linear modelling approach using these metrics to explain functions, so it is unclear whether these metrics are useful across seasons or comparable between years.

Here a linear modelling approach is presented that used ecosystem function data from a climate change experiment in temperate lowland grassland, where the functional diversity

of species was manipulated (see Chapters 2 & 3). Abiotic factors were initially used to find the best model using a standard model simplification technique and likelihood ratio tests, it then added biomass variables, then CWMs of trait values, and then FDvars of trait values in a similar vein to Dìaz *et al.* (2007). Simple models were created to predict a range of ecosystem functions including mineralisation rates, net ecosystem exchange (NEE) and leaching losses over two years of an experiment. The hypothesis is that abiotic and trait/function relationships show consistent patterns over time, and predictive factors vary according to season.

5.3 METHODS

5.3.1 STUDY SITE

The data were taken from an experiment set up on mesotrophic grassland in South-East England in October 2008, running till September 2010. This experiment investigated how the functional diversity of grassland vegetation can alter ecosystem responses to climate change. The study site was ploughed in October 2007 and dominated by *Holcus mollis* and *Agrostis* spp. Species were classified into functional effects groups in order to categorise the native species at the field site into groups that would be most homogeneous in their effects upon processes relating to processing and stocks of carbon, nitrogen and water. Five replicates of each species were grown from seed in a greenhouse and at maturity, a set of functional effects traits were measured, which were used in a hierarchical cluster analysis. The analysis identified three groups of species; perennials grasses and forbs (FG1), caespitose grasses and tall forbs (FG2), and annuals forbs and legumes (FG3), (for more details see Chapter 2). These traits were used later in the modelling method.

The field site was divided into four blocks to account for site differences. The experiment was a two way randomised block design. The first variable was climate, which had two levels, climate change and ambient. The second variable had seven levels, comprising every permutation of the functional groups. The climate change regime consisted of a rainfall regime based upon IPCC projections (IPCC 2007), which describe a 70% reduction in volume in summer in 2099, with lower frequency and higher intensity. This was achieved using rainout shelters which were in place in the summer (JJA). In winter (DJF), a

15% increase in rainfall was projected, so water butts 15% of the plot size were used to collect rainfall for reapplication. There were 56 plots in total (2 climate x 7 diversity x 4 blocks). See Chapter 3 for more details.

5.3.2 ECOSYSTEM FUNCTIONS MEASURED

The measures taken are detailed in Table 5.1 (for further details, see Appendix 3.8-3.11). For the analyses presented here, a set of abiotic measures was used. In October 2008 soil pH was measured in every plot with Mettler Toledo pH meter, Leicester, UK, and was significantly different in one block, in the shadier end of the field at the lowest point of the incline ($F_{3,51}$=5.15, $p<0.01$). The site is surrounded on two sides by trees, resulting in plot-to-plot variation in shading. To account for this, all PAR measures taken for the NEE measures were averaged across all time points (hereafter named "Average light"). The NEE measures were taken random plots every sampling month, to minimise sampling bias. In each plot soil moisture content (SMC) was measured weekly, and then averaged for each month.

5.3.3 BIOMASS CALCULATION

Biomass of each functional group was included as a proxy for the potentially differing physical effects of each group. The groups consisted of perennial plants, bunch grasses and tall forbs, and annual species. To calculate biomass in each plot, a formula was used that differentiated weights of grass and forbs (Everwand & Manning, unpublished data). This metric was obtained from weighing biomass harvested from $1m^2$ plots in Silwood Park, on a variety of grassland plots in a number of different months. It was calculated using the LME function in R2.11.1 (R Development Core Team, 2009). The metric gave the formula $y= -0.58 + 0.93x$ for forbs, and $y= -1.47 + 2.51x$ for grasses, where x was the estimated cover of each species in each plot. Biomass was calculated for each species in each plot according to their type, and negative values were given the value of 0.01g. For each plot, a biomass total for each functional group was then calculated. This was calculated using percentage cover values obtained from the vegetation surveys in May, July and September. When used in models, the biomass values from the closest month to that being tested would be used. For example, if soil N in February 2009 were being tested, the biomass values from May 2009 would be used.

Table 5.1: All ecosystem function measures taken in this study, their frequencies and covariates.

Measure	Method	Abiotic variables measured	Frequency
Species cover	Visual estimation, $1m^2$ quadrat	N/A	May and September, and July in 2010
NEE, Evapotranspiration (ET) and Ecosystem respiration (R_{eco})	Measured with a Ciras-2 Infra-Red Gas Analyser (IRGA) for four minutes in 2009 and with a Ciras-1 IRGA for two minutes in 2010. A chamber cuvette was attached measuring $299cm^2$ area, $8959cm^3$ volume. This was done in the light for NEE and ET and dark for Reco.	SMC (ThetaProbe Soil Moisture Meter HH2 with ML2x probe, Delta-T, Cambridge, UK), soil temperature (Hanna HI 98501, Bedfordshire, UK), photosynthetically active radiation (PAR, Skye Instruments, Wales).	March 09 – Sept 10 Monthly during the summer, every other month for the rest of the year.
Decomposition rates	Litter bags containing dried cut *Holcus mollis* and *Arrhenatherum elatius* left for three, six and nine months.		December 08 - March 09, June 09 and September 09.
Mineralisation and Nitrification	Comparison of extractable N in tubes incubated for three months with a sample taken at the beginning of this period. N was extracted using Allen (1989)'s KCl method.	SMC measured by calculating the proportion of weight lost after soil was dried compared to wet soil.	Every three months between Dec 08 and Sept 10.
Total soil N and P	Soil digestion using the Kjeldahl method (1883) modified with selenium catalyst, colourimetric analysis with Skalar SAN^{++} Continuous Flow Analyser (CFA) (Skalar, York, UK).		Annually in September 2008-2010.
Extractable NH_4, NO_3 and PO_x	Wet soil using Allen (1989)'s KCl method for nitrogen species, and Truogs solution for PO_x. Analysed colourimetrically using a CFA.	SMC calculated by weight as above.	Nov 08 – Sept 10 Monthly through the summer, quarterly the rest of the year.
Cations (Al^{3+}, Ca:Mg)	Edgell's (1993) extraction method, then analysis using inductively coupled plasma atomic emission spectroscopy (ICP-AES Agilent 7500c Series, Wokingham, UK).		July 2009.
Leaching of NH_4 and NO_3	Suction cup lysimeters, analysed using a CFA. A rainfall sample was taken for comparison.		Apr 09 – May 10 Monthly, when conditions permitted.

5.3.4 FUNCTIONAL TRAITS

The functional traits measured originally in the greenhouse were as follows: (see Appendix 2.1 for measures). Root: Shoot, photosynthetic surface area (PSA), specific leaf area (SLA), total foliar N, aboveground biomass (AGB), belowground biomass (BGB) and leaf dry matter content (LDMC). These traits were used to generate CWMs, (Garnier *et al.* 2004; Dìaz *et al.* 2007) and FDvar, (Mason 2003) measures using the program *f-diversity* (Casanoves *et al.* 2011). These measures used the abundance estimations from each vegetation survey to create a plot-level weighted mean and variance measure of traits using Eq 5.1 and 5.2 in Appendix 5.1, and the closest vegetation survey to the timing of the function was used, as with biomass.

5.3.5 STATISTICAL ANALYSIS

A standardised modelling approach was designed to build models of all ecosystem functions measured throughout the experiment, at all individual time points using R2.12.0. This was based upon a stepwise procedure described by Dìaz *et al.* (2007) where variables were assigned one of three categories, which began with simple abiotic measures, and became increasingly complex with biomass and trait measures of the plant community. Each category was modelled and simplified in turn, each building upon the minimum adequate model remaining from the previous category. The models were generalised linear models (GLMs), these had Gaussian errors and an identity link where errors were constantly distributed and normally distributed. Where errors increased with the mean, negative binomial errors with an identity link function were used to meet the assumption of constant variance. Negative binomial errors are often used for overdispersed count data, but a number of authorities have explained that this is a recommendation given the type of fit, and given the finite precision of the continuous variables used in this experiment; this error structure is appropriate (Mood *et al.* 1974; Aitkin 1989).

In the first step of the modelling process, the relationship between function and abiotic factors were assessed. These consisted of the climate change treatment, baseline pH, soil temperature, SMC and average PAR reception throughout the year. For evapotranspiration rates and net ecosystem exchange, the PAR measures taken simultaneously with the flux measures were used instead. For each abiotic variable was tested

for significant main effects, first-order interactions and then quadratic relationships. The resulting model was then simplified using likelihood ratio tests so that only significant terms remained (Crawley 2005). This simplification began by removing higher order interactions and then main effects where model likelihood was not improved.

The terms remaining after the first phase were retained in all models throughout the next level of the analysis, which was to test for interactions and main effects of summed biomass of the three functional groups. A biomass measure for each FG in each plot was added to the model with all two-way interactions between biomass measures. This was simplified in the same manner. When the minimum adequate model was obtained, two-way interactions were tested between remaining biomass measures and any abiotic variables, and simplified again.

The third step of the analysis explored the relationship between the ecosystem functions and CWMs. CWMs were added individually to the model from the second step. They were then removed and another was tested. If more than one was retained in the model, the most appropriate CWM was determined using Akaike's Information Criterion (AIC) (Johnson & Omland 2004). The chosen CWM was then modelled with interaction terms with other terms of the model. Finally, the likelihood ratio procedure was carried out again so that only significant terms were kept.

The third step of the procedure was then repeated with FDvar of individual traits, and the minimum adequate model was derived (similarly to CWM). Finally, the significance of each parameter in the final model was assessed using likelihood ratio deletion tests in order to determine whether the ones added later partitioned the variance more effectively than the earlier ones, rendering them non-significant and therefore able to be removed. The final model retained only significant terms at the $p<0.05$ level or higher. The contribution of each parameter to the minimum adequate model was estimated by;

1. Calculating the variance of the null model (total sum of squares, Crawley 2005).
2. Subtracting the explained variance of the selected model with the parameter in question removed, from the explained variance of the minimal adequate model.
3. Calculating the percentage of the value of the change in variance from the null model deviance.

Model fit was generally rather poor, so models were presented graphically when they explained 40% or more of the variance. The values were derived from the model parameters, holding all factors constant at the mean apart from the interaction (or quadratic) term in question. Predicted values were generated using the ranges of both of the variables in the interaction to create a predicted value for every combination of the two variables.

5.4 RESULTS

Climate change treatment was included in the models as a factor, but was eliminated in favour of SMC in every instance. Some selected models displayed a good model fit, for example the September 2009 ET model explained 70% of the total variance. Generally the selected models were poorly fitting (mean fit 30%), so only the models with a model fit of over 40% have been presented graphically. In most cases abiotic variables explained more of the variance and were retained in the models more frequently than plant traits, with FDvar terms in particular explaining function on very few occasions. There was a difference between retained explanatory variables in the growing season and over the winter; in the winter, abiotic variables were very important, especially those that were sensitive to average light and pH levels. The models showed evidence of consistent seasonal trends in all functions measured. This was particularly evident in the case of R_{eco} (Table 5.3), and NEE (Table 5.2) which were both described by soil temperature alone through the summer of 2009, then more biotic factors later in the experiment.

Table 5.2: Predictive linear models with Gaussian errors of evapotranspiration rate (µmol H_2O $m^{-2}s^{-1}$) over the course of 18 months. When only the intercept was applicable, a null model was specified.

Date	Formula	F statistic	P value	R^2	d.f.	Likelihood ratio tests and percentage of variance explained by each parameter.
Mar 09	253.12 + 3.27 Soil temp^2 + 0.11 PAR - 0.60 SMC - 56.31 Soil temp - 1.90 All light - 0.01 CWM LNC + 0.30 Soil temp*Average light	12.88	p<0.001	0.65	7,48	CWM LNC: F=9.16, p<0.01, 6.6% Soil temp* Average light: F=10.26, p<0.001, 22.23% SMC: F=11.599, p<0.01, 8.4% PAR: F=20.41, p<0.001, 14.77% Soil temp^2: F= 26.78, p<0.001, 19.39%
May 09	19.05 + 24.03 PAR	16.18	p<0.001	0.23	1,54	
June 09	-1.55 + 1.1 FDvar AGB	4.62	p<0.05	0.08	1,54	
Sept 09	16.67 - 1.9PAR - 1.38 Soil temp + 0.14 Soil temp*PAR	39.63	p<0.001	0.70	3,52	Soil temp*PAR: F=14.95, p<0.001 8.8%
Oct 09	181.40 - 3.28 Average light - 32.86 pH + 0.02 Biomass FG1 + 35.02 Biomass FG2 + 0.14 Biomass FG3 + 0.58 Average light*pH - 0.13 Biomass FG1*Biomass FG2 - 2.47 Biomass FG2*Biomass FG3	2.257	p<0.05	0.28	8,47	Biomass FG2*Biomass FG3: F=3.296, p<0.05, 14.25% Biomass FG1*Biomass FG2: F=3.35, p<0.05, 14.74% Average light*pH: F= 4.17, p<0.05 6.4%
Nov 09	-496.72 + 46.72 Soil temp + 88.70 pH + 4.89 PAR - 8.32 Soil temp*pH - 0.88 pH*PAR	8.67	p<0.001	0.46	5,50	PAR*pH: F=11.04, p<0.001, 35.5% Soil temp*pH: F= 7.05, p<0.001, 22.65%
Feb 10	-1.66 + 0.04 PAR	5.87	p<0.05	0.1	1,54	
May 10	Null model					
June 10	-15.3 + 2.9 pH + 0.5 CWM Root:Shoot	7.51	p<0.01	0.22	2,53	pH: F=9.42, p<0.01, 13.86% CWM Root:Shoot: F= 4.65, p<0.05, 6.84%
July 10	Null model					
Aug 10	-84.23 + 16.89 pH	9.43	p<0.01	0.16	1,50	
Sept 10	0.042 - 0.004 CWM BGB	5.26	p<0.05	0.09	1,54	

CWM = Community weighted mean. FDvar = Functional divergence. PAR = Photosynthetically active radiation. All light= Average light reception over the course of a year, as a proxy for shading. LNC = Leaf nitrogen content. AGB = Aboveground Biomass BGB = Belowground Biomass SLA = Specific leaf area, FG1 = perennial grasses, forbs and legumes, FG2 = caespitose grasses and tall forbs, FG3 = annual grasses, forbs and legumes PSA = Photosynthetic surface area LDMC = Leaf dry matter content SMC = Soil moisture content

Table 5.3: Predictive linear models with Gaussian errors of net ecosystem CO_2 exchange (mg CO_2 $m^{-2}s^{-1}$) over 18 months. When only the intercept was applicable, a null model was specified. Note that because NEE has negative values, a negative relationship means higher NEE is occurring under low values of that term.

Date	Formula	F statistic	P value	R^2	d.f.	Likelihood ratio tests and percentage of variance explained by each parameter.
March 09	0.86 - 0.004 PAR - 0.03 SMC	11.42	p<0.001	0.30	2,53	PAR: F=15.93, p<0.001, 21% SMC: F=11.64, p<0.01, 15.35%
May 09	3.31 - 0.14 Soil temp - 0.07Biomass FG2	5.08	p<0.01	0.13	2,52	Soil temp: F=6.87, p<0.05, 11.07% Biomass FG2: F=4.93, p<0.05, 7.94%
June 09	-126.044 + 4.15 Soil temp	4.27	p<0.05	0.07	1,54	
Sept 09	-599.07 + 23.23 Soil temp + 103.08 pH + 3.51 Average light - 4.0 Soil temp*pH - 0.6 pH* Average light	3.86	p<0.01	0.28	5,50	Soil temp*pH: F=4.52, p<0.05, 6.5% pH* Average light: F=5.72, p<0.05, 8.3%
Oct 09	Null model					
Nov 09	-1.33 + 0.15 Soil temp + 0.11 PAR - 0.01 Soil temp*PAR	23.41	p<0.001	0.67	4,46	Soil temp*PAR: F=30.77, p<0.001, 45.7%
Feb 10	0.33 - 0.02 CWM LDMC	6.09	p<0.05	0.10	1,54	
May 10	-19.98 + 0.38 PAR + 1.43 Soil temp + 0.23 CWM SLA - 0.03 PAR*Soil temp - 0.004 PAR*CWM SLA	8.75	p<0.001	0.47	5,50	PAR*CWM SLA: F= 20.296, p<0.001, 21.65% PAR*Soil temp: F=12.25 p<0.001, 13.07%
June 10	-0.86 - 0.016 PAR + 0.0006 CWM LNC	6.85	p<0.01	0.21	2,51	CWM LNC: F=4.80, p<0.05, 7.4% PAR: F=10.15, p<0.01, 15.7%
July 10	Null model					
Aug 10	18.428 - 34.67 SMC - 26.99 pH - 0.58 Average light + 5.03 SMC*pH + 0.099 SMC* Average light	2.67	p<0.05	0.22	5,48	SMC*pH: F= 6.86, p<0.05, 11.18% SMC* Average light: F= 6.43, p<0.05, 10.48%
Sept 10	Null model					

Table 5.4: Predictive linear models with Gaussian errors of ecosystem respiration (mg CO_2 $m^{-2}s^{-1}$) over the course of 18 months. When only the intercept was applicable, a null model was specified.

Date	Formula	F statistic	p	R^2	d.f.	Likelihood ratio tests and percentage of variance explained by each parameter.
March 09	Null model					
May 09	-6.97 + 1.08 Soil temp - 0.03 Soil temp^2	4.87	p<0.05	0.16	2,53	Soil temp: F= 7.6, p<0.01, 12.12%, Soil temp^2: F= 7.06, p<0.05, 11.25%
June 09	-61.90 + 5.91 Soil temp	7.55	p<0.01	0.12	1,54	
Sept 09	-0.1 + 0.037 Soil temp	6.07	p<0.05	0.11	1,51	
Oct 09	0.39 - 0.0003 Biomass FG1 + 0.08 Biomass FG3 - 0.009 Biomass FG2 - 0.0004 Biomass FG1*Biomass FG3	7.08	p<0.001	0.36	4,51	Biomass FG1*Biomass FG3: F= 9.06 p<0.001, 34.3% Biomass FG2: F= 7.58 p<0.01, 9.6%
Nov 09	0.16 - 0.06 Biomass FG2 - 0.001 Biomass FG3 + 0.008 Biomass FG2*Biomass FG3	5.72	p<0.01	0.25	3,52	Biomass FG2*Biomass FG3: 5.72 p<0.01, 24.8%
Feb 10	1.03 - 0.03 SMC - 0.02 All light - 0.001 Biomass FG2 - 0.0018 Biomass FG3 + 0.0007 SMC*Average light + 0.0004 Biomass FG2*Biomass FG3	8.79	p<0.001	0.53	6,47	SMC* Average light: F= 11.98, P<0.01, 11.95% Biomass FG2*Biomass FG3: F=6.42, P<0.05, 6.4%
May 10	-0.26 + 0.25 SMC - 0.01 SMC^2	5.8	p<0.01	0.18	2,52	SMC: F= 4.45 p<0.05, 7.0%, SMC^2: F= 6.51, p<0.05, 10.23%
June 10	1.65 - 0.09 Soil temp + 1.68 FDvar LDMC	7.6	p<0.01	0.23	2,52	Soil temp: F= 10.72, p<0.01, 15.96% FDvar LDMC: F= 5.85, p<0.05, 8.7%
July 10	5.69 - 0.1 Biomass FG2 - 0.04 Biomass FG3 + 0.015 Biomass FG2*Biomass FG3	3.2	p<0.05	0.16	3,51	Biomass FG2*Biomass FG3: F= 7.02, p<0.05, 11.6%
Aug 10	10.01 - 0.00005 CWM PSA	5.62	p<0.05	0.1	1,50	
Sept 10	Null model					

Table 5.5: Predictive linear models with Gaussian errors of extractable soil nitrogen ($mg^{-1}kg^{-1}$) over the course of 22 months. When only the intercept was applicable, a null model was specified.

Date	Formula	F statistic	p	R^2	d.f.	Likelihood ratio tests and percentage of variance explained by each parameter.
Feb 09	-1.16 + 1.24 pH - 0.02 Biomass FG3	6.67	p<0.01	0.2	2,52	pH: F= 5.66, p<0.05, 8.7%, Biomass FG3: F= 6.45, p<0.05, 9.9%
Apr 09	-73.8 + 10.59 Soil temp + 14.67 pH - 0.02 Biomass FG3 - 1.95 Soil temp*pH	8.09	p<0.001	0.4	4,49	Soil temp*pH: F= 9.33, p<0.01, 11.47%, Biomass FG3: F= 5.96, p<0.05, 7.32%
June 09	11.56 - 0.23 Soil temp - 0.05 Average light	7.41	p<0.01	0.22	2,52	Soil temp: F=10.76, p<0.01, 16.1%, Average light: F=6.66, p<0.05, 10.0%
Aug 09	14.03 - 0.98 Soil temp + 0.41 Biomass FG3 + 0.35 CWM SLA	7.56	p<0.001	0.35	3,42	Soil temp: F=5.93, p<0.05, 9.2%, Biomass FG3: F=7.98, p<0.01, 12.35%, CWM SLA: F= 5.76, p<0.05, 8.9%
Sept 09	3.85 + 0.11 SMC	18.46	p<0.001	0.26	1,52	
Dec 09	162.8 - 2.69 Average light - 27.23 pH + 0.47 Average light*pH	4.00	p<0.05	0.19	3,51	Average light*pH: F= 5.55, p<0.05, 8.8%
Mar 10	-0.03 + 0.07 SMC	5.69	p<0.05	0.1	1,51	
May 10	0.31 + 0.47 SMC	4.41	p<0.05	0.08	1,53	
June 10	-425.44 + 28.16 Soil temp + 77.24 pH - 5.0 Soil temp*pH	3.94	p<0.05	0.2	3,48	Soil temp*pH: F= 6.81, p<0.05, 11.39%
July 10	Null model					
Aug 10	1.81 + 3.89 CWM Root:Shoot	5.01	p<0.05	0.09	1,52	
Sept 10	386.04 - 6.23 Average light - 62.38 pH + 1.07 Average light*pH	3.72	p<0.05	0.18	3,51	Average light*pH: F= 4.12, p<0.05, 6.6%

Table 5.6: Predictive linear models with Gaussian errors of extractable phosphate ($mg^{-1}\ kg^{-1}$) over the course of 22 months. When only the intercept was applicable, a null model was specified.

Date	Formula	F statistic	p	R^2	d.f.	Likelihood ratio and percentage of variance explained by each parameter.
Nov 08	184.838 - 65.262 pH + 5.87 pH^2 - 2.43 CWM Root:Shoot	4.63	p<0.01	0.22	3,49	CWM Root:Shoot: F= 4.38, p<0.05, 7.0% pH: F= 5.37, p<0.05, 8.5% pH^2: F= 5.53, p<0.05, 8.8%
Feb 09	0.96 - 0.24 Soil temp + 0.02 Soil $temp^2$ - 0.01 CWM.AGB	6.71	p<0.001	0.28	3,52	CWM AGB: F=10.08 p<0.01, 13.97% Soil temp: F=8.69 p<0.001, 12.05% Soil $temp^2$: F=7.37, p<0.01, 10.22%
Apr 09	11.39 - 0.47 Soil temp - 0.09 Average light - 0.55 CWM AGB + 0.0078 Average light*CWM AGB	9.34	p<0.001	0.43	4,50	CWM AGB* Average light: F= 5.85, p<0.05, 6.7% Soil temp: F= 17.72, p<0.001, 19.63%
Jun 09	2.46 - 0.097 CWM AGB	7.05	p<0.05	0.12	1,54	
Aug 09	10.39 + 0.02 Biomass FG1 + 0.76 Biomass FG3 - 0.49 CWM LDMC - 0.004 Biomass FG1*Biomass FG3	4.46	p<0.01	0.26	4,51	CWM LDMC: F= 4.12, p<0.05, 6.0% Biomass FG1*Biomass FG3: F= 11.24, p<0.01, 16.32%
Sept 09	23.48 - 0.5 SMC - 1.03 Soil temp - 0.40 Average light + 0.01 SMC*All light + 0.02 Soil temp* Average light	3.4	p<0.05	0.25	5,50	SMC* Average light: F= 10.45, p<0.01, 15.6% Soil temp* Average light: F= 5.63, P<0.05, 8.4%
Mar 10	3.49 - 0.02 Biomass FG2	9.62	p<0.01	0.15	1,53	
May 10	2.09 + 0.006 Biomass FG1 + 2.26 FDvar LNC	5.34	p<0.01	0.17	2,53	FDvar Foliar N: F= 4.59, p<0.05, 7.2% Biomass FG1: F= 4.62, p<0.05, 7.3%
Jun 10	96.12 - 2.06 Average light - 15.75 pH - 0.03 Biomass FG2 - 0.27 Biomass FG3 + 0.01 Biomass FG1 - 1.24 CWM Root:Shoot + 0.37 Average light*pH + 0.003 Biomass FG2*Biomass FG3 + 0.002 Biomass FG3*Biomass FG1	3.58	p<0.01	0.43	9,43	Average light*pH: F= 2.97, p<0.05, 6.8% Biomass FG2*Biomass FG3: F= 4.62, p<0.01, 16.77% Biomass FG1*Biomass FG3: F=4.94, p<0.01, 11.8% CWM Root:Shoot: F= 5.23, p<0.05, 6.89%
Jul 10	3.26 + 4.17 FDvar LNC	8.771	p<0.01	0.14	1,52	
Aug 10	3.85 + 0.05 Biomass FG2 + 0.02 Biomass FG3 - 0.004 Biomass FG2* Biomass FG3	2.05	p>0.05	0.11	3,49	Biomass FG2*Biomass FG3: F=5.45, p<0.05, 9.87%
Sept 10	8.50 - 0.02 Biomass FG2 - 0.17 Biomass FG3 + 0.02 Biomass FG2*Biomass FG3	8.82	p<0.001	0.34	3,51	Biomass FG2*Biomass FG3: F=10.95, p<0.01, 14.13%

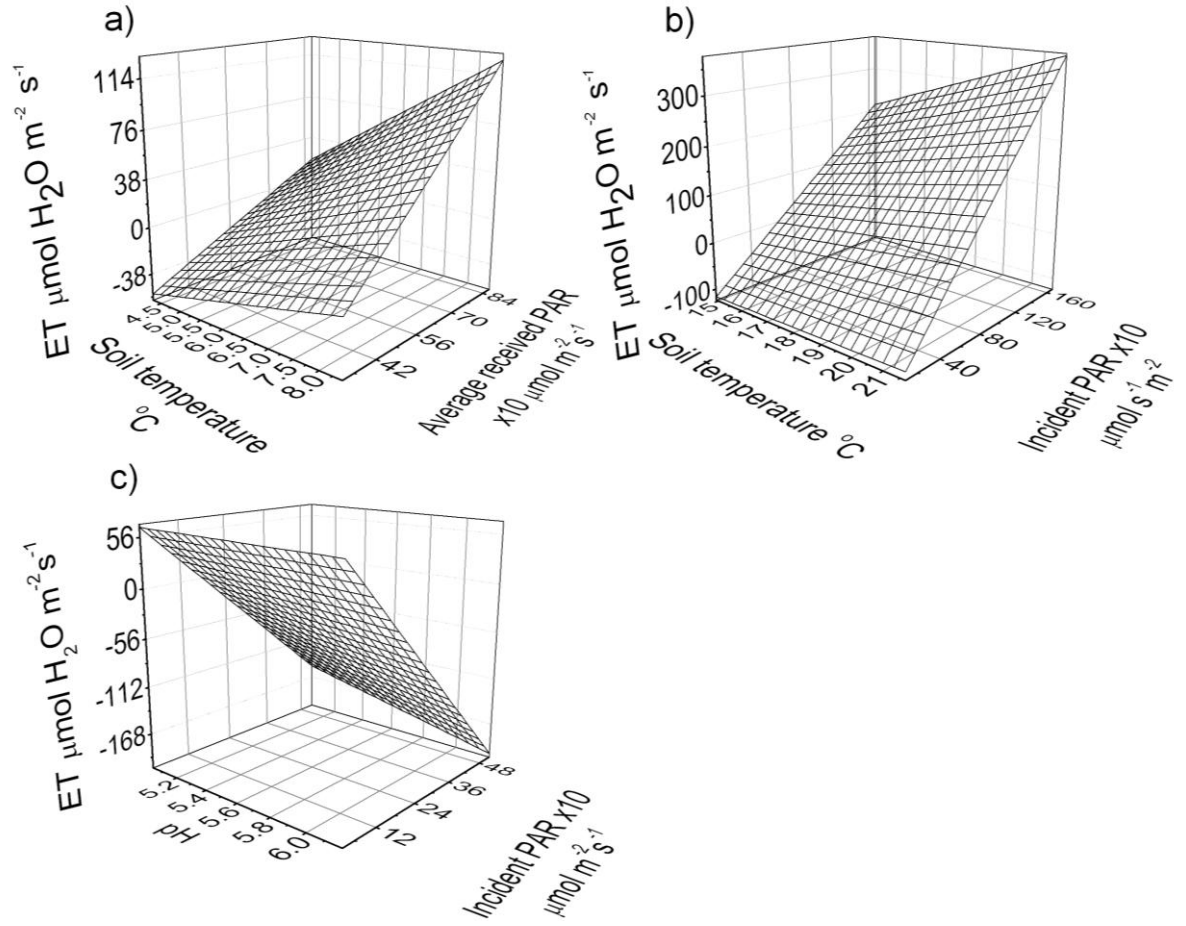

Figure 5.1: Predicted relationships of evapotranspiration rate with abiotic factors in a) March 2009 $F_{7,48}$=12.88, p<0.001, R^2=0.65, b) September 2009 $F_{3,52}$= 39.63, p<0.001, R^2=0.70, and c) November 2009 $F_{5,50}$=8.67, p<0.001 R^2=0.46. Average received PAR refers to the average yearly light received throughout the year, while incident PAR refers to light measured simultaneously with ET. Origin 8.5 (OriginLab Corporation, Northampton, MA, USA).

Throughout the experiment, ET was best explained by light (both yearly averages and simultaneous readings during ET measurement) and soil temperature. In the spring of 2009 and 2010 PAR was a key driver of ET, consistent with the beginning of the growing season and longer day lengths (Table 5.2, March 09, May 09, Feb 10). In March 2009, ET was highest over warm soils and in plots that received the highest average light through the year (Fig. 5.1a). Soil temperature and average light showed a positive interaction in determining ET, while other variables in this model, i.e. SMC and LNC were much less important (Table 5.2).

In the very dry summer period, traits describing allocation to roots or shoots explained variation of ET; a high CWM Root:Shoot ratio was linked with lower ET rates, especially in June 2010 (Table 5.2). This was followed in September 2010 with a negative correlation with belowground biomass when plants were senescing. pH also showed a positive relationship with ET during the summer, as it was the more important influence in June 2010, and was the only variable in the selected model for August 2010, when plants were recovering from a prolonged period of drought (Table 5.2). It is therefore likely that plot position was the strongest descriptor of ET at this time.

During the autumn in both years, soil temperature and light were again the most significant explanatory variables for ET; by September 2009, PAR explained more deviance than temperature, though warmer soils and higher sunlight together corresponded with higher ET (Fig. 5.1b). After senescence in October, there were functional group biomass interactions; which were negatively related to ET (Table 5.2), and negatively correlated with wetter, shadier locations in the site (Table 5.2). Generally, when FG2 was high in senesced biomass, in combination with one of the other groups, ET was low. Given that FG2 was usually a very sparse group, in plots where it was present in substantial amounts, there was unlikely to be any bare earth, meaning that there would have been a thick layer of senesced material. In November, at the beginning of the winter period, soil temperature ceased to be selected, with higher pH soils in the shadier, less elevated plots associated with lower ET (Fig. 5.1c). Whether this was due to decreased evaporation or less transpiration in this area is uncertain, although the change in trend of the relationship with light implies that plant driven ET had ceased and evaporation from the soil was the primary source of water vapour change.

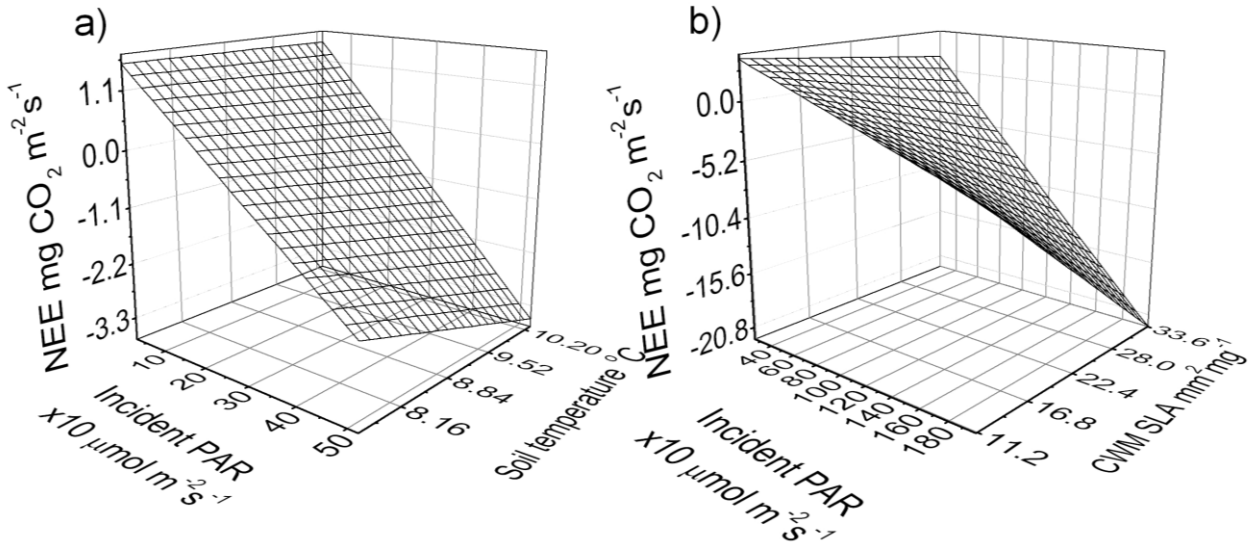

Figure 5.2: Predicted relationships of net ecosystem CO_2 exchange with abiotic and biotic variables in a) November 2009 $F_{4,46}$=23.41, p<0.001, R^2=0.67 and b) May 2010 $F_{5,50}$=8.75, p<0.001, R^2=0.47. More negative NEE implies photosynthesis is occurring.

NEE was much more sensitive to differences in weather across the two years of the study than ET, leading to the selection of very different model variables in spring 2009 and 2010. 2009 was warm and with regular rainfall. In spring 2009 the winter rainfall addition resulted in a marked negative relationship between NEE and SMC in March, and also PAR, meaning that more NEE was taking place under wetter, lighter conditions (Table 5.3). In the less water-stressed summer period of 2009, soil temperature appeared to be the primary driver of NEE; warmer soils correlated with more negative NEE in May, so were higher C sinks in June, but in September cooler soils correlated with more negative NEE values.

In 2010 the winter was exceptionally wet, followed by a hot dry spring and a sustained period of drought through June. During the spring of 2010, while PAR was still an important factor, leaf traits explained more variation, and were consistently the main drivers until June. In February 2010, high CWM LDMC correlated with more negative NEE (Table 5.3), and this continued into May when bright light and high SLA were associated with more negative NEE, implying more photosynthesis (Fig. 5.2).

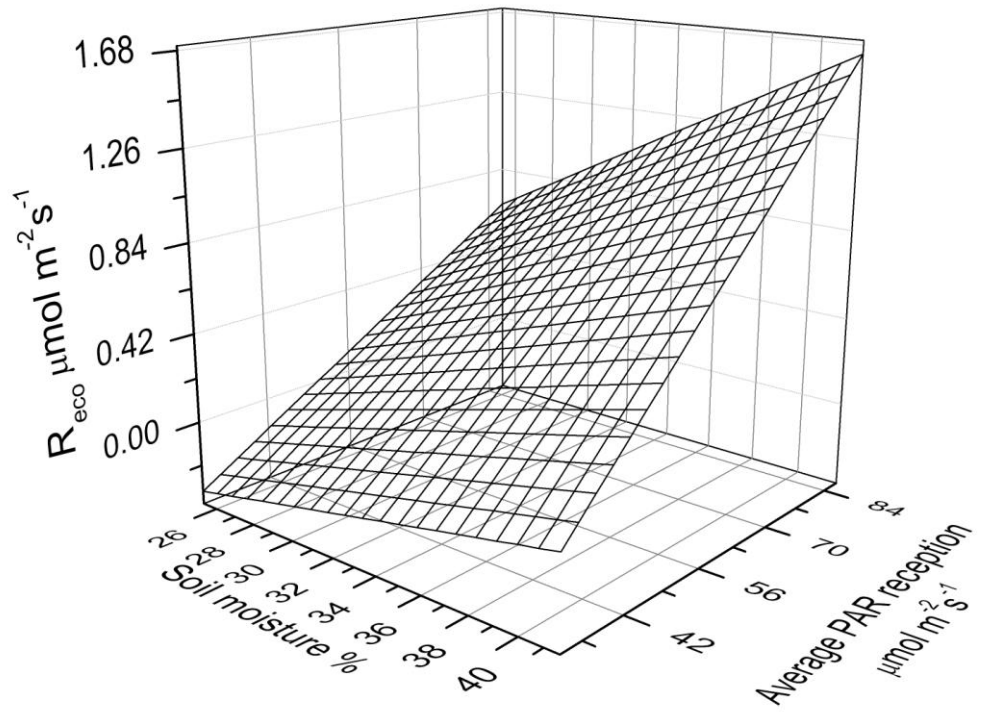

Figure 5.3: Predicted relationship between R_{eco}, SMC and average light in February 2010 ($F_{6,47}$=8.79, p<0.001, R^2=0.53, portion of explained variation: 11.98%).

R_{eco} retained similar variables across consecutive time points tested in 2009, including soil temperature and biomass (Table 5.4). In 2010, when the weather was extremely variable, trends of selected variables were less apparent across seasons. Through summer 2009, soil temperature was the most important variable; correlating positively with R_{eco} rates (the quadratic relationship of soil temperature in May was likely to be due to a link with SMC, where the highest R_{eco} was associated with intermediate values of temperature). In October and November 2009 and February 2010, biomass explained the highest proportion of deviance; the plots with higher accumulated biomass at the time of the vegetation survey in September 2009 had higher rates of R_{eco} through the following winter (Table 5.4). Through the early part of 2010, when water rapidly became limiting, R_{eco} had a strong positive association with SMC; in February 2010 R_{eco} was higher in wetter soils and those with lower average light (Table 5.4, Fig. 5.3). In May 2010, R_{eco} was low in both wetter and dry soils, with higher R_{eco} at intermediate SMC (14%), which is likely to be closely linked to soil temperature, though it was not selected in the model (Table 5.4).

In the summer of 2010, R_{eco} was best explained by community composition of biomass (measured in May 2010) and CWM of traits, which were similar to those selected for ET (Table 5.4). Higher community variation in leaf dry matter content, which can be considered a proxy for leaf decomposability, corresponded with higher R_{eco} in June 2010. June 2010 was exceptionally dry and there was extensive dieback, and in July 2010, higher living biomass (measured in July) was linked to higher R_{eco} rates. However, in August, communities containing high leaf surface area species had lower R_{eco}.

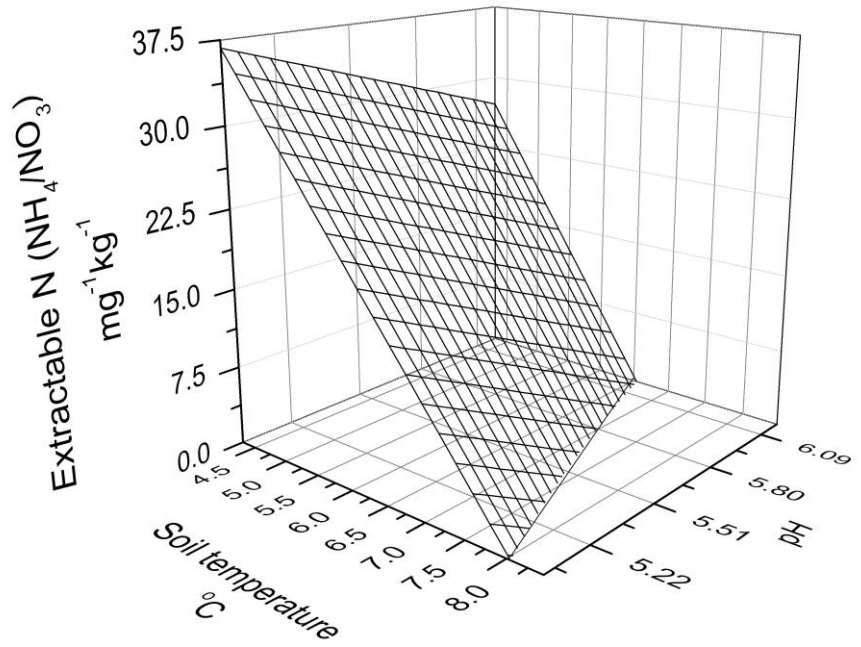

Figure 5.4: Predicted relationship of extractable N with soil temperature and pH in April 2009 ($F_{4,49}$=8.09, $p<0.001$, R^2=0.4).

Models for extractable soil N (NH_4 and NO_3) were much more varied in terms of abiotic variables retained than the flux measures, and fewer trends of selected variables were seen over time (Table 5.5). In spring 2009, high abundance of FG3 biomass (measured in May) was related to a depletion of extractable soil N in both February and April (Fig. 5.4). pH was also a key variable at these times; more basic soil correlated with higher N concentrations in February, but in April there was a negative interaction between soil pH and soil temperature, so N was more depleted when soil was warmer and more acidic (Fig. 5.4). There was a trend from April to August 2009 for high soil temperature to be associated with lower extractable soil N.

This changed in September 2009 when SMC became the main determinant of extractable soil N, which continued until May 2010, although in December 2009 pH and average light were positively related, suggesting that the drier more exposed plots were higher in N.

In August of both years, leaf traits were positively correlated with increased extractable soil N levels. In August 2009 CWM SLA, which is related to resource use strategy and allocation, was the trait with the best level of fit, and in 2010 Root:Shoot ratio described more variation in soil N, so higher root allocation was correlated with lower extractable N in the soil.

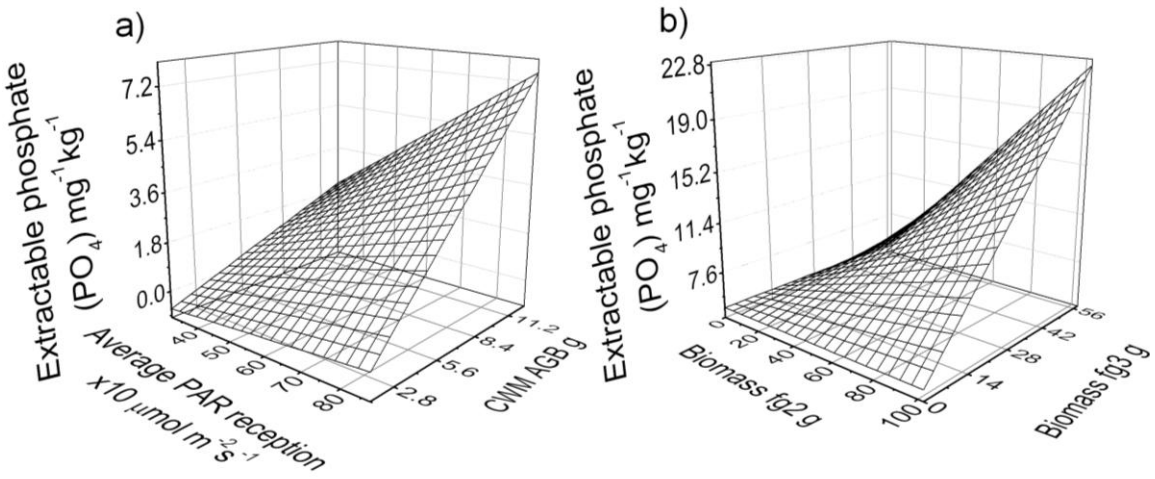

Figure 5.5: Predicted relationship of extractable phosphate with a) community weighted aboveground biomass and average received photosynthetically active radiation (a proxy of shading), in April 2009 ($F_{4,50}$=9.34, p<0.001, R^2=0.43) and b) Biomass of bunch grasses and tall forbs, and annual plants in June 2010 ($F_{9,43}$=3.58, p<0.01, R^2= 0.43).

Models for extractable phosphate (P) consistently contained more biotic variables than models for the other ecosystem functions (Table 5.6). Selected models for the majority of the time points included some measure of biomass, although the direction of the relationship changed through the time points. Between February and June 2009 the most important explanatory variable was CWM AGB. In February and June this was a negative relationship, while in April P was more abundant where CWM AGB was high but also where average light was high (Fig. 5.5a). From August 2009 onwards the main descriptor of extractable P in the soil was plant biomass, and in all models FG3 was present within an interaction with one of the other two functional groups (Table 5.6).

These changes in the direction of the CWM AGB – Extractable P relationship were consistent with periods of plant growth; when growth was occurring there was a positive relationship between biomass and extractable P, for example in June 2010 (Fig. 5.5b) as well as May and September 2010, but in August in both years, there was a negative relationship with biomass. CWM and FDvar traits were rarely retained in the models, but where present tended to correlate positively with more variable LNC, and negatively with traits associated with resource acquisition or provision such as Root:Shoot ratio and LDMC.

5.5 DISCUSSION

Abiotic variables and variables acting as proxies for site conditions (pH and average light) were retained in the models in winter, especially soil temperature, shadiness and plot position. In contrast, in the growing season biotic factors, particularly biomass estimates were better descriptors of ecosystem functions. CWM and FDvar traits were only retained in selected models in a small number of cases, but they suggest more detailed linkages between plant traits and function than the physical plant presence and chemical inputs described by biomass alone. The variables that described each ecosystem process were supported by the literature, and for the most part continued to be retained in the models for a few consecutive months. Unfortunately, a more concise synthesis was not possible because it was impractical to conduct vegetation surveys each month, so repeated measures analyses were inappropriate. The comparatively few occasions when CWM values or FDvar were significant suggests that while there is some support for Grime's mass-ratio hypothesis (Grime 1998) and Tilman's complementarity hypothesis (Tilman 1997), they may not be useful in experiments where there are no strong plant trait gradients to link community assemblages with functional changes.

This experiment used a method modified from Dìaz *et al.* (2007). Most papers attempting to predict ecosystem functions have proposed modelling methods, but have not applied them in the context of global change manipulations (Shipley *et al.* 2006; 2010), although a few have applied their modelling methods to examples of climate or land change (e.g. Dìaz *et al.* 2007, although see Schumacher & Roscher

2009). The field is beginning to move from the stage of methods testing towards seeking empirical answers, so this chapter offers both an evaluation of the method, and some predictions of the magnitude and directionality of ecological parameters which affect function in grasslands. There has been much support for using weighted traits to describe aspects of ecosystem processes (Garnier *et al.* 2004; Dìaz *et al.* 2007a; Mokany *et al.* 2008). Pakeman *et al.* (2008) carried out a test that explicitly demonstrated that weighting traits was much more powerful than using raw trait values when describing trait changes over environmental gradients. Using these traits in a linear modelling framework was appropriate in this study because it is a repeatable and transparent method, with all relationships between variables and their significances easily interpreted. Others have used ordination techniques such as PCA, which, while very rapid and simple to carry out, have no predictive capability, merely elucidating important traits (Craine *et al*. 2001; Gross *et al.* 2008).

In general model fit was rather low. Other studies using traits weighted by abundance had much higher R^2 values, such as Garnier *et al.* (2004)'s study on decomposition and SLA, where almost every model explained over 75% of variation. However, it is likely that the use of a strong environmental or disturbance gradient in studies such as this resulted in clearer patterns, while the more subtle differences in trait distributions in my study meant that even low residual deviation in the explanatory variables caused bigger picture trends to be confounded. The method used here was designed to capture effects of all species in case of idiosyncratic effects. Including all species may have reduced model fit but due to the good level of replication and key factors appearing consistently in consecutive months, there can be confidence in the broader messages from these analyses, which are described in turn below.

5.5.1 BIOMASS

Estimated biomass of functional groups was often an important variable, particularly in the autumn, as it represented many physical attributes that were more complex than individual traits could account for, including microclimate, litter inputs or thatch thickness and stand height. It has been noted that trait relationships with function are

often mediated by primary productivity at the site, so the inclusion of biomass was justified (Pakeman 2004). Therefore, there was a distinction between variables affected by biomass (composed of varying functional groups), and abundance weighted traits, which means that more detailed conclusions regarding functional traits versus mechanical drivers can be drawn.

The three FGs used in this experiment have very different growth forms. The FGs were split into perennial plants, bunch grasses and tall forbs, and annual plants; therefore, it can be assumed that FGs will be associated with different microclimates, life cycle lengths and therefore differing resource capture strategies, and subsequently different patterns of light penetration to the soil surface (more light would be expected with the sparser annual group). Increased biomass was positively related to R_{eco} but negatively associated with ET in the autumn, possibly because of the effects of thicker layers of decomposable material. It is likely that senescence of high biomass plots increased humidity at the soil surface leading to reduced evaporation (assuming all plant biomass was functionally obsolete in November), while maintaining R_{eco} throughout the winter (Bardgett *et al.* 2005; Baldocchi *et al.* 2006). This could be due to protection against the harsher effects of frost on microbial and root activity. Studies on the effect of mulch and thatch upon soil temperature and water evaporation are primarily concerned with crops such as winter wheat, and they generally support the results here; soil temperatures are lower under thatch over winter and evaporation is reduced which can be detrimental to plant germination and growth in future years (Chen *et al.* 2007).

FG3 annuals were retained in most models for both R_{eco} and extractable soil P; however, while R_{eco} consistently increased with higher FG3 biomass, its relationship with P was inconsistent through the growing season. While many have noted that increased living plant diversity enhances decomposition rates (Wardle *et al.* 2004; Cornwell *et al.* 2008), and a variety of leaf traits are always correlated, confounding factors could arise in diversity experiments through inappropriate species groupings (groupings not made with trait differences in mind). In grasslands, there tends to be a rather small pool of grass species, with a very large number of potential annuals and legumes. Therefore, when experiments sought to artificially increase diversity through random selections from a species pool, the probability was a large number of annual plants. By separating out these annual plants (rather than forbs, a common

categorisation), we have shown that this functional group has strong functional effects. The annual group had many legume species, which were common across the site. These are known to disproportionally deplete P from the soil, so the inconsistent relationship could correlate with abundance, nodulation or decomposition of legumes. This would result in lower soil P when legumes are abundant and nodulating in the summer and higher soil P in the autumn and winter when they are senescing (Hooper & Vitousek 1998; Spehn *et al.* 2002). Therefore, it is possible that high annual legume biomass accelerates P cycling through localised depletions of the rhizosphere, short life spans and rapid decomposability (Wardle *et al.* 2004). This is further collaborated by evidence in Chapter 3 that the site is rather soil P deficient (less than 5 $mg^{-1}kg^{-1}$ on average), which may increase the impact of very small changes in P in the soil (Hazelton 2007). Biomass is clearly an important factor in ecosystem processes, particularly with regard to nutrient resource allocation and alterations of physical conditions. In modelling biomass separately to individual traits, the assumption is tested that inputs into the system are directly proportional to biomass, in the forms of litter, water and nutrients. These inputs were, in many cases, stronger drivers of ecosystem processes than abundance weighted traits. By segregating the biomass component into functional groupings, some clear distinctions have been made regarding their effects on function, particularly in the case of legume effects on nutrient cycling.

5.5.2 CWM

The hypothesis that community weighted variables would have higher explanatory power during the growing season was supported, but they should be used with caution. In this case, the traits used were taken from plants grown in monoculture on soil from the site so that the traits would be as representative as possible, which is a method supported in the literature (Vile *et al.* 2006). The often low explained variation in this experiment suggests that traits measured from plants *in situ*, i.e. a taxon-free method (a method measuring the traits of plants in an area regardless of species identity), may enable a better model fit with function (Gaucherand & Lavorel 2007; Lavorel *et al.* 2008; Dìaz *et al.* 2007a). However, it is not widely used because

it is time and labour intensive and the results are only applicable to the individual site. This experiment aimed to test whether general predictions from *ex situ* trait values were possible, so that more cost and time effective methods can be evaluated for future use. On the whole predictions of function from trait databases are possible and have been demonstrated here, but they inevitably offer lower model fits than the *in situ* method described above.

Trait-function relationships can offer a mechanistic understanding of ecosystem processes. For example, ET was primarily driven by traits relating to root allocation. The importance of plant allocation to roots is not an intuitive driving variable for ET, though root traits are indicative of the general water use strategy of a plant, and deeper rooted species tend to be more competitor species than ruderals (Grime 1988), so grow slower and live longer (Rajaniemi & Reynolds, 2004). It has been noted that, as water becomes more limiting in a season, plant traits, particularly stomatal conductance, become the main drivers of ET (Chapin 2003). Stomatal closure, and rooting depth, density and allocation are general strategies to conserve water, and an indirect link between ET and root architecture has been drawn on many occasions (Sperry *et al.* 2002; Chaves *et al.* 2003), although it only seems to have been empirically demonstrated on xeric trees, where large root systems were strongly correlated with better water conservation and lower transpiration rates (Xu & Li 2008; Alsina *et al.* 2011).

In my experiment extractable soil P was explained mostly by negative relationships with above ground biomass, root to shoot ratio and LDMC, which could be caused by lower decomposability, resource depletion under larger plants or those with higher root allocation. Extractable P is supplied to the soil geochemically rather than biologically, so changes in its pool size are most likely to be as a result of plant uptake via mycorrhizal symbiosis (Aerts & Chapin 1999; Bardgett & Wardle, 2010). The relationship between different functional groups, particularly FG3 and extractable soil P could also be due to leaf N:P ratio of these groups. There is a known threshold of leaf N:P ratio where uptake of soil P is more necessary than soil N (~15), which could be the case in certain species of these functional groups (Aerts & Chapin 1999). However, when plants are water stressed, Chapin (1991) stated that uptake of P is vastly reduced compared with the control, so this could help explain the differences in relationship seen.

Extractable soil N was positively correlated with SLA and root to shoot ratio. SLA is inversely related to LDMC, so their effects on nutrient pools would be expected to be opposite. High SLA is understood to be associated with high productivity, short life span and high decomposability (Reich *et al.* 1992; Wilson *et al.* 1999; Corneliessen *et al.* 2003). Therefore, SLA could be an appropriate trait for describing high N inputs. SLA is an extremely plastic measure, and it has been identified as both a response and effect trait, although cause and effect are difficult to ascertain in this case (Gross *et al.* 2008; Klumpp & Soussana 2009). This indicates that in this experiment, soil nutrient pools were primarily determined by leaf traits in the summer, though traits were not useful descriptors outside of the growing season. A possible future avenue for this work could be to increase the set of plant traits used and tailor them more closely to the functions measured. For example, relative growth rate has not been tested here, and it has been shown in other work to be a very useful trait in explaining ecosystem function (Vile 2006).

5.5.3 FDVAR

FDvar has received support over a number of other metrics because it describes the range and relative abundance of traits, and close links to ecosystem function have been found (Dìaz *et al.* 2007a; Mokany *et al.* 2008), although in this study it explained little of the variation in ecosystem functioning. It is likely to be more appropriate for use across gradients with a large quantifiable change in conditions, which was likely not to be the case on the site here. Mokany *et al.* (2008) found it explained more variation than FD_Q (Rao's quadratic entropy, Botta-Dukat 2005), FRO (functional regularity, Mouillot *et al.* 2005) and FD (functional diversity, Petchey & Gaston 2002), in a site where contrasts between grassland composition had been maximised. A negative relationship between FDvar and a flux measure is likely to indicate reduced flux rates under high functional diversity, but with nutrient pools it almost certainly illustrates resource complementarity and more saturated resource use (Westoby & Wright 2006; Mokany *et al.* 2008). In this experiment all relationships with FDvar were positive; in summer ET was correlated with a higher diversity of aboveground biomass, which is likely to be due to partitioning of light and canopy

roughness between different species, which would comprise different heights and structures. This relationship has been the subject of many models (Penman-Monteith, Monteith 1973; ALEX, Anderson *et al.* 2000; FLUXNET, Baldocchi 2001). Mokany *et al.* (2008) asserted that a negative relationship between a trait FDvar and an ecosystem process was evidence that diversity reduces that process. This is rather a large generalisation; it implies that when a negative relationship with ecosystem processes occurs, one or a few dominants are more desirable than a community with a high trait distribution. This does not take into account that lower values of a function could imply higher flux rates in the case of NEE, or resource use and retention, e.g. leaching or soil nutrient pools. Most work carried out in the last twenty years has agreed that high species richness or functional diversity has positive effects on function (Tilman & Downing 1996; Weigelt *et al.* 2007; De Deyn *et al.* 2009), but results of these highly controlled experiments are likely to be very different to that of this study, which was strongly driven by abiotic effects.

The study of CWM and FDvar as predictors of ecosystem function is still in its early stages. There are still some issues with methodology and interpretation, but overall they are both broadly applicable to the explanation of ecosystem function. This experiment has offered some useful insights into how to predict ecosystem function, and which factors are important during certain seasons. This may also have caused the level of fit to be rather low on individual models, possibly caused by many species which had very low abundances being included (Garnier *et al.* 2004), or a lack of applicability of greenhouse measured traits.

The use of community weighted means can identify subtle controls of plant traits over ecosystem functioning. Ultimately, this linear modelling approach has demonstrated the importance of including seasonality on predictions of ecosystem processes, and shown that using CWM and FDvar metrics are often less important than biomass type or abiotic factors (Hector *et al.* 1999). It is a powerful method of evaluating variables before selecting the most descriptive set, and demonstrates that a generalised linear modelling procedure is appropriate to find the most appropriate descriptors of complex relationships between biotic and abiotic drivers of function. However, more work needs to be done before extrapolations to the larger scale are possible, including a comparison of greenhouse trait measures with *in situ* ones, and the effect the treatments have upon trait values and distributions.

Figure 6: Summer 2009

CHAPTER 6: DISCUSSION

6.1 OVERVIEW

This research aimed to investigate the link between plant functional diversity and climate change by creating a greenhouse experiment that used cutting edge techniques to group species by functional effects traits, and then combined these groups with climate change regimes, using the most recent climate projections, to create a large scale field experiment. The experiment ran over three years (2.5 of data collection), measuring a comprehensive suite of functions to accurately assess ecosystem process responses to these treatments. These measures were then applied to a modelling approach which attempted to predict ecosystem function based on a number of potential variables, both abiotic and biotic, with the long-term aim of applying these to a variety of ecosystems.

6.2 KEY FINDINGS AND CONTRIBUTION TO KNOWLEDGE

6.2.1 CLIMATE

The three implemented climate change regimes elicited different functional responses from one another, so changing frequency or intensity of drought caused ecosystem processes to respond in different ways. In general, changes in rainfall result in measurable changes in nutrient pools and slowing of processes through the summer. When soils are dry, plants begin to conserve water by stomatal closure and senescence, and root dieback (Chaves *et al.* 2003; McDowell *et al.* 2008; Farooq *et al.* 2009). This is associated with a dieback of grasses and a build-up of decomposable material and extractable nutrients in the autumn. The effect of reduced precipitation on soil moisture was lagged, and had a knock-on effect on all other measured processes, leading to most process responses occurring outside the climate change treatment phases. When plant available nitrogen was present in the soil, the lack of soil pore water reduced the ability of plant roots to access it, so it accumulated in the

soil (Chapter 3). This increased the risk of large winter rainfall pulses causing significant leaching losses, as occurred in December 2009 (Chapter 3).

Altering the frequency and intensity of rainfall led to effects that were specific to each regime, an outcome increasingly identified by studies on varying rainfall patterns (Knapp *et al.* 2002; Jentsch *et al.* 2007). In Chapter 4 I applied two different regimes; 1) a spring/summer drought period, and 2) highly variable rainfall. The consequences differed in effect size, particularly with regard to microbial driven processes; the variable treatment did not generally differ from the ambient, though there was substantial vegetation dieback, but processes under the spring/summer treatment were significantly suppressed. These differences highlight a need for climate change experiments to use a range of likely scenarios in order to be able to predict effects of different rainfall shifts on function. This thesis has demonstrated the value of tailoring regimes to fit the system in question, in order to gain a broader understanding of potential effects of climate change.

Combined spring and summer drought should be the subject of more attention, due to the substantial effects of the applied spring and summer drought (Chapter 4). For example, under this treatment mineralisation rates and soil respiration were significantly slower than the ambient. At present it is understudied, but there have been several incidences of extremely low rainfall in these seasons in the UK over the last decade (Met Office 2011), and these could increase in frequency in the future. In particular, the microbial driven processes were affected by long-term drought, and given the many areas where aboveground and belowground linkages are poorly understood, determining the effect of a sustained growing season drought should be of priority. Winter rainfall addition should not be underestimated either; it could be the reason that effect sizes of the climate regimes were not larger in the summer, particularly in the experiment described in Chapter 3; if reserves of groundwater had built up this could partially alleviate the effects of summer drought. Chimner & Welker (2005) explicitly tested this using drift fences to collect extra snow and showed that ecosystem respiration can be substantially buffered during summer drought if preceded by a large snowfall in winter.

There have been many calls for more investigation into extreme climate events (Smith 2011b), and, while there have been advances (Jentsch *et al.* 2007) this field is

still in its infancy. However, there have been some experiments that measured ecosystem processes when time between rainfall inputs was increased, such as Knapp *et al.* (2002) and Laporte *et al.* (2002). These both revealed that CO_2 efflux from the soil was reduced when intervals between rainfall events are increased, though this was not evident in my variable rainfall treatment. The notable dieback of the plants in the variable treatment, and particularly the loss of forbs, could indicate that when large infrequent rainfall inputs are the only water source, then only the species with adaptations for accessing deep groundwater are likely to prevail. Given that there tends to be a fairly small pool of potential grass species, and usually a larger number of annual plants in temperate grasslands, this could lead to a community with much lower functional trait diversity. It could also lead to available niches for invasives especially if some resource pools are unexploited (Elton 1958; Hooper & Vitousek 1998).

The implication for ecosystem functioning in temperate lowland grasslands is that there will be slower rates of nutrient cycling under droughted conditions. However, as the sensitivity of ecosystem respiration is less than the sensitivity of photosynthesis to water deficits, losses of carbon to the atmosphere could be less than feared. Moreover, the likelihood of more carbon being fixed is also low, because premature senescence of plant material is a very likely side-effect, particularly if rainfall were to become very infrequent or lower in volume.

6.2.2 DIVERSITY

Grasslands are sinks for large quantities of atmospheric carbon, and it is critical that they maintain high carbon sink strength (Poeplau *et al.* 2011; Yuan *et al.* 2011). In functionally diverse systems, ecosystem processes continue throughout the year, as there will be some plants that can take advantage of small water pulses and continue to photosynthesise during periods of drought (Sala *et al.* 1992).

This thesis has shown that altering plant functional diversity can lead to corresponding changes in responses to drought, which is a consideration for future land management policies. The functional grouping technique successfully differentiated plant species into groups that were associated with different functional

responses under climate change (Chapter 2). In doing this, it has validated a new approach to categorising species that highlighted differences in function, and created new groupings of species so far not explicitly tested. In particular, the groups described in Chapter 2 were notable by their differing growth and life cycle rates and rooting depths, resulting in differing soil process rates (Schwinning & Sala 2004; Schimel *et al.* 2007). Due to their differing characteristics, and the differing responses to stress reported in Chapter 3, there are likely to be scenarios where these groups are particularly vulnerable. Group 1 (perennial) species seem very vulnerable to drought and long periods where rainfall is very light. They are dominant when the soil is left undisturbed and when rainfall is heavy, so recommendations for land management could include irrigation, or cessation of ploughing or other maintenance, if this group is perceived to be sparse. Recommendations cannot be made for the group 2 (bunch grass and tall forb) species at this time, as this experiment has presented little indication of their requirements and vulnerabilities. The group 3 (annual) species are very opportunistic so can sprout and live their life cycle in a very short timeframe and their shallow roots are well placed to capitalise on small rainfall inputs. A reasonable hypothesis might therefore be that this group could be lost if a system were to go for a long period undisturbed so a grassy sward is likely to take over, or if rainfall inputs were extremely sporadic and heavy, so reserves of water in the top layers of soil were not maintained. Maintenance of this group would therefore include regular disturbance, or light water addition if severely droughted.

There has been much work testing how species partition their resources, mainly by grouping them into deep and shallow rooting species (Berendse 1979; Cross & Harte 2007; von Felten *et al.* 2009). These cluster loosely into life history groups, such as length of life cycle, which is linked to seed resource allocation and number, though not explicitly by perennation. Most biodiversity-ecosystem function studies have categorised species into grass-forb-legume (GFL), or by some characteristic such as C_3/C_4 photosynthetic pathway (Hector *et al.* 1999; He *et al.* 2002; Reich *et al.* 2004; De Deyn *et al.* 2009). In Chapter 2, I argue that traits should be chosen much more objectively, so groups can be created based on traits more closely linked to function. At the very least, grouping species into perennials and annuals results in discrete effects on ecosystem function, which I suggest are stronger than those of GFL. Therefore, the key recommendation for experiments using

functional traits groups in the future to describe biogeochemical cycling would be to emphasise perennation as a factor in their clustering technique, in order to encapsulate the differences in speed of tissue turnover and resource use necessary to complete the life cycle of these different species.

A great deal of literature has attempted to demonstrate that increasing species diversity leads to faster process rates, better resource use efficiency and resilience of primary productivity against disturbance (Tilman & Downing 1994; Tilman *et al.* 1996; Knops *et al.* 2001), the techniques used to show this has been widely criticised for having inherent design flaws (Grime 1997; Huston 1997; Bengtsson 1998; Loreau & Hector 2001; Naeem & Wright 2003). More recently, it became apparent that species identity, and more importantly, functional identity, is more descriptive, though finding general principles for grouping species are still undefined. There has remained a persistent assumption that species richness (superseded by functional diversity) can inform a number of key research questions in community ecology regarding extinction risk (rivet-popping hypothesis, Ehrlich & Ehrlich 1981), stability (Odum 1953), redundant species (Walker 1992), invasibility (Elton 1958) and keystone species (Power *et al*. 1996). While my experiment did not explicitly demonstrate that increasing functional groups is associated with higher flux and process rates, the differing responses of perennial and annual species, particularly through seasons, suggests that functional diversity may be a prerequisite for stability if that diversity included a range of root types and life spans. The experiment has indicated that when rain is scarce, certain characteristics are important in maintaining function; particularly lifespan which may be a proxy for rooting strategy and biotic turnover (e.g. annuals tend to be more shallow rooted and tissue turnover and decomposition is slower in perennials). Additionally, if climate change occurs as projected, each functional group has a role in maintaining function. The variation in rooting pattern and allocation between perennials and annuals creates more niches and substrates from species specific root exudates and litter inputs, which is also crucial for maintaining high microbial diversity (Bardgett 2005). The link between microbial diversity and function is still unclear, but microbial diversity has been empirically linked to processes including respiration (Bell *et al.* 2005).

Many species are known to be resource islands, where nutrients accumulate in high concentrations under individual plants, particularly caespitose grasses, although

they were not abundant enough to be able to demonstrate this in my study (Burke *et al.* 1998). I hope that in future years the species in group 2 will increase in abundance so their effect on function can be quantified and the conclusions from future field seasons will be more complex and subtle than basing them on plant longevity. Evidence suggests that plants are strong drivers of nitrogen cycling, and a variety of rooting patterns would allow for higher community level resource uptake, with less leaching losses; so more functionally diverse systems are likely to be more 'closed' systems in terms of nutrient retention (Tilman *et al.* 2006; Chapman *et al.* 2006). Describing rooting type could be a crucial next step in predicting soil functional processes.

6.2.3 PREDICTIONS

There are multiple methods used in evaluating the main drivers of ecosystem functions. However, many of these methods are not capable of predicting function, their purpose is to group and show directionality of important factors, but they cannot quantify effects (e.g. ordination techniques, Gross *et al.* 2008). Here I used a generalised linear modelling technique which is flexible, repeatable and offers robust predictions of function. While the majority of R^2 values were less than 50%, the retention of the same variables in the models over many months increases confidence in the method (Chapter 5). This temporal replication is an accepted statistical approach, used when spatial (plot level) replication is not possible (St Clair *et al.* 2009). I attributed the lack of retention of trait based variables in the model to lack of trait variation between the plots; more than one site is likely to be necessary to identify clearer relationships with function (Fortunel *et al.* 2009). The use of community weighted means for a number of different abundance measures has been statistically tested and verified in order to describe functional processes (Lavorel *et al.*1998); however, in this experiment weighted traits were less explanatory than abiotic variables, and only appeared at all during the growing season. Nevertheless, for finer scale processes, their use is justified for instances such as plot level processes at one point in time (Garnier *et al.* 2004; Dìaz *et al.* 2007a).

A method of predicting function which could result in better explanatory power is to use a taxon-free method, as recommended by Lavorel *et al.* (2008). With this, plants are sampled using a point-quadrat and their *in situ* traits are measured. This removes all doubt engendered by using greenhouse or hydroponically grown species in monoculture, or database data, and also removes the criticism that plants have such high plasticity, i.e. intra-specific variation that unless traits are measured from plants grown under a range of conditions, confidence in using them for predicting function is low (Albert *et al.* 2010). Unfortunately it involves a huge amount of effort and manpower. In this experiment it was inappropriate as harvesting the long-term plots would have affected function. Undoubtedly, an improvement on the accuracy of the method is required, as is more work to distinguish the effect of biomass from individual traits, particularly regarding processes directly affected by aboveground biomass such as decomposition.

6.3 LIMITATIONS

The DIRECT experiment took place on one site on lowland grassland in South-East England. Traditional statistics suggest that this is undesirable; a multisite comparison is often highly recommended, though often impractical. There are examples of experiments that compare many sites, for example BIODEPTH, which used a set of sites across Europe (Hector *et al.* 1999), but these are costly in terms of time and money, and are not without criticism. One potential problem is that under drought different soil textures favour grasses over shrubs, or vice versa; so the likelihood is that different sites would result in different assemblages under climate change (drainage capabilities and so on) (Sala *et al.* 1997). However, trait values of a given species are location sensitive measures, with the potential to change across soils, inclines and various other variables. Thus trait-based measures should be conducted across one site to minimise the intra-specific variability (Dìaz *et al.* 1999b).

A similar limitation is that of the potential difference in trait means and distributions between greenhouse and field, known as environmental filtering (an obvious example being aboveground biomass). One other way to overcome criticism regarding trait shifts from greenhouse to field is to conduct a validation test,

comparing the two. A test of this nature is underway, but could not be included in this thesis due to equipment constraints. This test uses an example of each species in each plot taken during the July 2010 vegetation survey, and measures three traits; aboveground biomass, specific leaf area and C:N ratio. A Bayesian analysis will then be carried out using the method described by Webb *et al.* (2010) using Bayesian multilevel models.

The treatments required manually removing unwanted species from the plots. Treatments that artificially create communities, ignoring natural rules of assembly, are difficult to maintain in a natural field site, thus weeding out plants that grew from the seedbank was a more viable option. This brings with it inevitable problems, as the functional groups were uneven in their dominance of the site. The process of weeding also created canopy gaps and alterations to microclimate, but these were filled very rapidly. The other issue with the treatments was the lack of presence of group 2 species. In other communities, especially MG1, *Dactylis glomerata* is often a dominant, but in the DIRECT experiment it has not filled the spaces in the plots. In future years there is a possibility that species in this group will increase in abundance, particularly *Lolium perenne*, which appeared late on in the experiment, but is generally a dominant species in MG6 grassland (Rodwell 1991).

Finally, the conclusions drawn here could not explicitly link changes in microbial activity to aboveground function, because it was only measured on two occasions, and no clear relationships were found. However, with the continuation of the experiment, this will become a key measure that will aid understanding of this system. Likewise, potential criticisms of the immaturity of this grassland will be countered as the project continues.

6.4 FUTURE RESEARCH

There are plans for this experiment to continue for at least another three years, allowing for further measurements of composition and function over a reasonable period of early succession. This thesis describes methods that a number of experiments are likely to follow in the future; functional groups created from

statistical clustering rather than taxonomic or morphological characteristics, several different climate change regimes, and a method of predicting ecosystem functions from simple measures of abiotic and biotic variables. Application of bespoke plant functional groups to climate change experiments is a rapidly growing field of study, and there is a proliferation of new possibilities to explore. The forthcoming IPCC report aims to offer more accurate regional projections with a greater emphasis on extreme weather, so future climate experiments will need to take this into account (IPCC 2007 www.ipcc.ch).

For the DIRECT experiment, there are a number of avenues for future research. Microbial biomass measures through the growing season are a top priority, with planned work on whole community characterisations, particularly describing fungal and bacterial biomass and identification of microbial taxa. This would offer more detailed perspectives on the drivers of ecosystem function, as roles of various soil microbial taxa become better described. Much more data is needed on the impact of extreme climates; the trends that appeared after 18 months of treatment between spring/summer rainfall and highly variable rainfall suggest that duration of drought is more detrimental to ecosystem processes than variability. The dearth of information on the impact of prolonged drought needs to be rectified, particularly since spring drought has become more frequent during the last decade in the South-East of England. There is also scope for studying higher trophic levels; some preliminary work on insect populations under drought is being carried out in the summer of 2011, and it may lead to more comprehensive experiments quantifying community interactions.

6.5 CONCLUSIONS

The experiment presented in this thesis has lent support to studies that suggest that functional diversity can modify effects of climate change in grasslands. There is evidence to suggest that prolonged drought through spring and summer will cause a strong decline in all belowground ecosystem functions, although in the long term these effects could be less damaging than suspected due to very rapid process recoveries after heavy rainfall inputs. Highly variable rainfall may have smaller

effects on function than previously thought, but the lack of recovery after the treatment ceased could indicate longer term, more damaging effects. By maintaining species functional diversity, ecosystem functions are less severely affected than in depauperate communities or monocultures. The possibility of prolonged drought or extremely rare rainfall events means that we could be ill-equipped to maintain services of temperate grasslands. Experiments of this nature are crucial to further our understanding of the complex linkages between plant community composition and ecosystem function; if the key predictors of ecosystem function can be identified then management schemes would be targeted to combat the effects of climate change. The DIRECT experiment is on a path to answer many critical questions about the effect of climate change on temperate grasslands, and the role of functional diversity in this response.

Figure 7: Climate change treatment with water collection system.

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Figure 8: DIRECT experiment, Winter 2009-2010

APPENDIX

APPENDIX 1: CLIMATE

Table 1A: Monthly and annual rainfall totals (mm) for the ten years before the experiment started, with mean values of these.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
1998	105.3	9.4	67.6	104.8	25.9	101.7	35	20.7	105.3	140.2	125.4	96.4	863.1
1999	113.6	31.7	36.9	56.8	35.9	74.2	14.4	105.3	124	67.4	61.7	85.5	834.9
2000	27	80.1	22.2	145.3	99.1	20.3	56.1	46	107.1	196.9	41.9	132.9	1104.3
2001	99.3	99.6	121.7	78.6	32.2	23.4	55.3	79.7	71.8	132.8	170.4	133.8	860.8
2002	85.4	101.7	46.5	43.3	92.2	59	73.4	47.3	40.3	92.6	40.7	25.8	1005.1
2003	86.8	32.4	25.1	36.1	45.4	46.8	58.3	18.1	12.3	51	183.5	140	641.8
2004	104.1	32.5	43.6	76.8	51.8	33.3	53.3	102.7	35.1	129.6	144.3	85.3	756.9
2005	39.3	24.9	51.4	50.2	33.1	34.5	61.6	55.1	40.4	98	34.8	59.3	612
2006	23.4	57.7	58.6	39	96	21	39.5	62.1	70.6	118	54.3	69.3	788.4
2007	79.7	103.6	55.6	2.5	103.8	102.4	120.1	53.5	30.6	45.7	96.7	105.9	845.2
2008	108.3	26.5	92.6	62.4	86.5	43.6	76.3	75.1	72.1	65.5	81.1	66.5	851.1
AVE	79.3	54.6	56.5	63.3	63.8	50.9	58.5	60.5	64.5	103.4	94.1	91.0	833.1

APPENDIX 2

APPENDIX 2.1: METHODS FOR OBTAINING TRAIT DATA

I obtained seed for 65 grassland plant species native to the field site area of Silwood Park (51°24'23.16"N, 0°38'55.33"W) (Crawley, 2005) from Herbiseed, (Twyford, England). These were sown onto moist John Innes Seed Compost in 19˚C±1˚C glasshouses with a 8/16h photoperiod, watered as required and allowed four weeks for germination. I then transplanted five replicates of each species to grow individually in 2 foot long drainpipes, covered with mesh at the bottom to allow free drainage, for 90 days in mesotrophic grassland topsoil. All plants were kept under the same conditions as before.

After 90 days (or at flowering for very short lived species), I used a Ciras-1 Infra-Red Gas Analyser (PP Systems, Hitchin, UK) with an Integrated Cuvette Air Supply Unit and standardised light emitting diodes (LED) to measure the evaporation ($mmoles^{-1}m^2s^{-1}$) and stomatal conductance ($mol^{-1}m^2s^{-1}$) of a healthy leaf, on each plant. I conducted all measurements between 10:00 and 14:00 hrs in full sunlight, recording measurements when values were stable.

After harvesting the plants and washing the roots, over a 1mm aperture sieve, we measured photosynthetic surface area (PSA) of the aboveground plant organs using a scanner and the "Leaf Area" program (Unit of Comparative Plant Ecology, Sheffield, 2005), which measured the area of the leaves to an accuracy of $0.001mm^2$. I dried the aboveground biomass (AGB) for 48 hours at 60˚C, and calculated specific leaf area using equation 2.1.

$$SLA\left(mm^2mg^{-1}\right) = \frac{PSA\ (mm^2)}{AGB(mg)} \quad \text{Eq. 2.1}$$

I ground the aboveground biomass using a ball mill and determined total foliar nitrogen concentration using Kjeldahl (acid digestion) analysis on a Foss-Tecator™ Digestion System (Warrington, UK) and analysed colourimetrically using a Skalar SAN^{++} Continuous Flow Analyser (York, UK).

APPENDIX 3

APPENDIX 3.1 SITE MAP

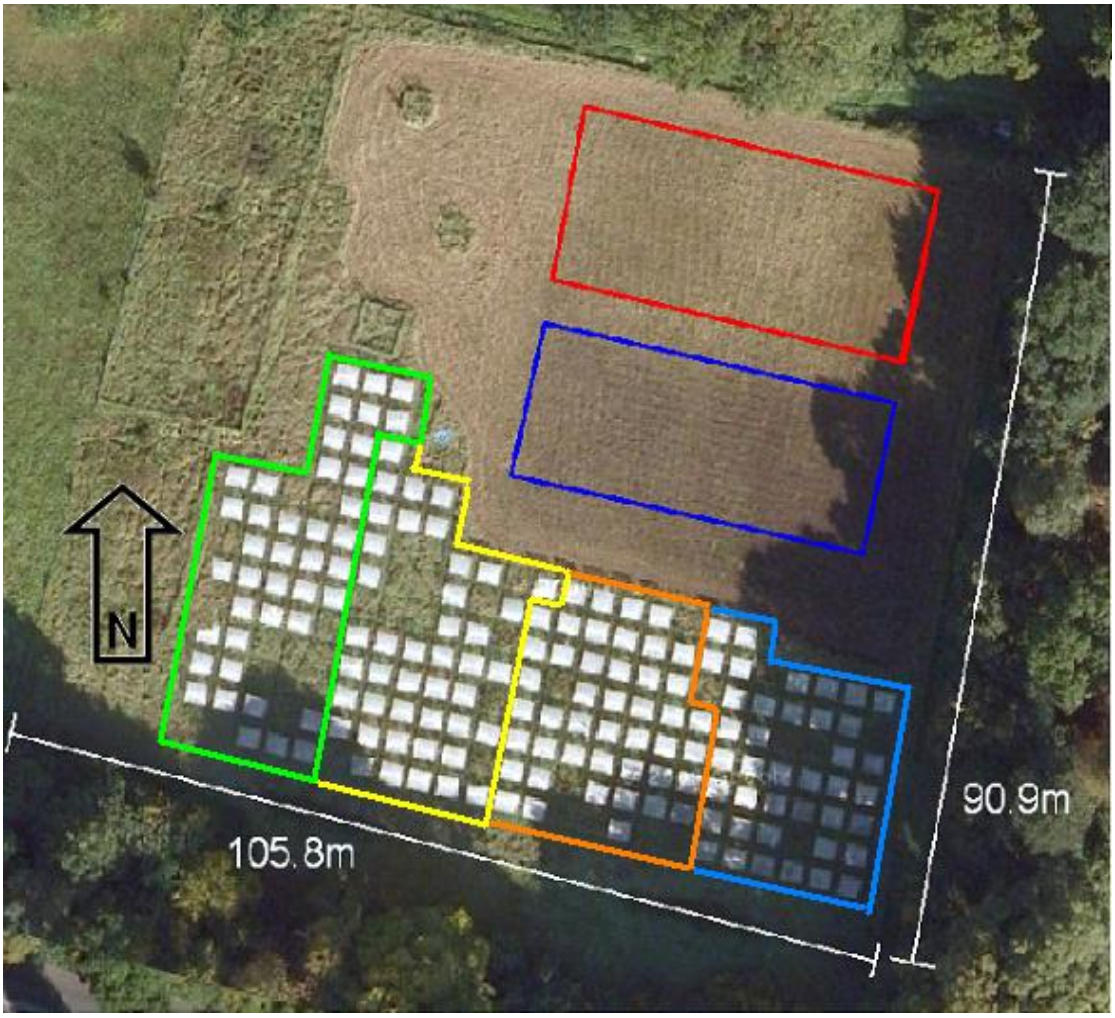


Figure 3A: Image of Four Acre Field in June 2009, accessed from Google maps 11/11/10. The blue and red rectangles delimit where fertiliser was applied in 2006 and 2007. The road runs parallel to the 105.8m long periphery of the field, ~20m away. The shelters slope into the prevailing wind. Block 1 is surrounded by blue, through to block 4 in green.

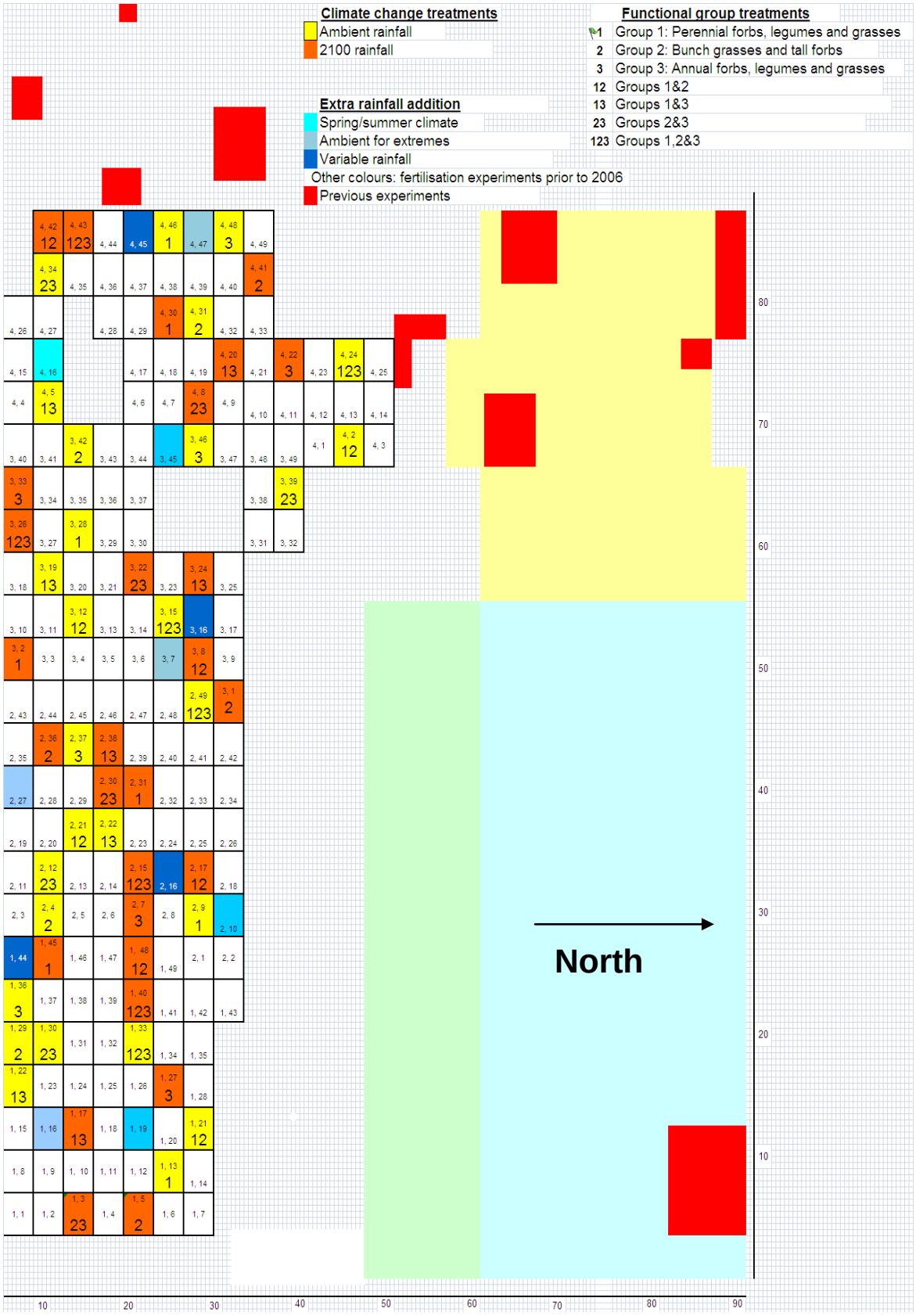


Fig. 3B: Schematic of the DIRECT experiment field site. Small numbers refer to block and plot, large numbers are functional group combinations. The main road runs down the left hand side of the diagram.

APPENDIX 3.2: INITIAL SITE CHARACTERISATION

Four soil samples from each plot were collected in October 2008 for analysis (n=56). The plot samples were homogenised and sieved, and 1:1 wet soil to dH_2O was mixed. The pH was measured with a pH meter (Mettler Toledo, Leicester, UK). They were then analysed using a one-way ANOVA with block as a factor, and then a post-hoc Tukey's HSD test to find that the first block (at the lowest point of the incline) had a significantly higher pH than the other three blocks (Block 1 mean = 5.87±0.05, Combined blocks 2-4 mean = 5.59±0.04, $F_{3,51}$=5.15, $p<0.01$).

A soil augur was used (10.5cm depth, 3cm diameter, 23.63cm^3 volume) to collect two soil samples from every plot. These samples were dried overnight at 60˚C before weighing. The bulk density was calculated using the equation weight/volume, and analysed using a one-way ANOVA as above, which found that there is no difference in compaction or pore space across the site ($F_{3,52}$= 2.36, $p>0.05$).

Four samples of soil from each plot were homogenised and dried, before being passed through sieves that had aperture sizes approximate to USDA particle size classifications (0.002µm, 0.063µm- proxy for 0.05µm, 0.09µm- proxy for 0.1µm, 0.425µm instead of 0.5µm, 1mm and 2mm, USDA 2010a). The USDA soil triangle was used to describe the site overall as loamy sand (93% sand, various grades, 7% silt). Each soil grade was arcsine transformed and blocks compared in an ANOVA with a post-hoc Tukey's HSD test. None of the soil grades changed across the site, except medium sized sand, which occurred significantly less (34.78%±2.93) in block 1 than in block 4 on the top of the incline (52.06%±3.21, $F_{3,10}$=3.46, $p<0.01$).

Two soil pits were dug, one at either end of the site, to ascertain the rooting and soil depth. The rooting and soil depths were shallower at the South-Eastern side (20cm rooting depth, 39cm soil depth in block 1, 35cm rooting depth, 51cm soil depth at block 4).

APPENDIX 3.3: CLIMATE CHANGE PROJECTIONS

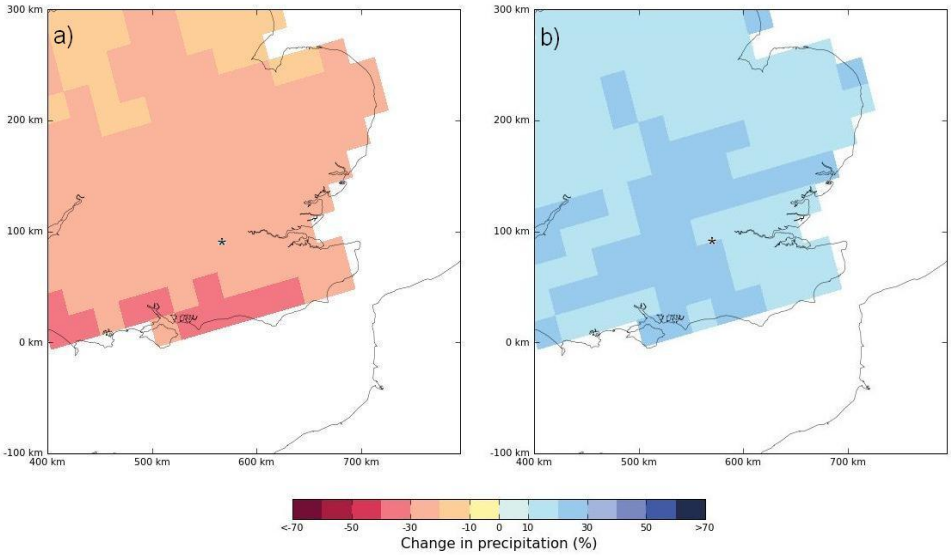


Figure 3C: Climate projections for 2070-2099 in the UK generated from the UKCP09 user interface (Murphy *et al* 2009). The projections are from the Medium emissions scenario (SRES A1B, Nakićenović *et al.* 2000) in a) summer (JJA) and winter (DJF) and predict that rainfall will decrease in volume between 20 and 30% from the 1961-1990 summer average in summer, and increase between 10 and 20% from the 1961-1990 winter level. The study site is marked with a star.

APPENDIX 3.4: DESIGN OF RAINOUT SHELTERS

In order to recreate the summer rainfall projection, individual rainout shelters were designed, which were intended to be durable, easily removed and replaced and to allow as much sunlight transmission as possible. The design of prototypes, showed transparent corrugated plastic was cost effective, easily replaced and very durable, mounted upon a square frame and at an angle of 12˚, enough to allow the water to run off. The frames were angled into the prevailing wind (lowest end SW) and 0.9m high at the front, 1.4m high at the back (Fig. 3D). These shelters were put up between June and August each year, and over both ambient and climate change treatments to control for differences in microclimatic and light conditions between treatments, as well as seed dispersal. The design

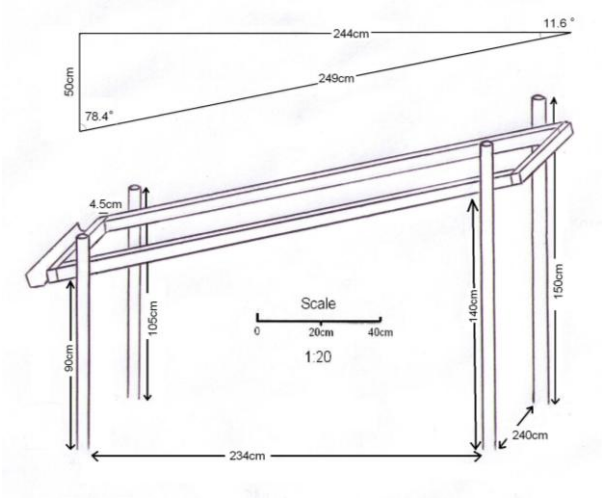


Figure 3D: Schematic of the dimensions of the rainout shelters.

is mainly based upon fixed-location shelters in Argentina created by Yahdjian and Sala (2006). The ambient plots also had plastic sheeting, but many holes were drilled in the grooves to allow all water to fall through.

APPENDIX 3.5: MEASUREMENTS OF LIGHT UNDER THE SHELTERS

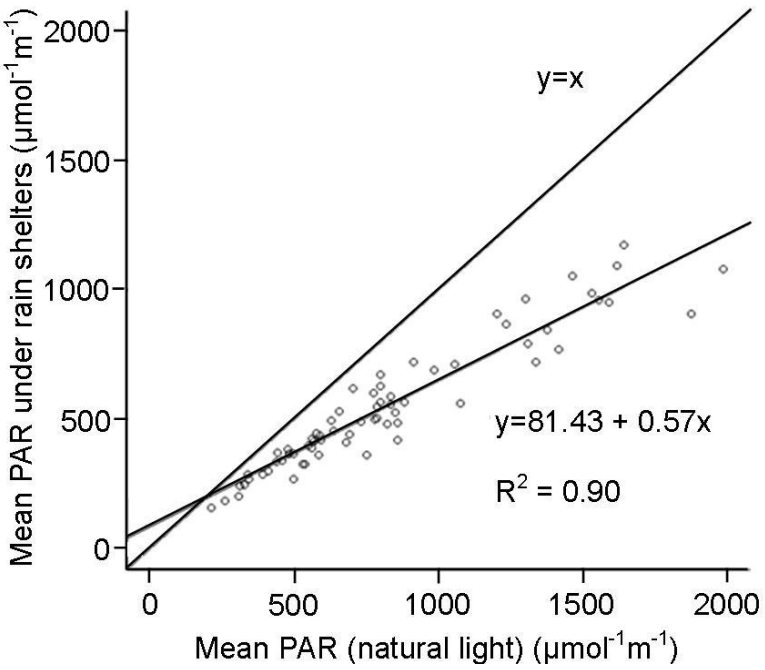


Figure 3E: As light intensity increases at the DIRECT field site, more light is excluded under the shelter, (t=24.576, d.f.= 65, $p < 0.0001$). The model explains 90.3% of the variation in the light data.

A PAR meter was used to measure the difference in light under and above each shelter, across a span of one day in June 2008 (10am-6pm). Three standardised points below the shelters were measured and a mean taken, and three above. A one-way ANOVA tested whether the holes in the ambient treatment caused an increase in light compared to climate change, but there was no significant difference ($F_{1,62}=0.28$, $p>0.05$). Finally, a linear modelling approach was used to test whether the light differed consistently throughout the day, which found that there was a higher intensity of light lost in full sunlight, but that this varied from full exposure in a linear manner (Fig. 3E, t=24.58, d.f.= 65, $p < 0.0001$). It should be noted that much of the interception occurs at intensities that are too intense for photosynthesis, and so the light intensity is effectively halved at these high levels by the shelters. Therefore more light is available on sunny days.

APPENDIX 3.6: MEASUREMENT OF DISCREPANCY OF iBUTTONS AND CORRECTION FACTOR

DS1923 Hygrochron temperature/humidity Dataloggers (Homechip, Milton Keynes) were deployed on the 56 core plots to take temperature and humidity measurements at half hourly intervals, at vegetation height. The data were assimilated using the Thermodata Viewer® 3.1.3 program (Thermodata Pty Ltd, Victoria, Australia). Paired t-tests using R version 2.12.0 (R Core Development Team, 2009) showed that there was significant deviance from measurements from a reliable source (Weatherlink weather station) when compared in the same locality over a nine day period (temperature: $t=11.90$, $d.f.=384$, $p<0.0001$, humidity: $t= 2.07$, $d.f.=384$, $p<0.05$).

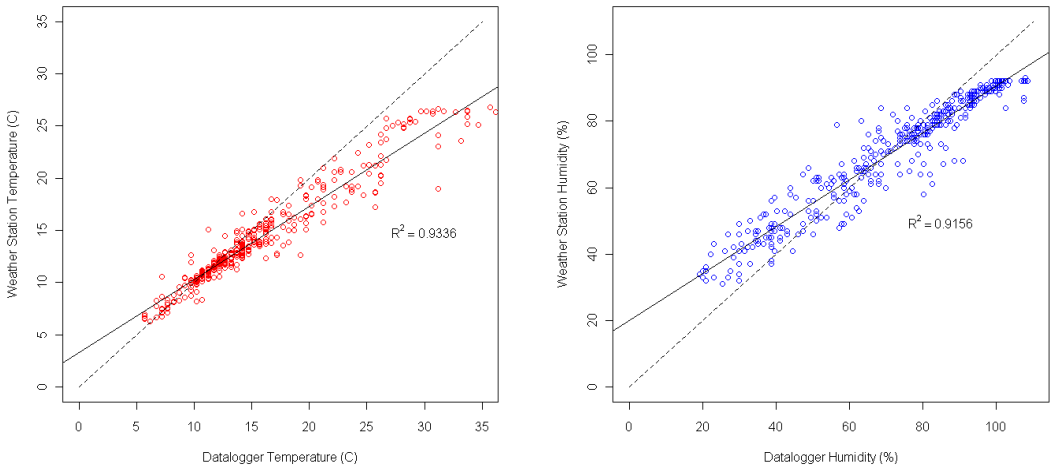


Figure 3F: Comparison of a iButton datalogger to a Weatherlink logger a) The temperature is most accurately measured at 10˚C, below which they overestimate the true temperature, and over which they underestimate it. The data from the iButtons can be recalculated using the formula: $y= 0.7010x + 3.2763$ ($t= 73.318$, $d.f.=384$, $p<0.0001$). b) The most accurate relative humidity is approximately 75% RH, below which it overestimates the true humidity and above which it underestimates it. The RH data can be recalculated using the formula: $y= 0.7055x + 20.002$ ($t= 64.45$, $d.f.= 384$, $p<0.0001$).

Linear regressions were conducted to find the equations of the line of best fit in figure 3Ea) and b). Once the intercept and the slope had been found, the correction factor given by this regression was applied to all the iButton data. The correction factor for temperature measures was $y= 0.7010x + 3.2763$, and the correction factor for humidity was $y= 0.7055x + 20.002$.

Patterns of data over the duration of this study are presented in Fig. 3G.

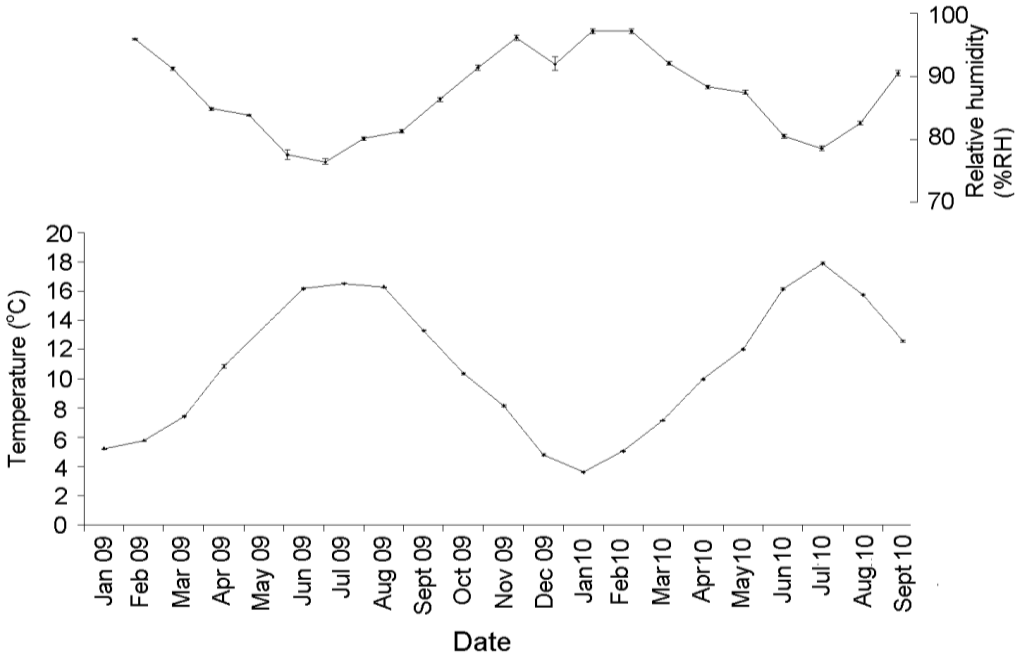


Figure 3G: Microclimate data measured using dataloggers, averaged across each month of the experiment. Error bars depict ±1 SEM.

APPENDIX 3.7: CALCULATION OF SEED REALLOCATION TO THE PLOTS.

$$\text{proportion of species } x \text{ in unweeded plots} = \frac{\left(\left(\frac{\text{sp. } x\ (\%)_{\text{plot 1}}}{\text{total spp. } (\%)}\right) + \left(\frac{\text{sp. } x\ (\%)_{\text{plot 2}}}{\text{total spp. } (\%)}\right) + \cdots \left(\frac{\text{sp. } x\ (\%)_{\text{plot } n}}{\text{total spp. } (\%)}\right)\right)}{n \text{ plots}}$$

Equation 3.1.

'sp. *x*' describes the percentage cover of a given species.

'total spp.' refers to the total cover of all species present of the functional group that sp. *x* belongs to.

'*n* plots' signifies all eight replicates of the control plots.

Eq. 3.1 was used to calculate the proportion of seed to be added to each plot according to how many groups were present (out of 2g for each group if all three groups were present, 3g if two groups were present, and 6g if only one). This was primarily so each plot would receive a total of 6g of seed. Seed was obtained from Herbiseed (Twyford UK), except in the case of *Arrhenatherum elatius* ssp. *bulbosum* which had bulbs from the field site added on a dry weight basis. The seed was added in May 2009.

APPENDIX 3.8: KJELDAHL METHOD FOR NITROGEN AND PHOSPHATE DIGESTION

Soil samples were dried at 80°C for 24 hours, before being ground using a ball mill. 0.25g±0.01g of ground soil was used, exact weight recorded, with one selenium tablet and 3ml 98% sulphuric acid (H_2SO_4, VWR, AnalaR grade for N analysis). The samples were digested (Gerhardt Kjeldatherm KB40S) for 30 minutes at 250˚C, then at 400˚C for a further two hours. Two blanks (the whole extraction occurring without the soil, to account for impurities) and two standard reference material (SRM- ISE 985, LGC Standards, Teddington, UK) samples per batch of 40 were included to increase precision. The block was cooled before each sample was diluted with distilled water and mixed on a Whirlimix (Fison's Scientific Apparatus, Loughborough). The samples were then filtered into 25ml volumetric flasks and diluted to volume.

APPENDIX 3.9: EXTRACTION OF INORGANIC NITROGEN AND PHOSPHATE FROM WET SOIL

Allen's (1989) method was used to extract inorganic nitrogen (ammonium, NH_4^+ and nitrate/nitrite, NO_3^-/NO_2^-) from wet soil using a weak extractant (20g soil:75ml 1M potassium chloride, KCl, VWR, AnalaR grade) and agitated for one hour on a Laboshake LS/RO500 (Gerhardt Königswinter, DE). Plant available phosphate (PO_4^+) was also extracted using 10g soil with 150ml Truogs solution (6g $(NH_4)_2SO_4$, 10ml 0.05M H_2SO_4, 2l dH_2O, AnalaR grade, VWR, UK). When the mixtures had been agitated, they were filtered using Whatman No.1 filter paper.

To ascertain soil moisture, a known amount of soil was weighed and dried overnight at 80˚C, before being reweighed. The moisture content calculated from this ([wet soil – dry soil]/wet soil) was used to calculate the exact weight of soil used in the extraction, which was carried out using wet soil so as not to change the microbial activity and substantially alter the nutrient contents.

APPENDIX 3.10: PRECISION AND USE OF THE SKALAR SAN^{++} CFA, FOR EXTRACTABLE AND TOTAL NUTRIENT DATA.

The CFA was calibrated using standards taken from stock solutions of ammonium chloride sodium nitrate, and potassium di-hydrogen o-phosphate, for ammonium, nitrate and phosphate, respectively. The middle standard was re-sampled after every 20 samples throughout the run to prevent drift, followed by a matrix blank. The blanks were set to zero to increase precision by removing any bias of the sample from the matrix (P. Visser, Skalar, pers. comm.). Analytical replicates were created (5% = 3 replicates) every six months to test the precision of the method (USEPA 2001).

Table 3A: 5% of samples were measured twice in the Skalar SAN^{++} CFA (n=3) during a number of analyses to test the precision of the machine.

Date	Nutrient	Difference between samples (%)
01/11/2008	Ammonium	2.44
01/11/2008	Nitrate	32.82
01/11/2008	Phosphate	49.54
01/02/2009	Ammonium	35.44
01/02/2009	Nitrate	50.66
01/04/2009	Phosphate	2.47
01/06/2009	Phosphate	13.78
01/08/2009	Nitrate	54.15
01/09/2009	Phosphate	49.26

APPENDIX 3.11: CATIONS

This technique partially digested dry soil in nitric acid and hydrogen peroxide to produce a digestate where the cations ^{27}Al, ^{24}Mg, ^{44}Ca, ^{39}K, ^{55}Mn and ^{47}Ti can be measured. 5% (n=2) analytical replicates were included, one blank per batch (two in total) and two samples of SRM per batch (NIST River Sediment 2704). The protocol USEPA 3050B for ICP-AES by Edgell (1988) was used for this analysis. Triplicate analysis of each digestate was carried out using ICP-AES (Agilent 7500c Series, Wokingham, UK), so the data analysed were means of those samples.

The soil was ground using a pestle and mortar (the metal ball mill could compromise the results), then 1g±0.001g was added to 10ml of a 1:1 solution of nitric acid (HNO_3, VWR, Aristar grade) and distilled water (dH_2O). The mixture was covered and heated to 95˚C±5˚C on a hot plate in a fume cupboard, refluxing for 10-15 minutes without boiling. The sample was cooled, then 5ml of concentrated HNO_3 was added and refluxed at 95˚C±5˚C for a further 40 minutes. The cover was removed and the digestate allowed to evaporate to approximately 5ml, before cooling. 2ml of dH_2O and 3ml of 30% hydrogen peroxide (H_2O_2, VWR, Aristar grade) were added and the samples gently warmed to minimise effervescence. After cooling again H_2O_2 was added in 1ml aliquots up to a total of 10ml while warming and cooling until effervescence was no longer seen. The vessels were covered and heated at 95˚C±5˚C for two hours until the digestate had been reduced to ~5ml. 10ml of concentrated hydrochloric acid was added (HCl, VWR, AnalaR grade), and the mixture was covered and refluxed at 95˚C±5˚C for a further 15 minutes. Finally the sample was filtered into a 100ml volumetric cylinder and diluted to volume with dH_2O. Limits of detection of the sampler are presented (Table B), and the precision of the analytical replicates (Table C).

Table 3B: Limits of detection of the ICP-AES analyser (ppb)

	^{24}Mg	^{27}Al	^{39}K	^{44}Ca	^{47}Ti	^{55}Mn
Limit of detection (ppb)	0.026158	0.074714	1.492034	0.458712	0.066391	0.016717

Table 3C: A measure of the precision of analytical replicates by direct comparison

Sample ID	^{24}Mg	^{27}Al	^{39}K	^{44}Ca	^{47}Ti	^{55}Mn
Replicate 3.19	505.01± 3.8	3992.48± 63.2	948.40± 4.5	538.42±21.6	89.54± 1.3	118.99± 0.3
Replicate 3.19	454.56± 2.4	3713.55± 29.7	811.30± 24.4	352.11±1.8	69.55± 1.8	115.29± 1.6
Difference (%)	**9.99**	**6.99**	**14.46**	**34.6**	**22.33**	**3.11**
Replicate 4.05	366.65± 4.4	3042.28± 18.8	650.28±15.6	233.65±6.4	77.78± 1.6	99.63± 0.3
Replicate 4.05	438.73± 4.6	3521.90± 56.9	823.54±6.8	248.66±9.5	121.18± 2.3	110.93± 0.6
Difference (%)	**16.4**	**13.62**	**21.04**	**6.04**	**35.81**	**10.19**

Table 3D: Average relative standard deviation (RSD) of samples for each cation tested, as a percentage of the original mean value. This tests the precision of the machine; its ability to produce consistent results for the same samples must be monitored.

	^{24}Mg	^{27}Al	^{39}K	^{44}Ca	^{47}Ti	^{55}Mn
%RSD	0.71	0.8	2.37	2.73	1.93	0.88

APPENDIX 3.12: STATISTICAL OUTPUT

APPENDIX 3.12.1: CO_2 AND WATER FLUX

Table 3E: Results of ANOVAs to determine treatment effects on net ecosystem exchange of CO_2. (Asterisks: *= 0.01-0.05, **=0.01-0.001, ***= <0.001). Block was included as an error term where significant.

Factor	d.f	Mar 09		May 09		Jun 09		Sep 09		Oct 09		Nov 09		Feb 10		May 10		Jun 10		Jul 10		Aug 10		Sep 10	
		F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Model		**2.84**	**	**2.22**	*	**5.19**	***	1.81	NS	1.30	NS	**4.68**	***	0.70	NS	**4.26**	***	0.85	NS	1.11	NS	0.77	NS	0.89	NS
Block	3	**6.36**	**	-	-	**26.06**	**	-	-	-	-	**19.00**	***	-	-	-	-	-	-	-	-	-	-	-	-
PAR	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**10.04**	**	-	-	-	-	-	-	-	-
Climate change	1	0.94	NS	1.46	NS	0.01	NS	1.18	NS	0.32	NS	0.02	NS	0.01	NS	**9.78**	**	0.54	NS	0.53	NS	0.87	NS	1.70	NS
Functional diversity	6	**3.77**	**	1.49	NS	**2.60**	*	2.03	NS	1.35	NS	2.00	NS	1.22	NS	1.74	NS	1.20	NS	0.62	NS	0.45	NS	0.99	NS
PAR*climate	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**13.31**	***	-	-	-	-	-	-	-	-
PAR*diversity	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**5.50**	***	-	-	-	-	-	-	-	-
Soil moisture*diversity	6	-	-	0.09	NS	**2.92**	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Climate*diversity	6	0.46	NS	**5.59**	***	1.21	NS	1.68	NS	1.40	NS	0.96	NS	0.29	NS	2.17	NS	0.56	NS	1.70	NS	1.07	NS	0.67	NS
Model R^2		0.54		0.56		0.79		0.36		0.29		0.66		0.18		0.72		0.21		0.26		0.19		0.22	

Table 3F: Results of ANOVAs to determine treatment effects on soil respiration (R_{eco}). (Asterisks: *= 0.01-0.05, **=0.01-0.001, ***= <0.001). Block was included as an error term where significant.

Factor	d.f	Mar 09		May 09		Jun 09		Sep 09		Oct 09		Nov 09		Feb 10		May 10		Jun 10		Jul 10		Aug 10		Sep 10	
		F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Model		**4.26**	***	**4.09**	***	**2.02**	*	**2.11**	*	0.91	NS	1.08	NS	1.06	NS	1.81	NS	**6.07**	***	0.95	NS	1.28	NS	0.77	NS
Block	3	**6.36**	**	-	-	**6.24**	**	**3.58**	*	-	-	-	-	-	-	**3.62**	*	-	-	-	-	-	-	-	-
Climate change	1	0.14	NS	0.03	NS	0.79	NS	**8.37**	**	2.24	NS	0.00	NS	0.14	NS	1.37	NS	4.27	NS	1.13	NS	1.52	NS	0.04	NS
Functional diversity	6	**3.50**	**	**8.26**	***	0.47	NS	0.99	NS	0.33	NS	0.48	NS	0.85	NS	0.67	NS	**5.21**	**	0.49	NS	0.88	NS	0.82	NS
Soil moisture*diversity	6	-	-	**3.03**	*	-	-	-	-	-	-	-	-	-	-	1.84	NS	**2.94**	*	-	-	-	-	-	-
Climate*diversity	6	**5.35**	***	**4.96**	**	1.67	NS	1.45	NS	1.27	NS	1.86	NS	1.41	NS	2.16	NS	**10.94**	***	1.38	NS	1.65	NS	0.84	NS
Model R^2		0.66		0.83		0.45		0.46		0.21		0.25		0.25		0.68		0.85		0.23		0.28		0.20	

Table 3G: Results of ANOVAs to determine treatment effects on evapotranspiration rate (ET). (Asterisks: *= 0.01-0.05, **=0.01-0.001, ***= <0.001). Block was included as an error term where significant.

Factor		Mar 09		May 09		Jun 09		Sep 09		Oct 09		Nov 09		Feb 10		May 10		Jun 10		Jul 10		Aug 10		Sep 10	
	d.f	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Model		**2.59**	**	2.06	*	1.80	NS	**2.41**	*	0.91	NS	**3.35**	**	0.86	NS	**5.34**	***	**4.06**	***	1.01	NS	0.44	NS	0.65	NS
Block	3	**9.13**	***	-	-	-	-	**14.35**	***	-	-	**13.59**	***	-	-	**17.14**	***	**14.54**	***	-	-	-	-	-	-
PAR	1	**4.89**	*	**14.84**	***	-	-	**45.65**	***	-	-	**10.96**	**	**7.80**	**	**22.58**	***	-	NS	-	-	-	-	-	-
Soil moisture	1	1.92	NS	-	NS	**5.64**	*	4.14	NS	-	-	0.00	NS	-	-	-	-	-	-	-	-	-	-	-	-
Climate change	1	0.01	NS	0.73	NS	0.03	NS	0.12	NS	2.24	NS	**5.84**	*	0.10	NS	2.51	NS	1.46	NS	1.79	NS	0.71	NS	0.31	NS
Functional diversity	1	0.53	NS	1.28	NS	**2.97**	*	0.89	NS	0.33	NS	1.05	NS	0.29	NS	1.74	NS	2.01	NS	1.60	NS	0.42	NS	0.39	NS
PAR*climate	1	-	-	-	-	-	-	-	-	-	-	**4.55**	*	-	-	-	-	-	-	-	-	-	-	-	-
Soil moisture*diversity	6	-	-	-	-	-	-	**3.44**	*	-	-	0.55	NS	-	-	-	-	-	-	-	-	-	-	-	-
Climate*diversity	6	1.53	NS	0.93	NS	0.95	NS	1.35	NS	1.27	NS	**4.09**	*	0.41	NS	0.64	NS	1.30	NS	0.30	NS	0.42	NS	0.98	NS
Model R^2		0.58		0.41		0.47		0.63		0.22		0.87		0.23		0.71		0.62		0.24		0.12		0.17	

APPENDIX 3.12.2: TOTAL NUTRIENTS

Table 3H: Results of ANOVAs to determine treatment effects on total soil nitrogen concentration. (Asterisks: *= 0.01-0.05, **=0.01-0.001, ***= <0.001). Block was included as an error term where significant.

Factor	d.f	Oct 08		Sep 09		Sep 10	
		F	p	F	p	F	p
Model		1.71	NS	**1.92**	*	1.38	NS
Block	3	**4.89**	**	**4.55**	**	**5.42**	**
Climate	1	3.21	NS	0.46	NS	0.24	NS
Functional diversity	6	1.50	NS	0.92	NS	0.61	NS
Climate*diversity	6	0.36	NS	1.86	NS	0.31	NS
Model R^2		0.44		0.45		0.37	
Model d.f		17,37		16,38		16,38	

Table 3I: Results of ANOVAs to determine treatment effects on total soil phosphorus concentration. (Asterisks: *= 0.01-0.05, **=0.01-0.001, ***= <0.001). Block was included as an error term where significant.

Factor	d.f	Oct 08		Sep 09		Sep 10	
		F	p	F	p	F	p
Model		1.17	NS	**2.35**	*	**2.44**	*
block	3	**3.12**	*	2.45	NS	**6.78**	***
Climate	1	0.20	NS	1.13	NS	1.28	NS
Functional diversity	6	0.78	NS	**2.74**	*	0.65	NS
Climate*diversity	6	0.86	NS	2.11	NS	2.26	NS
Model R^2		0.35		0.50		0.51	
Model d.f		17,37		16,38		16,37	

APPENDIX 3.12.3: MINERALISATION

Table 3J: Mineralisation rates between December 2008 and September 2009. (Asterisks: *= 0.01-0.05, **=0.01-0.001, ***= <0.001). Block was included as an error term where significant.

Factor	d.f.	Dec 08 - Mar 09		Mar 09 - Jun 09		Jun 09 - Sept 09		Sept 09 - Dec 09		Dec 10 - Mar 10		Mar 10 - Jun 10		Jun 10 - Sept 10	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Model	24,31	**2.09**	*	0.94	NS	1.75	NS	**3.08**	**	1.27	NS	**3.76**	***	1.45	NS
Block	3	-	-	-	-	-	-	**3.70**	*	-	-	-	-	-	-
Air temperature	1	3.66	NS	0.13	NS	-	-	0.69	NS	-	-	**28.25**	***	-	-
Soil moisture	1	-	-	-	-	0.96	NS	1.52	NS	-	-	3.15	NS	-	-
Climate change	1	0.03	NS	0.35	NS	0.02	NS	1.89	NS	0.23	NS	0.39	NS	0.00	NS
Functional diversity	6	2.15	NS	1.33	NS	1.80	NS	1.59	NS	0.25	NS	2.41	NS	0.81	NS
Air temp * soil moisture	1	-	-	-	-	-	-	**6.69**	*	-	-	-	-	-	-
Air temp * diversity	6	2.33	NS	1.32	NS	-	-	**6.35**	***	-	-	5.07	NS	-	-
Soil moisture * diversity	6	-	-	-	-	**3.40**	**	-	-	-	-	0.69	NS	-	-
Climate * diversity	6	2.11	NS	0.42	NS	0.48	NS	1.24	NS	0.37	NS	**3.45**	*	**2.33**	*
Model R^2		0.56		0.35		0.51		0.75		0.30		0.81		0.31	

Table 3K: Main effects of summed nitrification in 2009 and 2010. (Asterisks: *= 0.01-0.05, **=0.01-0.001, ***= <0.001). Block was included as an error term where significant.

Factor	d.f	Dec 08 - Mar 09		Mar 09 - Jun 09		Jun 09 - Sept 09		Sept 09 - Dec 09		Dec 10 - Mar 10		Mar 10 - Jun 10		Jun 10 - Sept 10	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Model	24,31	**2.59**	**	1.48	NS	**2.30**	*	1.17	NS	0.82	NS	**2.95**	**	**2.41**	*
Block	3	**3.23**	*	**2.91**	*	**5.81**	**	-	-	-	-	**8.93**	***	-	-
Air temperature	1	1.65	NS	-	-	0.02	NS	-	-	-	-	-	-	0.05	NS
Soil moisture	1	0.08	NS	**6.24**	*	0.03	NS	-	-	-	-	1.28	NS	0.43	NS
Climate change	1	**5.87**	*	1.10	NS	3.06	NS	1.66	NS	1.07	NS	3.40	NS	1.56	NS
Functional diversity	6	1.23	NS	1.24	NS	0.64	NS	1.48	NS	0.75	NS	0.49	NS	2.10	NS
Air temp * climate	1	-	-	-	-	**8.64**	**	-	-	-	-	-	-	-	-
Air temp * diversity	6	0.78	NS	-	-	1.72	NS	-	-	-	-	-	-	-	-
Soil moisture * climate	1	-	-	-	-	**4.32**	*	-	-	-	-	**8.76**	**	2.96	NS
Climate * diversity	6	**5.48**	***	0.29	NS	2.03	NS	0.79	NS	0.86	NS	1.64	NS	**3.59**	**
Model R^2		0.68		0.41		0.69		0.23		0.21		0.59		0.53	

APPENDIX 3.12.4: CATIONS

Table 3L: Results of ANOVAs to determine treatment effects on cation availability in the soil. (Asterisks: *= 0.01-0.05, **=0.01-0.001, ***= <0.001). Block was included as an error term where significant.

Factor	d.f.	Magnesium		Aluminium		Potassium		Calcium		Ca/Mg		Ca/Al	
		F	p	F	p	F	p	F	p	F	p	F	p
Model	13,42	0.67	NS	0.81	NS	0.96	NS	1.46	NS	2.03	*	1.14	NS
Block	3	-	-	-	-	-	-	-	-	7.08	***	3.84	*
Climate change	1	0.76	NS	0.37	NS	0.10	NS	3.0	NS	3.10	NS	0.90	NS
Functional diversity	6	0.53	NS	0.71	NS	0.44	NS	0.47	NS	0.48	NS	0.47	NS
Climate*diversity	6	0.79	NS	0.97	NS	1.63	NS	1.69	NS	1.71	NS	0.50	NS
Model R^2		0.17		0.20		0.23		0.46		0.59		0.32	

APPENDIX 3.12.5: EXTRACTABLE NUTRIENTS

Table 3M: Results of ANOVAs to determine treatment effects on extractable ammonium concentration in the soil. (Asterisks: *= 0.01-0.05, **=0.01-0.001, ***= <0.001). Block was included as an error term where significant.

		Nov 08		Dec 08		Feb 09		Apr 09		Jun 09		Jul 09		Aug 09		Sep 09		Dec 09		Mar 10		May 10		Jun 10		Jul 10		Aug 10		Sep 10	
Factor	d.f	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Model	16,39	1.7	NS	1.2	NS	4.6	***	1.2	NS	2.2	*	0.7	NS	1.3	NS	1.4	NS	1.2	NS	1.4	NS	1.6	NS	0.8	NS	2.2	*	2.1	NS	3.6	** *
Block	3	6.6	**	-	-	5.2	**	2.3	-	-	-	-	-	-	-	-	-	-	-	-	-	2.4	-	-	-	7.8	***	5.5	**	15	** *
Average air temperature	1	-	-	-	-	2.1	NS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.9	*
Climate	1	3.8	NS	0.6	NS	12	**	1.3	NS	1.4	NS	0.2	NS	1.5	NS	3.3	NS	2.1	NS	2.6	NS	1.5	NS	0.9	NS	0.1	NS	2.0	NS	4.7	*
Diversity	6	0.1	NS	0.9	NS	7.1	***	1.4	NS	2.6	*	0.3	NS	1.8	NS	1.2	NS	1.0	NS	1.7	NS	1.4	NS	0.5	NS	0.6	NS	1.1	NS	0.7	NS
Air temp*climate	1	-	-	-	-	4.4	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Air temp*diversity	6	-	-	-	-	3.8	**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Climate* diversity	6	0.6	NS	1.6	NS	1.4	NS	0.5	NS	2	NS	1.2	NS	0.6	NS	1.3	NS	1.3	NS	0.9	NS	1.5	NS	1.1	NS	1.4	NS	1.5	NS	0.4	NS
Air temp* climate* diversity	6	-	-	-	-	5.9 0	**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Model R^2		0.42		0.26		0.86		0.35		0.42		0.18		0.29		0.31		0.28		0.31		0.40		0.20		0.49		0.48		0.64	

Table 3N: Results of ANOVAs to determine treatment effects on extractable nitrate concentration in the soil. (Asterisks: *= 0.01-0.05, **=0.01-0.001, ***= <0.001). Block was included as an error term where significant.

		Nov 08		Dec 08		Feb 09		Apr 09		Jun 09		Jul 09		Aug 09		Sep 09		Dec 09		Mar 10		May 10		Jun 10		Jul 10		Aug 10		Sep 10	
Factor	d.f	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Model	16,39	1.5	NS	0.8	NS	2.4	*	2.6	**	2.7	**	0.8	NS	1.4	NS	0.6	NS	1.4	NS	3.1	**	1	NS	0.9	NS	4.9	***	1.3	NS	0.9	NS
Block	3	-	-	-	-	4.9	**	4.6	**	11	***	-	-	3.3	*	-	-	3.8	*	-	-	-	-	-	-	22	***	-	-	-	-
Average air temperature	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26	***	-	-	-	-	-	-	0.1	-	-	-
Climate	1	1.7	NS	0.2	NS	3.8	NS	1.6	NS	0.6	NS	0.7	NS	2.6	NS	0.0	NS	0.6	NS	0.2	NS	0.2	NS	1.2	NS	1.1	NS	0.2	NS	0.3	NS
Diversity	6	1.3	NS	0.6	NS	3.0	*	3.0	*	0.2	NS	1.1	NS	1.0	NS	1	NS	0.5	NS	1.1	NS	0.2	NS	0.9	NS	0.6	NS	0.3	NS	1.3	NS
Climate * diversity	6	1.8	NS	1.1	NS	0.4	NS	1.3	NS	1.2	NS	0.5	NS	0.7	NS	0.2	NS	1.4	NS	2.4	NS	1.9	NS	0.8	NS	1.4	NS	0.8	NS	0.6	NS
Air temp * climate * diversity	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.4	*	-	-
Model R^2			0.32		0.20		0.51		0.52		0.54		0.20		0.44		0.15		0.37		0.68		0.24		0.23		0.69		0.59		0.22

Table 3O: Results of ANOVAs to determine treatment effects on extractable phosphate concentration in the soil. (Asterisks: *= 0.01-0.05, **=0.01-0.001, ***= <0.001). Block was included as an error term where significant.

		Nov 08		Dec 08		Feb 09		Apr 09		Jun 09		Jul 09		Aug 09		Sep 09		Mar 10		May 10		Jun 10		Jul 10		Aug 10		Sep 10	
Factor	d.f	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Model	16,39	3.7	**	1.6	NS	2.8	NS	1.8	NS	1.3	NS	2.5	NS	3.2	**	1.3	NS	2.5	**	3	**	2.4	*	1.3	NS	1.6	NS	2.8	**
Block	3	14	***	2.8	NS	-	-	3.1	*	-	-	9.0	***	-	-	-	-	4.4	**	6.5	**	5.4	**	-	-	-	-	-	-
Average air temperature	1	-	-	-	-	1.7	NS	-	-	-	-	-	-	1	NS	-	-	-	-	-	-	-	-	-	-	0.2	NS	17	***
Climate	1	0.7	NS	0.0	NS	3	NS	0.1	NS	2.7	NS	0.4	NS	0.2	NS	0.7	NS	0.1	NS	0.5	NS	0.1	NS	0.4	NS	0.9	NS	2.9	NS
Diversity	6	0.8	NS	1.7	NS	3.8	**	1.8	NS	1.0	NS	0.4	NS	2.5	NS	1.3	NS	2.4	*	2.4	*	2.2	NS	2.1	NS	0.8	NS	1.2	NS
Air temp * climate	1	-	-	-	-	-	-	-	-	-	-	-	-	1.2	NS	-	-	-	-	-	-	-	-	-	-	5.5	*	-	-
Air temp * diversity	6	-	-	-	-	-	-	-	-	-	-	-	-	1.8	NS	-	-	-	-	-	-	-	-	-	-	2.6	*	-	-
Climate * diversity	6	1.9	NS	1.0	NS	2	NS	1.3	NS	1.3	NS	1.6	NS	1.8	NS	1.5	NS	2.2	NS	1.5	NS	1.6	NS	0.7	NS	1.0	NS	2	NS
Air temp * climate * diversity	6	-	-	-	-	-	-	-	-	-	-	-	-	8.1	***	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Model R^2			0.69		0.40		0.50		0.43		0.29		0.51		0.77		0.30		0.51		0.53		0.50		0.31		0.51		0.51

APPENDIX 3.12.6: LYSIMETERS

Table 3P: Results of ANOVAs to determine treatment effects on ammonium lost through leaching. (Asterisks: *= 0.01-0.05, **=0.01-0.001, ***= <0.001). Block was included as an error term where significant.

Factor	d.f.	Apr 09		May 09		Nov 09		Dec 09		Jan 10		Feb 10		Mar 10		Apr 10		May 10	
		F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Model	16,39	0.74	NS	3.31	**	0.74	NS	3.52	**	2.22	*	2.27	*	1.87	NS	1.22	NS	5.34	***
Block	3	-	-	-	-	-	-	3.96	*	-	-	5.74	**	4.97	**	-	-	-	-
Soil moisture	1	-	-	1.25	NS	-	-	1.68	NS	0.70	NS	NS	NS	-	-	-	-	6.70	*
Climate change	1	0.93	NS	0.94	NS	2.83	NS	0.00	NS	0.24	NS	0.92	NS	0.33	NS	3.10	NS	7.15	*
Functional diversity	6	0.35	NS	2.16	NS	0.67	NS	2.55	NS	1.61	NS	1.32	NS	0.44	NS	1.05	NS	3.34	*
Soil moisture*climate	1	-	-	5.47	*	-	-	0.01	NS	0.17	NS	NS	NS	-	-	-	-	5.27	*
Soil moisture*diversity	6	-	-	5.26	***	-	-	3.54	*	2.51	NS	NS	NS	-	-	-	-	9.99	***
Climate*diversity	6	1.11	NS	2.88	*	0.47	NS	4.75	**	2.37	NS	1.71	NS	2.01	NS	1.08	NS	1.31	NS
Soil moisture*climate diversity	6	-	-	-	-	-	-	4.50	**	3.31	*	NS	NS	-	-	-	-	6.38	***
Model R^2		0.19		0.68		0.20		0.85		0.73		0.49		0.43		0.29		0.84	

Table 3Q: Results of ANOVAs to determine treatment effects on leached losses of nitrate. (Asterisks: *= 0.01-0.05, **=0.01-0.001, ***= <0.001). Block was included as an error term where significant.

Factor	d.f.	Apr 09		May 09		Nov 09		Dec 09		Jan 10		Feb 10		Mar 10		Apr 10		May 10	
		F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Model	16,39	2.48	*	2.61	**	4.38	**	0.78	NS	1.92	NS	1.44	NS	1.70	NS	9.68	***	2.30	*
Block	3	6.27	**	4.29	*	-	-	-	-	5.53	**	3.68	*	5.73	**	63.07	***	8.77	***
Soil moisture	1	0.32	NS	0.81	NS	0.70	NS	-	-	-	-	-	-	-	-	0.05	NS	-	-
Climate change	1	0.17	NS	2.04	NS	49.13	***	0.50	NS	3.24	NS	0.35	NS	0.02	NS	1.25	NS	0.03	NS
Functional diversity	6	0.20	NS	1.05	NS	4.50	*	1.11	NS	0.27	NS	0.18	NS	0.62	NS	0.03	NS	1.37	NS
Soil moisture * climate	1	13.48	**	6.69	*	0.38	NS	-	-	-	-	-	-	-	-	-	-	-	-
Soil moisture*diversity	6	1.36	NS	3.89	**	0.79	NS	-	-	-	-	-	-	-	-	2.84	*	NS	NS
Climate * diversity	6	1.15	NS	0.65	NS	1.19	NS	0.49	NS	1.53	NS	1.77	NS	1.05	NS	1.46	NS	0.38	NS
Soil moisture * climate * diversity	6	4.20	**	3.73	**	4.84	*	-	-										
Model R^2		0.75				0.93		0.21		0.46		0.38		0.42		0.88		0.50	

APPENDIX 3.13: REPEATED MEASURES ANOVAS

Table 3R: Repeated measures ANOVAs for all ecosystem functions or compositions measured in the DIRECT experiment. All measures had at least five time points. † denotes a significant block effect. Asterisks denote *p<0.05, **p<0.01, ***p<0.001. ^{log} describes transformations applied.

Ecosystem function	Treatment	d.f.	F	p	Ecosystem function	Treatment	d.f.	F	p
Grass abundance	Climate†	1,39	0.05	0.82	**Mineralisation**	Climate	1,42	0.9	0.35
	Diversity	6,39	2.67	*		Diversity	6,42	1.58	0.18
	C*D	6,39	0.63	0.7		C*D	6,42	0.2	0.98
	Month	5,210	16.03	***		Month	6,252	17.82	***
	C*M	5,210	0.32	0.9		C*M	6,252	0.24	0.96
	D*M	30,210	2.66	***		D*M	36,252	1.07	0.36
	C*D*M	30,210	1.06	0.39		C*D*M	36,252	0.63	0.95
Forb abundance	Climate†	1,39	2.4	0.13	**Nitrification**	Climate	1,42	0.5	0.48
	Diversity	6,39	1.16	0.35		Diversity	6,42	0.5	0.8
	C*D	6,39	0.72	0.64		C*D	6,42	0.87	0.52
	Month	5,210	13.21	***		Month	6,252	68.09	***
	C*M	5,210	0.44	0.82		C*M	6,252	0.85	0.53
	D*M	30,210	1.54	*		D*M	36,252	1.13	0.3
	C*D*M	30,210	0.83	0.72		C*D*M	36,252	1.02	0.45
NEE 2009	Climate	1,42	0.14	0.71	**Extractable ammonium^{log}**	Climate	1,42	0.02	0.88
	Diversity	6,42	0.72	0.64		Diversity	6,42	0.77	0.6
	C*D	6,42	1.01	0.43		C*D	6,42	0.74	0.62
	Month	6,252	2.52	*		Month	12,504	147.87	***
	C*M	6,252	0.88	0.51		C*M	12,504	1.5	0.12
	D*M	36,252	0.9	0.64		D*M	72,504	1.13	0.24
	C*D*M	36,252	0.82	0.76		C*D*M	72,504	1.06	0.36

NEE 2010	Climate	1,42	2	0.16	**Extractable nitrate^{log}**	Climate†	1,42	3.14	0.08
	Diversity	6,42	0.58	0.74		Diversity	6,42	0.48	0.82
	C*D	6,42	0.68	0.67		C*D	6,42	1.03	0.42
	Month	4,168	4.23	**		Month	12,504	158.52	***
	C*M	4,168	0.74	0.57		C*M	12,504	0.77	0.68
	D*M	24,168	0.85	0.67		D*M	72,504	0.87	0.77
	C*D*M	24,168	1.14	0.31		C*D*M	72,504	1.11	0.27
Reco 2009	Climate†	1,39	4.15	*	**Extractable phosphate^{log}**	Climate	1,42	1.41	0.24
	Diversity	6,39	1.52	0.2		Diversity	6,42	2.87	*
	C*D	6,39	0.99	0.45		C*D	6,42	2.22	0.06
	Month	6,252	106.94	***		Month	11,462	97.94	***
	C*M	6,252	0.95	0.46		C*M	11,462	2.03	*
	D*M	36,252	0.57	0.98		D*M	66,462	2.69	***
	C*D*M	36,252	1.21	0.21		C*D*M	66,462	1.55	**
Reco 2010	Climate	1,42	3.14	0.08	**Leached ammonia^{log}**	Climate	1,39	0.2	0.66
	Diversity	6,42	0.43	0.85		Diversity	6,39	0.85	0.54
	C*D	6,42	1.71	0.14		C*D	6,39	1.42	0.23
	Month	4,168	12.9	***		Month	6,252	6.34	***
	C*M	4,168	0.67	0.61		C*M	6,252	0.09	1
	D*M	24,168	0.9	0.61		D*M	36,252	1.13	0.29
	C*D*M	24,168	1.17	0.28		C*D*M	36,252	1.32	0.12
ET 2009^{log}	Climate	1,42	0.57	0.46	**Leached nitrate^{log}**	Climate†	1,39	0.06	0.81
	Diversity	6,42	0.62	0.71		Diversity	6,39	0.27	0.95
	C*D	6,42	0.96	0.46		C*D	6,39	0.5	0.8
	Month	6,252	29.45	***		Month	6,252	10	***
	C*M	6,252	1.23	0.29		C*M	6,252	0.06	1
	D*M	36,252	1.15	0.26		D*M	36,252	0.59	0.97
	C*D*M	36,252	0.76	0.83		C*D*M	36,252	0.69	0.91

ET 2010^{sqrt}	Climate†	1,39	3.98	0.05	
	Diversity	6,39	1.26	0.3	
	C*D	6,39	0.33	0.92	
	Month	4,168	30	***	
	C*M	4,168	0.08	0.99	
	Diversity * month	24,168	0.85	0.66	
	C*D*M	24,168	0.67	0.88	

APPENDIX 4

APPENDIX 4.1: CLIMATE PROJECTIONS FOR SOUTH EAST UK

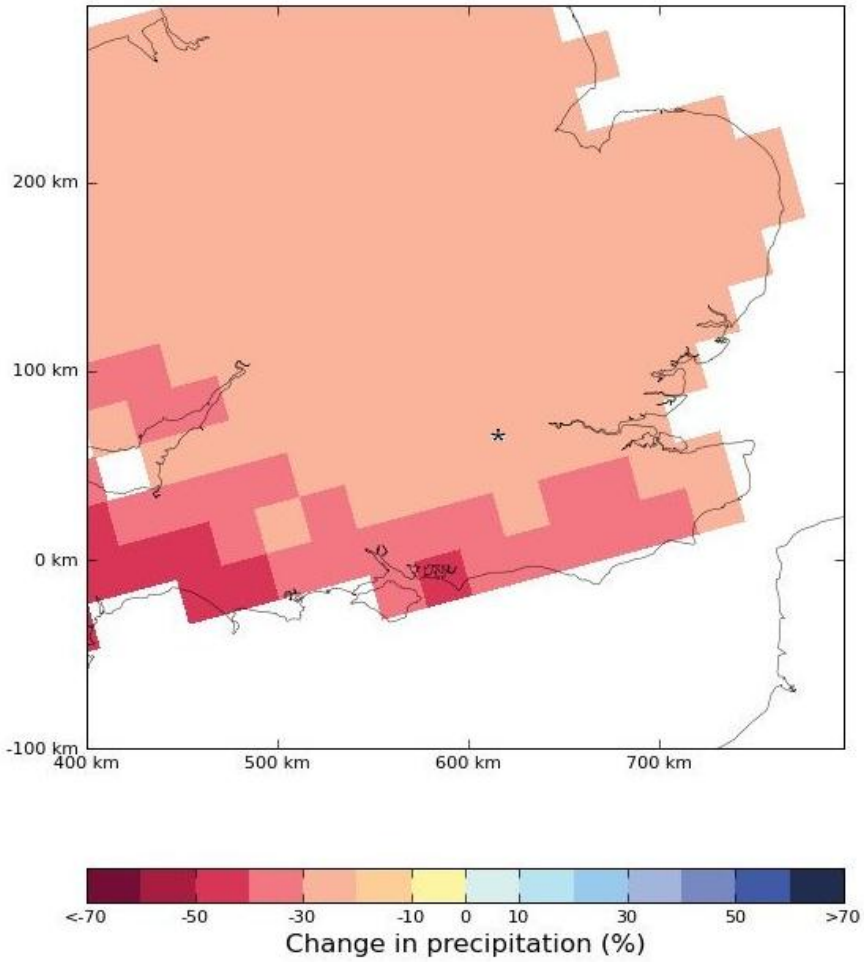
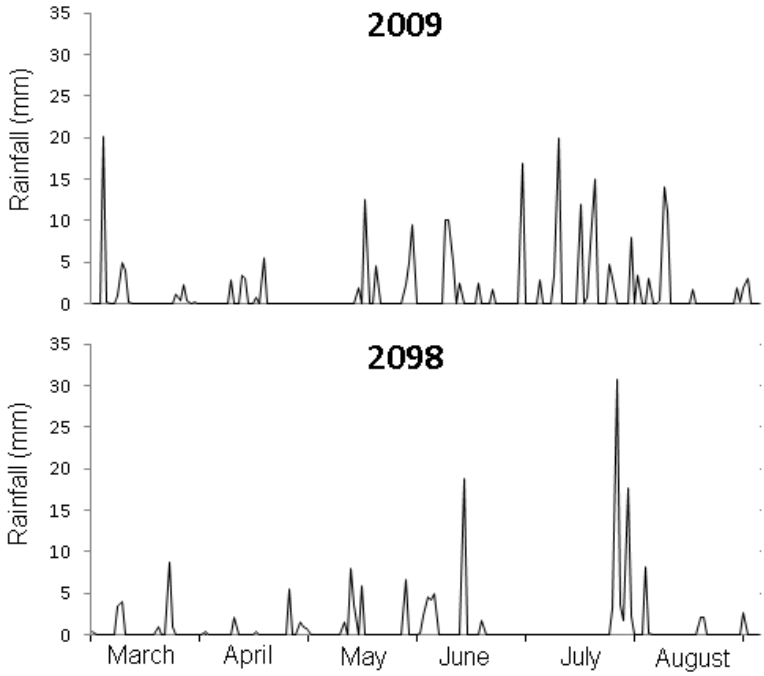


Figure 4A: Map drawn from UKCP09 data (Murphy *et al.* 2010) of summer (JJA) projections for the 2070-2099 high emissions scenario (A1F1, Nakićenović & Swart, 2000). The study site at Silwood Park, Berks is marked with a star.

APPENDIX 4.2: MODELLING RAINFALL SIMULATIONS USING THE GLIMCLIM PROGRAM

The two rainfall projection models used in this experiment were supported by a modelling process using generalised linear models (GLIMCLIM 2002) and daily rain gauge data from Heathrow airport (within 10 miles of the field site) dating from 1961-1990 to produce projections of daily and monthly rainfall from five internationally recognised coupled atmosphere-ocean GCMs (CCCma-CGCM2, CSIRO MK2, ECHAM4, HADCM3 and HADCM3 B2, data supplied by R. Chandler).

Data from the A1 scenario of these models were not available, so A2 was used (these are both high-end scenarios with atmospheric CO_2 concentrations that do not stabilise, but A2 anticipates a lower population with more renewable technology, Nakićenović & Swart 2000). A logistic regression model generated projections of occurrence of rainfall (zero or non-zero series) and a gamma model generated data describing volume of rainfall (the skew of non-zero distribution).These models were combined to create a projection for each day between January 2000 and December 2010, and January 2080 to December 2099. The simulations were run ten times for each GCM and one simulation from each time frame randomly selected. The output for 2000-2010 was used to test which GCM was most accurate at describing the climate compared with daily data from Silwood Park Weather Station (unpublished data). A paired t-test was used to compare the actual data with the projected data to find the most accurate model. All GCM models were significantly different to the observed data except CSIRO MK2 ($t = 0.95$, d.f. = 131, $p = 0.35$) and CGCM2 ($t = 1.42$, d.f. = 131, $p = 0.16$). The most similar model during a time-frame where the actual amounts are known can be assumed to be the most accurate at projecting future trends as well. The CSIRO model was used to create a future rainfall projection based upon the 2098 projections, which predict spring reductions of rainfall from 2009 by approximately 30% (Fig. B). This year was chosen as it has one of the more droughted spring periods between 2080 and 2099, while retaining a similar reduction in summer.



	% rainfall in 2098 compared with 2009
March	0.53
April	0.75
May	0.79
June	0.70
July	0.84
August	0.19

Figure 4B: Actual rainfall in 2009 at Silwood Park compared with projected rainfall in 2098 at Heathrow airport using the CSIRO MK2 rainfall projections. The table describes absolute volume changes over the March-August period. This projection was used as a basis for the 'spring/summer climate change' treatment.

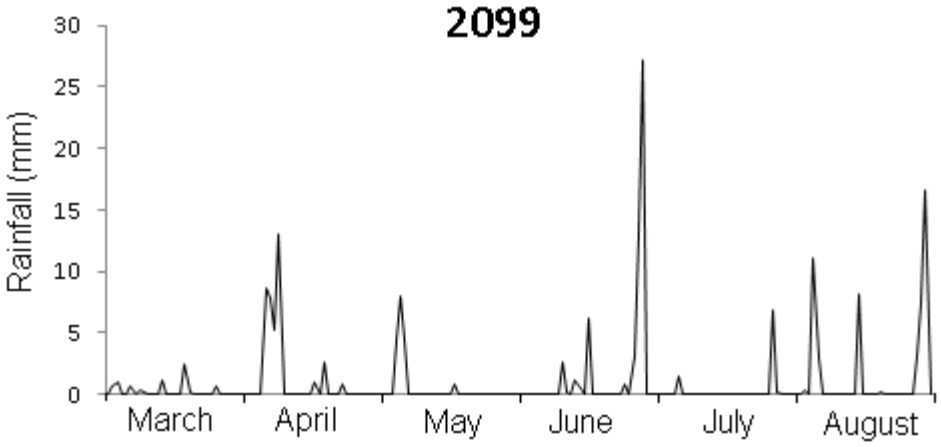


Figure 4C: Projection of summer rainfall 2099 from CSIRO MK2. It shows very severe drought periods in summer, followed by periods of heavy rainfall with little intermediate. This projection was used as the basis for the 'variable' treatment.

APPENDIX 4.3: RAINFALL PULSES APPLIED TO VARIABLE RAINFALL PLOTS

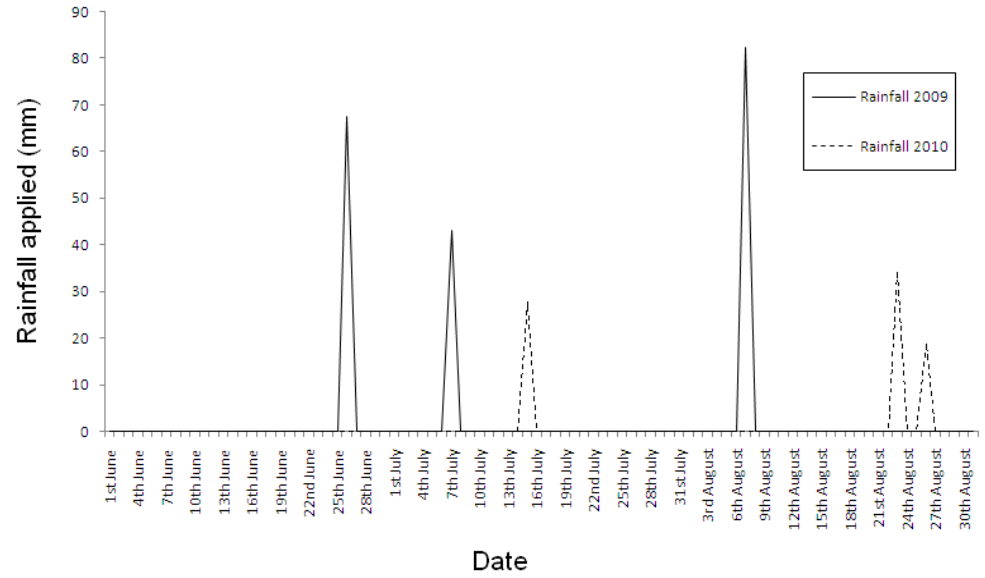


Figure 4D: Application of rainfall in variable treatment in 2009 and 2010.

APPENDIX 4.4: LEACHED NITROGEN

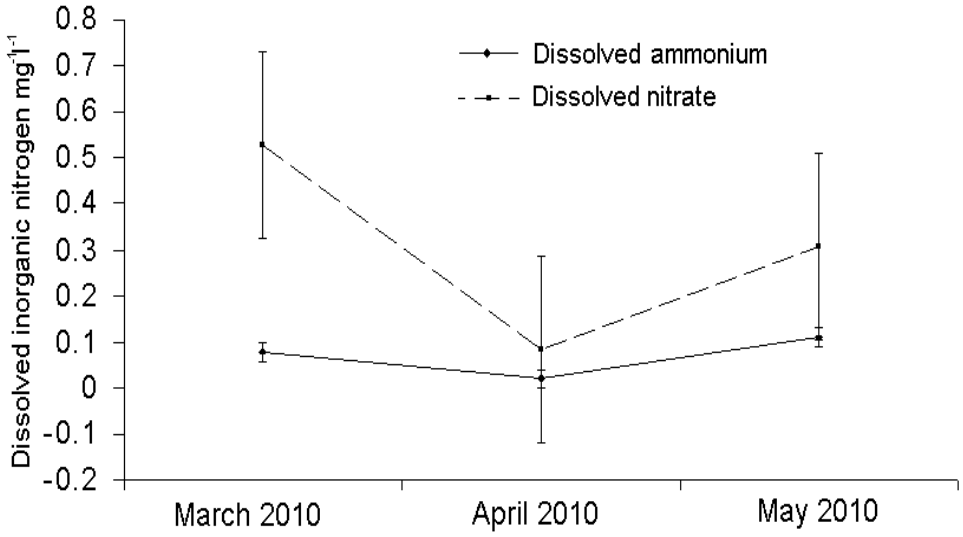


Figure 4E: Leached nitrogen lost from the system in spring 2010. Error bars depict ±1 SEM.

APPENDIX 4.5: ANOVAS FOR INDIVIDUAL TIME POINTS

APPENDIX 4.5.1: VEG SURVEY

Table 4A: One-way ANOVAs testing the effect of two climate change treatments upon grass abundance at individual time points. Block was initially included, but has been removed due to lack of significance. The model F value is therefore equal to factor F values.

Factor	d.f.	Jun 09		Oct 09		May 10		Jul 10		Sep 10	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Climate change	2,9	0.10	NS	2.16	NS	4.15	NS	3.85	NS	2.48	NS
Model R^2		0.02		0.32		0.48		0.46		0.36	

Table 4B: One-way ANOVAs testing the effect of two climate change treatments upon forb abundance at individual time points. Block was removed when non-significant.

Factor	d.f.	Jun 09		Oct 09		May 10		Jul 10		Sep 10	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Model	5,6	4.22	*	-	-	-	-	-	-	-	-
Block	3	5.34	*	-	-	-	-	-	-	-	-
Climate change	2	2.53	NS	0.56	NS	1.63	NS	0.87	NS	3.14	NS
Model R^2		0.78		0.11		0.27		0.16		0.41	

Table 4C: One-way ANOVAs testing the effect of two climate change treatments upon grass:forb ratio at individual time points. Block was initially included, but has been removed due to lack of significance. The model F value is therefore equal to factor F values.

Factor	d.f.	Jun 09		Oct 09		May 10		Jul 10		Sep 10	
		F	p	F	p	F	p	F	p	F	p
Climate change	2,9	0.43	NS	0.35	NS	2.04	NS	1.55	NS	0.83	NS
Model R^2		0.09		0.07		0.31		0.26		0.16	

APPENDIX 4.5.2: CO_2 FLUX

Table 4D: ANCOVAs testing the effect of climate change treatments and important covariates upon net ecosystem exchange (NEE). The block factor and the covariates were removed when non-significant.

Factor		Feb 10		May 10		Jun 10		Jul 10		Aug 10		Sep 10	
	d.f.	F	p	F	p	F	p	F	p	F	p	F	p
Model	7,3	12.5	*	-	-	4.98	*	2.38	NS	4.43	NS	-	-
Block	3	16.1	*	-	-	8.69	*	1.09	NS	3.57	NS	-	-
PAR	1	21.7	*	12.6	**	-	-	-	-	-	-	-	-
Logged soil temperature	1	13.1	NS	-	-	-	-	9.13	*	8.51	*	-	-
Climate change	2	2.19	NS	1.13	NS	2.06	NS	0.94	NS	3.68	NS	0.10	NS
Model R^2		0.97		0.65		0.86		0.74		0.84		0.02	

Table 4E: ANCOVAs testing the effect of climate change treatments and important covariates upon ecosystem respiration (R_{eco}). The block factor and the covariates were removed when non-significant.

Factor		Feb 10		May 10		Jun 10		Jul 10		Aug 10		Sep 10	
	d.f.	F	p	F	p	F	p	F	p	F	p	F	p
Model	8,3	-	-	4.32	*	7.91	**	-	-	-	-	-	-
Soil moisture	1	-	-	6.13	*	-	-	-	-	-	-	-	-
Soil temperature	1	-	-	10.9	*	15.0	**	-	-	-	-	-	-
Climate change	2	0.21	NS	0.11	NS	4.39	NS	0.13	NS	1.01	NS	1.97	NS
Model R^2		0.04		0.71		0.75		0.03		0.18		0.30	

Table 4F: ANCOVAs testing the effect of climate change treatments and important covariates upon evapotranspiration (ET). The block factor and the covariates were removed when non-significant.

Factor		Feb 10		May 10		Jun 10		Jul 10		Aug 10		Sep 10	
	d.f	F	p	F	p	F	p	F	p	F	p	F	p
Model	8,3	-	-	23.0	***	9.90	**	1.28	NS	14.4	*	-	-
Block	3	-	-	-	-	16.0	**	2.98	NS	16.6	*	-	-
Soil temperature	1	-	-	6.87	*	-	-	8.64	*	39.9	**	-	-
PAR	1	-	-	83.3	***	-	-	-	-	0.95	NS	-	-
Climate change	2	0.11	NS	0.92	NS	0.83	NS	7.20	*	11.9	*	0.77	NS
Model R^2		0.02		0.93		0.89		0.86		0.97		0.15	

APPENDIX 4.5.3: MINERALISATION

Table 4G: ANCOVAs testing the response of mineralisation rate to climate change treatments, with soil as a covariate. Block effects were removed as they were consistently non-significant.

Factor	d.f.	Dec 09 - Mar 10		Mar 10 - Jun 10		Jun 10 - Sept 10	
		F	p	F	p	F	p
Model	3,7	4.61	*	-	-	-	-
Soil moisture	1	7.38	*	-	-	-	-
Climate change	2	3.22	NS	0.04	NS	4.11	*

APENDIX 4.5.4: NITRIFICATION

Table 4H: ANCOVAs testing the response of nitrification rate to climate change treatments, with soil as a covariate. Block effects were removed as they were consistently non-significant.

Factor	d.f	Dec 09 - Mar 10		Mar 10 - Jun 10		Jun 10 - Sept 10	
		F	p	F	p	F	p
Model	3,7	2.97	-	-	-	0.8453	NS
Soil moisture	1	6.45	*	-	-	2.67	NS
Climate change	2	1.24	NS	0.31	NS	4.35	NS
Soil moisture * climate change	2	-	-	-	-	11.45	*

APPENDIX 4.5.5EXTRACTABLE NUTRIENTS

Table 4I: ANCOVAs testing the effect of climate change treatments upon extractable ammonium. Block and covariates were removed when non-significant.

		Aug 09		Sep 09		Dec 09		Mar 10		May 10		Jun 10		Jul 10		Aug 10		Sep 10	
Factor	d.f	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Model	5,6	905.30	**	-	-	7.96	*	73.58	**	-	-	-	-	-	-	-	-	4.66	NS
Block	3	860.97	**	-	-	-	-	107.2	**	-	-	-	-	-	-	-	-	6.93	NS
Soil moisture	1	174.59	**	-	-	2.36	NS	1.46	NS	-	-	-	-	-	-	-	-	-	-
Climate	2	1920.7	***	0.97	NS	10.1	*	54.99	**	0.92	NS	0.41	NS	0.11	NS	0.52	NS	1.25	NS
Soil moisture * climate	2	321.59	**	-	-	8.67	*	77.79	**	-	-	-	-	-	-	-	-	-	-
Model R^2		0.99		0.20		0.89		0.99		0.17		0.08		0.03		0.11		0.80	

Table 4J: ANCOVAs testing the effect of climate change treatments upon extractable nitrate. Block and covariates were removed when non-significant.

		Aug 09		Sep 09		Dec 09		Mar 10		May 10		Jun 10		Jul 10		Aug 10		Sep 10	
Factor	d.f	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Model	5,6	-	-	-	-	-	-	20.65	*	-	-	11.23	**	3.64	NS	0.52	NS	0.62	NS
Block	3	-	-	-	-	-	-	24.65	*	-	-	-	-	5.43	NS	-	-	-	-
Soil moisture	1	-	-	-	-	-	-	1.30	NS	-	-	19.66	**	-	-	-	-	-	-
Climate	2	3.80	NS	0.26	NS	1.31	NS	4.32	NS	0.65	NS	11.14	**	0.95	NS	0.52	NS	0.62	NS
Soil moisture * climate	2	-	-	-	-	-	-	40.66	*	-	-	7.10	*	-	-	-	-	-	-
Model R^2		0.46		0.05		0.23		0.99		0.14		0.90		0.75		0.12		0.12	

Table 4K: ANCOVAs testing the effect of climate change treatments upon extractable phosphate. Block and covariates were removed when non-significant.

		Aug 09		Sep 09		Mar 10		May 10		Jun 10		Jul 10		Aug 10		Sept 10	
Factor	d.f	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Model	5,6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.62	*
Block	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11.97	*
Climate	2	0.98	NS	0.1	NS	1.23	NS	0.8	NS	0.74	NS	4.2	NS	0	NS	9.3	*
Model R^2		0.18		0		0.22		0.2		0.14		0.5		0		0.9	

APPENDIX 5

Appendix 5.1: Formulae for calculation of CWM and FDvar

Eq. 5.1 CWM:

$$\sum_{i=1}^{s} w_i \, x_i$$

Where *s* is the total number of species, w_i is the relative abundance of the *i*th species and x_i is the trait values of the *i*th species.

Eq. 5.2 FDvar:

The relative abundance of species *i* is

$$w_i = \frac{a_i}{\sum_{i=1}^{s} a_i}$$

Where *a* is the abundance of the *i*th species.

$$\overline{lnx} = \sum_{i=1}^{s} w_i \, lnx_i$$

V is the weighted variance of trait *x*:

$$V = \sum_{i=1}^{s} w_i \left(lnx_i - \overline{lnx}\right)^2$$

This is then bounded between 0-1 using 5 as a scaling factor:

$$\frac{2}{\pi} \arctan\,(5V)$$

APPENDIX 5.2: STATISTICAL ANALYSES

Table 5A: Models describing the effect of different sets of variables on decomposition of *Arrhenatherum elatius* litter over nine months.

Date	Error	Formula	F statistic	p	R^2	d.f.	Likelihood ratio
Dec-Mar 09	Gaussian	171.33 - 1.78 Soil temp - 2.42 All light - 22.48 pH - 0.44 biomass FG2 - 0.1 biomass FG3 - 0.005 CWM LNC + 0.42 All light*pH	7.99	p<0.001	0.54	7,48	biomass FG3: F=10.79, p<0.01 CWM LNC: F=6.54, p<0.05 biomass FG2: F=8.51, p<0.01 All light: F=4.72, p<0.05 Soil temp: F=10.54, p<0.01
Mar-Jun 09	Gaussian	0.69 + 0.09 Soil moisture - 0.002 Soil moisture^2 + 0.04 Soil temp - 0.012 All light - 0.26 pH - 0.001 Soil temp*All light + 0.005 All light*pH	11.54	p<0.001	0.63	7,47	Soil moisture: F=7.59, p<0.01 All light*pH: F=6.85, p<0.05 Soil temp*All light: F=12.99, p<0.001
Jun-Sept 09	Negative binomial	exp(3.72 - 0.02 Soil moisture + 0.0003 Soil moisture^2)				53	Soil moisture: X^2=3.99, p<0.05

Table 5B: Models describing the effect of different sets of variables on decomposition of *Holcus mollis* litter over nine months.

Date	Error	Formula	F statistic	p	R^2	d.f.	Likelihood ratio
Dec-Mar 09	Gaussian	21.41 - 0.13 biomass FG3 - 6.02 Fdvar AGB	11.86	p<0.001	0.31	2,53	biomass FG3: F= 15.15, p<0.001, FDvar AGB: F= 5.63, p<0.05
Mar-Jun 09	Gaussian	9.84 - 2.23 Soil moisture - 4.34 Soil temp - 3.38 Fdvar Root:Shoot	10.94	p<0.001	0.35	3,52	Soil moisture: F=4.97, p<0.05 Soil temp: F=18.85, p<0.001 FDvar Root:Shoot: F=11.44, p<0.01
Jun-Sept 09	Negative binomial	exp(6.95 - 0.08 Soil moisture - 0.05 Soil temp - 0.11 CWM LDMC + 0.004 Soil moisture*Soil temp)				51	Soil moisture*Soil temp: X^2=5.51 p<0.05, CWM LDMC: X^2=10.81, p<0.01

Table 5C: Models describing the effect of different sets of variables on mineralisation rates over 21 months.

Date	Error	Formula	F statistic	p	R^2	d.f.	Likelihood ratio
Dec-Mar 08		Null model					
Mar-Jun 09	Negative binomial	exp(-7.38 - 0.48 Soil moisture - 0.16 pH - 0.27 All light+ 0.06 biomass FG2 + 0.13 CWM LDMC + 0.01 Soil moisture*All light)				48	CWM LDMC: X^2=6.35, p<0.05 biomass FG2: X^2= 6.76, p<0.01 Soil moisture*All light: X^2=7.35, p<0.01 pH: F= 9.44, p<0.01
Jun-Sept 09		Null model					
Sept-Dec 09	Gaussian	-2.49 + 0.00007 CWM PSA	5.32	p<0.05	0.09	1,54	
Mar-Jun 10	Gaussian	14.93 - 3.39 Soil moisture + 3.79 Soil temp - 1.17 All light - 0.04 biomass FG1 + 0.1 Soil moisture*All light	5.48	p<0.001	0.36	5,48	Soil moisture*All light: F= 6.39 p<0.05 Soil temp: F= 13.83, p<0.001 biomass FG1: F= 6.68, p<0.05
Jun-Sept 10	Gaussian	-52.733+ 3.29 CWM LDMC	7.36	p<0.01	0.12	1,53	

Table 5D: Models describing the effect of different sets of variables on nitrification rates over 21 months.

Date	Error	Formula	F statistic	p	R^2	d.f.	Likelihood ratio
Dec-Mar 08	Gaussian	20.37 - 0.60 Soil moisture - 2.82 Soil temp - 0.002 biomass FG1 + 0.1 Soil moisture*Soil temp	5.26	p<0.01	0.3	4,50	Soil moisture*Soil temp: F=5.11, p<0.01, biomass FG1: F= 7.35, p<0.01
Mar-Jun 09	Negative binomial	exp(-2.21 + 0.2 Soil moisture - 0.37 pH - 0.02 All light + 0.17 CWM LDMC)				50	Soil moisture: X^2=15.75, p<0.001 pH: X^2=6.85, p<0.05 All light: X^2=7.9, p<0.01 CWM LDMC: X^2= 8.1, p<0.01
Jun-Sept 09	Gaussian	-3.17 + 2.78 Soil moisture + 1.75 Soil temp - 0.68 All light - 5.69 pH + 0.02 biomass FG1 - 0.17 Soil moisture*Soil temp + 0.12 All light*pH	4.81	p<0.001	0.42	7,47	biomass FG1: F= 11.84, p<0.01 All light*pH: F= 4.98 p<0.05 Soil moisture*Soil temp: F= 12.35, p<0.001
Sept-Dec 09	Negative binomial	exp(-0.57 + 0.09 All light + 0.00006 CWM PSA - 0.0000009 All light*CWM PSA)				52	All light: CWM PSA: X^2= 6.62 p<0.01
Dec-Mar 10	Negative binomial	exp(0.13 - 0.27 All light - 0.001 All light^2 - 1.1 Soil moisture + 0.4 pH + 0. 9 FDvar Total P + 0.02 Soil moisture*All light)				48	Soil moisture*All light: X^2= 19.13, p<0.001 pH: X^2= 11.26, p<0.01 Fdvar Total P: X^2= 5.43, p<0.05 All light^2: X^2= 6.38, p<0.05
Mar-Jun 10		Null model					
Jun-Sept 10	Negative binomial	exp(6.93 - 0.46 Soil moisture - 0.29 Soil temp - 0.002 biomass FG2 - 0.001 biomass FG3 + 0.08 CWM LDMC + 0.02 Soil moisture*Soil temp - 0.001 biomass FG2*biomass FG3)				45	CWM LDMC: X^2= 4.50 p<0.05 biomass FG2*biomass FG3: X^2= 17.88, p<0.001 Soil moisture*Soil temp: X^2= 10.68 p<0.05

Table 5E: Models describing the effect of different sets of variables on total soil nutrients.

Date	Error	Variable	Formula	F statistic	p	R^2	d.f.	Likelihood ratio
Nov 08	Gaussian	Total soil N	1615.5 - 701.3 FDvar AGB	7.45	0.008	0.12	1,54	
Sept 09	Gaussian	Total soil N	343.26 + 40.95 Soil temp + 1.19 biomass FG1 - 195.95 biomass FG2 + 1.59 biomass FG1 * FG2	3.65	0.011	0.23	4,50	Soil temp: F= 4.18, p<0.05, Biomass FG1*FG2: F= 8.17, p<0.01
Sept 10	Negative binomial	Total soil N	exp(58.721 - 7.17 Soil temp + 0.249 Soil $temp^2$)				52	Soil temp: X^2= 18.15, p<0.001, $temp^2$: X^2= 18.40, p<0.001
Nov 08	Gaussian	Total soil P	Null model					
Sept 09	Gaussian	Total soil P	-36.91 + 48.86 Soil temp - 1455.33 FDvar LDMC - 0.88 biomass FG1 - 151.33 biomass FG2 + 1.21 biomass FG1*FG2	3.48	0.009	0.26	5,49	Soil temp: F= 8.55, p<0.01, biomass FG1*FG2: F=7.93, p<0.01, FDvar LDMC: F=5.31, p<0.05
Sept 10	Negative binomial	Total soil P	exp(23.88 -1.08 Soil moisture - 1.24 Soil temp + 0.63 FDvar LNC + 0.07 Soil moisture * Soil temp)				50	Soil moisture*Soil temp: X^2= 6.35, p<0.05, FDvar LNC: X^2= 4.12, p<0.05

Table 5F: Models describing leaching losses during two spring periods.

Date	Error	Formula	d.f.	Likelihood ratio
April 09	Negative binomial	exp(15.54 + 1.46 Soil temp - 0.08 Soil moisture - 0.06 Biomass FG1 - 30.46 FDvar SLA - 0.18 Soil temp*Soil moisture + 0.006 Soil temp*Biomass FG1 + 1.84 Soil temp*FDvar SLA + 0.002 Soil moisture*Biomass FG1 + 1.35 Soil moisture*FDvar SLA - 0.04 Biomass FG1*FDvar SLA)/100	10,45	Biomass FG1*FDvar SLA: X^2=10.7, p<0.01, Soil moisture*FDvar SLA: X^2=19.24, p<0.001, Soil temp*FDvar SLA: X^2=9.84, p<0.01, Soil temp*biomass FG1: X^2= 7.02, p<0.01, Soil temp*Soil moisture: X^2=12.44, p<0.001
May 09	Negative binomial	exp(3.06 + 0.18 Biomass FG2 + 0.018 Biomass FG3- 0.01 Biomass FG2*Biomass FG3)/100	3,52	Biomass FG2*Biomass FG3: X^2= 4.25, p<0.05
Jan 10	Negative binomial	exp(0.14 - 0.38 Biomass FG3 + 0.15 CWM LDMC + 0.017 Biomass FG3*CWM LDC)/100	3,44	Biomass FG3*CWM LDMC: X^2= 7.58, p<0.01
Feb 10	Negative binomial	exp(4.91 + 0.005 All light - 0.067 Soil moisture + 3.51 pH + 0.01 All light*Soil moisture - All light*pH)/100	5,50	All light*pH: X^2= 7.55, p<0.01, All light*Soil moisture: X^2=15.94, p<0.001
Mar 10	Negative binomial	exp(2.59 - 0.009 Biomass FG2 + 0.011 Biomass FG3)/100	2,44	Biomass FG2: X^2= 10.13, p<0.01, Biomass FG3: X^2= 39.14, p<0.001
Apr 10	Negative binomial	exp(6.49 - 0.14 Soil moisture - 0.004 Biomass FG1)/100	2,50	Biomass FG1: X^2= 5.58, p<0.05, Soil moisture: X^2= 11.67, p<0.001
May 10	Negative binomial	exp(-36.32 + 0.02 Soil moist^2 + 2.32 Soil moisture + 7.99 pH - 0.004 Biomass FG1 - 0.52 Soil moisture*pH)/100	5,47	Biomass FG1: X^2= 5.19, p<0.05, Soil moisture*pH: X^2= 11.75, p<0.001, Soil moisture^2: X^2= 4.88, p<0.05