The response of grassland carbon cycling to drought events and changes in nutrient availability

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Abstract

In grasslands, climate change has the potential to disrupt a range of ecosystem services, including agricultural production, carbon (C) storage and nutrient cycling. In particular, climate change is likely to increase the frequency and severity of extreme climate events, such as drought and the subsequent rewetting event. Yet the effect of drought events will not be consistent across grassland communities, instead likely varying with grassland properties. One such property may be the level of nutrient availability, which brings about changes in plant productivity, plant community composition, and soil microbial composition and function. In this thesis, the effect of reduced precipitation on C cycling in UK species-rich grasslands is investigated in two field experiments, with varying long-term grassland restoration treatments and short-term nutrient addition, and a glasshouse experiment with reduced soil moisture. It was hypothesised that changes in plant and soil microbial communities, brought about by differences in nutrient availability, would modulate above and belowground C cycling responses to drought. This thesis found that the level of nutrient availability was important for modulating how C is cycled in response to drought in plants, soil microbial communities and whole ecosystem CO_2 fluxes. For plants, the effect of drought and nutrient availability differed between functional groups, species and due to intraspecific trait variation. For soil microbial communities, the effect of drought on carbon use efficiency was modulated by short-term nutrient addition. Increased nutrient availability and drought therefore interact to determine how C is cycled and stored in plants and soil microbial communities, revealing the importance of agricultural practices in modulating whole community responses to climate change. Overall, this thesis shows the mechanisms by which drought may alter C cycling and its potential feedbacks to climate are complex, but at least in part, depend on the level of nutrient availability.

Declaration

I declare that this thesis is my own work and has not been submitted for a degree elsewhere. Contributions from supervisors and collaborators are properly acknowledged.

Andrew Joseph Cole

Lancaster University, December 2016

Statement of authorship

This thesis has been prepared in the alternative thesis format, as a set of four papers intended for submission to peer-reviewed journals. The papers are presented as intended for submission, except with a consolidated bibliography at the end of the thesis. All four papers have multiple authors and their contributions are detailed and certified by my supervisors below.

Chapter 2 is intended for submission as: Cole, A.J., Griffiths, R.I., Thomson, B.C., Ward, S.E., Whitaker, J., Ostle, N.J, Bardgett, R.D. (2017). Grassland restoration alters resistance and recovery of carbon and nitrogen cycling in plants and soil microbes to summer drought. *In preparation*.

A Cole planned the experiment with advice from N Ostle, R Bardgett, B Thomson and S Ward. A Cole carried out data collection, with assistance from B Thomson and R Griffiths for T-RFLP analysis. A Cole led the data analysis and the writing of the paper, with contributions from co-authors.

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A Cole planned the experiment with advice from N Ostle, R Bardgett, and J Whitaker. A Cole carried out full data collection, data analysis and led the writing of the paper, with contributions from co-authors.

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A Cole planned the experiment with advice from N Ostle, R Bardgett, J Whitaker and B Thomson. A Cole carried out data collection and analysis, but with H Grant for isotopic sample analysis. R Griffiths advised on analysis of T-RFLP data. A Cole led the writing of the paper, with contributions from co-authors.

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N Ostle and A Cole conceived and planned the experiment, with contributions from .R. Bardgett, R. Griffiths, and J. Whitaker for framing the research questions. A Cole carried out data collection and data analysis, but with H Grant for isotopic sample analysis. A Cole led the writing of the paper, with contributions from co-authors.

I hereby agree with the above statements:

Nicholas J Ostle Date 9th December 2016

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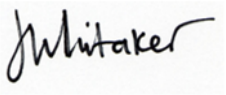

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Table of Contents

List of figures

List of tables

1 General Introduction

Ecological communities underpin many ecosystem services, such as agricultural productivity, water resources, nutrient cycling, and carbon (C) sequestration (NEA, 2011). However these services are at risk from both human activities and resulting changes in climate. In particular, human activities can lead to biodiversity loss, pollution, land use change and habitat fragmentation, which can in turn disrupt the provision of ecosystem services (Pimm, 1984; Tilman *et al.*, 2002; Foley *et al.*, 2005; Tylianakis *et al.*, 2008). Additionally, changes in climate may also alter the composition of ecological communities and the services they provide (Tilman *et al.*, 2006; Isbell *et al.*, 2011). However, human impacts and climate change will not alter ecological communities independently, but are likely to interact, such that a community's ability to resist and recover from climate change may also depend on human activities. This thesis considers how climate change will alter grassland biogeochemical cycles, and whether these changes will depend on the management of grasslands. In particular, it considers how drought events, which are predicted to become more frequent under climate change, may interact with in nutrient availability, which is altered by agricultural practices.

The global climate is changing, with increasing atmospheric CO_2, and predictions that at the end of the 21st century, temperatures will be at least 1.5°C higher than the previous century (IPCC, 2014). Projections also suggest that changes in precipitation extremes will exceed changes in mean precipitation (Kharin *et al.*, 2007; O'Gorman & Schneider, 2009). In the UK, for example, it is predicted that summer precipitation will reduce by 20% and potentially by up to 60% (Met Office, 2015). The importance of extreme drought events, in combination with subsequent rewetting, has required ecological experiments to investigate how such events may structure ecological communities (Jentsch *et al.*, 2007). Drought events therefore represent an important aspect of climate change with potentially widespread ecological effects, yet they are not the only factor that may influence the future provision of ecosystem services. One such factor is nutrient availability, which depends on both agricultural practices and atmospheric

deposition. There has been a near doubling of global nitrogen (N) inputs into terrestrial ecosystems, with N fertiliser use increasing seven-fold from 1960 to 1995 (Tilman *et al.*, 2002). This has led to large-scale changes in plant community composition, species richness and soil microbial community structure (Stevens *et al.*, 2004; Smart *et al.*, 2005; Smith *et al.*, 2008; Wesche *et al.*, 2012).

Terrestrial ecosystems are particularly important as they provide 99% of calories for food (Pimentel & Burgess, 2013) and hold very large C stores (Jobbagy & Jackson, 2000). In particular, grasslands, which cover 30% to 40% of the land surface (Shantz, 1954; FAO, 2005), are important for food production, biodiversity, and C sequestration. In Europe, grasslands are typically maintained by agricultural activities (Lemaire *et al.*, 2005; Chang *et al.*, 2016), cover 33% of European land area (Schnyder *et al.*, 2010), contain large stores of C, and may act as a large net soil C sink of 20 Tg C yr^{-1} (Chang *et al.*, 2015). Yet, these C stores are sensitive to agricultural practices and climate, which may alter grassland biogeochemical cycles and therefore alter feedbacks to climate (De Deyn *et al.*, 2011b; Bradford *et al.*, 2016; Ward *et al.*, 2016). As grassland communities include complex interactions between plants and soil biota, the potential for C storage depends on both plant and microbial physiology, with plants also modulating the C and nutrients available for soil communities (Bardgett & Wardle 2010). This thesis therefore focusses on the effect of drought on above and belowground C cycling in UK grasslands, particularly investigating how changes in nutrient availability may modulate the effect of drought.

1.1 Plants: Drought and nutrient availability

1.1.1 Plant productivity

Plants have morphological and physiological adaptations to survive drought conditions, which includes increasing water uptake and reducing water loss (Chaves, 2002; Chaves *et al.*, 2003; Zwicke *et al.*, 2015). These adaptations mean that extreme drought typically reduces plant

growth and C uptake, while also changing allocation of C belowground (Kahmen *et al.*, 2005; Fuchslueger *et al.*, 2014; Poorter *et al.*, 2015). In contrast, a sevenfold increase in N fertiliser use has dramatically increased agricultural yields (Tilman *et al.*, 2002) and shifted C allocation aboveground relative to belowground (Poorter *et al.*, 2015). Nutrient addition typically decreases competition belowground for nutrients, and increases competition aboveground for light (Tilman, 1990; Harpole *et al.*, 2016; DeMalach *et al.*, 2017), while plants exposed to water stress typically increases root biomass, enabling greater water acquisition (Poorter *et al.*, 2012). It may therefore be expected that nutrient addition, through increasing water demand while reducing potential for water acquisition, will reduce the ability of plants to survive extreme summer drought events.

The interactive effect of nutrient addition and drought on plant productivity has been shown in heathland species, where increased nutrient availability caused greater reduction in shoot growth under drought (Gordon *et al.*, 1999). In grasslands, the greatest drought-induced declines in plant biomass were found when pre-drought biomass was greatest, and this occurred regardless of plant species diversity (Wang *et al.*, 2007). However, other studies show that high species richness increases the resistance of plant productivity to drought (Isbell *et al.*, 2015). This suggests, increases in nutrient addition have the potential to increase the vulnerability of grasslands to drought, yet the relative importance of plant biomass, species richness and plant community composition, for understanding the resistance of plant productivity to drought, is not fully known.

1.1.2 Plant community composition and plant traits

In grasslands, changes in plant functional group composition may modulate how grasslands respond to drought. For example, perennial species may be more susceptible to drought, in comparison to annual species (Fry *et al.*, 2013), while mosses are particularly sensitive to changes in water availability yet have high desiccation tolerance (Turetsky, 2003; Cornelissen *et al.*, 2007; Proctor *et al.*, 2007). Furthermore, after drought ends, plant species can show

considerable variation in their recovery, suggesting a role for plant composition in modulating grassland community resilience to drought (Zwicke *et al.*, 2015). In European grasslands, increases in nutrient availability, due to increasing fertiliser use and atmospheric N deposition, have led to large declines in plant species richness, with communities becoming increasingly dominated by fast growing grass species with high N demand (Wesche *et al.*, 2012), while moss abundance typically declines (Virtanen *et al.*, 2000). In UK grasslands, increased nutrient loads have reduced the plant species pool over two decades (Smart *et al.*, 2005), with reductions in species richness also occurring due to N deposition (Stevens *et al.*, 2004). Additionally, experiments show multiple addition of nutrients decreases species richness, with greater reductions as nutrient addition persists for longer (Harpole *et al.*, 2016).

To combat these long-term declines in plant diversity, there has been a large body of research looking at how to restore grassland plant diversity (Smith *et al.*, 2000; Pywell *et al.*, 2002; Bullock *et al.*, 2007). In particular, cessation of fertiliser addition and addition of seed of a high diversity target plant community, have been found to lead to the restoration of species rich grassland (Smith *et al.*, 2008; Kirkham *et al.*, 2014). In summary, the effect of drought on plant growth may differ between grassland plant functional groups, the composition of which will, in part, depend on the level of nutrient availability, and conversely on the use of grassland restoration treatments to restore plant diversity. Nutrient addition may therefore have short-term effects on grassland vulnerability to drought, but also longer-term effects, modulated through changes in plant community composition.

Difference in drought tolerance may not always cut across commonly used groupings of grassland plants, such as mosses, grasses, perennials or annuals, but instead be related to a range of plant functional traits (Fry *et al.*, 2014). For example, high specific leaf area (SLA) may confer greater maintenance of growth and photosynthesis, but reduce the ability to avoid dehydration (Zwicke *et al.*, 2015), while traits associated with low nutrient stress tolerance may also be found in species with the greatest resistance of biomass to drought (Macgillivray &

Grime, 1995). Additionally, certain plant traits may correlate with reduced soil moisture, such that they exacerbate drought events (Gross *et al.*, 2008), while high root-to-shoot ratios may aid access to water resources (Poorter *et al.*, 2015). Plant traits can also show differences in how they recover after rewetting, with the recovery also differing between species (De Vries *et al.*, 2016). Yet plant traits also vary within species, due to intraspecific trait variation and plasticity in response to changes in resource availability (Siefert *et al.*, 2015). For example, changes in intraspecific trait variation in response to drought may account for more of the change in community traits, than is due to species turnover (Jung *et al.*, 2014). However the role of intraspecific trait variation in modulating plant responses to either drought or nutrient addition is poorly understood

1.1.3 Photosynthesis, respiration and net C uptake

Drought has been found to reduce photosynthesis, soil respiration and net ecosystem exchange (NEE), but not reduce ecosystem respiration, when analysed across ecosystems (Wu *et al.*, 2011). Yet in grasslands, there have been a wide range of responses to drought, with studies showing similar declines in photosynthesis and ecosystem respiration (Fry *et al.*, 2013), but, more commonly, greater reductions in photosynthesis compared with ecosystem respiration, suggesting proportionally more C lost through respiration (Bloor & Bardgett, 2012; Li *et al.*, 2016). After rewetting, the size of precipitation pulse can also determine the relative response of photosynthesis and respiration such that a large rain pulse may favour C sequestration (Chen *et al.*, 2009a). These ratios of ecosystem respiration to photosynthesis are particularly important as they can determine the amount of C not lost in respiration which is then available for longer term C storage in either plants or soil (Bradford & Crowther, 2013). It is unclear whether drought consistently increases the proportion of C lost in respiration relative to C uptake, and whether larger rewetting pulses reverses this, thereby promoting C storage. As such, we are unable to predict the relative responses of C uptake and ecosystem respiration to drought in grasslands, limiting our understanding of ecosystem feedbacks to climate.

Plant growth in grasslands is typically limited by one or more nutrients, such that nutrient addition is likely to reduce the amount of C invested in nutrient acquisition (Fornara *et al.*, 2013; Harpole *et al.*, 2016). Stoichiometric theory suggests that increasing nutrient availability is likely to be associated with more efficient growth, for example fertile forests have been found to produce biomass more efficiently, which suggests proportionally greater C storage relative to respiration (Vicca *et al.*, 2012). It would therefore be predicted that nutrient addition should increase C uptake relative to ecosystem respiration, while in grasslands the response of NEE and ecosystem respiration to nutrient addition is varied (Bloor & Bardgett, 2012; Xu *et al.*, 2014). Over the short-term, nutrient addition will bring about phenotypic plasticity with resulting intraspecific trait variation (Macgillivray & Grime, 1995). Over longer time-scales, nutrient addition will reduce species richness and shift the community to be dominated by fast growing grasses (Smith *et al.*, 2000; Kirkham *et al.*, 2014). Both short-term responses and longer-term shift in the plant community may alter the response of CO_2 fluxes and particularly the ratio of C uptake and ecosystem respiration to drought.

1.1.4 Plant carbon use efficiency

When studying photosynthesis and plant respiration, without incorporating soil respiration, plants have been found to use a relatively constant proportion of C for new biomass, often termed plant carbon use efficiency (CUE; Gifford 1995; Dewar, Medlyn & McMurtrie 1998; Cheng *et al.* 2000). However plant CUE, equivalent to 1 minus the proportion of respiration relative to photosynthesis, can change with type of crop species, plant size and forest age (Albrizio & Steduto, 2003; Van Iersel, 2003; DeLucia *et al.*, 2007). Recently, plant CUE has been estimated using the proportion of ^{13}C retained in plant biomass over time, following ^{13}C-CO_2 pulse-labelling (Bradford & Crowther, 2013; Street *et al.*, 2013). The methodology has suggested that arctic mosses have higher CUE than co-existing vascular plants (Street *et al.*, 2013), while grassland species have shown species specific C retention, which did not differ based on grassland restoration treatments (De Deyn *et al.*, 2011a). Changes in plant community

composition could therefore alter C storage and turnover in plant biomass; however, our understanding of how grassland plants may alter the efficiency of their growth in response to climate change and nutrient availability is limited. In particular, the recent development of using the retention of ^{13}C to estimate plant CUE allows further investigation of plant responses, and potential feedbacks, to C cycling and climate change.

1.2 Soil biology: Drought and nutrient availability

Drought changes the physical soil environment; water content decreases and soil pores shift from being filled with water, to being filled with air (Schimel *et al.*, 2007; Manzoni *et al.*, 2012a). However, the effect of drought on the physical soil environment will interact with nutrient availability, as lack of water in the soil reduces nutrient mobility, but conversely increases nutrient concentrations in the remaining water-filled pores (Schimel *et al.*, 2007). Yet plants will also change the soil environment through their response to both drought and nutrient availability. This may include changes to both C and nutrient inputs belowground; for example drought and nutrient addition can alter C allocation to roots (Kahmen *et al.*, 2005; Fuchslueger *et al.*, 2014; Poorter *et al.*, 2015), water stress can change the metabolites exuded from roots (Badri & Vivanco, 2009), and nutrient addition can increase organic C release from roots (Henry *et al.*, 2005). As such drought will alter the soil physical environment and the accessibility of C and nutrients for soil microbes.

1.2.1 Changes in soil food webs

The soil microbial community composition is sensitive to both drought and nutrient availability. Drought events tend to promote groups such as fungi or gram-positive bacteria, in part due to their thicker cell walls enabling greater survival of water stress (Yuste *et al.*, 2011; Fuchslueger *et al.*, 2014), while increased nutrient addition can shift soil microbial community structure, especially to increase the abundance of bacteria relative to fungi (Bardgett & McAlister, 1999; De Vries *et al.*, 2006; Smith *et al.*, 2008). More generally, soil food webs are sensitive to

changes in agricultural practices, with increasing intensification reducing soil biodiversity (Tsiafouli *et al.*, 2015), but they are also sensitive to changing precipitation, with increasing abundance of soil biota as precipitation increases (Blankinship *et al.*, 2011). Yet research has shown relatively few interactive effects between drought and nutrient addition, for example the abundance of the majority of soil biota in a North American grassland depended on the interaction between N addition and elevated CO_2, rather than N addition and drought (Eisenhauer *et al.*, 2012).

1.2.2 Fungi and bacteria: Acquiring nutrients and surviving water stress

The soil microbial community has morphological and physiological adaptations to survive drought events and efficiently acquire nutrients. However in contrast to plants, soil microbes typically respond to drought on shorter time scales and to spatially smaller changes in water and nutrient availability (Borken & Matzner, 2009). Fungi are generally more resistant to drought than bacteria, and may promote increased soil C and N retention (Schimel *et al.*, 2007; De Vries *et al.*, 2012a). This is in part because fungi create large hyphal networks that allow water transfer over large areas, and therefore access to water resources in soil pores (Manzoni *et al.*, 2012a). Mycorrhizal fungi may additionally increase water supply for plants (Wardle *et al.*, 2004; Schimel *et al.*, 2007). Although generally more sensitive to drought, bacteria may survive by switching from an active state to dormancy (Lennon & Jones, 2011), and be protected from drought for longer due to being in small soil pores which retain some water (Moyano *et al.*, 2013).

Both fungi and bacteria survive the increasing osmotic potential associated with drought through osmoregulation. Bacteria use amino compounds, and fungi use polyols (Csonka, 1989; Schimel *et al.*, 2007), such that the solutes used for osmoregulation can account for 7% to 20% of total bacterial C, and over 10% of cell mass for fungi (Koujima *et al.*, 1978; Killham & Firestone, 1984; Tibbett *et al.*, 2002). Yet for bacteria the accumulation of solutes for osmoregulation may impair metabolism, while for fungi there is no adverse effect (Manzoni *et*

al., 2012a). Additionally solutes used by bacteria contain N, while for fungi they do not, thereby altering the N-cost associated with osmoregulation for bacteria and fungi (Schimel *et al.*, 2007). The contrasting morphological and physiological traits of fungi and bacteria, suggests that changes in microbial community composition could subsequently bring about changes in microbial C and N cycling. There is clear potential for increased nutrient availability to shift microbial community composition and therefore alter the resistance to drought and recovery after rewetting.

1.2.3 Microbial growth and carbon use efficiency

Evidence is growing regarding the key role of soil microbes in processing plant inputs and ultimately promoting soil organic matter (SOM) formation (Schmidt *et al.*, 2011; Cotrufo *et al.*, 2013; Kallenbach *et al.*, 2016). It is thought that for SOM formation both the size of the microbial biomass and the efficiency with which it produces new biomass, will be important (Manzoni *et al.*, 2012b; Miltner *et al.*, 2012; Lange *et al.*, 2015; Kallenbach *et al.*, 2016). In soil microbial communities, the amount of C used in new biomass relative to C uptake, is termed microbial CUE, and averages around 50% across diverse studies (Manzoni *et al.*, 2012b; Geyer *et al.*, 2016). Yet reported values vary considerably from close to 0% to 85%, meaning that there is a very poor understanding of how changes in microbial CUE may determine the amount of C available for sequestration and ultimately how terrestrial C stores may feedback to climate (Manzoni *et al.*, 2012b; Sinsabaugh *et al.*, 2013, 2016). CUE of soil microbial communities typically increases as nutrients become non-limiting (Manzoni *et al.*, 2012b; Spohn *et al.*, 2016b), however the effect of drought on microbial CUE is not clearly understood (Herron *et al.*, 2009; Tiemann & Billings, 2011), while rewetting has been found to increase microbial CUE (Zeglin *et al.*, 2013b). Despite the importance of nutrient availability for microbial CUE, and the potential increase in drought events, no study has investigated the interactive effects of nutrient availability and drought on microbial CUE.

The large range in estimates of microbial CUE suggests that some of the variation may be due to the different methodologies used in previous research (Manzoni *et al.*, 2012b). At least part of this uncertainty comes from the use of different labelled substrates to investigate microbial CUE. For example the addition of glucose leads to high estimates of CUE, while oxalic acid results in very low estimates of CUE (Frey *et al.*, 2013). To avoid this substrate-dependency, recent research has used ^{18}O, and its incorporation in cell division, to estimate microbial CUE independently of particular C substrates (Spohn *et al.*, 2016a,b). The type of substrates used to investigate microbial CUE is particularly important as root exudation, a key source of C substrates, may be dependent on both nutrient availability and levels of water stress (Henry *et al.*, 2005; Badri & Vivanco, 2009). However, the further use of substrate-independent methods to measure microbial CUE are needed, particularly to utilise plant root exudation and turnover.

Many biogeochemical models of terrestrial ecosystems assume constant CUE, which does not change in response to climate (Manzoni *et al.*, 2012b). These models with constant CUE make several assumptions, for example that plant and microbial CUE must counterbalance each other in response to climate or environmental drivers. However this counterbalance assumption between different parts of an ecosystem is untested (Bradford & Crowther, 2013). Therefore linking plant and soil responses, and particularly their respective CUE, is of central importance to understand how communities cycle C, how it may change in response to climate, and whether it may act as a positive or negative feedback to climate.

1.3 Thesis aims and objectives

This thesis aims to investigate how C and N cycling in plants and soils responds to drought, and how this may depend on changes in nutrient availability, as brought about by changes in agricultural practices. It investigates the effect of long-term grassland restoration treatments, short-term nutrient addition and intraspecific trait variation on the resistance of C and N cycling to drought, and recovery after rewetting. This thesis is composed of four experimental chapters which address the following questions:

Chapter 2: How does restoration of grassland plant communities alter the resistance and recovery of C and N cycling to drought?

The increase in fertiliser use to bring about greater agricultural yields, has reduced plant species richness and changed plant community composition. To counteract this, restoration of grassland plant diversity has been investigated in long-term field experiments. This chapter investigates if shifts in plant and soil microbial community composition brought about by long-term restoration, alter how grassland C and N cycling responds to experimental summer drought. Specifically, it was hypothesised that grassland restoration would alter the resistance of C and N cycling to drought.

Chapter 3: How does intraspecific plant trait variation, brought about in part through N addition, modulate the response of CO_2 fluxes and C and N leaching to reduced soil moisture?

Although the effect of drought may differ between plant functional groups and species, it may also differ within species, due to intraspecific trait variation. This chapter uses N addition and shade conditioning, in a glasshouse pot experiment, to bring about intraspecific trait variation, in particular changing plant biomass and root-to-shoot biomass ratio. This was done to test the hypothesis that increased aboveground plant biomass and reduced ratio of root-to-shoot biomass would reduce resistance to, and recovery after, reduced soil moisture. The response of CO_2 fluxes and C and N leaching to reduced soil moisture and subsequent rewetting is used to assess the role of intraspecific trait variation relative to interspecific variation.

Chapter 4: Are responses to nutrient addition and drought similar for plant and soil microbial CUE?

When CUE is low, proportionally more C is respired suggesting less is available for sequestration. Yet, how CUE in plants and soils respond to drought and nutrient addition is unknown, which has implications for biogeochemical models of terrestrial C cycling because

they typically assume constant CUE. This chapter investigates how plant and microbial CUE, measured simultaneously using ^{13}C-CO_2 pulse-labelling, respond to drought and nutrient addition. This study tests the hypothesis that drought would decrease plant and soil microbial CUE, while nutrient addition would increase plant and soil microbial CUE.

Chapter 5: Do responses of moss CUE to nutrient addition and drought match responses of vascular plant CUE?

Research suggests moss CUE may be higher than vascular plant CUE. Yet mosses are sensitive to water availability and typically decrease in abundance as nutrient availability increases. The response of moss CUE to drought and nutrient addition is therefore unknown. This chapter investigates if moss CUE differs to vascular plant CUE in their response to drought and nutrient addition, while also improving estimates of plant CUE to take into account previously untested assumptions. Specifically it was hypothesised that moss CUE would not be greater than vascular plant CUE, due to previous overestimates of moss CUE, and that CUE in both plant functional groups would be decreased by drought and increased by nutrient addition.

1.3.1 Species-rich hay meadows

This thesis investigates the effect of drought and nutrient availability on C cycling in grasslands, and focusses on species-rich hay meadows in northern England to test the hypothesis outlined above. These species-rich hay meadows are used for grazing in autumn, winter and early spring, and hay production in the summer. They have been the focus of a large body of research, including grassland restoration, to identify management practices, which promote plant species diversity (Smith *et al.*, 2000; Kirkham *et al.*, 2014). Research has shown changes in plant community composition can alter soil microbial community composition (Smith *et al.*, 2008), and increase soil C accumulation (De Deyn *et al.*, 2011b) and soil N cycling (Bardgett *et al.*, 2006). This body of research shows that C cycling in plant and soil microbial communities are closely linked, depending on agricultural practices which also influences the composition of

plant and soil microbial communities. However the effect of climate change in these grasslands has received less focus (but see: Paz-Ferreiro *et al.*, 2012), and in particular, the effect of water stress on these communities, which often receive high mean annual precipitation, is unclear.

2 Grassland restoration alters resistance and recovery of carbon and nitrogen cycling in plants and soil microbes to summer drought

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2.1 Abstract

Evidence suggests that the restoration of plant diversity in grasslands not only brings benefits for biodiversity conservation, but also the delivery of ecosystem services. While biodiversity-function experiments show that greater plant diversity increases resistance of plant productivity to climate extremes, it is not known whether real-world management options for the restoration of grassland biodiversity likewise stabilise ecosystem responses to extreme climate events. We used a long-term field experiment in northern England to test the hypothesis that grassland biodiversity restoration increases the resistance and recovery of carbon (C) and nutrient cycling to drought. This was tested by measuring plant, soil and microbial responses to a simulated drought in experimental plots where fertiliser application and plant seeding have been manipulated for more than 20 years to enhance plant diversity. Long term cessation of fertiliser application decreased plant biomass and changed the abundance of plant functional groups, while seed addition altered the abundance of particular species. Plant productivity was resistant to the experimental drought across all grassland restoration treatments. Cessation of fertiliser

application increased the resistance of grasslands to drought, in terms of ecosystem respiration. However after rewetting, which coincided with regrowth following the hay cut, grasslands with cessation of fertiliser, showed smaller net C uptake indicating slower regrowth and recovery. Drought conditions promoted soil fungi relative to bacteria, but recovery of fungal community structure took more than three weeks after rewetting. In contrast, C and nitrogen in microbial biomass which were reduced by drought, recovered within three weeks. This study shows that drought can disrupt C cycling and soil microbial community structure even when plant productivity is not reduced. However the effect of drought depended on grassland restoration with seed and fertiliser treatments having contrasting effects on the resistance and recovery of grasslands to extreme drought events.

Keywords: grassland restoration, drought, fertiliser, seed addition, ecosystem respiration, soil microbial community.

2.2 Introduction

The restoration of plant diversity in grasslands, and the management practices required to bring it about, has been a major focus of research (Smith *et al.*, 2000; Pywell *et al.*, 2002; Bullock *et al.*, 2007; Kirkham *et al.*, 2014). In addition to increasing plant diversity, grassland restoration can bring benefits for the delivery of ecosystem services such as increasing soil carbon (C) accumulation (De Deyn *et al.*, 2011b), increasing agricultural hay yields (Bullock *et al.*, 2001) and increasing nutrient retention (Maron & Jefferies, 2001). While biodiversity-function experiments show that greater plant diversity increases resistance of plant productivity to climate extremes (Isbell *et al.*, 2015), it is not known whether real-world management options for the restoration of grassland biodiversity likewise stabilise ecosystem responses to extreme climate events. One such extreme climate event is drought, which is not only predicted to become more frequent under climate change (Kharin *et al.* 2007; O'Gorman & Schneider 2009; Met Office 2016), but can also disrupt C and nitrogen (N) cycling in grasslands (Harper *et al.* 2005; Smith 2011; Jentsch *et al.* 2011; De Boeck *et al.* 2011).

Drought will have direct effects on plant and soil microbial communities as soils become drier, however the response of plants to drought can also bring about indirect effects belowground (Bardgett *et al.*, 2013). Plants have morphological and physiological adaptations to survive drought conditions, in particular, enabling them to increase water uptake, reduce water loss and as the drought becomes more severe to ensure plant survival (Chaves, 2002; Chaves *et al.*, 2003; Zwicke *et al.*, 2015). These adaptations mean that extreme drought typically reduces plant growth and C uptake, alters allocation of C belowground (Kalapos *et al.*, 1996; Kahmen *et al.*, 2005; Fuchslueger *et al.*, 2014) and may change the metabolites exuded from roots (Badri & Vivanco, 2009). Drought may also alter the effect of grassland management on soil C cycling, for example the removal of plant biomass for hay can release a pulse of C belowground, however drought may change the partitioning of that C into microbial biomass or other organic pools (Fuchslueger *et al.*, 2016). Restoration of grassland plant diversity also has the potential to influence the effect of drought on plant and soil processes. One outcome of restoration will be to alter the composition of plant functional groups (Smith *et al.*, 2003), which in turn may alter grassland responses to drought (Evans *et al.*, 2011), potentially due to differences in biomass (Wang *et al.*, 2007), morphology or growth strategies (Fry *et al.*, 2013). Yet when investigated, research suggests no difference between restored and unrestored grasslands in terms of changes in plant productivity or community composition in response to drought (Carter & Blair, 2012).

The soil microbial community also has morphological and physiological adaptations to survive drought events, however in contrast to plants, they typically respond on short time scales and to spatially smaller changes in water availability (Borken & Matzner, 2009). The range of adaptations include microbes switching from being active to a dormant state (Schimel *et al.*, 2007), using osmolytes to regulate osmotic potential (Killham & Firestone, 1984; Tibbett *et al.*, 2002), and reducing growth to survive reduced substrate availability due to reduced diffusion (Schimel *et al.*, 2007). In the same way that drought can, over time, alter the plant community composition, the composition of soil microbial community can also shift, for example

promoting drought-tolerant groups such as fungi or gram-positive bacteria (Yuste *et al.*, 2011; Fuchslueger *et al.*, 2014). Overall, extreme drought typically reduces microbial activity with lower heterotrophic respiration (Manzoni *et al.*, 2012a). As water stress can alter plant C allocation belowground and change exudation from roots, drought can have indirect effects on the microbial community mediated by plant responses (Bardgett *et al.*, 2013; Fuchslueger *et al.*, 2016). This suggests grassland restoration may increase resistance to, or recovery after, drought. For example grassland restoration can shift the soil microbial community structure, especially to increase the abundance of fungi relative to bacteria (Smith *et al.*, 2008). As fungi are thought to be more resistant to drought than bacteria (Schimel *et al.*, 2007), and may promote increased C and N retention (De Vries *et al.*, 2012a), restored grasslands have the potential to be more resistant to extreme drought events.

This study investigates how long-term management treatments used to restore plant diversity may also confer greater resistance to drought and recovery after drought. This study utilises a 23-year grassland restoration experiment in northern England which has successfully brought about increased plant diversity through a combination of seed addition and absence of inorganic fertiliser application (Smith *et al.*, 2000, 2003). From 1990 to 2002 long-term seed addition increased species richness from 18.7 to 22.0 per $4m^2$, while inorganic fertiliser cessation increased diversity from 19.3 to 21.4 (Smith *et al.*, 2008). Changes in plant community coincided with changes in the soil microbial community, in particular promoting fungi relative to bacteria (Smith *et al.*, 2008), and an increase in soil C accumulation when seed addition and cessation of fertiliser were in combination with legume addition (De Deyn *et al.*, 2011b). A summer drought event was superimposed on these contrasting grassland communities to investigate how the resistance of C and N cycling to drought, and recovery after drought, was altered by grassland restoration.

We hypothesised that: 1) drought would reduce plant productivity, ecosystem respiration, net C uptake and microbial biomass and would have a greater negative effect on bacteria than fungi.

However the effect of drought would also depend on grassland restoration treatment, such that: 2) long-term fertiliser cessation would increase resistance and recovery of plant productivity to drought as the resulting smaller plant biomass would decrease water demand and therefore decrease water stress; and 3) restored grasslands with increased fungal abundance relative to bacteria would be more resistant to drought, because bacteria are likely to be less resistant than fungi to drought.

2.3 Methods

2.3.1 Experimental system

The study was conducted on selected plots of a long-term (23-year) grassland diversity restoration experiment at Colt Park meadows, Ingleborough National Nature Reserve, northern England (latitude 54°12'N, longitude 2°21'W; Bardgett & McAlister 1999; Smith *et al.* 2000; De Deyn *et al.* 2011b). The experiment was set up in 1990 on agriculturally improved *Lolium perenne-Cynosorus cristatus* grassland on shallow brown earth soils over limestone bedrock, in order to identify optimal management strategies for restoration of botanical diversity. The experiment includes four treatments in a fully factorial design: inorganic fertiliser addition, farm yard manure addition, seed addition and *Trifolium pratense* seed addiiton in three replicates blocks (Smith *et al.*, 2000, 2003, 2008). In this study we superimposed drought treatments on to a subset of plots (3m x 3m), namely plus or minus inorganic fertiliser addition and plus or minus seed addition (Appendix 1). These treatments were selected because the combination of seed addition and absence of inorganic fertiliser addition has resulted in the greatest increase in plant diversity (Smith et al 2008).

Before the long-term grassland restoration started the whole meadow received mineral fertiliser addition (Smith *et al.*, 2000). The fertiliser cessation treatment started in 1990 with the alternate treatment being continued fertiliser application (NPK 20:10:10 fertiliser: 25 kg ha^{-1} N, 12.5 kg ha^{-1} P and 12.5 kg ha^{-1} K) which has since been applied to plots by hand annually in spring (on

21 May in 2013), except in 2009 and 2010. Seed addition has been applied since 1990 with seed coming from two sources: locally collected seed and commercially bought seed. Seed collected from local traditionally managed, species-rich hay meadows was applied in autumn 1990-1992 (0.05 to 1.5 kg ha^{-1}), whereas commercially bought seed of 19 species was applied at 6.9 kg ha^{-1} in autumn 1990-92, and seed of three target species (*Lotus corniculatus*, *Briza media* and *Ranunculus bulbosus*) in August 1998 at 15.4 kg ha^{-1}. Locally collected seed of *Geranium sylvatucum* applied in September 1999/2000 at 0.5 kg ha^{-1} (Smith et al. 2003; Smith et al. 2008). All plots used in this study received *T. pratense* seed in 2004 but there was no establishement (De Deyn *et al.*, 2011b). Since 1999 all plots have been cut for hay between mid July and early August (on 16 July in 2013), and then grazed by sheep and cattle until May, when grazing ceases for the production of hay (Smith et al. 2000; Smith et al. 2003; Smith et al. 2008; De Deyn et al. 2011).

2.3.2 Drought treatments

To investigate the effect of drought on C and N cycling in grasslands, we set up 3 levels of the drought treatment in each long-term experimental plot, with drought treatments therefore imposed at the subplot level and seed and fertiliser treatments applied at the plot level. The three treatments were: control (no rain shelter), drought (rain shelter) and roofed control (rain shelter with holes). Rain shelters were open sided, constructed of transparent corrugated PVC, 0.8mm thick (Corolux, UK). Shelters were 90cm x 105cm with a height of 38cm to 63cm, giving a sloped roof of 16 degrees. Roofed control shelters were identical to those used in the drought treatment, except they contained holes to allow rainfall to reach the plot. This roofed control enabled us to test for unanticipated roof artefacts, such as increased air and soil temperatures which have been suggested to influence the results seen in previous research (Vogel et al. 2013). The roofed control treatment was included for measurements of species richness, total and plant functional group biomass and CO_2 fluxes, but not soil microbial community measurements. Drought manipulation was carried out from 5 June to 10 July 2013 (35 days). The effect of rain

shelters on soil moisture was assessed using a ThetaProbe soil moisture meter (Delta-T, UK), and soil and air temperature under rain shelters and in control plots was measured using Onset Hobo Pendant temperature loggers (Onset, USA) at a depth of 5cm for soil temperature and 5cm above the surface for air temperature.

The drought treatment excluded 180.8mm of rainfall which was equivalent to 7.5% of average annual precipitation at Colt Park meadows field site (mean: 2404mm, SD: 82mm). To assess drought severity and potential recurrence, a Gumbel I distribution was fitted to annual drought extremes, representing the number of days with less than 1mm of precipitation during the primary growth period (April-September). Due to insufficient number of years of precipitation data for the field site, data from Malham Tarn field centre was used, 18km southeast of the field site, and showed that a 100-year drought equated to 27 days <1mm rainfall. Similarly data from Hazelrigg Field station, 31km southwest, showed a 100-year drought equated to 34 days of <1mm rainfall (Bloor & Bardgett, 2012). These results suggest a 100 year drought event may be between 27 and 34 days, while in this study the drought treatment was *in situ* for 35 days. However the drought treatment in this study may not equate to the same level of water stress as a 100-year recurrence drought event, as to avoid disturbance to the plant community, plots were not trenched so lateral flow of water in the soil will have still occurred. Although the drought treatments was removed on 10 July 2013 significant rewetting from rainfall did not occur until 23 July 2013 (Fig 2.1a).

2.3.3 Plant community

Plant biomass was harvested on 10 July, from all plots at the end of the drought treatment, with aboveground biomass dried at 60°C for 48 hours and split into functional groups of grass, legumes, forbs and parasitic plants. The vegetation was ball milled before measurement of C and N content in 0.1g of plant material for each functional group on a Tru-spec CN analyser (Leco, St. Joesph, MI, USA). Plant species surveys were carried out between 29 June and 4 July 2013 on the central 706cm^2 of each plot, which was the same area as used to sample plant

biomass and measure CO_2 fluxes. To quantify belowground biomass, roots were removed from soil cores sampled during the drought (July) for microbial community analysis (see below). Roots were sieved, washed and dried at 60°C for 48 hours.

2.3.4 CO_2 flux measurements

Net ecosystem exchange (NEE) and ecosystem respiration were measured using static chambers following Ward *et al.* (2007, 2013), linked to an infra-red gas analyser (EGM 4, PP Systems, Herts, UK). Two minute headspace closures were used with NEE measured with transparent chambers and ecosystem respiration with opaque chambers covering an area of 706cm^2. Measurements were made between 10:15 and 16:30 with the order of blocks and plots alternated to avoid changes in temperature over the course of the day consistently impacting certain blocks and plots. Fluxes were adjusted for temperature, headspace volume and chamber area (Holland *et al.*, 1999). Three flux measurements were made before the drought treatment began (9 May, 17 May, 24 May), six during drought conditions (13 June, 20 June, 24 June, 27 June, 5 July, 8 July), one after rain shelters were removed but before significant rewetting (19 July) and four after re-wetting (26 July, 30 July, 7 August, 16 August).

2.3.5 Soil microbial community

At four time-points in 2013, three soil cores (2.4cm diameter, 10cm depth) per subplot were bulked together and sieved (2mm). Sample times were in June (before the drought), July (during the drought), August (3 weeks after rewetting) and for PLFA and T-RFLP analysis additionally in November (three months after rewetting). Before the drought, samples were only taken from 12 main plots to determine treatment effects of seed and fertiliser addition.

Drought effects on the soil microbial community structure across grassland treatments were assessed using the terminal restriction fragment length polymorphism (T-RFLP) method. This approach informs on the structure of bacterial and fungal community structure. Total genomic

DNA was extracted from soil samples using previously described procedures (Plassart et al. 2012) and the MoBio power soil kit. In addition, broad-scale changes in microbial community composition were assessed by phospholipid fatty acid analysis (PLFA) of soils from each subplot. Briefly, PLFA's were extracted from freeze-dried soil using a modified Bligh-Dyer extraction and separated from other lipids using aminopropyl solid phase extraction cartridge (Phenonenex, US; White *et al.* 1979). Gas chromatography was carried out on Agilent 6890 GC with CP-Sil 5 CB fused silica capillary column (Agilent, US). Biomarkers were used for bacteria (i15:0, a15:0, 15:0, i16:0, 17:0, i17:0, cy-17:0, 18:1ω7 and cy-19:0) and saprotrophic fungi (18:2ω6,9) (Bardgett *et al.*, 1996; Smith *et al.*, 2008).

Microbial biomass C and N were measured on 5g fresh soil subsamples using the chloroform fumigation-incubation method (Brookes et al. 1985) in soil cores taken before (June), during (July) and after (August) the drought. Briefly, one subsample was fumigated with chloroform for 16 hours before extraction with 25 mL 0.5 M K_2SO_4. The resulting filtrate was analysed for microbial C using a TOC analyser (5000A, Shimadzu, Milton Keynes, UK). For microbial N, filtrate was oxidised with $K_2S_2O_8$ before colorimetric analysis on an autoAnalyser (Bran and Luebbe, Northampton, UK). Microbial C and N were calculated as the difference between fumigated and unfumigated soil with adjustment factors applied for final analysis, using K_c=0.35 for microbial C and k_n=0.54 for microbial N (Sparling et al. 1990). DOC and DON were extracted from 5 g subsamples using 35mL of water with DOC analysed on a TOC analyser and DON extract oxidised with $K_2S_2O_8$ before analysis (see above). For pH 10g subsample was mixed with 25 mL deionised water, left to stand for 30 minutes and then tested with 210 pH Meter (Hanna Instruments, RI, USA).

2.3.6 Resistance and recovery indices

Resistance of CO_2 fluxes to drought was calculated using the index of Kaufman (1982) and Macgillivray & Grime (1995): resistance = P0/C0, where P0 is the drought treatment response during the drought and C0 is the response from control treatment (unroofed control) at the same

time point. Resistance is therefore the effect of drought proportional to the control. A 50% reduction caused by drought gives a resistance index of 0.5 while an increase of 50% gives an index of 1.5. No change under drought, i.e. resistant community processes/properties, gives an index of 1.

We investigated the recovery of CO_2 fluxes following removal of rain shelters using an index for recovery. Between the end of the drought and rewetting the annual hay cut took place removing aboveground biomass from all plots. Measurements after rewetting are therefore not only taking account of rewetting, but also regrowth after the hay cut. Many resilience indices incorporate resistance to drought and subsequent recovery (Orwin & Wardle, 2004). Because the hay cut changed many elements of C and N cycling across the whole community we do not use the term resilience, but instead recovery following drought and hay cut. We calculate recovery in the same way as resistance such that: recovery = Px/Cx, where Px is the drought treatment response after rewetting and Cx is the control value (unroofed control) at the same time point.

2.3.7 Statistical analysis

Linear mixed effects models (LME) were constructed for C and N cycling responses to seed, fertiliser and drought treatments. For each LME model the fixed effects were seed, fertiliser, drought and all interactions. The random effect was split-plot nested within block to take account of the experimental split-plot design. Where data was from repeated measures, plot ID was added as a random effect nested within split-plot. For all LME models, assumptions of normality and equal variances were checked graphically and response variables logged to improve normality. Weight functions were used to account for unequal variances, when necessary, following Zuur *et al.* (2009). We determined the significance of fixed effects by comparing models with and without the factor of interest using likelihood ratio tests (LRT). Outliers were removed where they were more than three times beyond the interquartile range. All statistical analysis was carried out in the R programming language 3.3.1 (R Core

Development Team, 2016) using the additional packages *nlme* (Pinheiro & Bates, 2013) and *plyr* (Wickham, 2011).

To investigate the interactive effects of drought and grassland management on soil microbial community structure each T-RF peak from T-RFLP analysis was converted to relative abundance as compared to the total of all fragments for that sample. Differences in microbial community structure between drought, seed and fertiliser treatments were assessed using multivariate statistical analysis using between-sample Bray-Curtis dissimilarities and non metric multidimensional scaling (NMDS). T-RFLP data was analysed for each sampling date separately and the significance of drought, seed and fertiliser treatments assessed using permutational multivariate analysis of variance with the R package *vegan* (Oksanen *et al.*, 2013).

2.4 Results

2.4.1 Rain shelter effects on soil moisture and temperature

The drought treatment reduced soil moisture from 58.8% to 33.3% while the roofed control reduced soil moisture from 58.8% to 49.8% (LRT=108.8, $P<0.0001$, Fig 2.1a). Soil moisture was also slightly reduced in plots with continued fertiliser application compared with long-term fertiliser cessation (LRT=7.03, P=0.008). Mean soil temperature at 5cm depth, averaged for each plot during the drought treatment, was 14.9°C with no difference between control (mean=14.8°C), roofed control (mean=14.9°C) and drought treatments (mean=15.0°C; LRT=1.72, $P=0.4239$, Fig 2.1d). Mean air temperature 5cm aboveground, averaged for each plot during the drought treatment, was 14.0°C with no difference between control (mean=14.0°C), roofed control (mean=13.9°C) and drought treatments (mean=14.2°C; LRT=2.53, $P=0.2828$, Fig 2.1c).

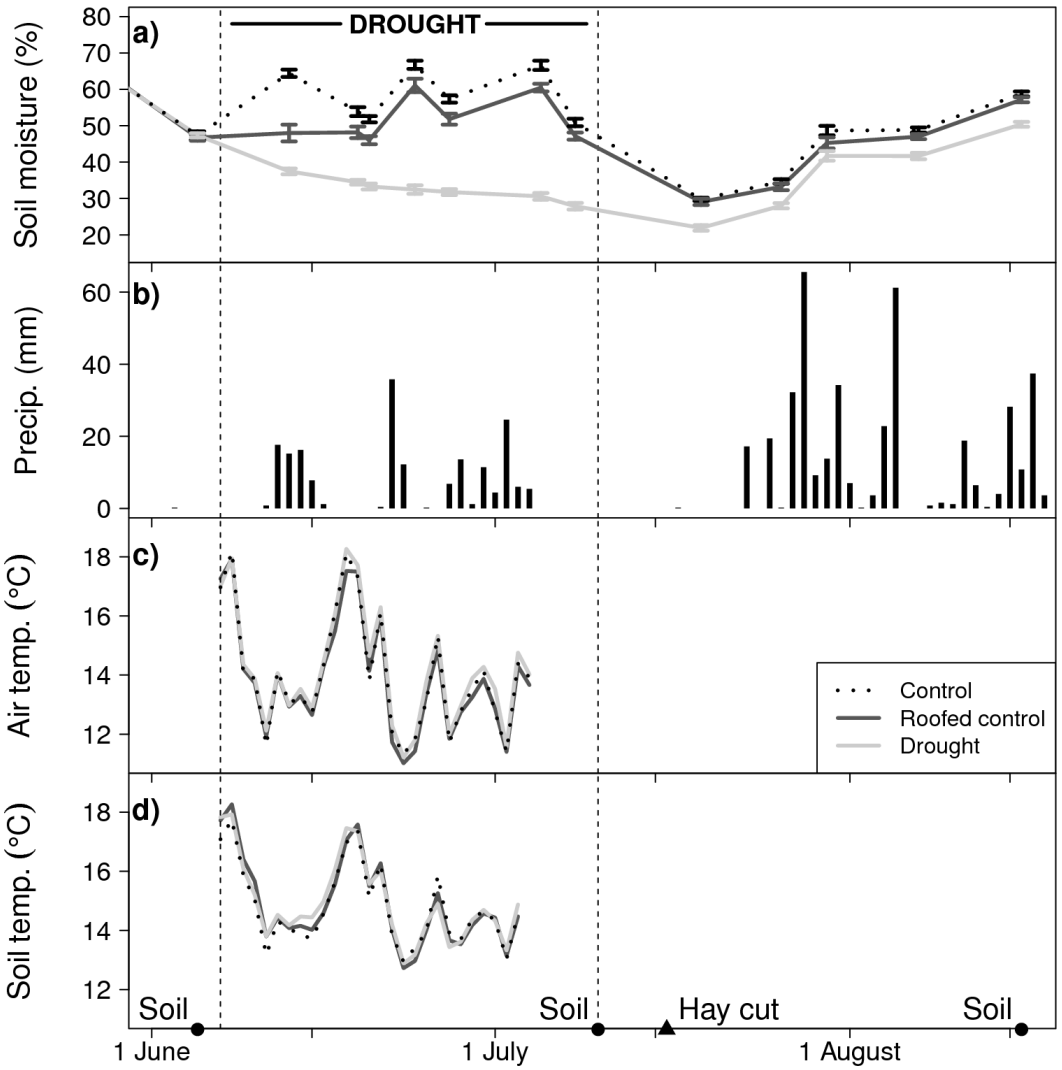

Figure 2.1 Meterological conditions during and after the drought treatment in 2013 a) soil moisture, b) daily precipitation, c) air temperature and d) soil temperature. Circles on x-axis show dates of soil sampling and triangle shows date of hay cut when aboveground biomass was removed. Vertical dashed lines show start and end date of rain shelters in place to simulate drought.

2.4.2 Plant community

Total aboveground plant biomass, harvested from all plots in July coinciding with the end of the drought period, was reduced in plots with long-term cessation of fertiliser application (LRT=17.95, d.f.=2, P<0.0001, Fig 2.2a), with no effect of the drought superimposed across the grassland restoration treatments. The effect of fertiliser cessation varied between plant functional groups (plant functional group x fertiliser: LRT=65.32, d.f.=3, P<0.0001), such that in plots with cessation of fertiliser, grass and forb biomass was decreased, legume biomass did not change, and parasitic plant biomass increased. Belowground biomass, sampled in July coinciding with the end of the drought period, was decreased in seed addition plots but only with cessation of fertiliser application (fertiliser x seed: LRT=5.74, d.f.=1, P=0.0166, Fig 2.2b). The proportion of root biomass relative to shoot biomass was increased in fertiliser cessation plots (LRT=11.76, d.f.=1, P=0.0006, Fig 2.2c).

Although plots with seed addition did not differ in abundance of plant functional groups there were species specific differences (Fig 2.3). For *Rhinanthus minor*, plots with seed addition had reduced percentage cover, although the reduction was smaller in drought plot (seed x drought: LRT=6.43, d.f.=2 P=0.0402). For *Holcus lanatus*, plots with seed addition had a small increase in percentage cover but the increase occurred primarily in non-drought plots (seed x drought: LRT=14.36, d.f.=2, P=0.0008). The number of vascular plant species ranged from 9 to 16 in 706cm^2 and was increased in fertiliser cessation plots (LRT=9.80, d.f.=1, P=0.0018, Fig 2.2d). Seed addition increased species richness but only in the unroofed control rainfall treatment (seed x drought: LRT=7.57, d.f.=1, P=0.0227).

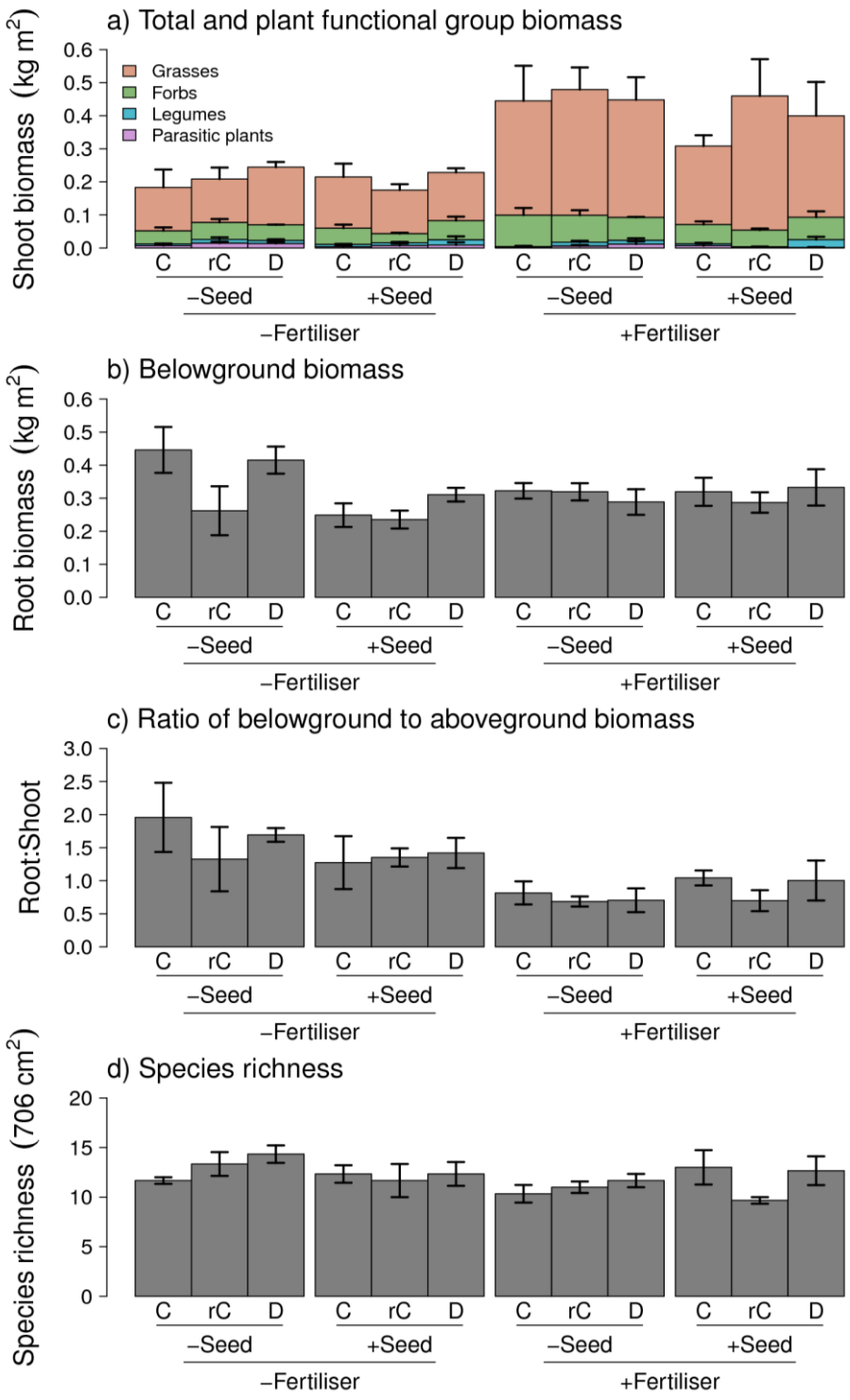

Figure 2.2 Plant biomass and species richness in response to grassland restoration treatments of nutrient and seed addition and drought treatments for, a) aboveground total biomass and for the plant functional groups of grass, forbs, legumes and parasitic plants, b) belowground bimass, c) ratio of belowground to aboveground biomas and d) species richness. C=control, rC=roofed control and D=drought. –Fertiliser represents the cessation of fertiliser treatment, while +Fertiliser represents continued fertiliser application. Bars indicate mean ±SE (n=3).

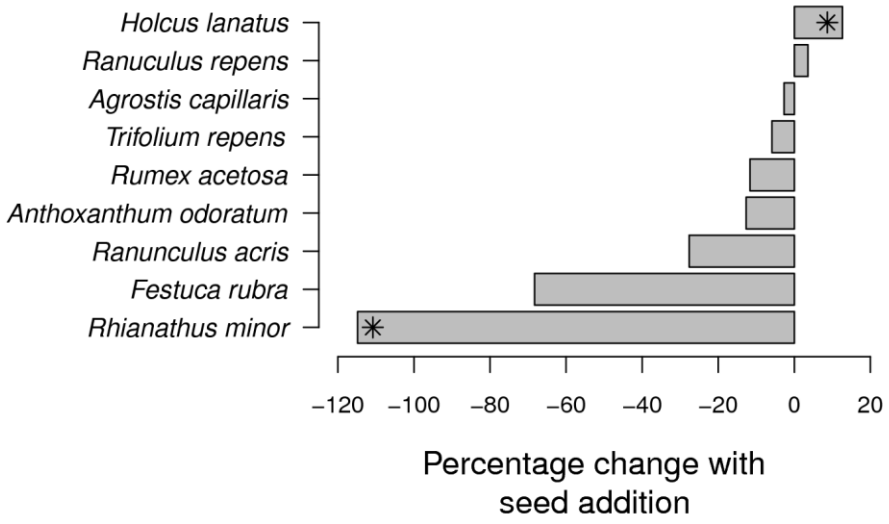

Figure 2.3 Percentage change in species cover with the seed addition treatment for the nine most common species. Bars with an asterisk show significant effect (P<0.05) of seed treatment, although interacting with drought treatment.

The ratio of C to N content in aboveground biomass, harvested at the end of the drought, was highest in grasses, then forbs and then legumes (LRT=150.02, d.f.=2, P<0.0001, Fig 2.4, Table 2.1). Drought increased C:N content in all functional groups, however the increase brought about by drought was smaller with the grassland restoration treatment of seed addition (seed x drought: LRT=5.55, d.f.=1, P=0.0185, Fig 2.4, Table 2.1). Parasitic plant C:N content, measured from a subset of 10 plots and statistically analysed separately, was significantly increased by drought (LRT=6.34, d.f.=1, P=0.0188, Fig 2.4d).

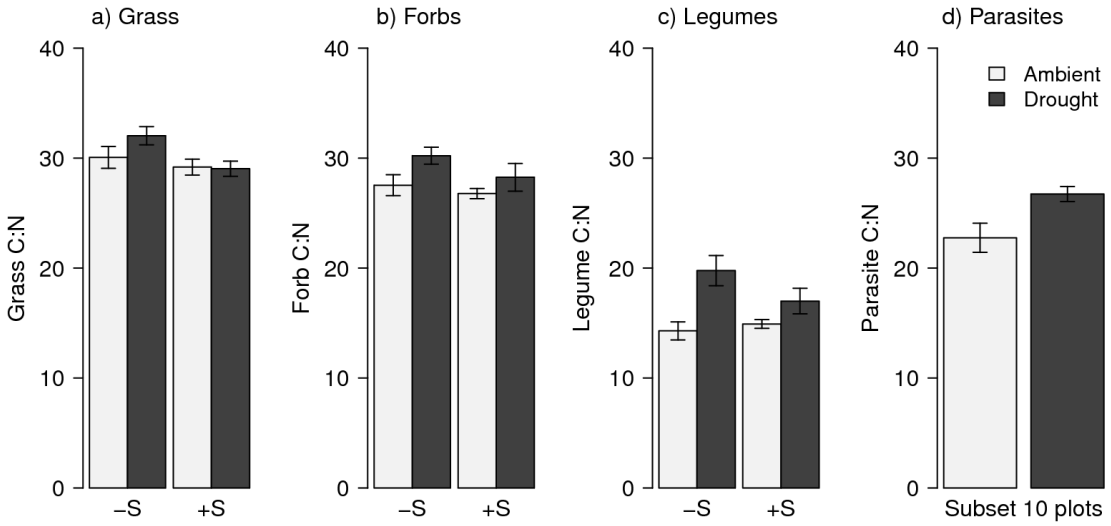

Figure 2.4 Ratio of C content to N content in plant biomass under drought conditions, a) grass C:N, b) forb C:N, c) legume C:N and d) parasitic plant C:N. –S = without seed addition; +S = with seed addition. Bars indicate mean ±SE (n=6), except for panel d where n=5.

Table 2.1 The effect of drought, fertiliser and seed treatments and plant functional group (PFT) on C%, N% and C:N in aboveground biomass. The PFTs analysed were grass, forbs and leguumes while parasitic plants were analysed separately. Significance of parameters calculated using Likelihood ratio tests (LRT).

		%C		**%N**		**C:N**	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
PFT	2	**54.06**	**<0.0001**	**57.69**	**<0.0001**	**150.02**	**<0.0001**
Fertiliser cessation (F)	1	**10.39**	**0.0013**	0.05	0.8271	0.29	0.5905
Seed addition (S)	1	0.36	0.5470	**5.05**	**0.0246**	3.79	0.0514
Drought (D)	1	0.66	0.4163	**11.49**	**0.0007**	**14.88**	**0.0001**
PFT x F	2	4.25	0.1193	4.82	0.0900	3.47	0.1766
PFT x S	2	0.66	0.7173	0.29	0.8629	0.63	0.7301
PFT x D	2	**12.09**	**0.0024**	**9.26**	**0.0010**	5.30	0.0706
F x S	1	0.25	0.6188	0.36	0.5489	0.08	0.7716
F x D	1	1.25	0.2628	1.14	0.2860	1.01	0.3157
S x D	1	1.52	0.2183	**5.23**	**0.0222**	**5.55**	**0.0185**
PFT x F x S	2	1.34	0.5670	0.64	0.7269	1.84	0.3986
PFT x F x D	2	5.51	0.0637	2.12	0.3460	2.75	0.2522
PFT x S x D	2	1.01	0.6033	2.16	0.3397	0.71	0.7022
F x S x D	1	0.46	0.4959	0.48	0.4902	1.07	0.3008
PFT x F x S x D	2	3.48	0.1758	4.07	0.1305	2.77	0.2500

2.4.3 CO_2 fluxes

Ecosystem respiration, averaged across sampling dates during the drought period, was decreased in fertiliser cessation plots, but the decrease was smaller in combination with drought (fertiliser x drought: LRT=11.06, d.f.=2, P=0.0040, Fig 2.5). After the drought, ecosystem respiration on average remained lower in fertiliser cessation plots (LRT=5.01, d.f.=1, P=0.0252, Fig 2.5). The resistance of ecosystem respiration to drought decreased over time and was greater in fertiliser cessation plots (date: LRT=15.08, d.f.=1, P=0.0001; fertiliser: LRT=7.00, d.f.=1, P=0.0081, Fig 2.6a). The reduction in ecosystem respiration under drought conditions correlated with plant biomass particularly towards the end of the drought, such that plots with greater plant biomass had greater reductions in ecosystem respiration (Fig 2.7). After the drought treatment ended, ecosystem respiration recovery increased over time but did not differ with fertiliser or seed treatments (date: LRT=17.51, d.f.=1, P<0.0001, Fig 2.6b).

The NEE sink strength was smaller in fertiliser cessation plots both during and after the drought, although the increase was much smaller after drought (during drought: LRT=17.24, d.f.=1, P<0.0001; after drought: LRT=5.56, d.f.=5.56, P=0.0184, Fig 2.5). The resistance of NEE to drought was the same over time and across experimental treatments (Fig 2.6c), while after drought recovery was reduced in fertiliser cessation plots and increased over time (date: LRT=3.96, d.f.=1, P=0.0466; fertiliser: LRT=4.58, d.f.=1, P=0.0323, Fig 2.6d).

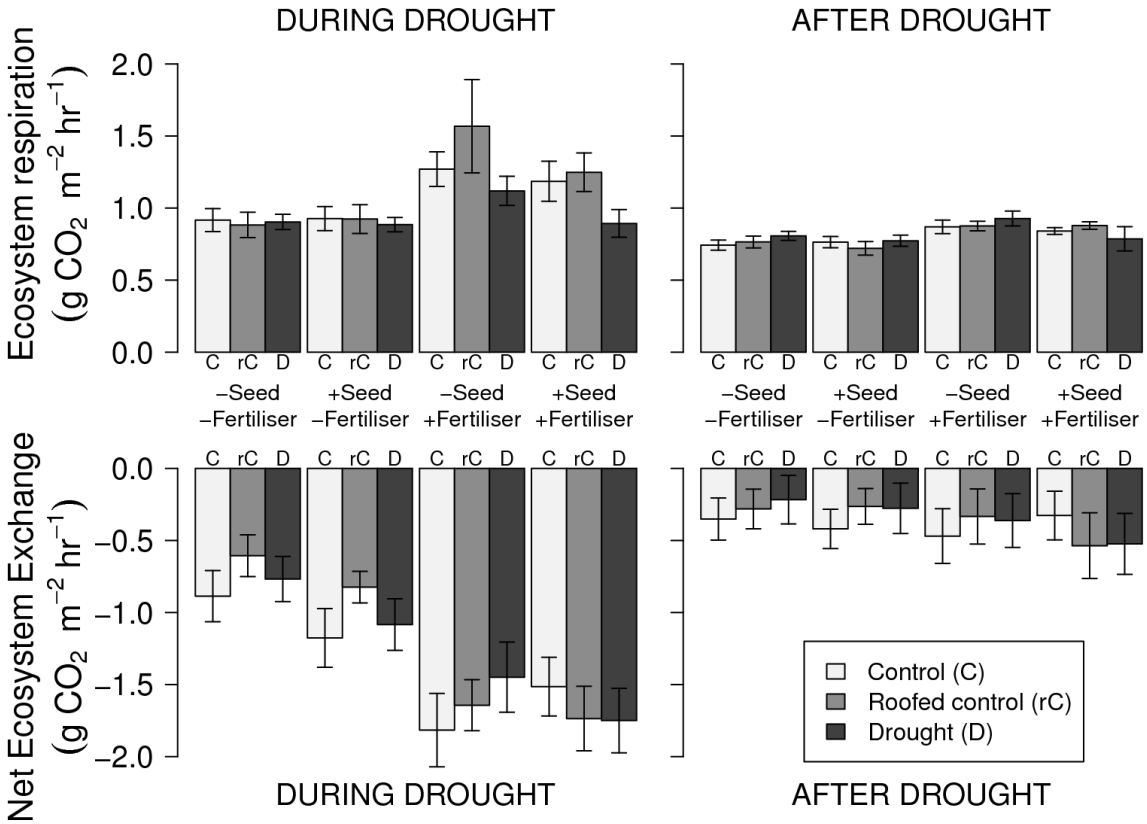

Figure 2.5 Ecosystem respiration and NEE in response to seed and fertiliser treatments and averaged across all sampling dates during and after the drought. Negative NEE values represent net C uptake. C=control, rC=roofed control and D=drought. –Fertiliser represents the cessation of fertiliser treatment, while +Fertiliser represents continued fertiliser application. Bars indicate mean ±SE (n=3).

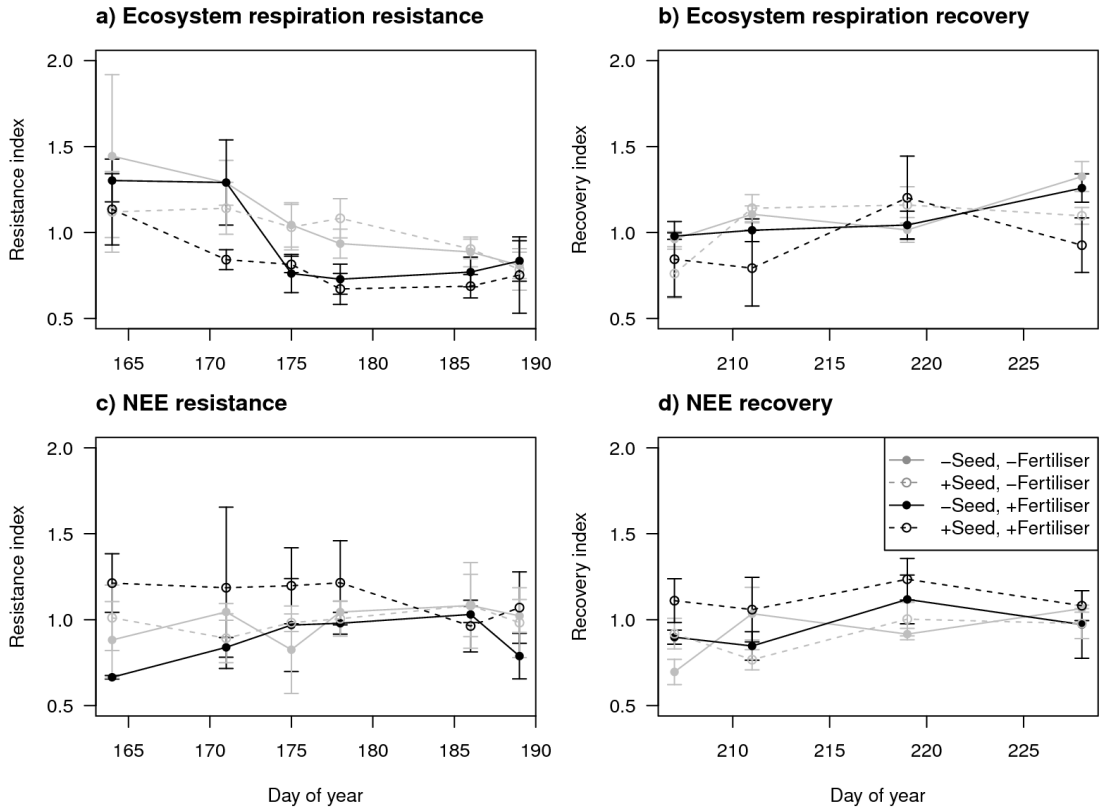

Figure 2.6 Resistance and recovery indeces for ecosystem respiration and NEE during the drought and after the drought with seed and fertiliser addition treatments, a) ecosystem respiration resistance, b) ecosystem respiration recovery, c) NEE ressitance and d) NEE recovery. –Fertiliser represents the cessation of fertiliser treatment, while +Fertiliser represents continued fertiliser application. Symbols indicate mean ±SE (n=3).

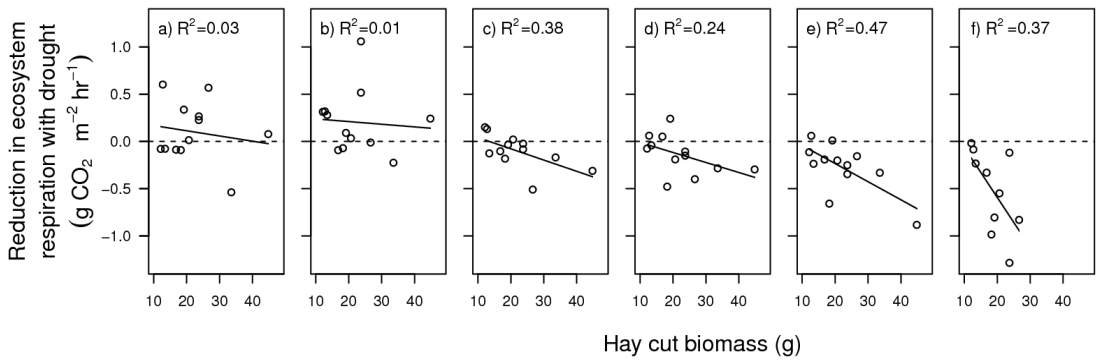

Figure 2.7 Reduction in ecosystem respiration with drought across six sampling dates. Dashed horizontal line indicates the same level of ecosystem respiration with and without drought, points below the line indicate lower ecosystem respiraton with drought; a) julian day 164, b) 171, c) 175, d) 178, e) 186 and f) 189.

2.4.4 Soil microbial community

Before the drought, the grassland restoration treatments of fertiliser cessation and seed addition had no effect on C or N content or C:N of the microbial biomass (Fig 2.8). During the drought, microbial biomass C was decreased in drought plots but only in combination with seed addition (seed x drought: LRT=10.51, d.f.=1, P=0.0014, Fig 2.8b). After the drought, microbial biomass C was the same across all treatments (Fig 2.8c). During the drought, microbial biomass N was reduced under the drought treatment (LRT=4.51, d.f.=1, P=0.0337, Fig 2.8e), but after the drought it was the same across all treatments (Fig 2.8f). The resulting ratio of microbial biomass C relative to N was not significantly changed by experimental treatments during the drought (Fig 2.8h). However after drought the ratio of microbial biomass C to N was increased in plots previously under drought but only in fertiliser cessation plots (fertiliser x drought: LRT=7.51, d.f.=1, P=0.0061, Fig 2.8i).

In drought conditions, the concentration of fungal PLFAs was increased and this occurred primarily in plots with either seed addition or continued fertiliser application (fertiliser x seed x drought: LRT=4.26, d.f.=1, P=0.0391, Fig 2.9f), while the concentration of bacterial PLFAs was not altered by drought, fertiliser or seed addition. The resulting ratio of fungal PLFA to bacterial PLFA was increased under drought conditions (LRT=6.61, d.f.=1, P=0.0101, Fig 2.9j).

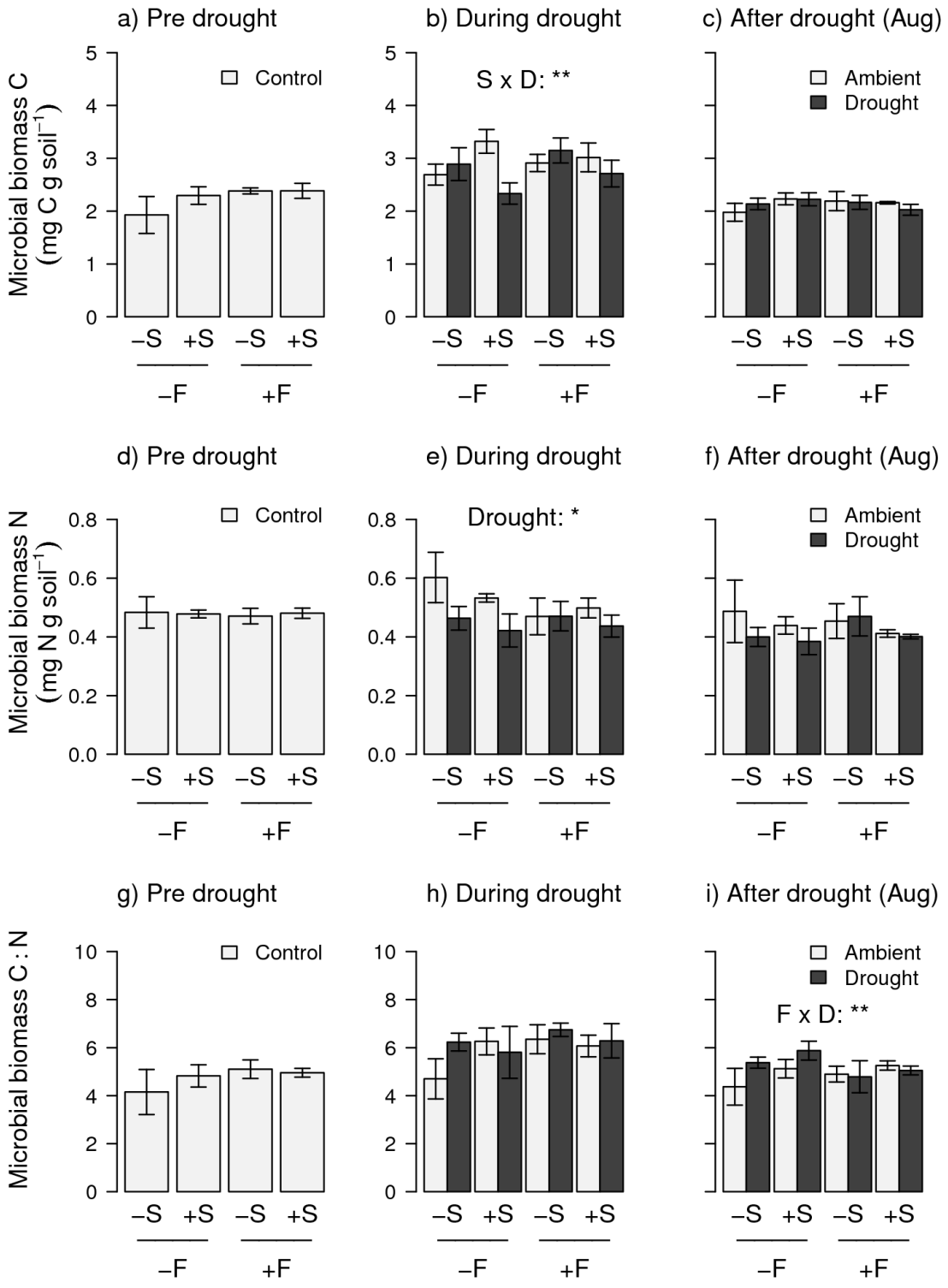

Figure 2.8 The effect of drought, seed and fertiliser addition on C and N in microbial biomass, d-f) microbial biomass N before, during and after drought, g-i) microbial biomass C:N before, during and after drought. The significance of main effects and interactions indicated by ** = P<0.01, * = P<0.05. –S = no seed addition, +S = seed addition, –F represents the cessation of fertiliser treatment, while +F represents continued fertiliser application. Bars indicate mean ±SE (n=3).

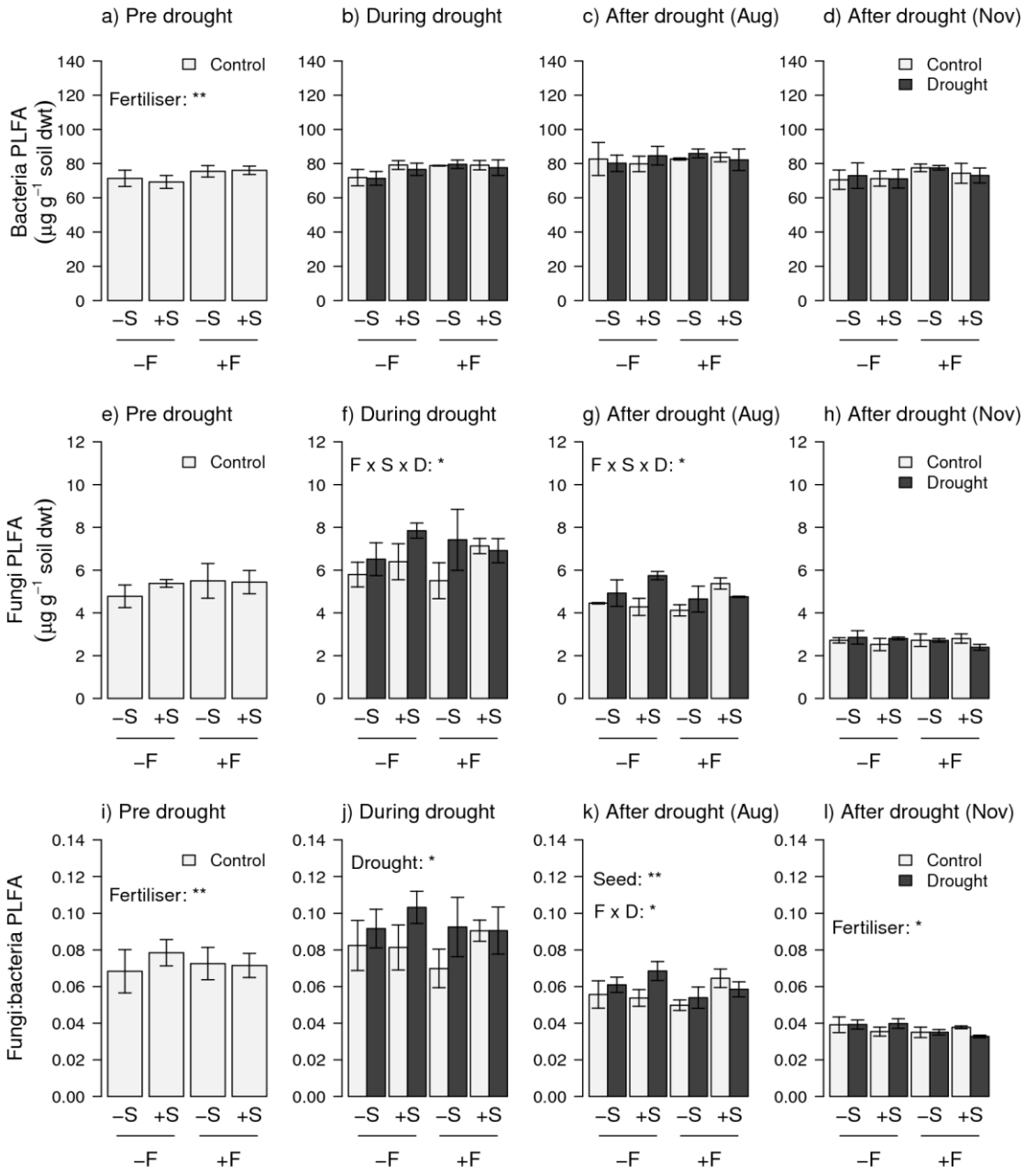

Figure 2.9 The effect of drought, seed and fertiliser addition on bacterial and fungal PLFA concentrations, a-d) bacterial PLFA concentrations before, during and after drought in August and Novemebr, d-h) fungal PLFA concentrations before, during and after drought in August and Novemebr, i-l) fungal:bacterial PLFA concentrations before, during and after drought in August and Novemebr. The significance of main effects and interactions indicated by ** = P<0.01, * = P<0.05. –S = no seed addition, +S = seed addition, –F represents the cessation of fertiliser treatment, while +F represents continued fertiliser application. Bars indicate mean ±SE (n=3).

After the drought (in August), bacterial PLFA concentrations were not significantly different between any treatments, while fungal PLFA concentrations continued to depend on drought, seed and fertiliser treatments (fertiliser x seed x drought: LRT=5.82, d.f.=1, P=0.0158, Fig 2.9g). The resulting ratio of fungal PLFA to bacterial PLFA was increased in plots previously exposed to drought, but only in fertiliser cessation plots (fertiliser x drought: LRT=4.18, d.f.=1, P=0.0409, Fig 2.9k), while the fungal to bacterial ratio was also increased in plots with seed addition (LRT=7.12, d.f.=1, P=0.0076, Fig 2.9k). For samples in November, more than three months after rewetting, neither bacterial nor fungal PLFA concentrations were altered by drought, seed or fertiliser treatments. However the resulting ratio of fungal PLFA to bacterial PLFA was increased in fertiliser cessation plots (LRT=4.29, d.f.=1, P=0.0382, Fig 2.9l).

Drought changed the bacterial community structure, as assessed by T-RFLP (Fig 2.10a). After the drought, the bacterial community structure assessed by T-RFLP in August and November did not depend on the previous drought treatment, but did differ with seed addition (Fig 2.10b,c). The fungal community assessed by T-RFLP was unaffected by drought, seed or fertiliser treatments.

2.4.5 Soil properties

During the drought, DOC in soil was not significantly affected by drought, seed or fertiliser treatments, however after the drought in August DOC was lower in plots with seed addition but only in fertiliser cessation plots (fertiliser x seed: LRT=4.43, d.f.=1, P=0.0354). During the drought, DON was lower in plots with seed addition, but only with cessation of fertiliser addition (fertiliser x seed: LRT=4.04, d.f.=1, P=0.0444), additionally DON was reduced under drought particularly in plots without seed addition (seed x drought: LRT=4.57, d.f.=1, P=0.0326).

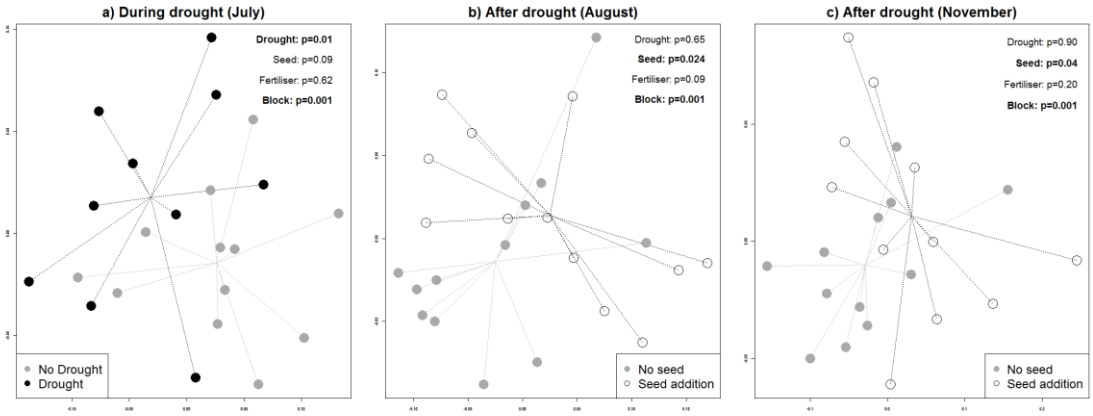

Figure 2.10 The effect of drought, seed and fertiliser addition on bacterial community structure, as assessed by T-RFLP analysis. Structure of bacterial community at three time points: a) during drought (July), b) after drought (August), and c) long-term recovery from drought (November). Different coloured points used to highlight significant treatment effects.

2.5 Discussion

The aim of this study was to investigate if real-world management options for the restoration of grassland biodiversity brought about changes in resistance and recovery to drought events of C and N cycling in plant and soil microbial communities. The long-term grassland restoration treatments of seed addition and fertiliser cessation brought about changes in plant communities, with differences in species composition and biomass, which in turn altered the effect of drought on CO_2 fluxes, plant C:N content, microbial biomass C, and soil microbial community structure. These effects of drought occurred despite the overall resistance of plant productivity to drought.

The long-term grassland restoration treatments brought about changes in total plant biomass, abundance of plant functional groups and species composition. Plots with continued fertiliser addition had greater biomass primarily due to the large increase in grass biomass (Fig 2.2). This confirms conclusions from the same experiment where fertiliser addition promoted grass biomass (Smith *et al.*, 2000, 2003, 2008; De Deyn *et al.*, 2011a), and more broadly with research showing fertiliser alters both species and plant functional group composition (Fornara *et al.*, 2013; Kirkham *et al.*, 2014). It shows that long-term fertiliser cessation is an important treatment to promote restoration of grassland plant diversity. In contrast, seed addition did not result in changes in total plant biomass or abundance of plant functional groups, but it did change individual species abundance (Fig 2.3) and soil bacterial community structure (Fig 2.10). In particular, *R. minor* had reduced abundance in plots with seed addition, although the size of reduction depended on the drought treatment. This matches previous research from the same experiment in which *R. minor* had lower occurrence in plots with seed addition (Smith *et al.*, 2000). The root hemi-parasite *R. minor*, is an important species as it can reduce plant productivity and increase soil N cycling, potentially through altering root turnover and exudation in the rest of the plant community (Bardgett *et al.*, 2006).

Although grassland restoration treatments did bring about changes in plant species richness, the changes were relatively small and suggest that differences in species number were unlikely to

explain the observed differences in grassland C and N cycling responses to drought (Fig 2.2d). The negative effect of fertiliser addition on species richness found in this study, although small, matches previous results from the same long term experiment (Smith *et al.*, 2003, 2008) as well as research into the effect of fertiliser and N deposition (Stevens *et al.*, 2004; Kirkham *et al.*, 2014). Seed addition brought about a small increase in species richness but only in control plots, with the opposite effect in roofed control and drought treatments. This small and inconsistent effect of seed addition may be because this study was carried out on a subset of plots from the long-term experiment with smaller survey area and additionally because the effect of long term treatments such as seed addition may differ between years. Previous results from the same experiment show seed addition increased species richness (Smith *et al.*, 2003, 2008), and does so across a wide range of grassland studies (Bullock *et al.*, 2001, 2007; Pywell *et al.*, 2002). Analysis of multiple biodiversity and ecosystem function experiments shows that increases in richness from 1 up to 64 species can increase resistance of plant productivity to extreme precipitation events (Isbell *et al.*, 2015). This suggests the change in species richness, seen in this study, from on average 11 to 15 species per 706cm^2 is unlikely to have a major role in grassland resistance to, or recovery after, drought.

2.5.1 Effect of drought on plant community

Contrary to the first hypothesis, plant biomass was not reduced under drought conditions, suggesting plant productivity was resistant to drought (Fig 2.2a). Additionally belowground biomass and the ratio of belowground to aboveground biomass did not change under drought conditions (Fig 2.2b,c). As the drought treatment intercepted 7.5% of average annual precipitation and brought about changes in CO_2 fluxes, soil microbial community composition and plant C:N content, it was severe enough to alter some community processes even though plant biomass was resistant to drought. Although drought can reduce plant productivity (Wang *et al.*, 2007; Vogel *et al.*, 2012; Isbell *et al.*, 2015), other studies have shown that even severe drought can have no effect (Mirzaei *et al.*, 2008; Jentsch *et al.*, 2011; Carter & Blair, 2012),

with a meta-analysis showing that multiyear reduced precipitation experiments do not regularly alter the productivity-precipitation relationship (Estiarte *et al.*, 2016). Belowground biomass responses to drought have been less studied than aboveground plant productivity, however both increased C allocation to roots to aid exploitation of reduced water availability (Kalapos *et al.*, 1996; Kahmen *et al.*, 2005; Poorter *et al.*, 2012), and reduced belowground C allocation have been reported (Sanaullah *et al.*, 2012b; Fuchslueger *et al.*, 2014; Hasibeder *et al.*, 2014).

Despite the resistance of plant productivity to drought, plant C content relative to N content was increased with drought, particularly in plots with seed addition and was primarily due to reductions in shoot N content (Fig 2.4). A similar decrease in shoot N was found following drought in two European grasslands (Walter *et al.*, 2012; Fuchslueger *et al.*, 2016), and may be due to reduced N mobility in drier soils (Chaves *et al.*, 2003). In this study the smaller increase in shoot C:N with seed addition suggests that changes in plant community composition can modulate the effect of drought on the relative N content in plant leaves. This is broadly consistent with a meta-analysis across ecosystem types which showed that in semi-arid ecosystems C:N ratios generally decreased in response to drought, but in wet-temperate ecosystems there were mixed responses (Sardans *et al.*, 2012). Our results showed that in grasslands with mean annual precipitation above 2000mm, drought increased aboveground C:N in all plant functional groups. These changes in leaf C:N content suggest that a very severe drought, bringing about leaf senescence could lead to changes in plant litter quality and therefore potentially decomposition rates.

2.5.2 Effect of drought on CO_2 fluxes

Drought reduced ecosystem respiration, but did not reduce NEE which suggests an equivalent reduction in photosynthesis to maintain no change in NEE (Fig 2.5, 2.6). This agrees with a study of experimental drought in UK grasslands which showed an overall net balance in reduction of photosynthesis and ecosystem respiration in response to drought (Fry *et al.*, 2013). However this contrasts with research which suggests greater reductions in photosynthesis than

ecosystem respiration under drought conditions in model grassland communities (Bloor & Bardgett, 2012), with similar results in semi-arid grasslands (Li *et al.*, 2016) and the same conclusion from a global meta-analysis of drought studies (Wu *et al.*, 2011). Greater drought sensitivity of photosynthesis compared with ecosystem respiration indicates that increased frequency and length of drought events could reduce the potential for ecosystem C storage and could act as a positive feedback to climate change. In contrast the results in this study suggest no change in net C uptake with drought.

Grasslands with the greatest plant biomass were expected to be less resistant to drought, due to increased water demand, reduced soil moisture, and therefore greater water stress. In support of this we found that in grasslands with continued fertiliser application, which brought about a near two-fold increase in plant biomass, ecosystem respiration was less resistant to drought and that the level of resistance decreased over time (Fig 2.6, Fig 2.7). This confirms previous results where a severe drought reduced CO_2 fluxes but without an overall change in biomass (Mirzaei *et al.*, 2008). Our results suggest that resistance of ecosystem respiration was biomass dependent such that grasslands with low nutrient inputs and low biomass may therefore be more resistant to drought.

Grasslands with fertiliser cessation had the smallest plant biomass and therefore the lowest water demand, which did not additionally decrease soil moisture and had the smallest drought-induced reduction in ecosystem respiration. It is therefore likely that plants in these restored grasslands, without fertiliser addition, experienced the least water stress under drought conditions. However after rewetting and hay cut the recovery of CO_2 fluxes was lower in plots with fertiliser cessation suggesting slower re-growth of plants (Fig 2.5, 2.6). This suggests fertiliser cessation may have decreased water stress but also decreased recovery after drought. The removal of aboveground biomass which occurred just before rewetting may have produced a pulse of rhizodeposition from roots, which can lead to greater N mineralization, enabling N acquisition by plants and therefore supporting regrowth (Hamilton 2008, Henry 2008). Plants

undergoing the least water stress may be expected to have more non-structural C available for release from roots to promote N mineralisation (Fuchslueger *et al.*, 2014). This would imply regrowth following rewetting and the hay cut should be fastest with fertiliser cessation, however as the opposite was found in this study, it suggests that the greater regrowth may have been because initial fertiliser addition was still increasing N availability and therefore the absence of this with fertiliser cessation led to slower recovery.

2.5.3 Effect of drought on soil microbial community

We hypothesised that an increase in the abundance of fungi relative to bacteria brought about by grassland restoration treatments, would increase resistance of soil C and N cycling to drought. Fungi are expected to be more resistant to water stress than bacteria (Schimel *et al.*, 2007; Bapiri *et al.*, 2010), and may therefore confer greater resistance of soil C and N cycling in drought conditions (De Vries *et al.*, 2012a). In support of the greater resistance of fungi to drought, we found that the fungal-to-bacterial PLFA ratio was increased with drought across all grassland restoration treatments (Fig 2.9j) primarily due to an increase in fungi, rather than a decrease in bacteria (Fig 2.9b,f). Three weeks after rewetting, fungal PLFAs and the fungal-to-bacterial PLFA ratio still showed differences brought about by drought, but both showed complete recovery 3 months after rewetting (Fig 2.9). This suggests recovery of the soil microbial community structure was slower than the recovery of C and N content of the microbial biomass, which recovered within three weeks of rewetting. The speed of recovery observed in this study is intermediate when compared to previous research were soil microbial community structure following drought recovered one week after rewetting in sub-alpine grassland (Hasibeder *et al.*, 2014), while resilience of fungal and bacterial biomass to a second drought event differed for up to 77 days after rewetting (De Vries *et al.*, 2012a). The study points to some redundancy in the soil microbial community, in that C and N pools could be the same even when there were differences in underlying soil microbial community structure.

In restored grasslands with seed addition, drought reduced microbial biomass C, but this did not occur in the absence of seed addition. This indicates changes in plant community composition, the primary change associated with seed addition (Fig 2.3), had an indirect effect of drought on microbial biomass C (Fig 2.8b). In this study, seed addition brought about grassland communities with reduced root biomass (although primarily in plots without fertiliser addition) but also had decreased cover of the root hemi-parasite *R. minor*. *R minor* can reduce plant productivity and increase soil N cycling potentially changing plant root turnover and exudation (Bardgett *et al.*, 2006). The importance of the plant community in determining the resistance of microbial biomass C to drought has previously been shown in model grasslands where resistance was lower with increased species richness (Bloor & Bardgett, 2012). A meta-analysis showed that although drought generally reduced microbial biomass C, the reductions were small compared with changes in microbial biomass C in response to plant diversity (Thakur *et al.*, 2015). Recent research shows that increased microbial activity and biomass can promote C sequestration (Miltner *et al.*, 2012; Lange *et al.*, 2015), however the fast recovery of microbial biomass C in this study suggests there is unlikely to be a lasting effect on C sequestration.

2.6 Conclusion

This study set out to investigate if real-world grassland restoration treatments stabilise responses to extreme climate events. It was found that two commonly used grassland restoration treatments, namely the cessation of fertiliser application and mixed seed addition, had differing effects on the resistance and recovery of C and N cycling in plants and soil microbes in response to drought. Despite overall resistance of plant productivity, cessation of fertiliser increased resistance of ecosystem respiration to drought primarily due to the resulting smaller biomass having a lower water demand. In contrast, grasslands with seed addition had reduced stability of microbial biomass C to drought, suggesting changes in plant community composition partly altered the effect of drought on the soil microbial community. Overall, resistance to drought may be best promoted in low biomass grasslands, without fertiliser addition. While biodiversity-

ecosystem function experiments show that increasing species richness can increase resistance of plant productivity to drought, this study shows the effect of real-world grassland restoration can differ to those of biodiversity-ecosystem function experiments. Additionally, the commonly used grassland restoration treatments of fertiliser cessation and seed addition had contrasting effects on the resistance to, and recovery after, drought events.

3 Intraspecific variation in plant traits alters grassland carbon cycling in response to reduced soil moisture

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3.1 Abstract

Plant species will differ in how they respond to climate change. Additionally individual plants of the same species can show considerable variation in traits, yet is unclear whether such intraspecific trait variation is important for determining how plant carbon (C) cycling responds to extreme climate events. Reduced precipitation and drought, leading to water stress for plants, is predicted to become more frequent under climate change. This study investigates whether predicted reductions in C uptake and respiration with reduced soil moisture, and increases in leaching losses upon rewetting are consistent irrespective of intraspecific trait variation. This study used four grassland species, with varying traits and growth strategies, grown in monoculture and a four-species community. Monocultures and the mixture received combinations of nitrogen (N) addition and shade conditioning, to bring about intraspecific variation in plant morphology. Half were then subjected to reduced soil moisture for four weeks followed by rewetting. Across all monocultures and mixture, reduced soil moisture was found

to lower C storage and retention in plants and soil through three processes: i) net CO_2 uptake was smaller with reduced soil moisture, ii) the proportion of C respired from plants and soil was increased relative to C uptake under reduced soil moisture, and iii) upon rewetting of dry soil, dissolved organic C (DOC) leachate losses were increased. However intraspecific trait variation, in all monocultures and the mixture, modulated these changes in C storage, such that the greatest reductions in net C uptake under reduced soil moisture occurred with high plant biomass and low specific leaf area (SLA). In contrast, the greatest DOC losses were found when plant biomass was low but with differences between the species in monoculture and the mixture. The results show that intraspecific variation in plant traits can alter how grassland species respond to predicted changes in precipitation, and that carbon cycling in grasslands with high plant biomass may be more sensitive to changes in precipitation regimes. Together, these results demonstrate that differences in plant traits within species can be as important as differences between species for modulating responses to climate change.

Keywords: climate change, reduced precipitation, plant functional traits, intraspecific trait variation, grasslands, CO_2 fluxes, nutrient leaching.

3.2 Introduction

Plant species are expected to differ in how they respond to climate change (Chaves, 2002; Craine *et al.*, 2012), but it is less certain how variation within species may lead to altered responses to different climates (Albert *et al.*, 2010; Lepš *et al.*, 2011; Ravenscroft *et al.*, 2014). Reduced precipitation and the water stress it can impose on plants is predicted to become more frequent under future climate scenarios (Kharin *et al.*, 2007; O'Gorman & Schneider, 2009). Intraspecific variation in plant traits can account for 25% of total trait variation within plant communities (Siefert *et al.*, 2015). In grasslands, drought has been found to alter intraspecific trait variation across a range of species (Jung *et al.*, 2014), while for some species populations from origins contrasting in level of precipitation show differences in resistance to drought

(Beierkuhnlein *et al.*, 2011; Poirier *et al.*, 2012). However research is needed to understand how intraspecific trait variation influences plant responses to changes in precipitation.

As precipitation regimes change and potentially become more extreme, plants and soils will be exposed to reduced precipitation, but also rewetting events at the end of reduced precipitation. In grasslands, both reduced precipitation and rewetting can alter carbon (C) and nitrogen (N) cycling through changes in plant growth, soil structure and microbial community activity (Kalbitz *et al.*, 2000; Sanderman *et al.*, 2008). Reduced precipitation often results in reduced plant biomass, and can also alter short term C and N cycling, biomass allocation and leaching losses from soil (Harper *et al.*, 2005; Jentsch *et al.*, 2011; Smith, 2011; Hagedorn & Joos, 2014). Rewetting of dry soils typically increases losses of dissolved organic matter (DOM), while plant responses to rewetting can show compensatory growth (Kalbitz *et al.*, 2000; Mirzaei *et al.*, 2008; Bloor & Bardgett, 2012). The relative importance of intraspecific plant trait variation for modulating these plant responses to reduced precipitation is unknown.

Plant biomass, and its allocation above or belowground, has the potential to determine how plants respond to water stress. Grasslands with the greatest plant biomass at the start of a drought have been found to have the greatest drought-induced reduction in plant biomass, suggesting responses to water stress in grasslands may be biomass-dependent (Wang *et al.*, 2007). However a strong effect of plant biomass has not been found in all studies: increased plant biomass, brought about through increased N availability, has been found to have very little effect determining CO_2 fluxes and C and N leaching (Bloor & Bardgett, 2012). Additionally the relative allocation of biomass either above or belowground, which can vary intraspecifically (Martin-Olmedo *et al.*, 2002), may modulate plant responses to reduced precipitation. Plants can allocate more C belowground during drought to aid exploitation of reduced water resources (Kalapos *et al.*, 1996; Kahmen *et al.*, 2005; Poorter *et al.*, 2012; Backhaus *et al.*, 2014). It may therefore be expected that plants with high root biomass relative to shoot biomass may maintain growth under reduced soil moisture to a greater extent than plants with proportionally less

biomass allocated belowground. As the ratio of root-to-shoot biomass can vary within species, intraspecific variation in this plant trait may be important for modulating plant responses to reduced precipitation.

In addition to biomass and its allocation either below or aboveground, intraspecific variation across a range of traits may explain differences in plant responses (Jung *et al.*, 2014; Siefert *et al.*, 2015). Cross-ecosystem comparisons suggest plants can be characterised as having an exploitative growth strategy, with high levels of photosynthesis, transpiration and specific leaf area (SLA), or a conservative growth strategy, typically with slower growth and lower SLA (Reich *et al.*, 1999; Diaz *et al.*, 2004). In grasslands, differences in growth responses to drought were predictable across grassland species on the basis of plant traits (Macgillivray & Grime, 1995). Although grassland plant traits have been used to understand the drivers of CO_2 fluxes in grasslands (Everwand *et al.*, 2014; Milcu *et al.*, 2014), the retention of N in plants and soil (De Vries & Bardgett, 2016) the plasticity in root traits in response to drought (De Vries *et al.*, 2016) and feedbacks to soil moisture (Gross *et al.*, 2008), studies typically only account for interspecific trait variation. The role of intraspecifc trait variation in modulating CO_2 fluxes and C and N leaching is currently unknown.

The aim of this study was to investigate the effect of reduced soil moisture on C and N cycling in grassland species and in particular the role of intraspecific variation in plant traits, including plant biomass and ratio of root-to-shoot biomass, in modulating the response to reduced soil moisture. We hypothesised that i) reduced soil moisture will decrease CO_2 fluxes and increase C and N leaching, and ii) changes in C and N cycling as a consequence of reduced soil moisture will be exacerbated as plant biomass increases and the ratio of root-to-shoot biomass decreases. To test these hypotheses a glasshouse experiment was conducted using four grassland species, of varying growth strategies and plant functional types, and one mixed community. Planted mesocoms differing in plant biomass, biomass allocation and plant traits were created through application of nitrogen (N) and shading treatments singly and in combination. The effects of

drought and rewetting on C and N cycling processes were then assessed through responses of CO_2 fluxes and C and N lost in leaching.

3.3 Methods

3.3.1 Experimental design and plant communities

To understand the mechanisms controlling species' level responses to reduced soil moisture a 'drought-rewetting' mesocosm experiment was established in a controlled temperature glasshouse at Lancaster University, UK. Mesocosms were plant pots (length=11cm, width=11cm, height=12cm) set out in a split-plot design with 4 blocks. The experiment consisted of 4 monocultures and one mixed species community, in a fully factorial design with and without N addition, with and without shade conditioning and two levels of soil moisture. The 5 plant species combinations, 8 treatment combinations and 4 replicates resulted in a total of 160 mesocosms. Seeds were sown in January 2014 and after 2 weeks growth, transferred to mesocosms containing loamy-sand soil (pH=5.6; %C=7.9; %N=0.28; Finch Aggregates, Blackburn, UK; Fig 3.1).

Four species were grown in monoculture (*Plantago lanceolata*, *Festuca rubra*, *Dactylis glomerata*, *Holcus lanatus*) which are found co-existing in grasslands (Rodwell 1993), but differ in plant functional group (grasses or forbs) and have different growth strategies (resources acquisitive vs. resource conservative) with varying plant traits. The forb *P. lanceolata* and the grass *F. rubra* may be considered more resource conservative while the grasses *D. glomerata* and *H. lanatus* may be considered more resource acquisitive (Baxendale *et al.*, 2014). An additional mixture community was included which had one individual from each of the four species grown in monoculture. To match planting density across all communities, four individuals were grown in each mesocosm.

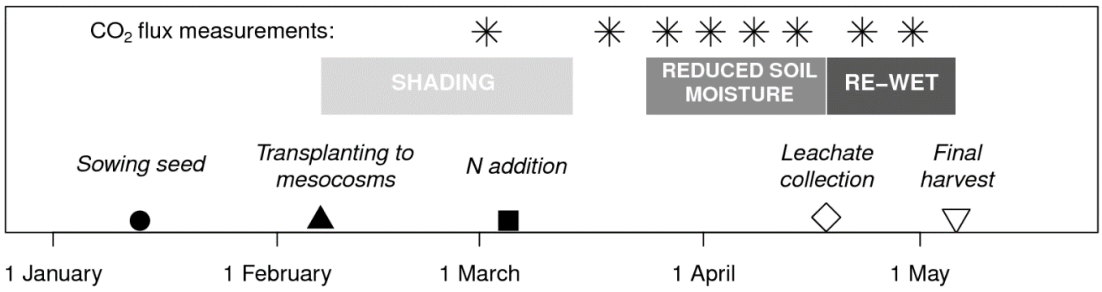

Figure 3.1 Experimental timetable showing mesocosms setup, experimental treatments and measurements. Sowing seed (closed circle), transplanting plants to mesocosms (closed triangle), N addition (closed square), leachate collection (open diamond), final harvest (open triangle) and CO_2 flux measurements (star). Each mesocosm for a given species received the same treatments and measurements on the same day, however not all measurements and treatments could be carried out on all species on the same day. The dates shown in the figure may therefore differ slightly depending on species.

3.3.2 Nitrogen addition, shade and soil moisture treatments

To test the hypothesis that biomass would determine plant responses to reduced soil moisture, we imposed two treatments that independently alter plant biomass. The first was N addition applied as ammonium nitrate (25kg N ha^{-1}) which was added in solution to half the mesocosms (applied week beginning 5 February 2014). The second treatment was shading of half of the mesocosms, for 6 weeks (2 February – 14 March 2014), to bring about different biomass levels independently of N addition (Fig 3.1). Neutral density plastic mesh was used above and to the sides of sub-blocks receiving shade treatment, which was confirmed by a measured reduction in PAR of 55%.

To simulate reduced rainfall, mesocosms were watered to maintain a set water holding capacity (WHC). This was calculated as a percentage of a fully saturated mesocosm assessed gravimetrically on a subset of mesocosms not otherwise used in the experiment. The control treatment had soil moisture maintained at 60% WHC and the reduced soil moisture treatment (RSM) was maintained at 30% WHC (started 24 March 2014). At the end of the reduced soil moisture treatment all mesocosms were rewetted to 80% WHC with the leachate being collected (rewetting in week beginning 21 April 2014, Fig 3.1). By maintaining mesocosms at a set WHC we reduced the potential effect of larger plants using more water and therefore inducing a greater water stress over time.

3.3.3 CO_2 fluxes and C and N leaching

Net ecosystem exchange (NEE) and ecosystem respiration were measured using an infra-red gas analyser (EGM 4, PP Systems, Hitchin, UK). Each mesocosm was enclosed in a static chamber (30cm diameter, 35cm height) which was either transparent (for NEE) or opaque (for ecosystem respiration). Measurements were made between 09:00 and 14:30 with all mesocosms of the same community measured consecutively. CO_2 fluxes were measured twice before

reduced soil moisture was imposed, four times during reduced soil moisture and twice after rewetting (Fig 3.1).

Soil was rewet to 80% WHC, and all resulting leachate was collected and analysed. Dissolved organic carbon (DOC) content was measured on a TOC analyser (Shimadzu 5000A, Milton Keynes, UK). DON was calculated as the difference between total N and DIN, where DIN was measured by running the leachate on an autoanalyser (Bran and Luebbe, Northampton, UK) for ammonium and nitrate. Total N was measured by oxidising the leachate with potassium persulphate ($K_2S_2O_8$) before running on an autoanalyser in the same way.

3.3.4 Plant traits and soil properties at final harvest

The final harvest destructively sampled all aboveground and belowground biomass (week beginning 5 May 2014, Fig 3.1). DOC and DON remaining in the soil was extracted from 5g soil using 35mL deionised water. Extracts were then run in the same way as leachate DOC and DON. Inorganic N was extracted using 25mL KCl with 5g soil and analysed using autoanalyser procedures (Bran and Luebbe, Northampton, UK). Leaf area was measured on the youngest leaf fully unfurled and without damage for each individual. These same leaves were weighed while fresh, dried at 50°C and reweighed. Specific Leaf Area (SLA) was calculated as leaf area divided by dry weight. Leaf dry matter content (LDMC) was calculated as fresh weight divided by dry weight. Total C and N content of ground aboveground plant leaves was analysed on a CN analyser (Tru-spec, Leco, St. Joesph, MI, USA).

Roots were removed when sieving soil samples (2mm sieve) then washed and stored in 40% ethanol and analysed for diameter and length using EPSON flatbed scanner and WinRhizo software (Regent Instruments Inc., Sainte-Foy-Sillery-Cap-Rouge, QC, Canada). Roots were weighed fresh, dried at 50°C and reweighed. Specific root length (SRL) was calculated as root length divided by dry weight. Root dry matter content (RDMC) was calculated as fresh weight

divided by dry weight. Root:shoot allocation was the ratio of belowground biomass to aboveground biomass.

3.3.5 Statistical approach

First, the proportion of variation in plant biomass, root:shoot allocation and leaf and root traits which could be accounted for by differences between species, within species and residual variation between replicates was investigated. To compare the different sources of variability, nested linear models were used to separate the total variance into different components, following Messier, McGill & Lechowicz (2010) Jung *et al.* (2014). For each plant measurement the different components were: between species (interspecific trait differences), between treatments but within species (i.e. intraspecific variability explained by treatments of N addition, shade conditioning and drought), and between replicates (i.e. unexplained residual variation). Principal component analysis of all plant traits across the four monocultures revealed the primary two axes of variation.

Second, the effect of experimental treatments on plant properties, CO_2 fluxes and soil C and N leaching and retention were examined. Linear mixed effects (LME) models were constructed for which the explanatory variables were: species, N addition, shade and reduced soil moisture (and all interactions). The response variables were: NEE and ecosystem respiration during reduced soil moisture (averaged across four sampling dates) and after rewetting (average across two sampling dates), DOC, DON and DIN leaching, DOC, DON and inorganic N retained in soil at the final harvest, plant biomass, root:shoot allocation and other plant traits (Fig 3.2, Table 3.1). The random intercept term was split-plot nested within block to take account of the split-plot design and weight functions were used to take account of unequal variances (Zuur *et al.* 2009). Response variables were logged where it improved normality of residuals. Significance of interactions and main effects were assessed using likelihood ratio tests (LRT) comparing models with and without the variable of interest. For DIN leaching only data from *F. rubra* and *H. lanatus* could be analysed due to multiple zero values in DIN leaching for other species.

The third element of statistical analysis was to investigate whether changes in CO_2 fluxes and C and N leaching in response to reduced soil moisture was determined by variation in plant traits. Paired mesocosms, which only differed in soil moisture treatment, were used to calculate the difference between control and reduced soil moisture mesocosms for all measurements of NEE, ecosystem respiration, and C and N leaching and retention in soil. NEE and ecosystem respiration were averaged across sampling dates during reduced soil moisture treatment (four sampling dates) and after rewetting (two sampling dates). Inorganic N, because of very large differences in variation between species, did not meet model assumptions. Model selection was based on the procedures proposed by Díaz *et al.* (2007) and follows those used by De Vries *et al.* (2012) and Everwand *et al.* (2014). In this method parameters are added in a set hierarchical order such that the measurements added first are hypothesised to have the greatest control on ecosystem function. Parameter combinations found to be important at one stage are retained to be included in the model to which the next set of parameters are added. Firstly, biomass, root:shoot and plant species identity were added, followed by all two way interactions and retained based on AIC. This process was then repeated for plant traits and then all two-way interactions. As plant biomass and plant traits were sometimes highly correlated, we used generalised variance inflation factors (VIF) to select parameters which had low levels of collinearity (VIF<3). The r-squared of the final model was reported for fixed effects only termed, 'marginal r-squared' (Nakagawa & Schielzeth, 2013) and comparison of the different species was assessed using Tukey post hoc comparisons. All statistical analysis was carried out in the R programming language 3.3.1 (R Core Development Team, 2016) using the additional packages *nlme* (Pinheiro & Bates, 2013), *plyr* (Wickham, 2011) and *vegan* (Oksanen *et al.*, 2013).

3.4 Results

3.4.1 Plant responses to experimental treatments

The experimental treatments of N addition, shade conditioning and reduced soil moisture brought about intraspecific variation in biomass, root:shoot allocation and plant traits, which was in addition to differences between species (Figs. 3.2 and 3.4). For some plant traits (plant biomass and leaf C) intraspecific variation was far greater than interspecific differences, for other traits it was the opposite (SLA, SRL and RDMC), while others showed similar levels of intra- and interspecific variation (root:shoot, LDMC). The two dominant axes of PCA for plant traits explained 68.0% of total variation in plant traits. Axis one explained 43.1% of variance, with high loadings for plant traits related to biomass which showed high intraspecific variation, while axis two explained 24.9% of variance with high loadings for plant height, RDMC and root diameter which showed more interspecific variation (Fig 3.3).

Total plant biomass was increased approximately 3-fold by N addition, although the increase varied across species (Species x N addition: LRT=20.98, d.f.=4, P=0.0003), and was marginally larger in combination with shade conditioning (N addition x Shade: LRT=14.42, d.f.=1, P=0.0001; Table 3.1). Reduced soil moisture independently reduced total biomass (LRT=12.28, d.f.=1, P=0.0005; Table 3.1) while total biomass correlated very highly with both aboveground biomass (Pearson correlation: r=0.93) and belowground biomass (r=0.94, Fig 3.3). Total biomass in the mixed species community was greater than the monocultures, although this was primarily the case for mesocosms with N addition. For other plant traits such as root:shoot, SLA, LDMC and leaf N the mixed species community results were intermediate between those seen in the monocultures (Fig 3.4).

Root:shoot allocation was generally reduced by shade conditioning, although the magnitude of the reduction differed between species (Species x shade: LRT=14.60, d.f.=4, P=0.0056, Fig 3.3, Table 3.1) and with N addition (N addition x shade: LRT=6.22, d.f.=1, P=0.0126). However N

addition also decreased root:shoot allocation in the majority of species, and when in combination with reduced soil moisture caused root:shoot allocation to be reduced further (Species x N addition x RSM: LRT:13.03, d.f.=4, P=0.0111).

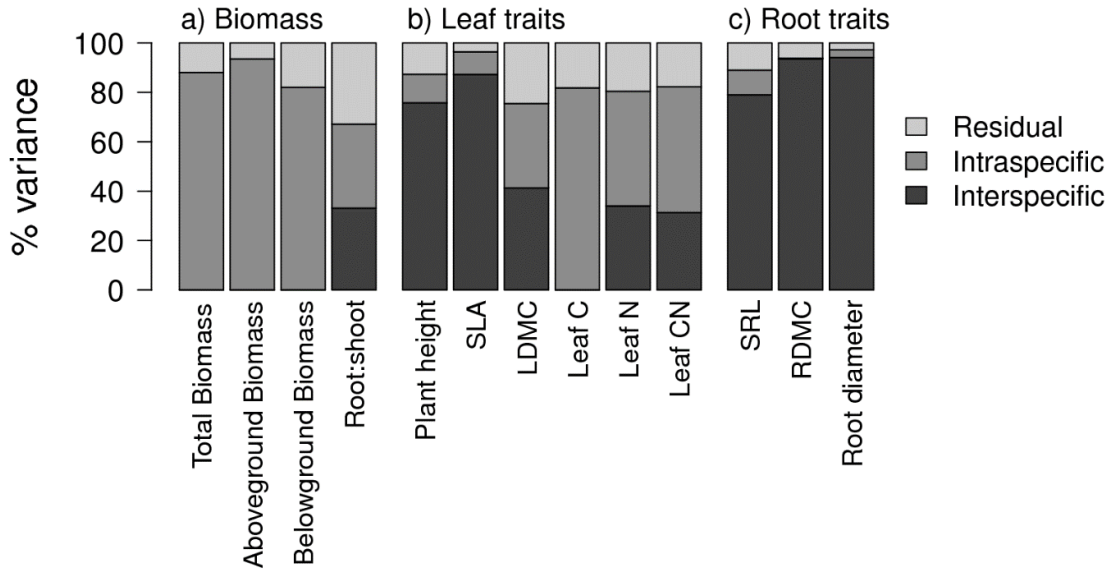

Figure 3.2 Source of variance for plant biomass, root:shoot allocation and leaf and root traits. Variance can be interspecific (variation between four species grown in monoculture), interspecific (variation brought about by experimental treatments) or residual variation at the replicate level. Calculated using variance component analysis (Messier *et al.*, 2010; Jung *et al.*, 2014). Plant traits grouped in a) biomass related plant traits, b) leaf traits, c) root traits.

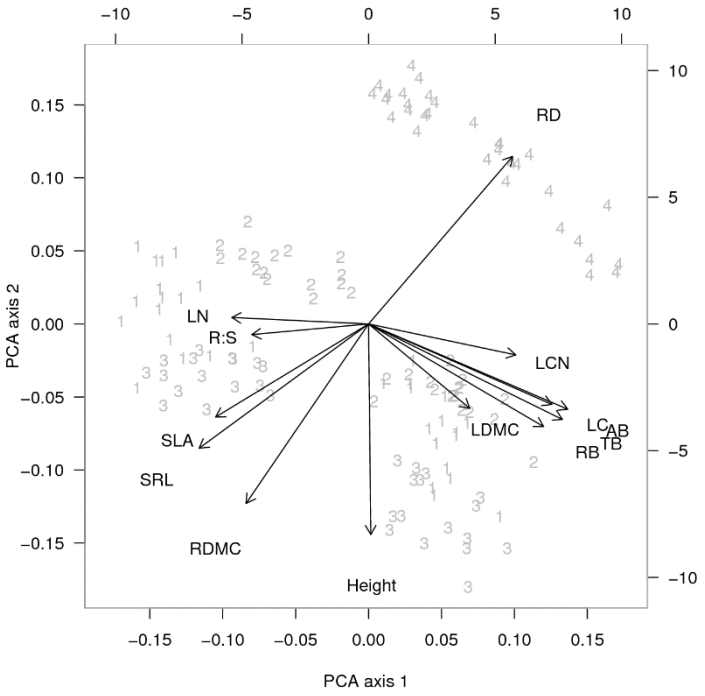

Figure 3.3 Principal components analysis (PCA) of plant traits across all four monocultures in response to N addition, shade conditioning and reduced soil moisture. Number in grey indicate species, where: 1 = *D. glomerata*, 2 = *F. rubra*, 3 = *H. lanatus*, 4 = *P. lanceolata*. Plant traits represented by initials as: TB=total biomass, AB=aboveground biomass, RB=belowground biomass, R:S=root-to-shoot biomass ratio, SLA=specific leaf area, LDMC=leaf dry matter content, Height=maximum plant height, RD=root diameter, SRL=specific root length, RDMC=root dry matter content, LC=leaf C content, LN=leaf N content, LCN=leaf C:N.

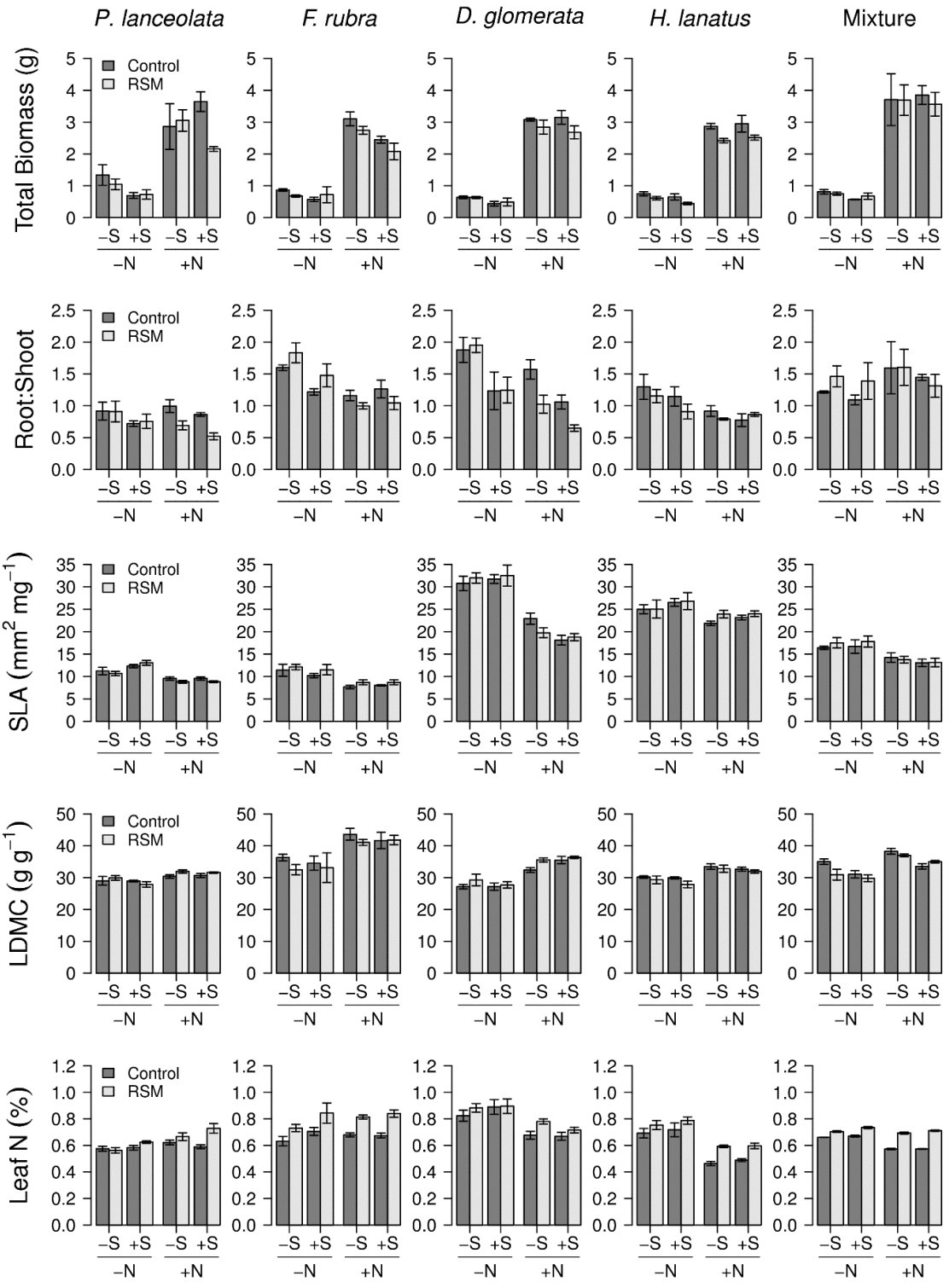

Figure 3.4 Interactive effect of reduced soil moisture (RSM), shade conditioning (+S), N addition (+N) and species identity on total plant biomass, root:shoot allocation, SLA, LDMC and Leaf N. Means and standard errors are presented (*n*=4).

Table 3.1 Effects of species identity, N addition, shade conditioning and reduced soil moisture and all interactions on all measures of plant morphology and chemical composition. a) total biomass, b) aboveground biomass, c) belowground biomass, d) root:shoot, e) max plant height, f) SLA, g) LDMC, h) leaf C, i) Leaf N), j) leaf CN, k) SLR, l) RDMC) and m) root diameter. Significance tested using likelihood ratio tests (LRT) comparing models with and without parameter of interest where degrees of freedom (d.f.) shows the difference in degrees of freedom between the models. Significant effects (P<0.05) are shown in bold type.

		a) Total biomass		**b) Aboveground biomass**		**c) Belowground biomass**	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
Species (Sp)	**4**	**26.30**	**<0.0001**	**38.08**	**<0.0001**	**33.30**	**<0.0001**
N addition (N)	**1**	**242.07**	**<0.0001**	**283.51**	**<0.0001**	**209.99**	**<0.0001**
Shade (Sh)	**1**	**6.78**	**0.0092**	2.28	0.1311	**10.35**	**0.0013**
Reduced soil moisture (RSM)	**1**	**12.28**	**0.0005**	**8.57**	**0.0034**	**20.30**	**<0.0001**
Sp x N	**4**	**20.98**	**0.0003**	**29.61**	**<0.0001**	**16.09**	**0.0029**
Sp x Sh	4	5.81	0.2136	**19.43**	**0.0006**	7.46	0.1136
Sp x RSM	4	5.35	0.2536	**10.13**	**0.0384**	2.90	0.5744
N x Sh	**1**	**14.42**	**0.0001**	**11.88**	**0.0006**	**15.53**	**0.0001**
N x RSM	1	0.12	0.7338	2.00	0.1569	1.03	0.3101
Sh x RSM	1	0.43	0.5115	0.08	0.7711	0.00	0.9969
Sp x N x Sh	4	3.86	0.4246	4.67	0.3225	0.71	0.9496
Sp x N x RSM	4	2.90	0.574	5.43	0.2458	6.97	0.1373
Sp x Sh x RSM	4	2.85	0.5827	8.86	0.0646	2.82	0.5887
N x Sh x RSM	1	1.24	0.2646	1.37	0.2433	0.17	0.6783
Sp x N x Sh x RSM	4	2.60	0.2315	3.21	0.5232	4.57	0.3343
		d) Root:Shoot		**e) Max plant height**		f) **SLA**	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
Species (Sp)	**4**	**85.37**	**<0.0001**	**178.64**	**<0.0001**	**265.53**	**<0.0001**
N addition (N)	**1**	**25.20**	**<0.0001**	**100.10**	**<0.0001**	**77.91**	**<0.0001**
Shade (Sh)	**1**	**9.76**	**0.0018**	**6.12**	**0.0134**	0.37	0.5456
Reduced soil moisture (RSM)	**1**	**9.56**	**0.002**	2.53	0.1114	0.03	0.8623
Sp x N	**4**	**23.27**	**0.0001**	**34.74**	**<0.0001**	**42.88**	**<0.0001**
Sp x Sh	4	**14.60**	**0.0056**	7.48	0.1125	**10.82**	**0.0286**
Sp x RSM	4	8.91	0.0633	**13.60**	**0.0087**	**12.26**	**0.0155**
N x Sh	**1**	**6.22**	**0.0126**	0.05	0.8271	**5.29**	**0.0214**
N x RSM	1	**11.84**	**0.0006**	0.02	0.9012	1.73	0.188
Sh x RSM	1	0.12	0.7341	**4.99**	**0.0255**	0.51	0.4769
Sp x N x Sh	4	6.42	0.1701	6.59	0.1594	8.58	0.0724
Sp x N x RSM	4	**13.03**	**0.0111**	2.17	0.7053	3.31	0.5076
Sp x Sh x RSM	4	1.15	0.8864	**11.08**	**0.0257**	3.69	0.4492
N x Sh x RSM	1	0.14	0.7119	1.37	0.2425	0.78	0.3777
Sp x N x Sh x RSM	4	1.76	0.7801	1.67	0.7962	2.44	0.6547

continued

		g) LDMC		h) **Leaf C**		i) **Leaf N**	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
Species (Sp)	**4**	**80.88**	**<0.0001**	7.32	0.1199	**88.79**	**<0.0001**
N addition (N)	**1**	**82.84**	**<0.0001**	**254.17**	**<0.0001**	**27.31**	**<0.0001**
Shade (Sh)	**1**	**4.52**	**0.0335**	2.06	0.1515	**6.53**	**0.0106**
Reduced soil moisture (RSM)	**1**	0.36	0.5474	**76.33**	**<0.0001**	**118.58**	**<0.0001**
Sp x N	**4**	**39.36**	**<0.0001**	**11.71**	**0.0196**	**89.14**	**<0.0001**
Sp x Sh	4	**17.37**	**0.0016**	2.07	0.7222	3.49	0.4793
Sp x RSM	4	**17.48**	**0.0016**	5.34	0.2544	**13.47**	**0.0092**
N x Sh	**1**	1.83	0.1765	**48.00**	**<0.0001**	**5.44**	**0.0197**
N x RSM	1	**6.13**	**0.0133**	**4.20**	**0.0404**	**43.87**	**<0.0001**
Sh x RSM	1	0.43	0.5134	0.20	0.6521	**7.04**	**0.008**
Sp x N x Sh	4	4.56	0.3356	**10.42**	**0.0339**	5.04	0.2829
Sp x N x RSM	4	1.72	0.7872	9.48	0.0501	1.74	0.7841
Sp x Sh x RSM	4	**10.97**	**0.0269**	5.55	0.2352	**9.60**	**0.0477**
N x Sh x RSM	1	0.30	0.5814	**14.05**	**0.0002**	0.00	0.9466
Sp x N x Sh x RSM	4	0.68	0.954	6.56	0.161	0.56	0.9678
		j) **Leaf CN**		**k) SRL**		**l) RDMC**	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
Species (Sp)	**4**	**92.15**	**<0.0001**	**167.08**	**<0.0001**	**287.98**	**<0.0001**
N addition (N)	**1**	**36.17**	**<0.0001**	**38.52**	**<0.0001**	**8.37**	**0.0038**
Shade (Sh)	**1**	**6.96**	**0.0084**	0.20	0.6553	0.02	0.8815
Reduced soil moisture (RSM)	**1**	**84.88**	**<0.0001**	0.04	0.837	**11.18**	**0.0008**
Sp x N	**4**	**98.28**	**<0.0001**	**46.82**	**<0.0001**	4.70	0.32
Sp x Sh	4	4.98	0.2895	2.79	0.5943	4.04	0.4002
Sp x RSM	4	**22.92**	**0.0001**	2.66	0.6154	2.13	0.7119
N x Sh	**1**	0.53	0.4656	**19.44**	**<0.0001**	0.34	0.5619
N x RSM	1	**64.21**	**<0.0001**	1.29	0.2569	**18.62**	**<0.0001**
Sh x RSM	1	0.48	0.4876	1.52	0.2183	0.12	0.7263
Sp x N x Sh	4	5.59	0.2326	**10.93**	**0.0273**	3.53	0.4731
Sp x N x RSM	4	8.59	0.0722	3.90	0.4193	5.10	0.2775
Sp x Sh x RSM	4	9.18	0.0569	2.14	0.7098	2.29	0.6824
N x Sh x RSM	1	0.48	0.4887	0.04	0.8425	0.44	0.5053
Sp x N x Sh x RSM	4	2.11	0.7147	1.21	0.876	9.27	0.0547
		m) Root diameter					
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
Species (Sp)	**4**	**275.67**	**<0.0001**				
N addition (N)	**1**	**68.49**	**<0.0001**				
Shade (Sh)	**1**	0.31	0.5749				
Reduced soil moisture (RSM)	**1**	0.44	0.5089				
Sp x N	**4**	**29.53**	**<0.0001**				
Sp x Sh	4	3.74	0.442				
Sp x RSM	4	2.18	0.7031				
N x Sh	**1**	2.85	0.0913				
N x RSM	1	0.38	0.5383				
Sh x RSM	1	**4.60**	**0.032**				
Sp x N x Sh	4	6.46	0.1671				
Sp x N x RSM	4	4.71	0.3188				
Sp x Sh x RSM	4	2.21	0.6971				
N x Sh x RSM	1	0.14	0.7105				
Sp x N x Sh x RSM	4	3.37	0.4987				

The response of all plant traits depended on at least one interaction between experimental treatments suggesting complex controls on intraspecific variation (Table 3.1). For example, SLA differed between species, while the main intraspecific variation was brought about by N addition which reduced SLA, although depending on species (Species x N addition: LRT=42.88, d.f.=4, P<0.0001, Fig 3.4). Small changes in SLA also depended on the combination of N addition and shade, species identity and shade and reduced soil moisture (Table 3.1). Variation in LDMC was equally due to both differences between species and variation within species (Fig 3.2). N addition primarily increased LDMC, although the size of the increase varied between species (Species x N addition: LRT=39.36, d.f.=4, P<0.0001, Fig 3.4) and was larger when in combination with reduced soil moisture treatment (N addition x shade: LRT=6.13, d.f.=1, P=0.0133). Leaf N content was more variable within species than between species (Fig 3.4), with reductions in leaf N content with N addition and increases with reduced soil moisture which were greater when in combination with N addition (N addition x RSM: LRT=43.87, d.f.=1, P<0.0001).

3.4.2 CO_2 fluxes

The NEE sink strength was larger with N addition and generally smaller with reduced soil moisture, although the effect of reduced soil moisture was small or absent without N addition and varied between species (Species x N addition x RSM: LRT=17.17, d.f.=4, P=0.0018, Fig 3.5; Table 3.2). Shade conditioning generally brought about small reductions in NEE sink strength although the size of the reduction was dependent on species and soil moisture (Species x Shade x RSM: LRT=10.74, d.f.=4, P=0.0297). The difference in NEE sink strength between control and reduced soil moisture treatment was explained by total plant biomass and SLA (Table 3.3, Fig 3.6a,b), such that higher plant biomass was associated with larger declines in NEE sink strength, however the effect of biomass was greater with low SLA (Total biomass x SLA: LRT=6.35, d.f.=1, P=0.0117).

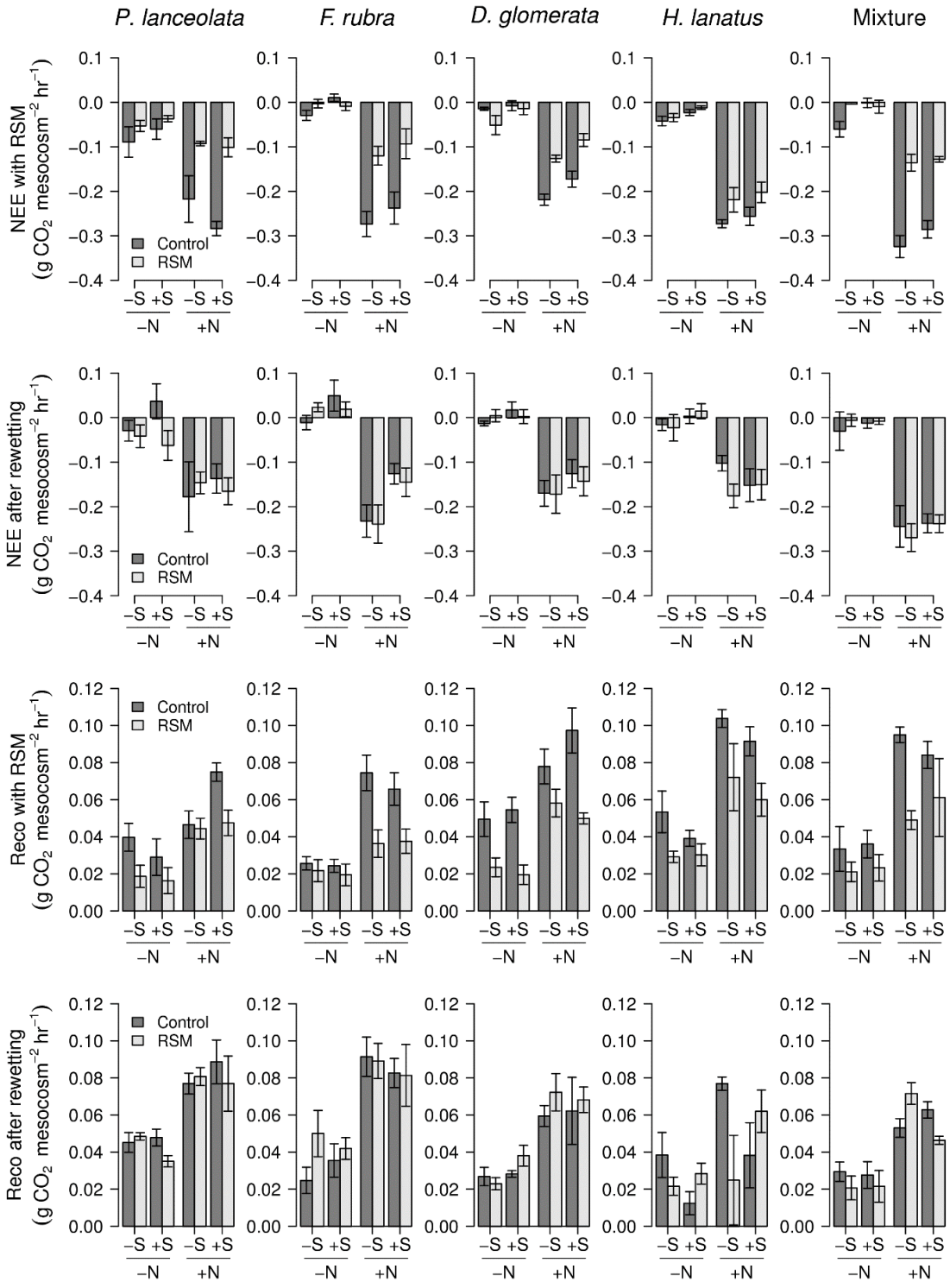

Figure 3.5 Interactive effect of reduced soil moisture (RSM), shade conditioning (+S), N addition (+N) and species identity on NEE and ecosystem respiration (Reco) while soil moisture was manipulated and after rewetting when soil moisture was constant across treatments. Means and standard errors are presented (*n*=4).

Table 3.2 Effects of species identity, N addition, shade conditioning and reduced soil moisture (RSM) and all interactions on CO_2 fluxes during and after the reduced soil moisture treatment: a) NEE with RSM, b) NEE after rewetting, c) Ecosystem respiration (Reco) with RSM, d) Reco after rewetting. Significance tested using likelihood ratio tests (LRT) comparing models with and without parameter of interest where degrees of freedom (d.f.) shows the difference in degrees of freedom between the models. Significant effects ($P<0.05$) are shown in bold type.

		a) NEE with RSM		**b) NEE after rewetting**		**c) Reco with RSM**		**d) Reco after rewetting**	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*
Species (Sp)	**4**	**23.64**	**0.0001**	**16.18**	**0.0028**	**34.01**	**<0.0001**	**46.68**	**<0.0001**
N addition (N)	**1**	**144.88**	**<0.0001**	**147.98**	**<0.0001**	**111.53**	**<0.0001**	**104.83**	**<0.0001**
Shade (Sh)	**1**	**11.57**	**0.0007**	**8.40**	**0.0037**	0.37	0.5419	0.16	0.6884
Reduced soil moisture (RSM)	**1**	**11.01**	**0.0009**	0.31	0.577	**63.13**	**<0.0001**	0.01	0.9096
Sp x N	**4**	**33.39**	**<0.0001**	**22.78**	**0.0001**	7.38	0.1173	4.50	0.3423
Sp x Sh	4	1.49	0.8278	2.62	0.6237	2.99	0.5599	3.73	0.4433
Sp x RSM	**4**	**17.72**	**0.0014**	3.76	0.4389	7.07	0.1325	7.47	0.1129
N x Sh	1	0.00	0.9471	0.59	0.4421	0.69	0.4072	0.07	0.7865
N x RSM	**1**	**67.13**	**<0.0001**	1.05	0.3045	**6.81**	**0.0091**	0.01	0.9401
Sh x RSM	1	0.90	0.342	2.15	0.1424	0.01	0.9121	0.62	0.4315
Sp x N x Sh	4	4.43	0.3514	5.35	0.2531	4.67	0.3226	3.24	0.5182
Sp x N x RSM	**4**	**17.17**	**0.0018**	2.52	0.6419	6.02	0.1976	3.53	0.4736
Sp x Sh x RSM	**4**	**10.74**	**0.0297**	6.68	0.1539	4.72	0.3174	**16.35**	**0.0026**
N x Sh x RSM	1	0.31	0.5763	1.67	0.1958	0.75	0.3873	0.87	0.3518
Sp x N x Sh x RSM	4	2.84	0.5847	0.25	0.9927	4.97	0.2905	7.05	0.1333

Table 3.3 The effect of plant species, plant biomass, root:shoot allocation and plant traits on the change in CO_2 fluxes and C and N leaching brought about by reduced soil moisture. Specific responses modelled were: a) NEE before rewetting, b) NEE after rewetting, c) ecosystem respiration (Reco) before rewetting, d) ecosystem respiration after rewetting, e) leached DOC, f) leached DON, g) leached DIN, h) DOC at final harvest, i) DON at final harvest and j) inorganic N at final harvest. Parameters were included in the final model on the basis of AIC. Significance tested using likelihood ratio tests (LRT) comparing models with and without parameter of interest where degrees of freedom (d.f.) shows the difference in degrees of freedom between the models. Significant effects ($P<0.05$) are shown in bold type. Marginal r-squared estimated on the full model and for fixed effects only (Nakagawa & Schielzeth, 2013).

	a) NEE with reduced soil moisture			b) NEE After rewetting			c) Reco with reduced soil moisture			d) Reco After rewetting		
	LRT	d.f.	*P*	LRT	d.f.	*P*	LRT	d.f.	*P*	LRT	d.f.	*P*
Total biomass (TB)	**45.79**	**1**	**<0.0001**	Intercept only model			**11.10**	**1**	**0.0009**	Intercept only model		
Root:shoot (RS)	2.98	1	0.0844									
SLA	**7.04**	**1**	**0.008**				**5.72**	**1**	**0.0168**			
TB x SLA	**6.35**	**1**	**0.0117**									
Marginal r-squared	R^2_M = 0.60			R^2_M = NA			R^2_M = 0.13			R^2_M = NA		

	e) Leached DOC			f) Leached DON			g) Leached DIN		
	LRT	d.f.	*P*	LRT	d.f.	*P*	LRT	d.f.	*P*
Species	**30.78**	**4**	**<0.0001**	**45.05**	**4**	**<0.0001**	**44.34**	**3**	**<0.0001**
Total biomass	**4.28**	**1**	**0.0385**						
Root:shoot				**4.44**	**1**	**0.0352**	2.98	1	0.0845
Leaf N	**5.34**	**1**	**0.0208**						
Marginal r-squared	R^2_M = 0.40			R^2_M = 0.46			R^2_M = 0.52		

	h) DOC (final harvest)			i) DON (final harvest)			j) Inorganic N (final harvest)		
	LRT	d.f.	*P*	LRT	d.f.	*P*	LRT	d.f.	*P*
Total biomass				**10.73**	**1**	**0.0011**	Data did not meet model assumptions		
LDMC	**10.47**	**1**	**0.0012**						
Marginal r-squared	R^2_M = 0.13			R^2_M = 0.13					

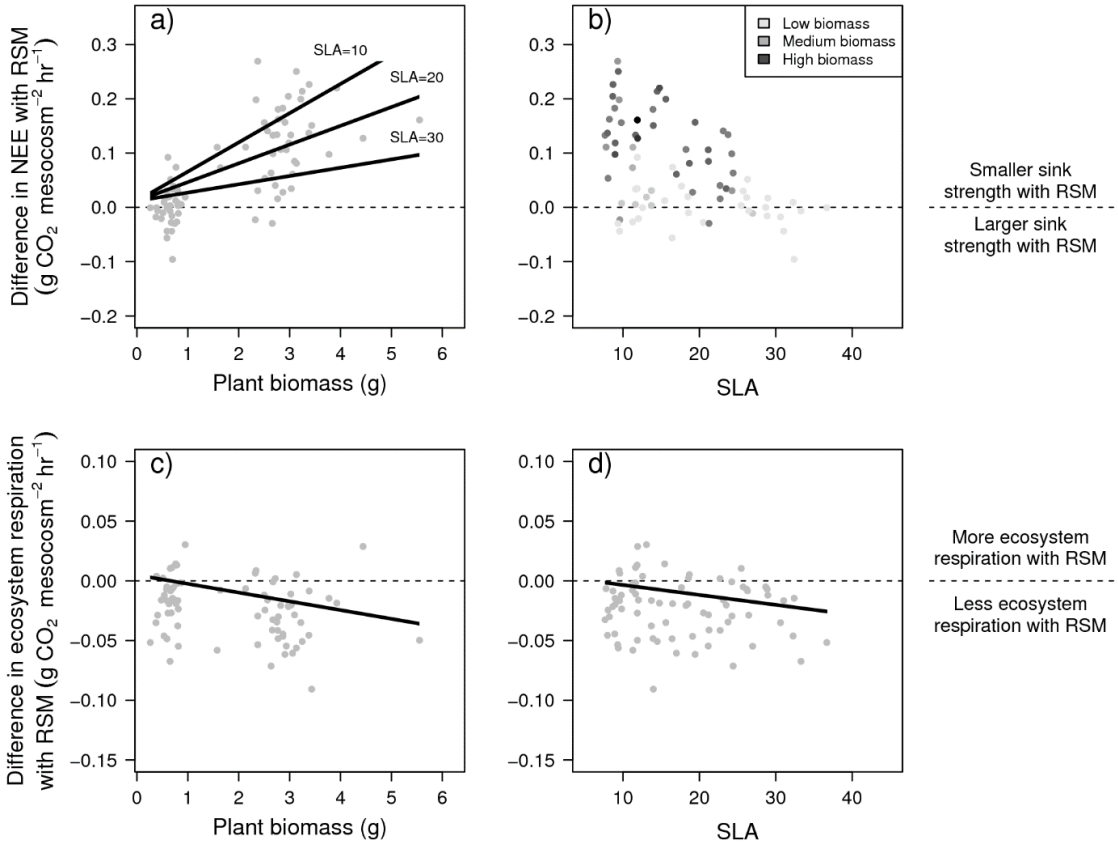

Figure 3.6 The difference in NEE and ecosystem respiration under reduced soil moisture (RSM) and the relationship with plant biomass and SLA; a) the difference in NEE with RSM and plant biomass with modelled relationship for SLA when it is 10, 20 and 30mm^2 mg^{-1}, b) the difference in NEE with RSM and SLA where colour of data points shows plant biomass on a continuous scale from black (high biomass) to light grey (low biomass), c) the difference in ecosystem respiration with RSM and plant biomass with model output shown in solid black line, d) the difference in ecosystem respiration with RSM and SLA with model output shown in solid black line.

After rewetting, the NEE sink was no longer reduced by the reduced soil moisture treatment, while the NEE sink was still greater with N addition, although varying with plant species (Species x N addition: LRT=22.78, d.f.=4, P=0.0001), and slightly reduced by shade (LRT=8.40, d.f.=1, P=0.0037, Fig 3.4). The difference in NEE between control and reduced soil moisture treatments following rewetting, was best explained by an intercept only model, without either biomass or SLA which had best explained the difference before rewetting (Table 3.3).

Ecosystem respiration was increased by N addition and generally decreased by reduced soil moisture, although the effect of reduced soil moisture was greater when in combination with N addition (N addition x RSM: LRT=6.81, d.f.=1, P=0.0091), with additional small differences between species (Species: LRT=34.01, d.f.=4, P<0.0001, Fig 3.5). The difference in ecosystem respiration between control and reduced soil moisture treatment was greater as plant biomass and SLA both increased, although models only had a marginal r-squared of 0.13 suggesting limited explanatory power (Table 3.3, Fig 3.6c,d).

After rewetting, ecosystem respiration was still increased by N addition as it had been before rewetting (LRT=104.83, d.f.=1, P<0.0001). Ecosystem respiration also showed complex responses to previous reduced soil moisture, shade conditioning and plant species (Species x Shade x RSM interaction: LRT=16.35, d.f.=4, P=0.0026, Fig 3.5). Specifically, for *H. lanatus* shade conditioning in combination with reduced soil moisture increased ecosystem respiration, while in contrast, for *P. lanceolata* ecosystem respiration was reduced by previous reduced soil moisture but only with shade conditioning. The difference in ecosystem respiration after rewetting between control and reduced soil moisture treatment was best explained by an intercept only model, suggesting differences in recovery were not due to differences in plant biomass, root:shoot allocation or plant traits (Table 3.3).

The CO_2 fluxes from the mixed species treatment were generally similar to those of the monocultures, however reduced soil moisture in combination with N addition had a particularly negative effect on NEE for the mixture. This reduction in NEE was equal to the reduction found in *P. lanceolata* and *F. rubra* and greater than that found in *D. glomerata* and *H. lanatus* (Fig 3.5). After rewetting, NEE in the mixed species treatment with N addition was more negative than for any of the monocultures (Fig 3.5). For mixed species communities, ecosystem respiration during the reduced soil moisture treatment was broadly similar to that of the monocultures (Fig 3.5). However, after rewetting, ecosystem respiration from the mixed species community was generally lower than ecosystem respiration from the monocultures (Fig 3.5).

3.4.3 Leachate losses of C and N

DOC leaching after rewetting was approximately 20 times greater than DON leaching, which was itself greater than DIN leaching (Fig 3.7). DOC, DON and DIN leaching were generally greater in mesocosms subjected to the reduced soil moisture treatment. Increases in DOC leaching with reduced soil moisture were not as large when in N amended mesocosms, but varied between plant species (Species x N addition x RSM interaction: LRT=14.81, d.f.=4, P=0.0051). For DON the increase in leaching with reduced soil moisture varied between plant species (Species x N addition: LRT=37.40, d.f.=4, P<0.0001) and to a lesser extent with shade and N addition (Table 3.4, Fig 3.7). For DIN leaching, analysis of *F. rubra* and *H. lanatus* mesocosms (other species excluded due to multiple zero values) showed that the increase with reduced soil moisture differed between species (Species x RSM: LRT=5.07, d.f.=1, P=0.0243). Leachate of DOC and DON from the mixed species community was similar to leachate losses from the monocultures, while DIN leachate from the mixture was intermediate when compared with the monocultures (Fig 3.7). However, DON and inorganic N in soil at the final harvest were lower in the mixed species community than any of the monocultures (Fig 3.9).

The differences in DOC, DON and DIN leaching between control and reduced soil moisture mesocosms were associated with differences between species (Table 3.3, Fig 3.8), but

additionally decreases in DOC leaching were associated with increasing plant biomass and decreasing leaf N (Fig 3.8d), while increases in DON leaching were associated with increasing root:shoot allocation (Fig 3.8e).

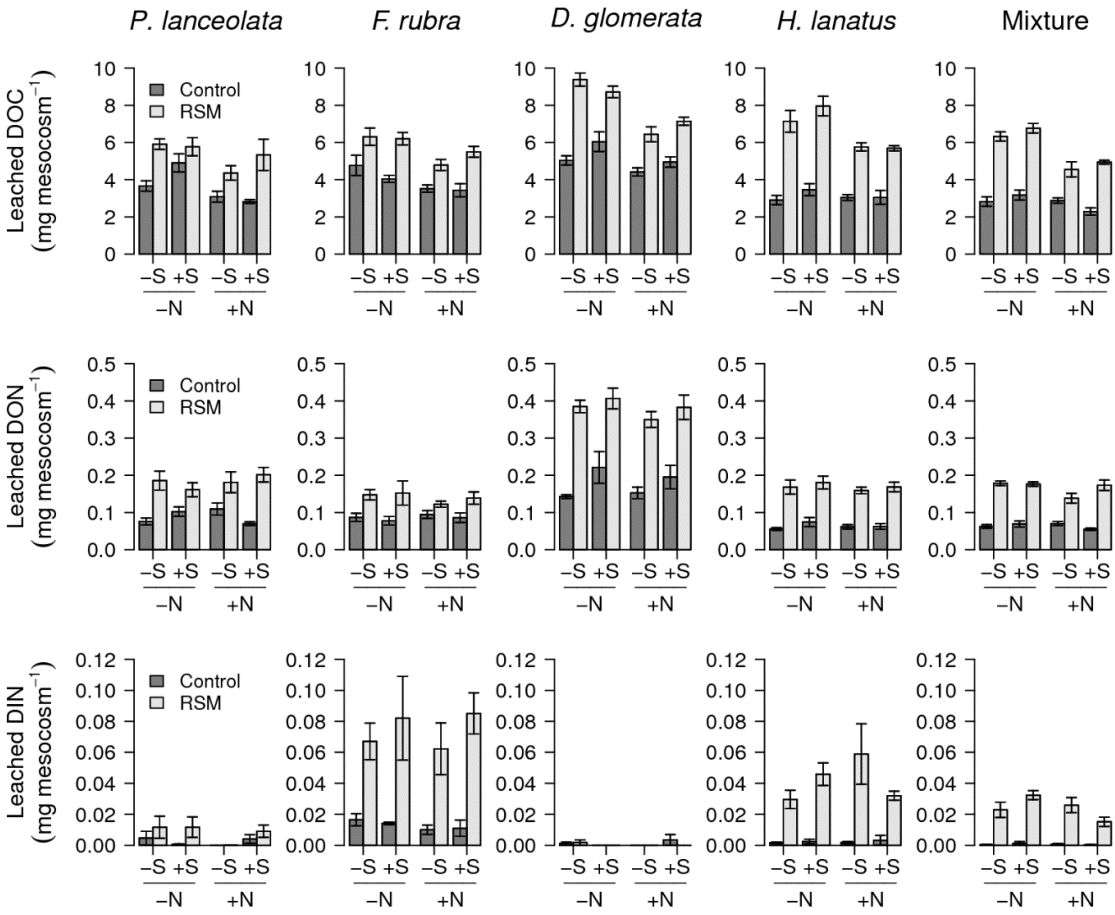

Figure 3.7 Interactive effect of reduced soil moisture (RSM), shade conditioning (+S), N addition (+N) and species identity on DOC, DON and DIN leaching upon rewetting. Means and standard errors are presented (*n*=4).

Table 3.4 Effects of species identity, N addition, shade conditioning and reduced soil moisture (RSM) and all interactions on C and N leaching upon rewetting and retention in the soil at the final harvest : a) leached DOC, b) leached DON, c) leached DIN, d) DOC retained in the soil at the final harvest, e) DON retained in the soil at the final harvest, f) Inorganic N retained in the soil at the final harvest. Significance tested using likelihood ratio tests (LRT) comparing models with and without parameter of interest where degrees of freedom (d.f.) shows the difference in degrees of freedom between the models. Significant effects (P<0.05) are shown in bold type.

	a) Leached DOC			b) **Leached DON**			c) **Leached DIN**		
	LRT	d.f.	*P*	LRT	d.f.	*P*	LRT	d.f.	*P*
Species (Sp)	**123.70**	**4**	**<0.0001**	**63.91**	**4**	**<0.0001**	**30.23**	**1**	**<0.0001**
N addition (N)	**60.21**	**1**	**<0.0001**	2.06	1	0.1508	0.38	1	0.5357
Shade (Sh)	**4.62**	**1**	**0.0316**	1.61	1	0.2047	0.20	1	0.651
Reduced soil moisture (RSM)	**144.72**	**1**	**<0.0001**	**150.54**	**1**	**<0.0001**	**45.31**	**1**	**<0.0001**
Sp x N	4.57	4	0.3339	2.13	4	0.7114	2.93	1	0.0871
Sp x Sh	5.22	4	0.2654	9.04	4	0.0601	0.14	1	0.7067
Sp x RSM	**26.78**	**4**	**<0.0001**	**37.40**	**4**	**<0.0001**	**5.07**	**1**	**0.0243**
N x Sh	0.99	1	0.3188	1.74	1	0.187	0.03	1	0.8579
N x RSM	**14.77**	**1**	**0.0001**	3.09	1	0.0785	0.52	1	0.4722
Sh x RSM	1.29	1	0.2563	2.20	1	0.1381	0.02	1	0.8784
Sp x N x Sh	8.20	4	0.0847	2.57	4	0.6314	0.46	1	0.496
Sp x N x RSM	**14.81**	**4**	**0.0051**	2.81	4	0.5897	0.04	1	0.8479
Sp x Sh x RSM	5.26	4	0.2612	2.96	4	0.5639	2.24	1	0.1345
N x Sh x RSM	**6.53**	**1**	**0.0106**	**9.69**	**1**	**0.0019**	3.24	1	0.0717
Sp x N x Sh x RSM	7.44	4	0.1144	4.08	4	0.3952	1.86	1	0.1727
	d) **DOC at final harvest**			e) **DON at final harvest**			f) **Innorganic N at final harvest**		
	LRT	d.f.	*P*	LRT	d.f.	*P*	LRT	d.f.	*P*
Species (Sp)	**107.44**	**1**	**<0.0001**	**57.70**	**4**	**<0.0001**	**234.34**	**4**	**<0.0001**
N addition (N)	**8.89**	**1**	**0.0029**	**100.18**	**1**	**<0.0001**	**105.15**	**1**	**<0.0001**
Shade (Sh)	0.08	1	0.7828	1.84	1	0.1746	0.94	1	0.3324
Reduced soil moisture (RSM)	**63.87**	**1**	**<0.0001**	**64.26**	**1**	**<0.0001**	**62.65**	**1**	**<0.0001**
Sp x N	8.51	4	0.0746	**9.61**	**4**	**0.0475**	**15.66**	**4**	**0.0035**
Sp x Sh	3.52	4	0.4747	7.64	4	0.1058	9.34	4	0.0531
Sp x RSM	9.19	4	0.0566	6.37	4	0.1731	**11.08**	**4**	**0.0257**
N x Sh	2.92	1	0.0875	1.57	1	0.2097	0.23	1	0.6314
N x RSM	**7.66**	**1**	**0.0056**	0.00	1	0.9455	0.06	1	0.8104
Sh x RSM	**3.94**	**1**	**0.0471**	1.04	1	0.3083	1.11	1	0.2921
Sp x N x Sh	6.75	4	0.1496	2.38	4	0.6669	1.58	4	0.812
Sp x N x RSM	**10.76**	**4**	**0.0294**	3.62	4	0.4602	4.73	4	0.3159
Sp x Sh x RSM	2.79	4	0.5937	2.88	4	0.5788	2.29	4	0.6826
N x Sh x RSM	0.42	1	0.5177	0.28	1	0.5992	0.43	1	0.5112
Sp x N x Sh x RSM	6.07	4	0.1941	4.83	4	0.3053	1.58	4	0.812

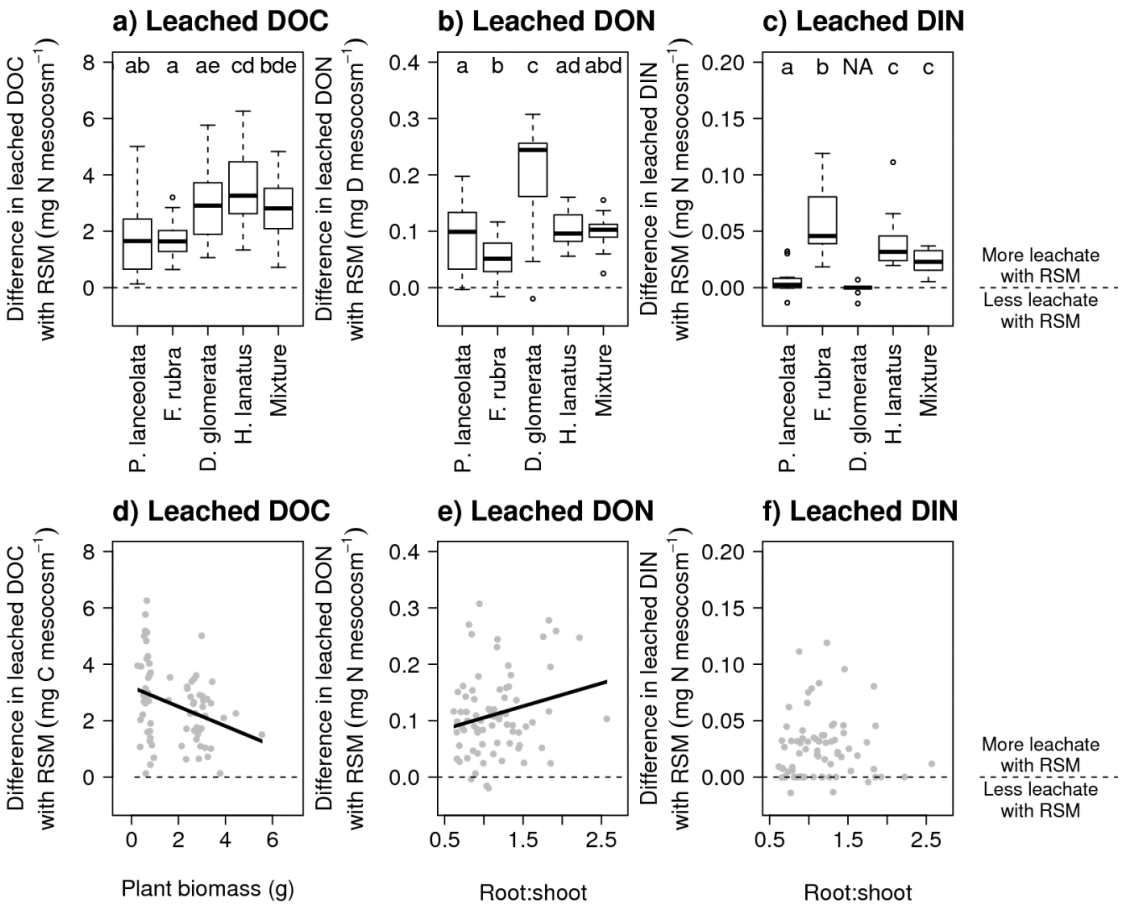

Figure 3.8 The difference in DOC, DON and DIN under reduced soil moisture (RSM) and the importance of plant species, biomass and root:shoot allocation to determine C and N leaching; a) the difference in leached DOC with RSM for each species, b) the difference in leached DON with RSM for each species, c) the difference in leached DIN with RSM for each species, the difference in leached DOC with RSM and plant biomass, b) the difference in leached DON with RSM and root:shoot allocation, c) the difference in leached DIN with RSM and root:shoot allocation. Different letters in panels a-c show significant differences between leaching in species. Solid black line in panel d and e is the modelled significant relationship.

3.4.4 C and N at the final harvest

DOC remaining in the soil at the final harvest was generally lower with reduced soil moisture, although the size of the change depended on species and N addition (Species x N addition x RSM: LRT=10.76, d.f.=4, P=0.0294; Fig 3.9). DON remaining in the soil at the final harvest was decreased by reduced soil moisture (LRT=64.26, d.f.=1, P<0.0001; Fig 3.9) and N addition although the reduction differed between (Species x N addition: LRT=9.61, d.f.=4, P=0.0475; Fig 3.9). Inorganic N in the soil at the final harvest was very dependent on plant species, with much higher inorganic N in *P. lanceolata* than the other species. N addition and reduced soil moisture both reduced inorganic N, but the extent of the reduction depended on species identity (Species x N addition: LRT=15.66, d.f.=4, P=0.0035; Species x RSM: LRT=11.08, d.f.=4, P=0.0257; Fig 3.9). Models using plant biomass, root:shoot allocation and plant traits to explain the difference in DOC and DON with reduced soil moisture only had r-squared of 0.13, suggesting that even significant plant traits had limited explanatory power.

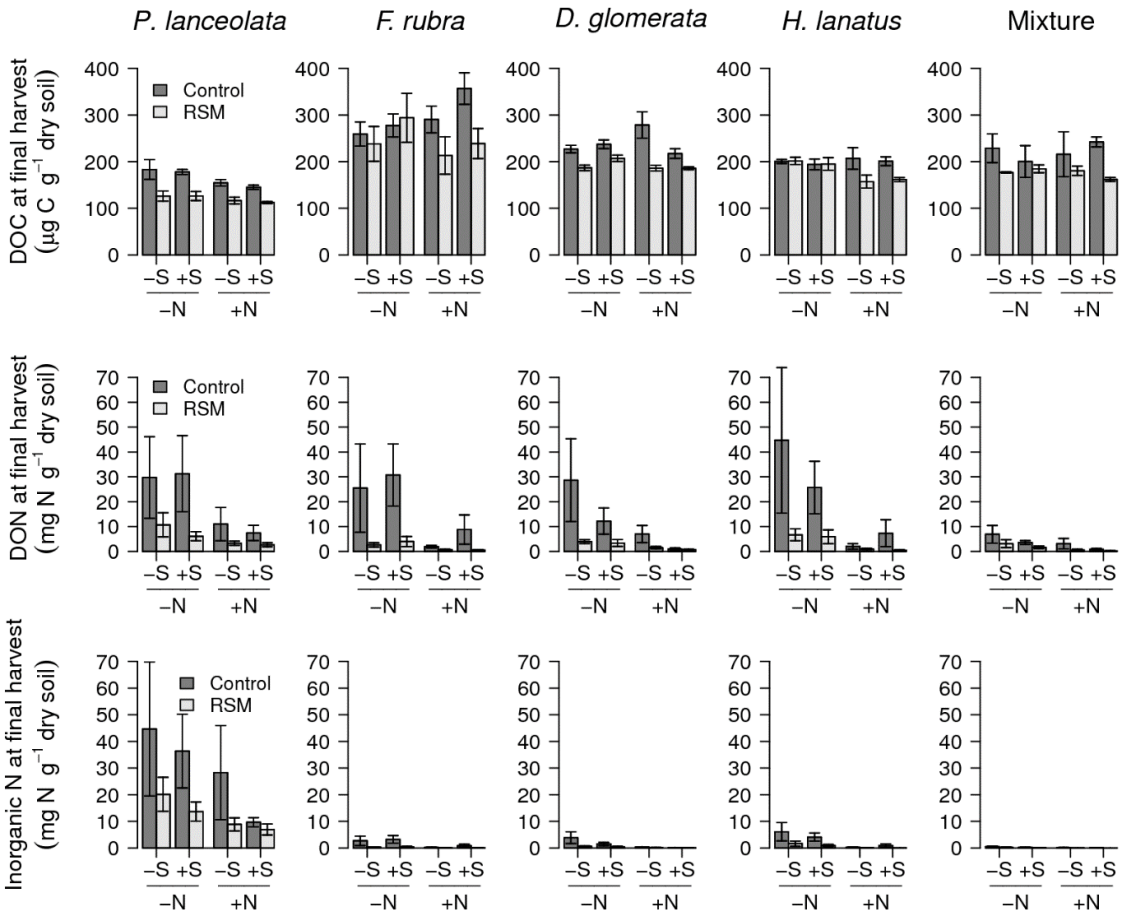

Figure 3.9 Interactive effect of reduced soil moisture (RSM), shade conditioning, N addition and species identity on DOC, DON and inorganic N retained in the soil at the final harvest. Means and standard errors are presented (*n*=4).

3.5 Discussion

This study tested the hypothesis that reductions in CO_2 fluxes in response to reduced soil moisture, and increased leaching of C and N upon rewetting, would be modulated by intraspecific plant trait variation, in particular, plant biomass and its relative allocation to root or shoot biomass. Reduced soil moisture was found to reduce C storage and retention in plants and soil through: i) reduced NEE, representing lower net C uptake, ii) proportionally more C respired relative to C uptake, iii) increased DOC leaching losses upon rewetting. However these changes were not consistent across all mesocosms of the same species, with differences dependent on intraspecific trait variation. In particular large plant biomass and low SLA were the best predictors of larger reductions in NEE sink strength, while large plant biomass predicted smaller DOC leaching losses (Table 3.3).

3.5.1 Variation in plant traits

Plant traits differed within and between species, for which the intraspecific variation was brought about by interactions between experimental treatments. Intraspecific variation was particularly high for plant traits such as total biomass and leaf C, while it was a minimal source of variation for traits such as SLA, RDMC and root diameter (Fig 3.2). The response of plant traits always included an interaction between N addition, shade and reduced soil moisture, suggesting complex controls on intraspecific trait variation (Table 3.1). Across studies, 25% of leaf trait variation within a community can be due to intraspecific variation, while the leaf traits used in this study have been found to show high levels of intraspecific variation (Siefert et al. 2015). However less is known regarding intraspecific variation in root traits (Albert *et al.* 2010), although in this study, variation in root traits was primarily due to differences between species (Fig 3.2). Given the different levels of intraspecific trait variation, not all plant traits may be expected to contribute equally to intraspecific differences in plant responses to climate change.

Intraspecific trait variation was primarily brought about by N addition, with smaller effects of shade conditioning and reduced soil moisture (Fig 3.4; Table 3.1). Plant biomass varied three-fold within species, while root:shoot allocation varied up to two-fold within and between species. The large increase in plant biomass with N addition was as anticipated (Fornara *et al.*, 2013; Farrer & Suding, 2016), while the general decrease in root:shoot allocation with N addition (although dependent on species and soil moisture), is likely because plants switch from competition for nutrients belowground to competition for light (Tilman, 1990; DeMalach *et al.*, 2017). Plants with shade conditioning had lower biomass and lower root:shoot allocation. The effect of shading has been less studied, although in mountain grasslands shading resulted in the transfer of recently assimilated C to roots at the expense of aboveground C (Bahn *et al.*, 2013), which differed to this study where shade reduced root:shoot allocation. This difference may be explained by differences in shading and methodology; Bahn *et al.* (2013) focussed on the short-term fate of ^{13}C, with shading only starting after pulse-labelling. In comparison this study used shade conditioning to bring about a gradient of biomass and its allocation above or belowground and focussed on whole biomass responses rather than fate of ^{13}C.

Although total plant biomass was lower in the reduced soil moisture treatment, as found in previous studies (Bloor & Bardgett, 2012; Vogel *et al.*, 2012), the decline did not interact with N addition or shading (Table 3.1). This contrasts with previous research which showed largest drought-induced reduction in plant biomass in grasslands with greatest initial plant biomass (Wang *et al.*, 2007). This suggests, that in this study, the resistance of plant productivity to reduced soil moisture was not biomass dependent. Root:shoot allocation did not increase with reduced soil moisture across all treatments, instead depending on N addition. This was in contrast to expectations as plants have typically been shown to allocate more C belowground during drought to aid exploitation of reduced water availability (Kalapos *et al.*, 1996; Kahmen *et al.*, 2005; Poorter *et al.*, 2012; Backhaus *et al.*, 2014).

3.5.2 CO_2 fluxes and reduced soil moisture

It was hypothesised that greater plant biomass would increase vulnerability to reduced soil moisture, due to greater water demand, causing greater water stress and therefore a greater reduction in CO_2 fluxes. In support of this, greater NEE and ecosystem respiration reductions were found with high biomass plants, however for NEE the decline in sink strength as biomass increased was greater when SLA was low (Fig 3.6a,b), while for ecosystem respiration the reduction was greater as SLA increased (Fig 3.6d). Cross-ecosystem comparisons suggest SLA is generally higher in wetter environments and associated with increased photosynthesis and transpiration, implying a general exploitative strategy (Reich *et al.*, 1999; Diaz *et al.*, 2004; Gross *et al.*, 2008). However, less is known about the potential importance of intraspecific SLA variation, which in this study correlated with high SRL and low root diameter (Fig 3.3). The impact of SLA on NEE in this study, suggests an exploitative growth strategy may confer greater resistance of NEE to reduced soil moisture, and is therefore opposite to expected. In contrast the effect of SLA on ecosystem respiration is consistent with previous findings. The observed effect of SLA on NEE may partly be due to the species used in this study, as *D. glomerata* had the highest SLA but also the smallest declines in NEE associated with reduced soil moisture (Fig 3.4 and 3.5). It is unclear if this pattern would be consistent for other grassland species with high SLA.

The decrease in NEE and ecosystem respiration with reduced soil moisture implies that photosynthesis declined more than ecosystem respiration. If photosynthesis had stayed constant with reducing ecosystem respiration the net balance, as represented by NEE, would have been a stronger sink (Fig 3.5). Research of the effect of water stress on CO_2 fluxes in grasslands has shown varying responses of photosynthesis and respiration. Grassland mesocosms with similar species to this study, suggested greater reductions in photosynthesis than ecosystem respiration under drought conditions (Bloor & Bardgett, 2012), with similar results in semi-arid grasslands (Li *et al.*, 2016). In contrast, while other studies have shown an overall net balance in

photosynthesis and ecosystem respiration reductions in responses to drought (Fry *et al.*, 2013), with the same conclusion from a global meta-analysis of drought studies (Wu *et al.*, 2011). It is unclear why under drought or reduced soil moisture the balance between photosynthesis and respiration differs between studies, however this study suggests that under reduced soil moisture conditions a greater proportion of C is released through ecosystem respiration relative to C uptake.

3.5.3 CO_2 fluxes after rewetting

Lower CO_2 fluxes under reduced soil moisture, generally recovered completely after rewetting. NEE had no lasting reduction or overcompensation after rewetting. However ecosystem respiration results suggested that for particular species and with particular combinations of treatments, there was overcompensation or incomplete recovery (Fig 3.4, Table 3.2 and 3.4). However, these responses were not explained by differences in plant biomass, root:shoot allocation or any plant traits (Table 3.3). A range of CO_2 flux responses to rewetting after drought or water stress have been reported previously. Model grassland communities had greater light use efficiency and NEE after drought (Mirzaei *et al.*, 2008), while other model grassland communities showed some overcompensation for both ecosystem respiration and NEE, but generally with faster recovery for respiration (Bloor & Bardgett, 2012). For grasslands in semiarid regions the reduction in CO_2 fluxes persisted for a long time without showing overcompensation (Li *et al.*, 2016), but also showed faster ecosystem respiration recovery to rewetting than plant CO_2 uptake (Chen *et al.*, 2009b). Our results suggest that both NEE and ecosystem respiration recovered quickly after rewetting and that there was only small and species specific overcompensation or incomplete recovery for ecosystem respiration.

The relationship between monocultures and diverse mixed species communities has been the focus of a wide range of research, particularly in terms of plant productivity (Tilman & Downing, 1994; Isbell *et al.*, 2015; Craven *et al.*, 2016), but also CO_2 fluxes and C and N leaching (Mirzaei *et al*., 2008; Jentsch *et al*., 2011; Bloor & Bardgett, 2012). The four-species

community used in this study, although simple and relatively species-poor, allows a comparison with the monocultures. Despite the low plant species richness, the mixed species community differed from being an average of the monocultures for CO_2 flux responses to rewetting. In particular ecosystem respiration after rewetting was lower than the average of the monocultures, while in contrast, NEE after rewetting was greater. This suggests the mixed community recovered more quickly, with greater growth relative to respiration. Previous research has shown differing effects of species diversity on CO_2 fluxes after rewetting. For example, it has been found that recovery of NEE in more diverse communities after rewetting was greater than in less diverse communities (Mirzaei *et al*., 2008), while in model grassland communities ecosystem respiration after rewetting was greater in high, rather than low, diversity communities, with no difference in net C uptake (Bloor & Bardgett, 2012). The faster recovery of CO_2 fluxes in the mixed community after rewetting suggests that there may have been complementarity between different species in accessing water resources after rewetting.

3.5.4 Controls on C and N leaching losses

Leaching of DOC, DON and DIN upon rewetting was greater in mesocosms with previous reduced soil moisture compared to control soil moisture (Fig 3.7). This agrees with a large body of research where rewetting following dry periods has been found to increase DOC losses (Kalbitz *et al.*, 2000; Hagedorn & Joos, 2014). The sources of DOC and DON can be complex, including: litter, humus, root exudation and turnover and microbial biomass (Kalbitz *et al.*, 2000; Sanderman *et al.*, 2008). It has been suggested that rewetting can increase DOC through: i) reduced microbial use of DOC before rewetting, ii) enhanced microbial mortality and turnover in dry soils, and iii) altered soil structure increasing DOC leachate (Lundquist *et al.*, 1999; Muhr *et al.*, 2010).

The best predictors of the increase in leaching brought about by reduced soil moisture were species identity, total biomass and root:shoot allocation (Table 3.3, Fig 3.8). Small increases in DOC and DON leaching were respectively found in mesocosms with the greatest plant biomass

and lowest root:shoot allocation. This suggests an important role for roots in regulating leaching from grasslands as total biomass correlated highly with root biomass. Other studies of grassland communities have attributed reductions in N leaching to increases in root biomass (De Vries *et al.*, 2015), with the amount of N leached dependant on root traits such as RDMC (De Vries & Bardgett, 2016). The mechanism by which root biomass affects leaching is not clear, however root exudates, one of the sources of DOC, can be reduced by fertilisation in grasslands suggesting that high plant biomass may lead to lower DOC (Henry *et al.*, 2005). As the increase in root biomass in this study was predominantly brought about by N addition, any resultant change in root exudation with fertilisation may be an important mechanism for changes in C leachate.

The observed increase in C and N leaching upon rewetting of previously dry soil was likely to be short in duration. DOC has been found to quickly return to baseline levels (Kalbitz *et al.*, 2000), while grassland communities exposed to drought have been found to have less DOC and DON leachate from two to 20 days after rewetting (Bloor & Bardgett, 2012). This is also supported by results, in this study, showing generally less DOC, DON and inorganic N remaining in the soil at the end of the experiment (Fig 3.9). Therefore, these studies suggest that for grassland species the spike of C and N leaching upon rewetting grasslands may last less than two days.

3.6 Conclusions

Reduced soil moisture was found to alter C and N cycling in grassland plant species. Reduced soil moisture reduced C storage and retention in plants and soil through: i) reduced NEE, representing lower net C uptake, ii) proportionally more C respired relative to C uptake, iii) increased C and N leaching losses upon rewetting. These responses generally followed predictions from previous research, however the size of the change in C and N cycling was modulated by intraspecific trait variation. Out of all traits, plant biomass was particularly important, as larger plant biomass predicted greater reductions in net C uptake (particularly in

combination with high SLA), predicted greater decreases in ecosystem respiration, and predicted decreases in DOC leaching upon rewetting. This study shows that the response of grasslands to climate change can be modulated by intraspecific trait variation. This suggests that carbon cycling in high biomass grasslands, regularly found under intensive agricultural systems, may therefore be more sensitive to future climate change that alters soil moisture conditions.

4 Differences in plant and soil microbial carbon use efficiency in response to drought

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4.1 Abstract

The proportion of carbon (C) used to form new biomass, relative to C released in respiration, in both soil microbial communities and plants, is key for determining the amount of C available for sequestration and potential feedbacks to climate change. Yet this carbon use efficiency (CUE) is rarely studied in plants and soil microbial communities in the same experiment. Although CUE is not constant, many biogeochemical models assume constant CUE forcing further assumptions on how plant and microbial CUE may counterbalance each other. We hypothesized that CUE in plants and soil microbes is decreased by summer drought and

increased by nutrient addition. This was tested using a ^{13}C-CO_2 pulse-labelling approach on species-rich temperate grasslands in northern England, applied to factorial treatments of experimental drought and fertiliser addition. This allowed us to determine how drought and nutrient addition influenced plant and soil microbial CUE simultaneously, in addition to overall ecosystem CUE. We found that microbial and plant CUE responded differently to drought and nutrient addition. Contrary to expectations, drought increased microbial CUE when combined with nutrient addition. However after rewetting microbial CUE was 11.8% lower than during the drought, suggesting that drought events have short term but reversible impacts on microbial CUE. In contrast plant CUE, when averaged across plant species, was reduced by nutrient addition but was not affected by drought, although there were species specific responses. This study shows that drought events, predicted to become more frequent under climate change, lead to differing responses in plant and microbial CUE, which do not counterbalance each other as assumed in many biogeochemical models of terrestrial ecosystems

Keywords: carbon use efficiency, soil microbial communities, bacteria, fungi, grassland, drought, nutrient availability.

4.2 Introduction

Greater respiratory loss of CO_2 from plants and soil microbes results in less carbon (C) available for long term storage and sequestration (Cotrufo *et al.*, 2013; Kallenbach *et al.*, 2016). However we have a poor understanding of the proportion of C lost through respiration relative to biomass production, and how losses may change in response to climate change (Allison *et al.*, 2010; Sinsabaugh *et al.*, 2016). In soil microbial communities, estimates of the amount of C used in new biomass relative to C uptake, termed carbon use efficiency (CUE), average around 50% across diverse studies, although can vary considerably from close to 0% to 85% (Manzoni *et al.*, 2012b). For plants it is often stated that 50% of photosynthetic C is lost through respiration, and that the ratio of respiration to photosynthesis is invariant, even under changes in temperature and CO_2 concentrations (Gifford, 1995; Dewar *et al.*, 1998; Cheng *et al.*, 2000; Van Oijen *et*

al., 2010). However, this ratio in plants has been shown to vary depending on several factors, including crop species (Albrizio & Steduto, 2003), plant size (Van Iersel, 2003) and forest age (DeLucia *et al.*, 2007). Despite the studies of plants and soil microbes separately, there has been a lack of research bringing together the efficiency with which biomass is produced above and belowground (Bradford & Crowther, 2013).

Despite the potential importance of CUE for C cycling, most biogeochemical models of terrestrial ecosystems assume constant CUE, which does not change in response to climate (Manzoni *et al.*, 2012b). Biogeochemical models which use constant CUE make several assumptions on ecosystem C use which are currently untested. For example as nutrient availability increases in terrestrial ecosystems it might be expected that soil microbial CUE will increase. For the models' assumption of constant CUE to hold it requires other parts of the ecosystem, particularly plants, to become less efficient C users to counterbalance the shift in soil microbial CUE. However this counterbalance assumption between different parts of an ecosystem is untested (Bradford & Crowther, 2013). Our understanding of plant and soil microbial CUE, and their potential importance for C cycling and feedbacks to climate, is further hindered as most studies measure plant and microbial CUE separately rather than simultaneously. The lack of studies exploring growth efficiency of plants and soil microbes simultaneously is in part due to the use of different methodologies. For soil microbes, CUE is often measured using ^{13}C labelled substrates, with CUE estimated as the proportion of C used in biomass relative to the total uptake (Geyer *et al.*, 2016). In contrast studies of plant CUE use the ratio of respiration to photosynthesis, which is equivalent to 1-CUE (Van Iersel, 2003), while studies of whole plant communities use the ratio of net primary production (NPP) to gross primary production (GPP; DeLucia *et al.* 2007; Van Oijen *et al.* 2010).

Despite the variability in measurements of microbial CUE, there may be fundamental controls on microbial growth, such as nutrient availability and temperature (Manzoni *et al.*, 2012b). Additionally, because microbial CUE can contribute to SOM formation, a change in microbial

growth efficiency in response to climate change has the potential to lead to positive or negative feedbacks (Cotrufo *et al.*, 2013). This suggests fundamental controls on microbial CUE, such nutrient availability, could interact with extreme climate events, such as drought, however the relative importance of these contrasting controls on microbial CUE is currently unclear. Increased nutrient availability has generally been found to increase microbial CUE (Steinweg *et al.*, 2008; Sinsabaugh *et al.*, 2013). As soil microbes have tightly constrained C:N:P ratios (60:7:1) and soils have increased ratios (186:13:1 Cleveland & Liptzin 2007), the resulting imbalance requires soil microbes to allocate C for acquisition of N and P. Therefore microbial communities in environments with more favourable substrate stoichiometry are likely to have increased CUE (Manzoni *et al.*, 2012b), which is supported by recent findings in European grasslands in which N addition increased microbial CUE (Spohn *et al.*, 2016b). In contrast to the range of research into the effect of nutrient availability on microbial CUE, much less is known about how drought events, and their subsequent rewetting, may alter microbial CUE. Studies show no clear effect of changes in soil moisture on microbial CUE (Herron *et al.*, 2009; Tiemann & Billings, 2011), but that rewetting may increase microbial CUE following longer periods without precipitation (Zeglin *et al.*, 2013b). Despite the inconclusive research results, drought may be expected to reduce microbial CUE for several reasons. Substrate diffusion is likely to be reduced in dry soil, thereby limiting access of substrates and nutrients to microbes (Schimel *et al.*, 2007). Additionally, drought can cause microbes to accumulate solutes to avoid dehydration, which may in turn inhibit both growth and growth efficiency (Borken & Matzner, 2009). Finally, if drought results in a shift in microbial community composition it could have an impact on CUE as microbes may differ in their growth efficiencies (Payne & Wiebe, 1978; Stark & Firestone, 1995). It may therefore be expected that microbial CUE is reduced under drought, but increased after rewetting. However it is unknown how drivers expected to have contrasting effects, such as drought and nutrient availability, may interact to determine microbial CUE.

The overall goal of this study was to determine the individual and combined effects of drought and nutrient availability on microbial and plant CUE in species-rich temperate grassland. Specifically we tested the following two hypotheses that: i) drought decreases both microbial and plant CUE as water stress requires the use of C to maintain water potential in microbes and access water in plants; and ii) alleviation of nutrient limitation via nutrient addition, increases microbial and plant CUE as less C is used for nutrient acquisition. The result of these hypothesis will be that microbial and plant CUE will correlate in their response to nutrient addition and drought, and that overall ecosystem CUE, the retention of C in microbes and plants relative to ecosystem respiration, will not remain constant but also increase in response to nutrient addition and decrease in response to drought. This was tested in species-rich temperate grassland in northern England using ^{13}C-CO_2 pulse-labelling, which enabled measurement of plant and microbial CUE.

This study focusses on grasslands because they rapidly cycle C from plants to soil microbes, can act as soil C sinks, but also contain plant species with differences in C uptake and retention (De Deyn *et al.*, 2011a,b). In contrast to previous studies of microbial CUE, the microbial community was enriched with ^{13}C through photosynthetic assimilation of ^{13}C-CO_2 in the plants and subsequent release of ^{13}C, through root exudation, into the soil. By using this novel approach, soil microbial CUE can be measured by tracing the fate of labelled plant inputs in order to predict how microbial CUE may feedback to ecosystem C dynamics in response to climate change and nutrient availability. Additionally it allowed a comparison of soil microbial CUE with plant CUE, which for the first time will enable investigating if CUE in plants and soil microbial community counterbalance each other, which is currently untested but assumed in biogeochemical models.

4.3 Methods

4.3.1 Experimental setup and pulse-labelling

The field manipulation experiment was conducted at Colt Park meadows in Ingleborough National Nature Reserve, northern England (latitude 54°12'N, longitude 2°21'W) on a *Lolium perenne-Cynosorus cristatus* grassland, similar to those used by De Deyn *et al.* (2011b) and Smith *et al.* (2008). The soil is a brown earth with shallow average depth of 28cm over limestone bedrock. Experimental plots were set up on a uniform grassland community which prior to the start of the experiment was grazed by cattle and sheep from autumn to May, before livestock removal to allow hay production with an annual hay cut after mid-July. Four experimental treatments were implemented in a randomized block design: control, nutrient addition, experimental drought and combination of nutrient addition and experimental drought. Experimental treatments were set up in six replicate blocks giving 24 plots of $4m^2$. Nutrient addition comprised a propriety NPK fertiliser (50Kg N ha^{-1}, 25Kg P ha^{-1}, 25Kg K ha^{-1}) and was applied at the start of the experiment on 20 May 2014. Drought conditions were created using rain shelters which intercepted all rainfall reaching the central plot region. Rain shelters were open sided, constructed of transparent corrugated PVC, 0.8mm thick (Corolux, Staveley, UK), and were 90cm x 105cm in size with a height of 38cm to 63cm giving a sloped roof of 16 degrees (Chapter 2). Rain shelters were set up on 28 May 2014 and removed on 14 July 2014. Soil moisture was assessed in each plot with a ThetaProbe soil moisture meter (Delta-T, UK). The annual hay cut which removed aboveground vegetation to approximately 5cm sward height, and was consistent with annual agricultural management, was applied across all experimental plots was on 22 July 2014.

The drought treatment excluded 157mm of rainfall which was equivalent to 6.5% of average annual precipitation at Colt Park meadows field site (mean: 2404mm, SD: 82mm). To assess drought severity and potential recurrence, a Gumbel I distribution was fitted to annual drought extremes representing the number of days with less than 1mm of precipitation during the

primary growth period (April-September). Due to insufficient number of years of precipitation data for the field site, data from two nearby weather stations were used. At Malham Tarn field centre, 18km southeast of the field site, a 100-year drought equated to 27 days <1mm rainfall. Similarly data from Hazelrigg Field station, 31km southwest, showed a 100-year drought equated to 34 days of <1mm rainfall (Bloor & Bardgett, 2012). These results suggest a 100 year drought event may be between 27 and 34 days, while in this study the drought treatment was *in situ* for 48 days. However the drought treatment in this study may not equate to the same level of water stress as a 100-year drought event, as to avoid disturbance to the plant community, plots were not trenched so lateral flow of water in the soil will have still occurred.

Pulse-labelling of each experimental plot was carried out on 31 June 2014. For each plot a transparent static chamber, 30cm in diameter and 19 litres in volume, was placed in the central plot area onto a matching base ring cut 5cm into the soil, for 20 minutes and 40ml of 99.9% atom ^{13}C-CO_2 was injected (Ward *et al.* 2009). This was repeated seven times in the day for each plot with 30 minutes between each labelling event. The fate of ^{13}C-CO_2 was assessed in the field by measuring ^{13}C in plant biomass and ^{13}C-CO_2 in ecosystem respiration (described below). The uptake and metabolism of ^{13}C by the microbial biomass from root exudation was assessed using a laboratory incubation of soil collected from field plots 24 hours after pulse-labelling. Samples were taken after 24 hours because previous research at a neighbouring field site demonstrated that ^{13}C in the microbial biomass peaks 24 hours after pulse-labelling with ^{13}C-CO_2 (De Deyn *et al.*, 2011a).

4.3.2 Sample collection and analysis

The loss of ^{13}C-CO_2 through ecosystem respiration was measured in the field using opaque static chambers, which were the same size as those used in pulse-labelling, and incorporated both autotrophic and heterotrophic respiration. Natural abundance measurements were taken before 'pulse-labelling' on three replicate blocks. Measurements after pulse-labelling were taken: +2 hours, +1 day, +3 days, +5 days, +7 days, +11 days , +16 days and +25 days on all 6

replicate blocks. Chambers were in place for 30 minutes and gas samples were taken immediately after enclosing and then every 10 minutes. For each time point two gas samples were taken in exetainer vials (Labco Ltd, UK), one for total CO_2 (10ml) and analysed using gas chromatography (PerkinElmer Autosystem Gas Chromatograph), and a second sample (25ml) for isotopic analysis.

The retention of ^{13}C in plant biomass over time was measured in leaf samples from five common grassland species from each plot. Leaf samples were taken at four time points after 'pulse-labelling': +2 hours, +1 day, +7 days and +14 days to assess ^{13}C incorporation into plant biomass. Natural abundance measurements were taken in the same way before the 'pulse-labelling' from three replicate blocks. Five plant species were selected to represent the three main plant functional groups and were the most abundant species in each group; grasses: *Anthoxanthum odoratum, Holcus lanatus;* forbs: *Ranunculus repens, Plantago lanceolata;* and legume: *Trifolilum repens*. Roots were collected from three soil cores per plot (diameter 2.4cm) and bulked together, and were sampled from field plots one day and 14 days after pulse-labelling. Leaf and root samples were freeze dried and ground in preparation for isotopic analysis.

The uptake of ^{13}C into soil microbial biomass and lost through soil respiration was measured using a laboratory incubation, following field pulse labelling with ^{13}C-CO_2. Three soil cores (2.4cm diameter) were collected from each field plot 24 hours after pulse-labelling and then bulked together. Roots were removed from the soil by hand to reduce any further plant inputs of ^{13}C. Two subsamples of soil from drought treatment plots were used: one was kept at field drought soil moisture (37.7% [SE:0.7] water content, assessed gravimetrically) while the second was brought back to control soil moisture content using deionized water (41.0% [SE:0.6] water content). In total there were 40 soil incubations: 24 for each field plot, 12 from field drought plots which were rewetted at the start of the incubation and 4 natural abundance incubations. For each incubation 10g of soil was incubated at 25ºC in 1.8L containers (Lock 'n' lock, UK).

Before incubations were sealed, they were flushed for 20 seconds with compressed air. Incubations were sealed for the first 4.5 hours with gas samples taken at +0h (immediately after sealing), +1.5h, +3h and +4.5h. Lids were removed after 4.5 hours and incubations flushed again with compressed air before re-sealing and further headspace samples taken at +4.5h (immediately after resealing), +7h, +9.5h and +24h. The reason incubations were opened and flushed partway through the incubation was to avoid headspace CO_2 becoming excessively high, and secondly to reduce the amount of air being removed for analysis relative to total headspace volume. At each time point two headspace gas samples were taken, one for total CO_2 concentration (PerkinElmer Autosystem Gas Chromatograph) and a second for isotopic analysis (see below).

Microbial biomass C was measured using chloroform fumigation extraction (Brookes *et al.*, 1985) with total C content of K_2SO_4 extracts measured on Elemental Analyser (5000A, Shimadzu, Milton Keynes, UK) for soil 5g subsamples before and after the incubation. Following this extracts were freeze-dried and ground in preparation for isotopic analysis. Additional soil properties were measured to assess the treatment effects of nutrient addition. Inorganic N concentrations in soil were assessed using KCl extracts with 5g soil with 25mL KCl before colorimetric analysis on an AutoAnalyser (Bran and Luebbe, Northampton, UK). Olsen's P, a measure of plant available phosphate in soil, used 0.5M sodium bicarbonate extraction on 5g soil before Autoanalyser analysis (Bran and Luebbe, Northampton, UK). pH was measured on 10g fresh soil with 25 mL deionised water, left to stand for 30 minutes and then tested with 210 pH Meter (Hanna Instruments, RI, USA).

4.3.3 Soil microbial community structure

Treatment effects on microbial community structure was measured using phospholipid fatty acids (PLFA) analysis, while bacterial and fungal community structure was assessed using terminal restriction fragment length polymorphism (T-RFLP) analysis. PLFAs were extracted from freeze-dried soil using a modified Bligh-Dyer extraction method, and separated from other

lipids using aminopropyl solid phase extraction cartridge (Phenonenex, US)(White *et al.*, 1979). Gas chromatography was carried out on Agilent 6890 GC with CP-Sil 5 CB fused silica capillary column (Agilent, US). Biomarkers were used for bacteria (i15:0, a15:0, 15:0, i16:0, i17:0, 17:0, cy-17:0, cy-19:0 and 18:1ω7) and fungi (18:2ω6,9) with the ratio of the two defined as the fungi-to-bacteria ratio (F:B), following Bardgett, Hobbs & Frostegard (1996) and Smith *et al.* (2008). For T-RFLP, total genomic DNA was extracted from soil samples using the procedures described by Plassart *et al.* (2012) in combination with the powersoil96 kit (MoBio, US). T-RF peaks were converted to relative abundance before analysis. ^{13}C-PLFA was extracted from soil at the start of the incubation, while T-RFLP was extracted from soil at the start and end of the incubation.

4.3.4 Stable isotope analysis and calculations

For isotopic analysis the freeze-dried and ground plant and microbial biomass samples were weighed into tin capsules and combusted in an elemental analyser (CarloErba, Italy). The resulting CO_2 from combustion was analysed for $\delta^{13}C$ using Isotope Ratio Mass Spectrometry (IRMS Dennis Leigh technology, UK). $\delta^{13}C$ of CO_2 for field ecosystem respiration and laboratory soil respiration were measured using IRMS (Isoprime Ltd, UK) coupled to a trace-gas pre-concentrator. After sample injection to the pre-concentrator, water was removed by a magnesium perchlorate chemical trap and the CO_2 was then cryogenically focused followed by IRMS analysis giving $\delta^{13}C$ values.

$\delta^{13}C$ notation represents the ratios (R) of ^{13}C:^{12}C relative to the VPDB (Vienna Pee Dee Belemnite) standard. For microbial biomass samples $\delta^{13}C$ was given by:

$$\text{Microbial biomass } \delta^{13}\text{C} = \frac{\left(\delta^{13}\text{C}_{\text{fum}} \times \text{mgC}_{\text{fum}}\right) - \left(\delta^{13}\text{C}_{\text{unfum}} \times \text{mgC}_{\text{unfum}}\right)}{\left(\text{mgC}_{\text{fum}} - \text{mgC}_{\text{unfum}}\right)}$$

where mgC_{fum} and mgC_{unfum} represents mg of C measured in fumigated and unfumigated samples respectively. $\delta^{13}C$ values were converted to isotopic ratio ($^{13}C/^{12}C$) for each sample (R_{sample}):

$$R_{\text{sample}} = \left(\frac{\delta^{13}\text{C}}{1000} \times R_{\text{PDB}}\right) \times R_{\text{PDB}}$$

where, R_{PDB} = 0.011237 is the ratio for Pee Dee Belemnite. R_{sample} was converted to $^{13}C_{atom\ \%}$:

$$^{13}\text{C}_{\text{atom \%}} = \left(\frac{R_{\text{sample}}}{R_{\text{sample}} + 1}\right) \times 100$$

For ecosystem and soil respiration samples, the $^{13}C_{atom\ \%}$ in respired CO_2 was calculated using a two-source mixing model:

$$^{13}\text{C}_{\text{atom \% resp}} = \frac{(\text{C}_{\text{sample}} \times \ ^{13}\text{C}_{\text{atom \% sample}}) - (\text{C}_{\text{air}} \times \ ^{13}\text{C}_{\text{atom \% air}})}{\text{C}_{\text{sample}} - \text{C}_{\text{air}}}$$

where C_{sample} is the CO_2 concentration at the end of the enclosure and C_{air} is CO_2 concentration immediately after enclosing. For ^{13}C in plant biomass, soil microbial biomass and ecosystem and soil respiration, the enrichment relative to natural abundance was calculated as $^{13}C_{atom\ \%\ excess}$:

$$^{13}\text{C}_{\text{atom \% excess}} = \ ^{13}\text{C}_{\text{atom \% of sample}} - \ ^{13}\text{C}_{\text{atom \% of natural abundance}}$$

To take account of the differing sizes of C pools and fluxes, $^{13}C_{atom\ \%\ excess}$ was converted to µg ^{13}C following Hafner *et al.* (2012):

$$\mu\text{g}\ ^{13}\text{C} = \frac{^{13}\text{C}_{\text{atom \% excess}}}{100} \times \mu\text{g C}$$

where µg C is the mass of C in plant biomass, soil microbial biomass and soil and ecosystem respiration.

Isotopic enrichment of individual PLFAs were expressed as $\delta^{13}C$ values corrected for the methyl group added during methanolysis:

$$\delta^{13}C_{PLFA} = \frac{\left[(N_{PLFA} + 1)\delta^{13}C_{FAME} - \delta^{13}C_{MeOH}\right]}{N_{PLFA}}$$

where N_{PLFA} is the number of C-atoms in the PLFA molecule, $\delta^{13}C_{FAME}$ is the $\delta^{13}C$ value after methanolysis and $\delta^{13}C_{MeOH}$ is the $\delta^{13}C$ value of the methanol (Tavi *et al.*, 2013). Absolute amount of ^{13}C in individual PLFA biomarkers was given by:

$$\mu g\ PLFA\ ^{13}C\ g^{-1}soil(dwt) = {}^{13}C\ atom\ \% \times \mu g\ PLFA\ g^{-1}soil(dwt) \times \frac{M(PLFA - C)}{M(PLFA)}$$

where M(PLFA-C) is the molar mass of the C in the PLFA molecule and M(PLFA) is the molar mass of the PLFA molecule.

4.3.5 Estimates of CUE

In this study, CUE was estimated for soil microbes, plants and at the ecosystem level. Microbial CUE was measured in soil incubations allowing separation of soil and plant respiration. Plant and ecosystem CUE were measured in field plots. Microbial CUE was calculated as the percentage of ^{13}C in microbial biomass at the end of the incubation, relative to total ^{13}C uptake.

$$\text{Microbial CUE} = \frac{{}^{13}C\ \text{microbial biomass}}{{}^{13}C\ \text{microbial biomass} + {}^{13}C{-}CO_2} \times 100$$

where total ^{13}C uptake was the sum of ^{13}C in microbial biomass and cumulative ^{13}C-CO_2 respired during the incubation (Geyer *et al.*, 2016). In contrast to previous studies the ^{13}C comes only from root exudation and turnover, rather than labelled substrate additions. Under the

framework set out by Geyer et al (2016), microbial CUE would be classified as community scale microbial CUE.

CUE in plants was estimated in two ways, both of which were based on the % of ^{13}C retained in plant biomass over a set length of time (Street *et al.*, 2013). Overall plant CUE was averaged across species and estimated as:

$$\text{Plant CUE}_d = \frac{^{13}\text{C shoot}_d + {}^{13}\text{C root}_d}{^{13}\text{C shoot}_{+2\text{ hours}}} \times 100$$

where *d* was the time point at which ^{13}C incorporation was measured, either 24 hours or 14 days after pulse-labelling, while ^{13}C incorporation in shoots two hours after pulse labelling was used as the baseline representing maximum ^{13}C uptake. ^{13}C incorporation in shoots was averaged across five species by their representative % cover while root samples represented roots from all species. Although ^{13}C incorporation in plant roots was not measured two hours after pulse labelling, this is unlikely to alter the results as ^{13}C incorporation in roots was very low compared to shoots and increased over time, suggesting there would have been very little ^{13}C in roots two hours after pulse-labelling.

The second estimate of CUE in plants, looked at species specific CUE. Because roots could not be split into species, species specific CUE only considered the retention on ^{13}C in shoot biomass. This was done for five species:

$$\text{Species specific shoot CUE}_d = \frac{^{13}\text{C species shoot biomass}_d}{^{13}\text{C species shoot biomass}_{+2\text{ hours}}} \times 100$$

where *d* was the time point at which ^{13}C incorporation was measured, either 24 hours, 7 days or 14 days after pulse-labelling. In the same way as plant CUE, ^{13}C incorporation in shoots two hours after pulse labelling was used as the baseline representing maximum ^{13}C uptake

Ecosystem CUE estimated the percentage of ^{13}C in soil microbial biomass and total plant biomass relative to total ^{13}C uptake, which was estimated as the total ^{13}C in plant and microbial biomass and lost through ecosystem respiration:

$$\text{Ecosystem CUE}_d = \frac{^{13}\text{C microbial biomass}_d + {}^{13}\text{C shoot}_d + {}^{13}\text{C root}_d}{^{13}\text{C microbial biomass}_d + {}^{13}\text{Cshoot}_d + {}^{13}\text{C root}_d + {}^{13}\text{C}-\text{CO}_{2\,d}} \times 100$$

where *d* was the time point at which ^{13}C incorporation was measured, either 24 hours or 14 days after pulse-labelling. ^{13}C-CO_2 respired is the cumulative loss of ^{13}C in respiration from pulse labelling to the time of measurement. This was done through interpolating ^{13}C-CO_2 ecosystem respiration between sampling dates, assuming measured ecosystem respiration were representative for the time between sampling.

4.3.6 Statistical analysis

To test the hypothesis that nutrient and drought altered microbial, plant and ecosystem CUE, linear mixed effects (LME) models were used with fixed effects of: nutrient addition, drought and the two-way interaction. The random effect was block to take account of the blocked experimental design. For analysis of ecosystem respiration and plant CUE over time, sampling date was added as a categorical fixed effect with plot ID as a random effect nested within block to take account of repeated measures. For microbial CUE, which included samples which had been rewetted, an additional fixed effect ("rewet") and interaction with nutrient addition was added, with an additional random effect of plot ID, nested within block, to take account of the fact that rewetted soil was taken from drought plots and therefore acted as repeated measures. For all LME models, assumptions of normality and equal variances were checked graphically and response variables logged to improve normality where necessary. Weight functions were used to account for unequal variances, following Zuur et al. (2009). We determined the significance of fixed effects by comparing models with and without the factor of interest using

likelihood ratio tests (LRT). All statistical analyses were carried out in the R programming language 3.3.1 (R Core Development Team, 2016) using the additional packages *nlme* (Pinheiro & Bates, 2013), *plyr* (Wickham, 2011) and *vegan* (Oksanen *et al.*, 2013).

4.4 Results

The drought treatment effectively reduced soil moisture from around 50% to 30%, although this reduction was greater at later sampling dates (Drought x Date: LRT=201.19, P<0.0001, Fig 4.1a). The time lag between the start of the drought treatment and reduction in soil moisture, was due to low rainfall in the first weeks of the experiment (Fig 4.1b). Artifacts of having rain shelters in place were not found for either air (Drought=14.7°C; Control=14.5°C ; T-test, t=-0.56, P=0.5972) or soil temperatures (Drought=15.1°C; Control=15.2°C ; T-test, t=0.31, P=0.776).

Drought increased soil inorganic N concentrations (LRT=5.06, P=0.0245, Fig 4.2), but did not alter soil pH, Olsen's P or aboveground plant biomass. The nutrient addition treatment brought about a 2.1 fold increase in aboveground plant biomass (LRT=28.45, P<0.0001, Fig 4.2d), which depending on sampling date led to lower soil moisture (Nutrient x Date: LRT=19.79, P=0.006, Fig 4.1a). Nutrient addition significantly increased Olsen's P (LRT=6.29, P=0.0121) and inorganic N availability (LRT=20.335, P<0.0001), and reduced soil pH (LRT=4.71, P=0.0299, Fig 4.2). Soil collected from field plots and used in laboratory incubations also had soil moisture content reduced by drought conditions and in nutrient addition plots (Fig 4.2f), matching changes measured directly in field plots (Fig 4.1a).

Despite changes in soil moisture and nutrient availability, there was no associated change in fungal microbial community structure in response to nutrient addition or drought as assessed by T-RFLP analysis (Appendix 2). However soil bacterial community structure was changed by nutrient addition, but this only occurred in soils that had been exposed to drought and then subsequently rewetted (Appendix 2). Similarly, analysis of total PLFA concentrations showed

no change in the abundance of fungi or bacteria PLFA or the ratio of fungi to bacterial PLFA in response to nutrient addition and drought (Fig 4.3a,b,c).

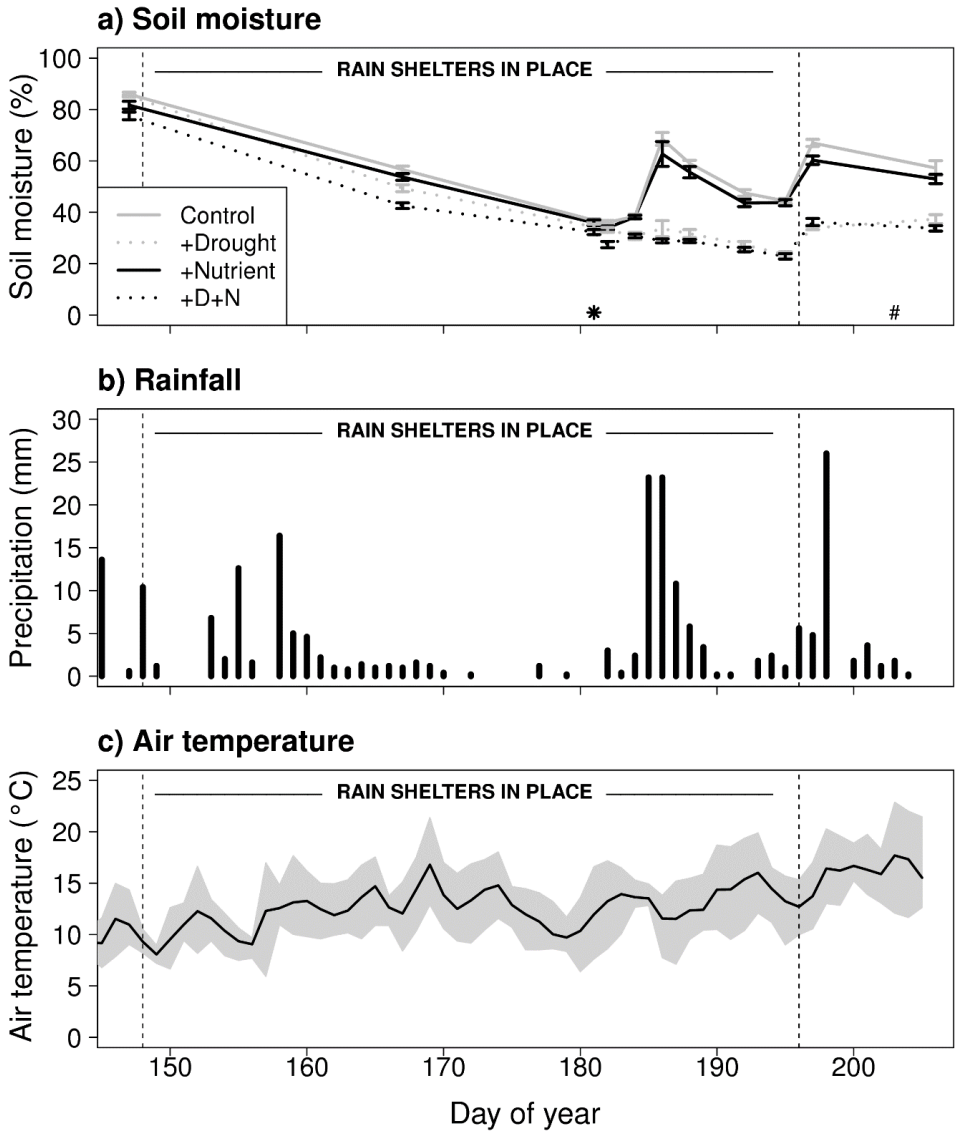

Figure 4.1 Abiotic properties from field experimental plots and on site weather station during the experiment: a) soil moisture for each combination of treatments. Day of pulse labelling (*****) and day of hay cut (#); b) rainfall and c) air temperature with daily mean (black line) and daily minimum and maximum (grey shading).

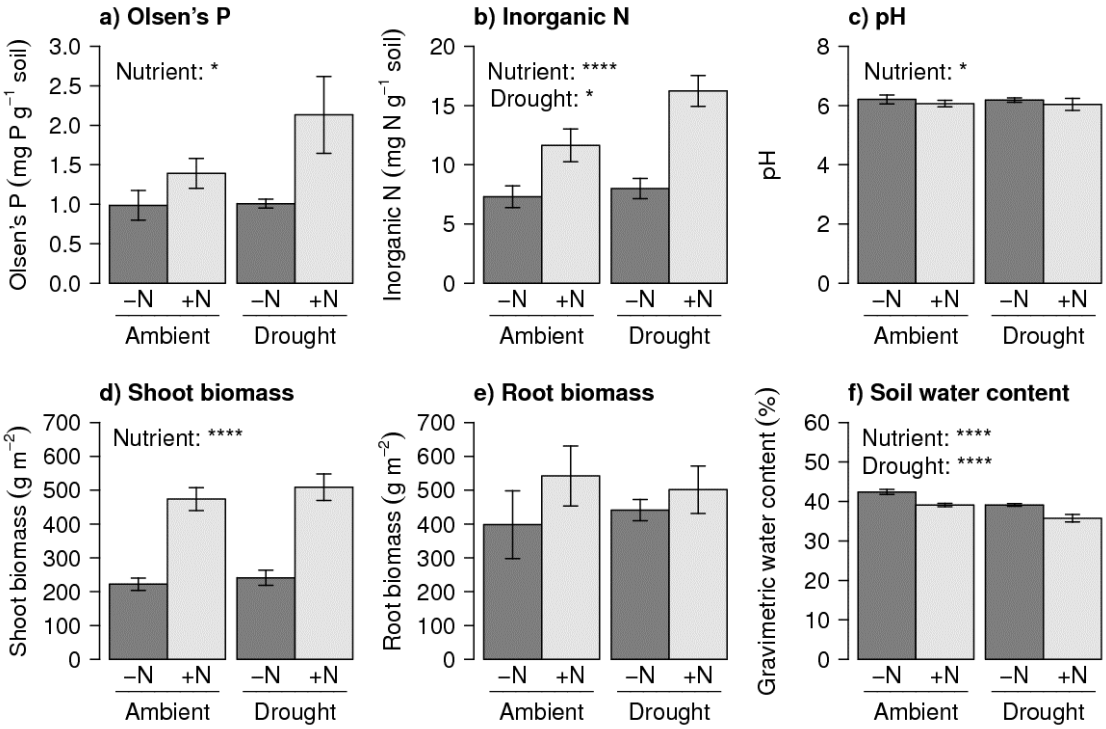

Figure 4.2 The effect of nutrient addition and drought on plant and soil properties: a) Olsen's P, b) inorganic N, c) soil pH, d) shoot biomass, e) root biomass and f) water content assessed gravimetrically in soil incubations. Significance of nutrient and drought treatments assessed using likelihood ratio tests with significance indicated as: P<0.0001****, P<0.001***, P<0.01**, P=<0.05*.

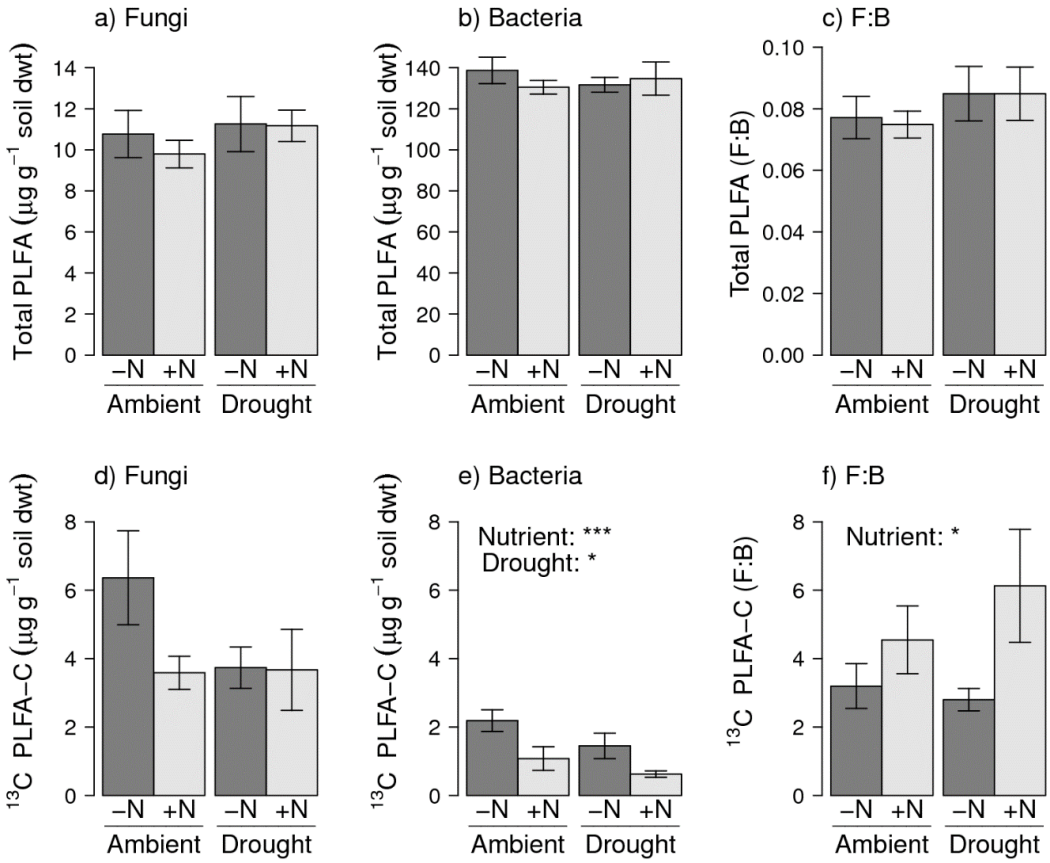

Figure 4.3 The structure of the soil microbial community and incorporation of ^{13}C into microbial PLFA biomarkers. Response of total PLFA biomarkers to drought and nutrient addition representing a) fungi, b) bacteria and c) fungi:bacteria. The incorporation of ^{13}C in PLFA-C biomarkers for d) fungi, e) bacteria and f) fungi:bacteria. Significance of nutrient and drought treatments assessed using likelihood ratio tests with significance indicated as: P<0.0001****, P<0.001***, P<0.01**, P=<0.05*.

4.4.1 Microbial CUE

Microbial CUE was increased by nutrient addition, but only when in combination with drought (drought x nutrient interaction: LRT=6.17, P=0.013, Fig 4.4), while rewetting of soil reduced microbial CUE (LRT=14.16, P=0.0008, Fig 4.4). However, microbial CUE did not correlate with either plant or ecosystem CUE when assessed over 24 hours (Fig 4.5).

The total CO_2 respired during soil incubations was increased by nutrient addition, but not under drought conditions (Drought x Nutrient, LRT=4.10, P=0.0429; Rewet x Nutrient, LRT=6.69, P=0.0097, Fig 4.6c). However, the amount of ^{13}C-CO_2 respired during the incubation was reduced by a third with nutrient addition (LRT=20.12, P<0.0001, Fig 4.6f). The size of the total microbial biomass C pool did not change over the course of the incubation and was not altered by drought or nutrient addition (Fig 4.6a,b). However, at the start of the incubation, more ^{13}C was assimilated by the microbial biomass when soils were subject to drought (LRT=7.18, P=0.0074, Fig. 4.12d). At the end of the incubation ^{13}C retained in microbial biomass was reduced by rewetting and nutrient addition, however, the reduction with nutrient addition was greater without drought (drought x nutrient interaction: LRT=5.12, P=0.0237, Fig. 4.12e).

The fate of ^{13}C in the soil microbial community was assessed using PLFA biomarkers, which showed ^{13}C was assimilated by fungi more than bacteria (Fig 4.3). ^{13}C in bacterial PLFAs were decreased in communities with nutrient addition (LRT=14.67, P<0.0001) and drought (LRT=5.30, P=0.0213, Fig 4.3e). The resulting ratio of ^{13}C in fungal to bacterial PLFA was increased in communities with nutrient addition (LRT=4.67, P=0.0307, Fig 4.3f).

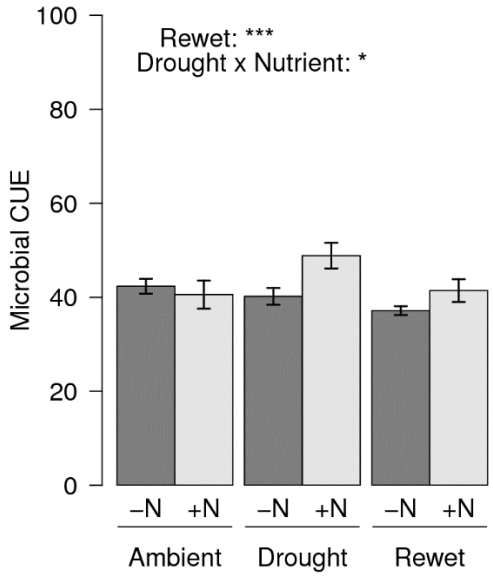

Figure 4.4 Microbial CUE response to drought, rewetting and nutrient addition. Calculated as the proportion of ^{13}C in microbial biomass relative to total ^{13}C uptake (calculated as ^{13}C in microbial biomass and soil respiration). Significance of nutrient and drought treatments assessed using likelihood ratio tests with significance indicated as: P<0.0001****, P<0.001***, P<0.01**, P=<0.05*.

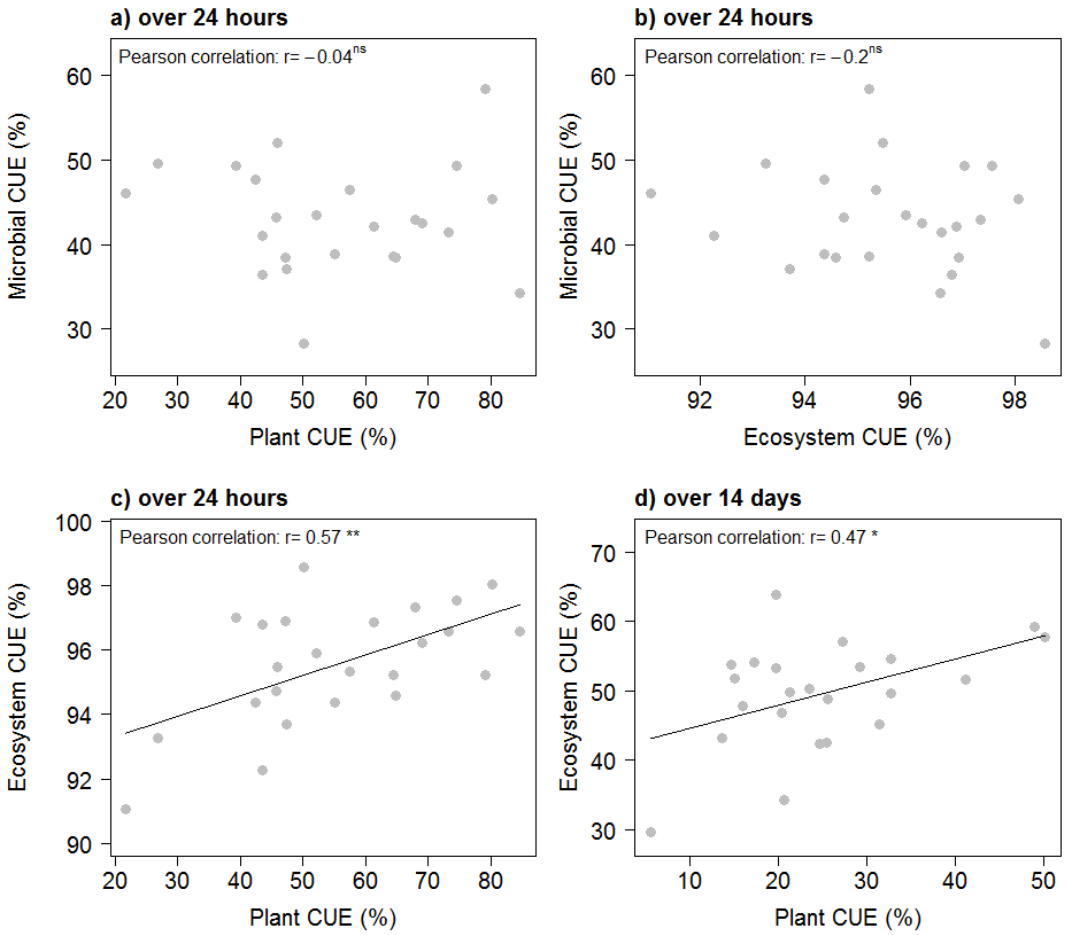

Figure 4.5 The relationship of CUE between microbes, plants and the whole ecosystem, a) microbial CUE and plant CUE assessed over 24 hours, b) microbial CUE and ecosystem CUE assessed over 24 hours, c) ecosystem CUE and plant CUE assessed over 24 hours, and d) ecosystem CUE and plant CUE assessed over 14 days. Black lines show significant correlations as tested by Pearson correlation where r shows strength of correlation. Significance of correlation indicated as: P<0.0001****, P<0.001***, P<0.01**, P=<0.05*.

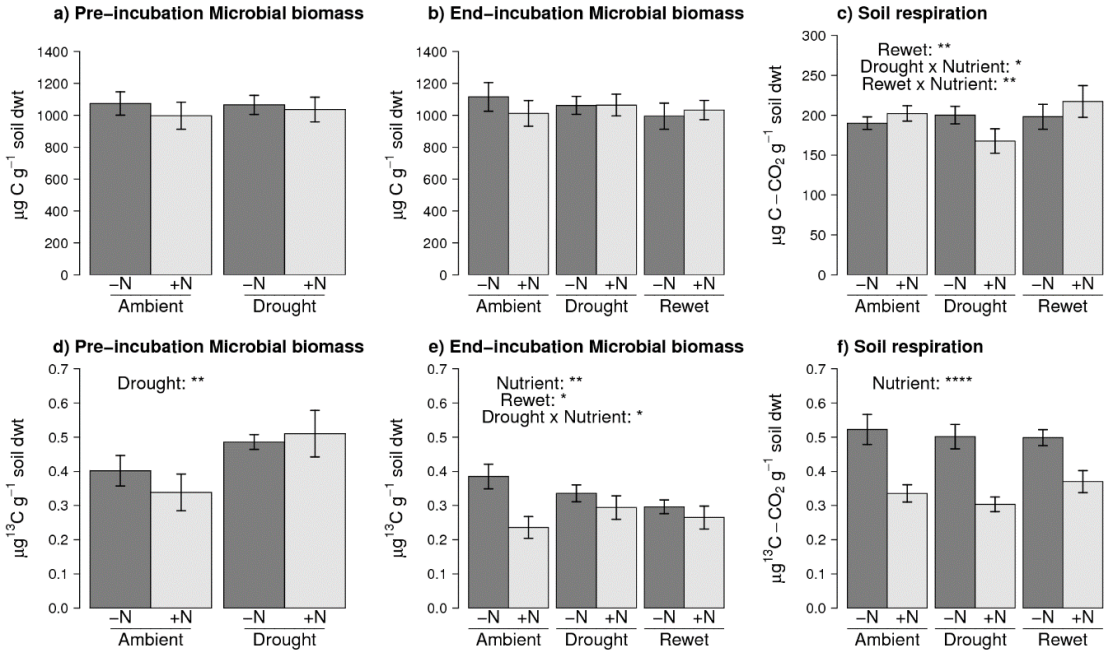

Figure 4.6 Pools of total C and ^{13}C in microbial biomass during the laboratory incubation and released through soil respiration. Total C in microbial biomass at the a) start of the laboratory incubation, b) end of the laboratory incubation and c) total C-CO_2 in soil respiration. The µg ^{13}C in microbial biomass at the d) start of the laboratory incubation, e) end of the laboratory incubation and f) µg ^{13}C-CO_2 in soil respiration. Significance of nutrient and drought treatments assessed using likelihood ratio tests with significance indicated as: P<0.0001****, P<0.001***, P<0.01**, P=<0.05*.

4.4.2 Plant CUE

When averaged across all five species, plant CUE was approximately 50-60% for the first 24 hours, and was not altered by nutrient addition or drought (Fig 4.7a). However after 14 days plant CUE decreased to 20-30% and was significantly lower in communities which had received nutrient addition (14 days nutrient: LRT=6.72, P=0.0095, Fig 4.7b).

Nutrient addition increased ^{13}C in shoot biomass when averaged across plant species (Fig 4.8a), primarily due to increased overall plant biomass. This increase in ^{13}C shoot enrichment was found two hours (LRT=19.87, P<0.0001), one day (LRT=15.13, P=0.0001) and 14 days (LRT=4.95, P=0.0261, Fig 4.8a) after pulse labelling. In contrast ^{13}C allocation to roots was not affected by nutrient addition or drought, either one day or 14 days after pulse labelling (Fig 4.8a). The ratio of ^{13}C incorporation in roots-to-shoots was less than 0.10 one day after pulse labelling, and was reduced by nutrient addition (LRT=9.92, P0.0016). The root-to-shoot ratio of ^{13}C allocation increased to 0.16-0.31, 14 days after pulse labelling but was not affected by nutrient addition or drought (Fig 4.8b).

Aboveground plant CUE, measured in specific species, decreased over time for all species (Fig 4.9). However there were species specific CUE responses to nutrient addition (Nutrient x species interaction: LRT=30.91, P<0.0001) and drought (Drought x species interaction: LRT=10.46, P=0.0334). For grasses, nutrient addition increased CUE in *H. lanatus* over short timescales (Nutrient x Time: LRT=9.16, P=0.0103, Fig 4.9a). For *A. odoratum*, nutrient addition reduced CUE but only non-drought conditions, however the strength of this interaction between nutrient and drought treatments reduced over time (Nutrient x drought x time: LRT=6.32, P=0.0424, Fig 4.9b). The two forb species had contrasting responses to nutrient addition and drought: for *R. repens* nutrient addition and drought reduced CUE (Nutrient: LRT=5.98, P=0.0144; Drought: LRT=6.91, P=0.0086), while for *P. lanceolata* nutrient addition and drought had no significant effect (Fig 4.9d). For the legume, *T. repens*, nutrient addition

decreased CUE, which over 14 days resulted in half the ^{13}C being assimilated in biomass with nutrient addition than in plants without (Nutrient: LRT=12.06, P=0.005).

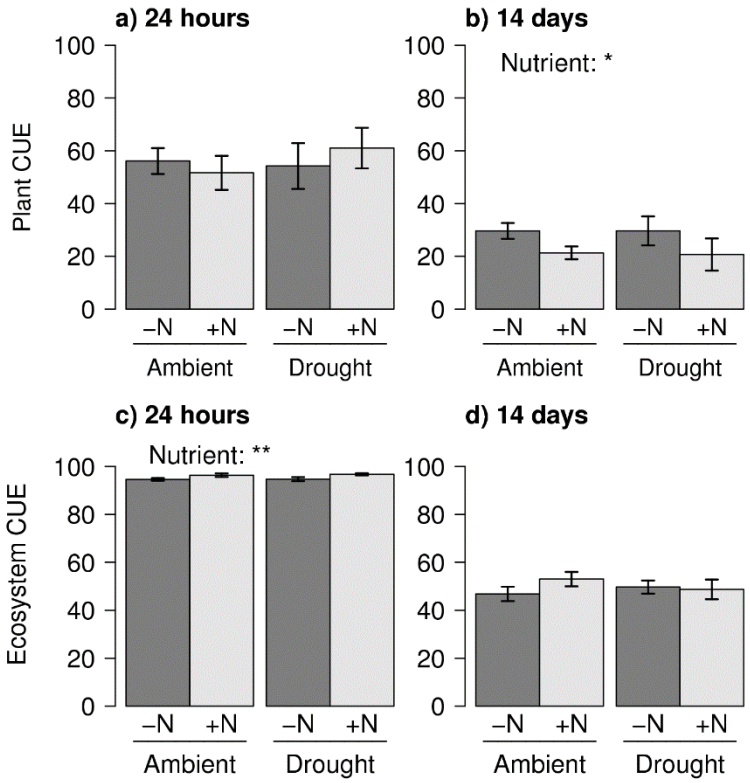

Figure 4.7 Plant and ecosystem CUE responses to nutrient addition and drought over two time periods a) plant CUE over 24 hours, b) 14 days, c) ecosystem CUE over 24 hours, and d) 14 days. Significance of nutrient and drought treatments assessed using likelihood ratio tests with significance indicated as: P<0.0001****, P<0.001***, P<0.01**, P=<0.05*.

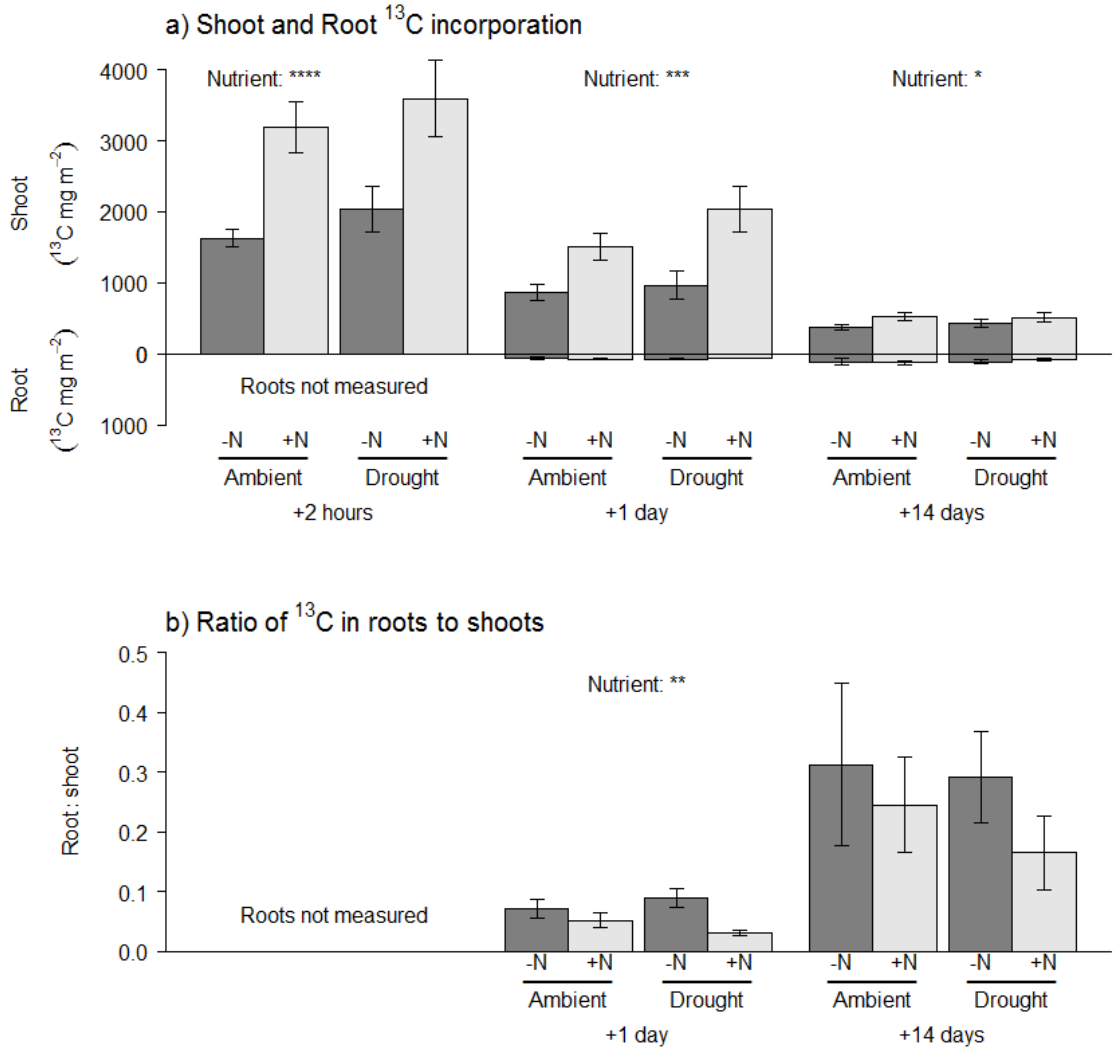

Figure 4.8 Incorporation of ^{13}C in plant shoots and roots over time ; a) shoot incorporation, above zero line, and root incorporation, below zero line, for samples taken 2 hours (shoot only), one day and 14 days after pulse labelling for each nutrient and drought treatment, b) Ratio of ^{13}C incorporation in roots to shoots, for sample taken one day and 14 days after pulse labelling for each nutrient and drought treatment. Significance of nutrient and drought treatments assessed using likelihood ratio tests with significance indicated as: P<0.0001****, P<0.001***, P<0.01**, P=<0.05*.

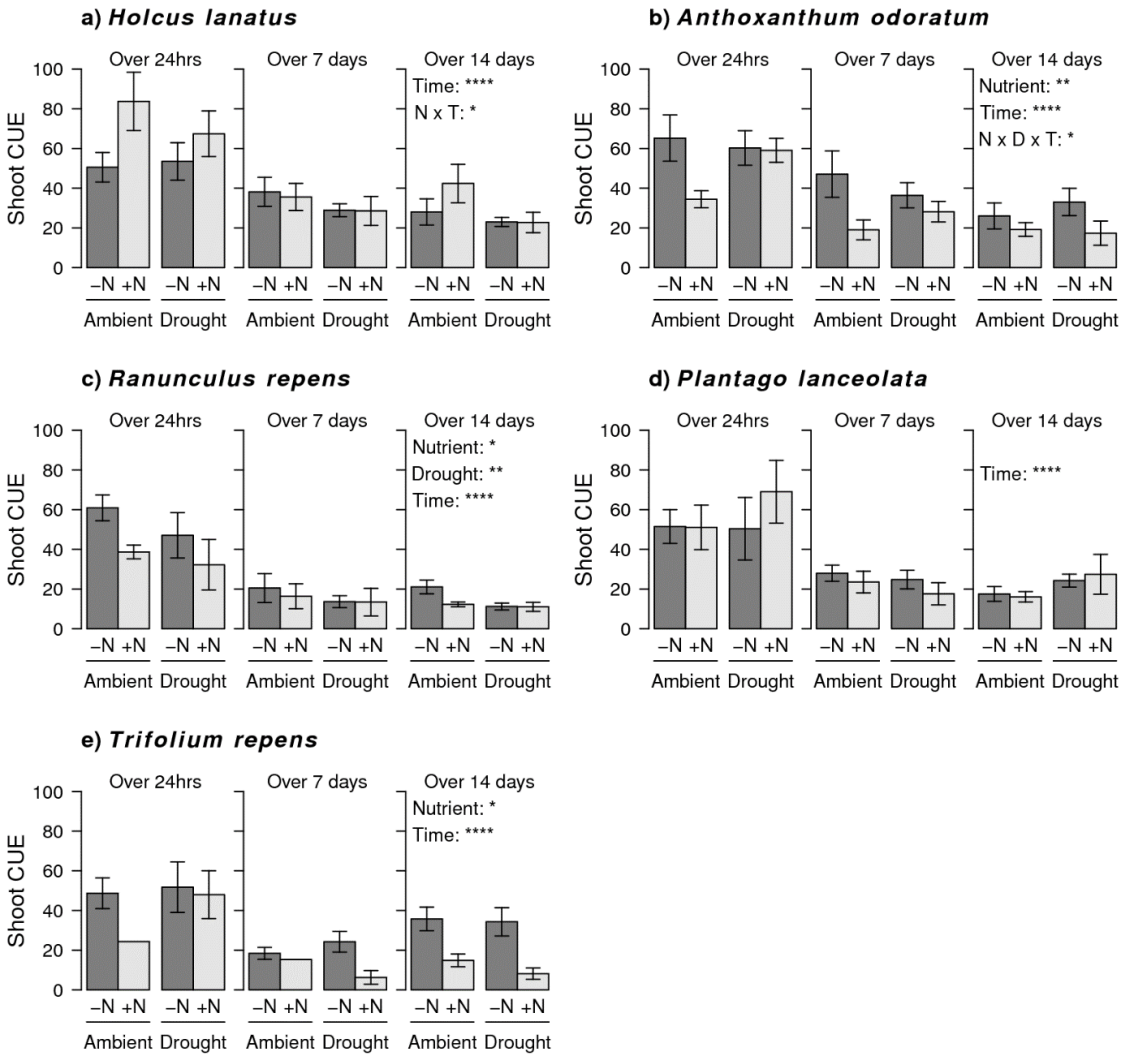

Figure 4.9 Species specific shoot CUE over 24 hours, 7 days and 14 days. Shoot CUE calculated as the % ^{13}C retained in plant biomass. Results shown for five species: a) *Holus lanatus*, b) *Anthoxanthum odoratum*, c) *Ranunculus repens*, d) *Plantago lanceolata* and e) *Trifolium repens*. Significance of nutrient and drought treatments assessed using likelihood ratio tests with significance indicated as: P<0.0001****, P<0.001***, P<0.01**, P=<0.05*.

4.4.3 Ecosystem CUE

Over 24 hours, ecosystem CUE was greater in plots receiving nutrient addition (96.5%) than those without (94.6%) nutrient addition (LRT=10.27, P=0.0014, Fig 4.7c). Ecosystem CUE was much lower when measured over 14 days, but there was no effect of nutrient addition or drought (Fig 4.7d). Ecosystem CUE positively correlated with aboveground plant CUE when measured over 24 hours and weakly correlated over 14 day (Fig 4.5c,d).

A key component of ecosystem CUE, was the amount of ^{13}C lost through ecosystem respiration. During the drought manipulation the total $C\text{-}CO_2$ ecosystem respiration flux was increased by nutrient addition and decreased by drought, although the size of the increase and decrease depended on sampling date (Nutrient x date: LRT=11.92, P=0.0358; Drought x date: LRT=19.30, P=0.0017, Fig 4.10a). However, ^{13}C content in ecosystem respiration declined very rapidly after pulse labelling (Fig 4.10b), but was consistently higher in treatments receiving nutrients (LRT=11.43, P=0.0006).

Although more $^{13}C\text{-}CO_2$ was respired in plots receiving nutrients, this did not result in lower ecosystem CUE (Fig 4.5 and 4.7). This was because plots with nutrient addition had greater plant biomass and therefore more ^{13}C stored in aboveground biomass (Fig 4.8). This greater storage of ^{13}C in plants was sufficient to offset the greater $^{13}C\text{-}CO_2$ lost through respiration and therefore ecosystem CUE was generally increased by nutrient addition (Fig 4.4).

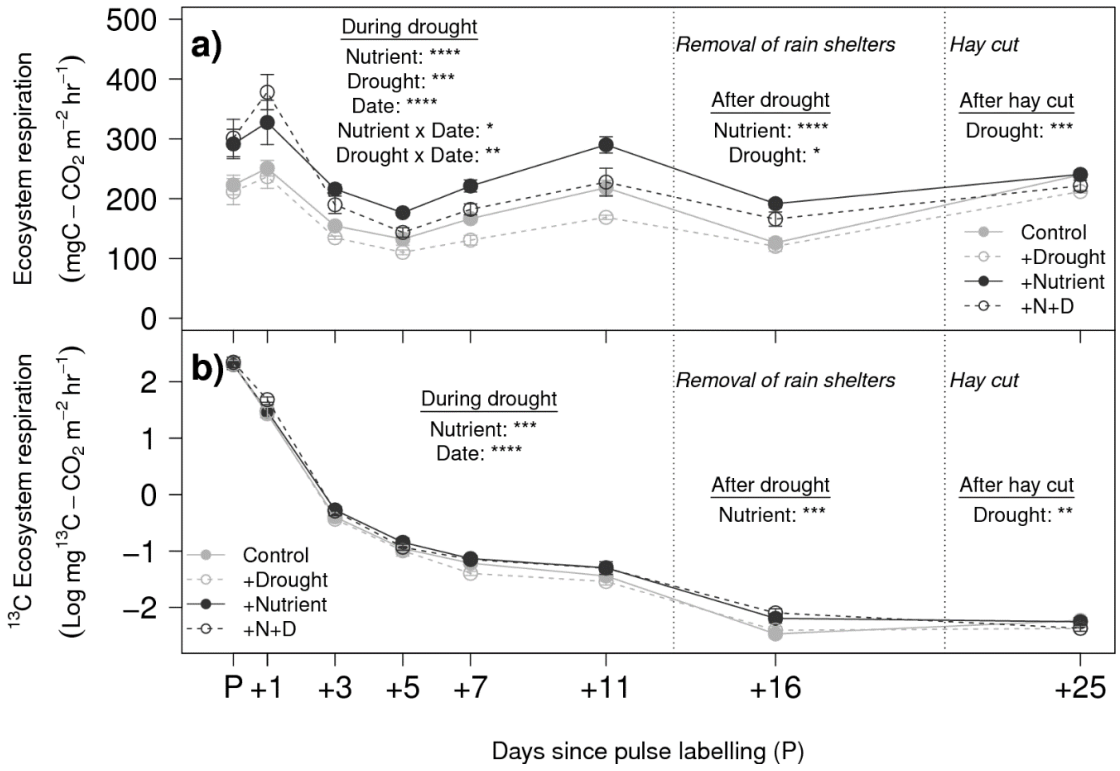

Figure 4.10 The release of CO_2 in ecosystem respiration and ^{13}C-CO_2 enrichment from field plots over the course of the experiment, a) total ecosystem respiration flux, b) total ^{13}C in ecosystem respiration as log µg ^{13}C-CO_2. Time since pulse labelling is shown in days and P is the day of pulse labelling. Sampling dates are split by vertical dotted lines into those from during the drought (left), after the drought (centre) and after the hay cut (right). Significance of nutrient, drought and date assessed using likelihood ratio tests, with significance indicated as: P<0.0001****, P<0.001***, P<0.01**, P=<0.05*.

4.5 Discussion

Many studies have considered either: microbial CUE (Frey *et al.*, 2013; Hagerty *et al.*, 2014; Spohn *et al.*, 2016b) or plant CUE (De Lucia *et al.*, 2007; Metcalfe *et al.*, 2010; Van Oijen *et al.*, 2010; Street *et al.*, 2013) in isolation. As such our understanding of how CUE may be related between plant and soil microbial communities is limited, which is particularly important as global terrestrial C models often assume constant CUE (Bradford & Crowther, 2013). The goal of this study was to test the hypothesis that in grasslands, the CUE of soil microbes and plants would decrease in response to summer drought and increase in response to nutrient addition. In contrast to our hypotheses it was found that microbial CUE increased under drought when in combination with nutrient addition, while plant CUE generally decreased with nutrient addition. As a result, there was no detectable relationship between microbial and plant CUE (Fig 4.5a,b). This suggests no counterbalance between microbial and aboveground plant CUE under changing drought and nutrient conditions.

The experimental drought increased in severity over time. Upon pulse-labelling and the measurement of microbial, plant and ecosystem CUE over 24 hours, soil moisture was reduced from 41.0% to 37.7%. This is likely to represent only a mild drought event, and potentially less severe than previous drought studies (Mirzaei *et al.*, 2008; Bloor & Bardgett, 2012). However as the experiment progressed the drought become more severe with greater differences in soil moisture (Fig 4.1a). Therefore, measurements of plant and ecosystem CUE over 14 days will represent responses to a more severe drought event.

4.5.1 Environmental controls of microbial CUE

Drought was hypothesized to decrease, and nutrient addition increase, microbial CUE. In partial support of this, drought did reduce microbial CUE in the absence of nutrient addition, but drought increased microbial CUE when in combination with nutrient addition. Additionally, soils which had been subjected to drought, had reduced microbial CUE when rewetted (Fig 4.4).

Both stoichiometric theory and previous research suggest that increasing nutrient availability should increase microbial CUE (Cleveland & Liptzin, 2007; Sinsabaugh *et al.*, 2008, 2016; Manzoni *et al.*, 2012b; Spohn *et al.*, 2016b). However our results show that this positive effect of nutrient addition on microbial CUE can be counteracted by small changes in soil moisture. In this study a reduction in soil moisture, from 41.0% to 37.7% water content, altered the effect of nutrient addition on microbial CUE. This contrasts with previous research showing that microbial CUE is generally resistant to changes in soil moisture, except in very dry soils (Herron *et al.*, 2009; Tiemann & Billings, 2011). This study shows that microbial CUE can be sensitive to changes in soil water content, and this can interact with nutrient addition.

There are a range of mechanisms that might explain the response of microbial CUE to drought and nutrient addition. The most likely mechanism underlying the increase in microbial CUE with nutrient addition and drought in combination is the accumulation of osmolytes, which microbes use to survive water stress (Borken & Matzner, 2009). To counteract dehydration and control water potential inside the cell, bacteria use amino compounds (such as proline, glutamine and glycine betaine) and fungi use polyols (such glycerol, erythritol and mannitol) as osmolytes (Csonka, 1989; Schimel *et al.*, 2007). In bacteria under water stress, solutes may account for 7 to 20% of total bacterial C, while for fungi it can be over 10% of cell mass (Koujima *et al.*, 1978; Killham & Firestone, 1984; Tibbett *et al.*, 2002). Accumulation of osmolytes enriched with ^{13}C, would increase ^{13}C in microbial biomass and explain how measures of CUE could increase under drought conditions. This is supported by the finding in this study that ^{13}C in microbial biomass was increased with the combination of drought (Fig 4.6d).

Osmoregulation may also explain why the increase in microbial CUE with drought only occurred when in combination with nutrient addition. Because bacteria and fungi differ in the N requirements associated with osmoregulation, there is potential for osmoregulation to differ depending on N availability. Solutes used by fungi, such as polyols, do not contain N, while

bacterial solutes do contain N, so that fungi will have lower N costs than bacteria in accumulating osmolytes (Schimel *et al.*, 2007). The N costs associated with osmoregulation for bacteria can be between 11% and 30% of total bacteria N (Schimel *et al.*, 2007), which suggests that in N-limited microbial communities, bacteria may be unable to meet the N cost associated with osmoregulation. This is supported by results in this study, that changes in ^{13}C assimilation in bacterial PLFAs were reduced by drought but not in fungal PLFAs (Fig 4.3). As PLFAs are found in cell membranes, this suggests that the small change in soil moisture in the drought treatment was sufficient to cause more stress in bacteria than fungi indicating a greater need for osmoregulation in bacteria than fungi. Evidence for osmoregulation being important in determining microbial CUE also comes from the prediction that a reduction in CUE would occur after rewetting due to the release of accumulated solutes (Fig 4.1; Schimel *et al.* 2007; Borken & Matzner 2009), with such a reduction found in this study (Fig 4.4). This suggests that CUE may increase under drought due to osmoregulation, particularly when bacteria can meet the associated N cost, but that microbial CUE is reduced upon rewetting as solutes are removed.

A second potential mechanism that could account for changes in microbial CUE is a change in plant root exudates in response to drought and nutrient addition. It is known that low nutrient availability can alter root system architecture and promote secretion of exudates to facilitate nutrient uptake, while mild drought may also increase secretion (Badri & Vivanco, 2009). If exudates shifted to be more labile and N-rich, microbial CUE would be expected to increase. A change in root exudates is supported by an increase in ^{13}C enrichment of microbial biomass in droughted soils at the start of incubation suggesting altered uptake of root derived substrates (Fig 4.6d). However, a change in root exudates does not explain the reduction in microbial CUE after rewetting, which occurred after plant roots were removed from the soil to limit any further C inputs. Rewetting would not have changed root exudates, but is likely to have increased substrate diffusion in the soil as water content increased (Stark & Firestone, 1995). If root exudation were entirely responsible for the change in microbial CUE, then upon rewetting

microbial CUE should have increased as microbes have more access to substrates due to increased diffusion, while in this study microbial CUE decreased upon rewetting.

Two mechanisms previously suggested for understanding microbial CUE responses to drought (Herron *et al.*, 2009), are however, unlikely to explain the response of microbial CUE in this study. First drought may alter microbial community composition, and as microbial species or functional groups can differ in their ability to cope with water stress and C utilization, there may be concomitant changes in CUE (Payne & Wiebe, 1978; Rinnan & Baath, 2009). However, this is unlikely to explain the changes in CUE found in this study as microbial community structure did not change, as assessed by total PLFA ratios and T-RFLP (Fig 4.3c; Appendix 2). A second potential mechanism is that drought will reduce substrate supply due to reduced diffusion in thin water films potentially bringing about lower CUE (Stark & Firestone, 1995). Although this reduced diffusion of substrate under drought probably occurred in this study, it is unlikely to explain the results, as microbial CUE was highest in the driest plots (those with drought and nutrient addition, Fig 4.1 and 4.2f). Therefore the mechanism explaining the response of microbial CUE in this experiment must be sufficient to overcome any negative effect of reduced substrate supply. Change in microbial CUE was therefore likely dependent on osmoregulation and the differential accumulation of solutes depending on nutrient availability.

Microbial CUE has been found to be very variable, in part due to the use of different labelled single substrates (Dijkstra *et al.* 2011; Frey *et al.* 2013; Hagerty *et al.* 2014). In this study microbial CUE was assessed using ^{13}C enrichment from labelled root exudates and potentially root turnover. Two previous studies that did not use labelled substrates, instead using ^{18}O and cell division, found microbial CUE ranged from 31% to 45% (Spohn *et al.*, 2016a,b). These estimates of microbial CUE were very similar to those in this study (38% to 50%), suggesting when methods do not rely on single substrates, microbial CUE is more restricted than the wide range of previous estimates.

4.5.2 Variation in plant CUE

We hypothesised that plant CUE would be increased by nutrient addition and decreased by drought. In contrast we found that species averaged plant CUE was resistant to changes over 24 hours and was then decreased by nutrient addition (Fig 4.7a,b). Previous research has suggested that if plant CUE is measured over weeks it is approximately 50% and invariant to environmental change, while over shorter timescales it shows greater variation (Gifford, 1995; Cheng *et al.*, 2000; Van Iersel, 2003). The estimates of plant CUE in this study, using a relatively new method (Street *et al.*, 2013), were both lower than 50% and varied depending on nutrient addition. However the change in plant CUE was almost entirely due to changes in ^{13}C retention in leaves; ^{13}C incorporation in roots was very low, although increased over time (Fig 4.8). This agrees with previous research in grasslands which found low allocation of recent photosynthate C to roots, compared with shoots, and that the proportion of C allocated belowground was not altered by drought (Hasibeder *et al.*, 2014). This, however, contrasts with research showing increased C allocation to roots under water stress (Kalapos *et al.*, 1996; Kahmen *et al.*, 2005; Backhaus *et al.*, 2014). The reduction in the proportion of ^{13}C allocation to roots with nutrient addition matches predictions that nutrient addition may reduce competition belowground for nutrient acquisition and increase competition aboveground for light (Tilman, 1990; Poorter *et al.*, 2012; DeMalach *et al.*, 2017). This suggests that changes in retention of C in leaves is most important for understanding changes in plant CUE, with only a limited role for roots, although their contribution to plant CUE increases over time.

In addition to changes in plant CUE brought about by nutrient addition over 14 days, there were also strong differences between species, which were dependent on drought, nutrient addition, and their interaction (Fig 4.9). Our findings support previous research in a neighbouring field site, that the uptake and retention of ^{13}C can depend on plant species identity with no clear plant functional group effects (De Deyn *et al.*, 2011b). However in contrast the previous research found that grassland treatments, including cessation of fertiliser application, did not alter the

retention of ^{13}C in plant biomass (De Deyn *et al.*, 2011a). In this study there were effects of nutrient or drought in four of the five species, although nutrient addition brought about both increases and decreases in CUE depending on the species (Fig 4.9). For low stature species such as *T. repens*, nutrient addition would have increased shading with potentially strong competition for light. Photosynthesis and growth in *T. repens* may have been much lower resulting in less ^{13}C retained in leaf biomass. Our results suggest differences in the retention of C in aboveground plant biomass are not due to plant functional groups, but species specific responses which also depend on the length of time over which CUE is measured.

4.5.3 Whole ecosystem CUE

As there was no evidence of counterbalancing between microbial and plant CUE, ecosystem CUE was expected to vary in response to nutrient addition and drought. As expected, ecosystem CUE did increase in response to nutrient addition over 24 hours which also correlated with aboveground plant CUE, but did not depend on drought (Fig 4.7c, 4.5c). This suggests in these grasslands, ecosystem CUE may be more strongly determined in the short term after photosynthesis by plant CUE, rather than microbial CUE (Fig 4.7c,d). Few studies have investigated ecosystem CUE, although in forests increased nutrient availability has been found to increase ecosystem CUE, although assessed using very different methods (Fernández-Martínez *et al.*, 2014).

Estimates of ecosystem CUE in this study are dependent on the size of the ^{13}C-CO_2 flux in ecosystem respiration, relative to the amount of ^{13}C retained in plant and microbial biomass (Fig 4.6, 4.8 and 4.10). However these are difficult to estimate across equivalent scales. It is necessary to interpolate ^{13}C-CO_2 ecosystem respiration fluxes, while the soil microbial biomass is hard to estimate accurately to depth. In this study assumptions had to be made regarding ecosystem respiration and soil microbial biomass. Estimates of ecosystem respiration are therefore liable to not capture the full ^{13}C dynamics over time for ecosystem respiration or in space for microbial biomass.

4.6 Conclusions

It is untested whether CUE in plants and soil microbial communities counterbalance each other in order to maintain an overall constant CUE. In this study, there was no evidence of such a counterbalance in response to nutrient addition and drought. Contrary to hypotheses drought increased microbial CUE when in combination with nutrient addition, potentially through the accumulation of solutes for osmoregulation, particularly when bacteria could meet the associated N costs. However rewetting led to an 11.8% reduction in microbial CUE suggesting increases in CUE brought about by drought will be short-term and reversible. Although drought had no effect on plant CUE when averaged across species, drought did alter CUE in specific plant species. CUE in microbial biomass and plants was therefore shown to respond differently to expected changes in climate, with small changes in soil moisture capable of bringing about significant changes in how efficiently the soil microbial community cycles C.

5 Carbon use efficiency of mosses and vascular plants differ in response to drought and nutrient addition

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5.1 Abstract

The uptake and storage of carbon (C) in grasslands can depend on the presence of particular plant functional groups. Although drought events, which are predicted to become more frequent under climate change, are known to disrupt grassland C cycling, it is unknown how plant functional groups differ in their response to drought in terms of C cycling. An important aspect of C cycling in plants is their carbon use efficiency (CUE), the proportion of C uptake which is used in new biomass. In particular mosses, which have distinct physiology and morphology, are likely to be especially sensitive to drought but also changes in nutrient availability. Yet it has been suggested that mosses have higher CUE than vascular plants. This study aims to

investigate if mosses differ in CUE to vascular plants, and if their response to drought and nutrient addition are also different. A ^{13}C-CO_2 'pulse-labelling' experiment, with both drought and nutrient addition treatments, was combined with transplanting moss communities to measure assimilation of ^{13}C-CO_2 during the experiment. Across the range of experimental treatments, moss CUE ranged from 36-86% and 12-64% depending on the method of CUE calculation. Regardless of methodology, moss CUE overlapped vascular plant CUE which ranged from 34-58% across experimental treatments. However the effect of drought and nutrient addition on moss CUE differed to that of vascular plant CUE. Moss CUE was increased under drought conditions, with no change in vascular plant CUE, while nutrient addition also increased moss CUE, but decreased vascular plant CUE. Nutrient addition decreased ^{13}C uptake in mosses by 80% suggesting less C was cycled through the moss biomass, even if that ^{13}C was retained at a higher proportion in moss biomass. This study shows that in grasslands the response of moss C cycling to drought and nutrient addition is distinct to that of vascular plants and could therefore lead to altered retention of C in moss biomass, in contrast to the rest of the plant community. However the amount of C photosynthesised by the moss community was reduced as vascular plant biomass increased, showing the extent to which mosses may impact ecosystem C storage is sensitive to the indirect effects of vascular plants.

Keywords: Bryophytes, mosses, carbon use efficiency, drought, nutrient availability, grasslands.

5.2 Introduction

Extreme climate events can alter carbon (C) cycling and the potential for C storage in terrestrial ecosystems (Chen *et al.*, 2009a; Jentsch *et al.*, 2011). However, in grasslands, the effect of climate change on C cycling and storage is likely to be dependent on the community composition (Mirzaei *et al.*, 2008; Isbell *et al.*, 2013), with particular plant functional groups responding differently drought (Knapp *et al.*, 2002; Fry *et al.*, 2013; Mariotte *et al.*, 2013; Shi *et al.*, 2014). In particular, drought events, which are predicted to increase in frequency and

severity with climate change, can reduce grassland plant productivity, photosynthesis and respiration (Kharin *et al.*, 2007; O'Gorman & Schneider, 2009; Zhao & Running, 2010). While plant functional groups might modulate effects of drought on C cycling and storage, the composition of plant functional groups in grasslands is also often affected by nutrient addition, whether from the use of fertiliser or from atmospheric depositions (Smart *et al.*, 2005; Wesche *et al.*, 2012). For example, plant species diversity was shown to be negatively related to atmospheric nitrogen (N) deposition in grasslands (Stevens *et al.*, 2004), and it is well established that the addition of inorganic fertilisers to grasslands suppresses plant species diversity and promotes more competitive grasses at the expense of slower growing herbs (Smith *et al.*, 2003; Kirkham *et al.*, 2008; La Pierre *et al.*, 2016). However, this, in turn might alter C cycle feedbacks to climate change (Vogel *et al.*, 2012). The potential interactive effects of climate and nutrient addition on grassland C cycling in different plant functional groups is not fully understood.

A distinct group of plants found in grasslands are the mosses, which can account for up to nearly 50% of aboveground biomass (Carroll *et al.*, 2000; Virtanen *et al.*, 2000; De Deyn *et al.*, 2011b). Mosses, in common with other Bryophytes, have unique physiology and ecology setting them apart from vascular plants (Turetsky, 2003; Cornelissen *et al.*, 2007; Orwin & Ostle, 2012). For example, mosses have been found to retain a greater proportion of photosynthesised C in their biomass over time than vascular plants (De Deyn *et al.*, 2011a; Street *et al.*, 2013). This suggests mosses have high carbon use efficiency (CUE), an estimate of the proportion of C used in new biomass relative to total C uptake (Manzoni *et al.*, 2012b; Sinsabaugh *et al.*, 2013). CUE is important as it can help to explain feedbacks to climate change as low CUE suggests less C storage and more respired as CO_2. However CUE is methodologically challenging to measure (Manzoni *et al.*, 2012b). For plants ^{13}C stable isotope methods have recently been used to calculate CUE, defined as the proportional decline of ^{13}C in plant biomass over a set length of time (Street *et al.* 2013; Bradford & Crowther 2013). However this method is dependent on no additional $^{13}CO_2$ being photosynthesised after the initial measurement, as this would increase

^{13}C enrichment over time. In ^{13}C 'pulse-labelling' experiments, ecosystem respiration is also enriched with ^{13}C-CO_2 (Johnson *et al.*, 2002; Ostle *et al.*, 2003; Street *et al.*, 2013), so if mosses, partly because of their low stature, photosynthesise recently respired ^{13}C-CO_2, one of the key assumptions of calculating CUE will not be met. This will have the effect of inflating estimates of CUE (Fig 5.1a). It is unknown if later uptake of ^{13}C-CO_2 by mosses in pulse-labelling experiments is responsible for the high moss CUE reported in arctic communities (Street *et al.*, 2013) and high C retention in grasslands (De Deyn *et al.*, 2011a). It is therefore unclear whether mosses do produce new biomass C more efficiently than vascular plants.

Mosses distinct physiology means that they are also very sensitive to water availability, yet desiccation tolerance allows them to survive very dry periods and undergo fast recovery upon rewetting (Proctor & Smirnoff, 2000; Turetsky, 2003; Proctor *et al.*, 2007). This sensitivity to water availability likely explains why moss cover in grasslands generally responds negatively to drought and reduced precipitation (Bates *et al.*, 2005; Ingerpuu & Kupper, 2007), while as moisture decreases, mosses in peatlands switch from net C uptake to C emission (Nijp *et al.*, 2014). The physiology of mosses therefore suggests they are likely to be sensitive to drought events, potentially shifting the proportion of C lost through respiration relative to photosynthesis. In addition to sensitivity to water availability, mosses also differ to many vascular plants in their generally low stature, altering light accessibility (Bisbee *et al.*, 2001), and their lack of roots altering nutrient and water acquisition strategies (Bates, 1994; Ayres *et al.*, 2006). In agricultural grasslands, the application of fertiliser has led to significant reductions in moss biomass as they are unable to compete with vascular plants that benefit from increased nutrient availability (Carroll *et al.*, 2000; Virtanen *et al.*, 2000; Arróniz-Crespo *et al.*, 2008). Although mosses do not have roots, through their rhizoids, some species may be able to access soil nutrients and respond with higher growth rates and efficient nutrient retention (Bates, 1994). The effect of nutrient addition on moss C cycling may therefore be mixed, and dependent on relationships with vascular plants. In particular, it is unknown how changes in nutrient availability will modulate responses to drought events in mosses.

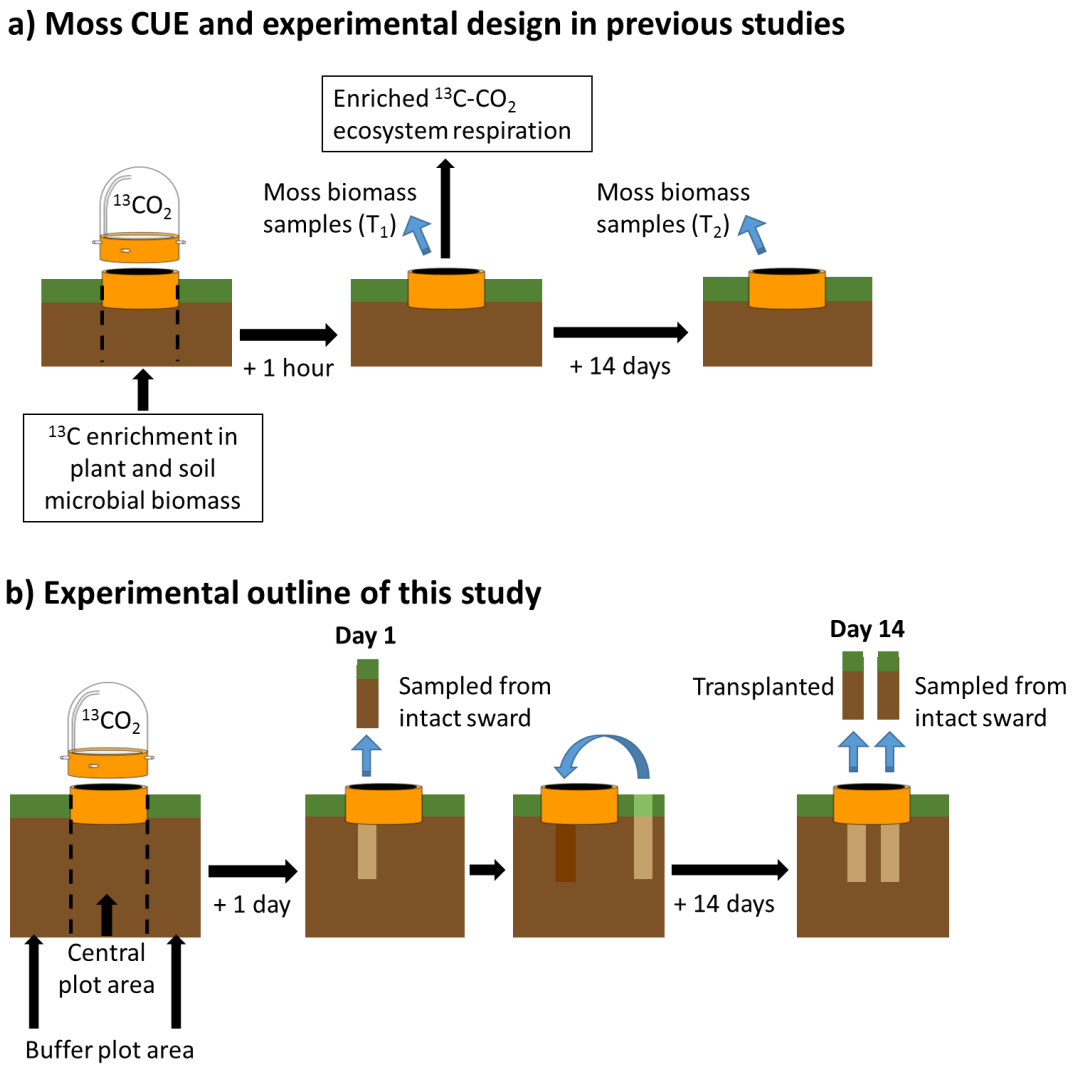


Figure 5.1 Experimental design of this study and previous research: a) Estimates of moss CUE in previous studies measure ^{13}C enrichment over time. Using this method, estimates of CUE are potentially overestimated as ^{13}C enrichment can be increased after initial pulse-labelling through uptake of recently respired ecosystem respiration; b) Experimental design showing three sets of moss and microbial biomass communities, 1) sample taken at day one, 2) transplanted communities from day 14 and, 3) in situ communities from day 14. Pulse labelling was carried out on 30 June 2014, day one samples on 1 July 2014 and transplanted and cores from the intact sward on 14 July 2014.

This study investigates how drought and nutrient addition alter C cycling and CUE in mosses and vascular plants. In particular, this study tests the following hypotheses: a) drought will reduce moss C uptake and CUE, due to moss growth being very dependent on water availability, b) nutrient addition will increase moss C uptake and CUE due to increased growth and reduced nutrient limitation, c) previous estimates of moss CUE are overestimates and that moss CUE does not differ to that of vascular plants. To test these hypotheses we established a drought-nutrient factorial experiment on species-rich temperate grassland in northern England. This was coupled with ^{13}C-CO_2 pulse-labelling and moss transplantation to investigate C uptake, turnover and CUE in mosses.

5.3 Methods

5.3.1 Experimental setup and pulse-labelling

The study was conducted at Colt Park meadows in Ingleborough National Nature Reserve, northern England (latitude 54°12'N, longitude 2°21'W) on a *Lolium perenne-Cynosorus cristatus* grassland. These grassland meadows were chosen as previous research on neighboring meadows has shown that mosses can have an important role in ecosystem carbon storage (De Deyn *et al.*, 2011a,b). The soil is a brown earth with shallow average depth (28cm) over limestone bedrock (De Deyn *et al.*, 2011b). In these grasslands, among the most common moss species are *Brachythesium rutabulum* and *Rhytidiadelphus squarrosus* (Rodwell 1993). Experimental plots were set up on a uniform grassland community which before the experiment had received the same management of autumn to early spring cattle and sheep grazing, followed by livestock removal to allow hay production, and an annual hay cut after mid-July. Four experimental treatments were implemented in a fully factorial design: control, nutrient addition, experimental drought, and a combination of nutrient addition and experimental drought (also see chapter 4). Treatments were set up in six replicate blocks, with a total of 24 plots of $4m^2$. The nutrient addition treatment involved adding propriety NPK fertiliser (50Kg N ha^{-1}, 25Kg P ha^{-1}, 25Kg K ha^{-1}) and was applied by hand on 20 May 2014. Experimental drought conditions

were created using rain shelters which intercepted all rainfall reaching the central plot region, as used by Cole *et al.* (Chapter 4). Rain shelters were open sided, constructed of transparent corrugated PVC, 0.8mm thick (Corolux, Staveley, UK), and were 90cm x 105cm in size with a height of 38cm to 63cm giving a sloped roof of 16 degrees. Rain shelters were set up on 28 May 2014 and removed on 14 July 2014. Soil moisture was assessed in each plot with a ThetaProbe soil moisture meter (Delta-T, UK). Pulse-labelling of each experimental plot with ^{13}C-CO_2 was carried out on 31 June 2014. This was done on a per plot basis by placing a transparent static chamber, 30cm in diameter, on an *in situ* base ring in the central plot area for 20 minutes and 40ml of 99.9% atom ^{13}C-CO_2 was injected into the chamber (Ward *et al.* 2009). This was repeated seven times in the day for each plot. All aboveground vegetation in the central plot area was removed 17 days after 'pulse-labelling' and was dried at 60°C for 48 hours.

The drought treatment excluded 157mm of rainfall which was equivalent to 6.5% of average annual precipitation at Colt Park meadows field site (mean: 2404mm, SD: 82mm). To assess drought severity and potential recurrence, a Gumbel I distribution was fitted to annual drought extremes representing the number of days with less than 1mm of precipitation during the primary growth period (April-September). Due to insufficient number of years of precipitation data for the field site, data from Malham Tarn field centre was used, 18km southeast of the field site, and showed that a 100-year drought equated to 27 days <1mm rainfall. Similarly data from Hazelrigg Field station, 31km southwest, showed a 100-year drought equated to 34 days of <1mm rainfall (Bloor & Bardgett, 2012). These results suggest a 100 year drought event may be between 27 and 34 days, while in this study the drought treatment was in situ for 48 days. However the drought treatment in this study may not equate to the same level of water stress as a 100-year drought event, as to avoid disturbance to the plant community, plots were not trenched so lateral flow of water in the soil will have still occurred.

5.3.2 Moss sampling and core transplantation

Moss was sampled from the intact grassland sward across all plots one day and 14 days after pulse labelling, using a 2.4cm diameter core to remove both moss biomass, and the top 10cm of soil for measurement of microbial biomass. These two sets of samples, from one and 14 days after pulse-labelling, showed the retention of ^{13}C in both moss and soil microbial biomass over two weeks. As the moss samples collected 14 days after pulse labelling were from the intact grasslands sward and had remained *in situ*, they therefore received ^{13}C from initial uptake during pulse-labelling, but also through subsequent indirect uptake of recently respired ^{13}C-CO_2.

In ^{13}C pulse-labelling experiments, mosses may photosynthesise recently respired ^{13}C-CO_2, meaning that not all ^{13}C in moss biomass is from the initial pulse-labelling and that therefore the assumptions in previously used estimates of CUE will not be met (Street *et al.*, 2013). To investigate the amount of ^{13}C in moss biomass from later photosynthesis of ^{13}C-CO_2 enriched respiration, moss was transplanted from areas of the plot which had not received pulse-labelling ('buffer region' in Fig 5.1b) to the area which had received pulse-labelling ('central region' in Fig 5.1b). The moss was transplanted one day after pulse-labelling, with the top 10cm of soil (diameter 2.4cm) and placed into the gaps in the grassland sward from the moss and soil samples collected one day after pulse-labelling (see above). Transplanted moss and soil was removed 14 days after pulse labelling and therefore only received ^{13}C from enriched ecosystem respiration and not directly from initial pulse labelling.

The experimental design produced three sets of cores as outlined above: i) samples taken from the intact grassland sward one day after pulse-labelling, termed 'day 1 intact sward', ii) samples taken from the intact grassland sward 14 day after pulse-labelling, termed 'day 14 intact sward', and iii) samples taken from transplanted moss and soil 14 days after pulse-labelling, termed 'day 14 transplanted cores'. There were 24 cores in each set, one for each field plot and ^{13}C enrichment was measured in moss biomass and soil microbial biomass in each core.

5.3.3 Harvesting and ^{13}C analysis

Moss biomass, from the intact grassland sward and transplanted cores, was frozen and freeze-dried before ball milling for ^{13}C analysis. Once dried, moss biomass was weighed to estimate how moss abundance had changed in response to nutrient and drought treatments. Microbial biomass C in soil cores was calculated using chloroform fumigation and K_2SO_4 extraction (Brookes *et al.*, 1985). Soil was sieved (2mm) to remove roots and split into two 10g subsamples. One subsample was fumigated with chloroform for 24 hours after which both subsamples were shaken with 25ml K_2SO_4. The resulting extract was run on TOC (5000A, Shimadzu, Milton Keynes, UK) for total C content before the extract was freeze-dried and ground for ^{13}C analysis.

To allow comparison of moss CUE and that of vascular plants, leaf samples were collected from five vascular plant species one day and 14 days after pulse labelling. The five plant species were selected to represent the three main vascular plant functional groups and the most abundant species in each group, grasses: *Anthoxanthum odoratum*, *Holcus lanatus*, forbs: *Ranunculus repens, Plantago lanceolata*, and legume: *Trifolilum repens*. Roots were collected from three soil cores per plot and bulked together (diameter 2.4cm), and were sampled from field plots one day and 14 days after pulse-labelling. Leaf and root samples were freeze dried and ground in preparation for ^{13}C analysis.

Moss, microbial biomass and vascular plant samples were weighed into tin capsules and combusted using an elemental analyser (CarloErba, Italy). The resulting CO_2 from combustion was analysed for $\delta^{13}C$ using isotope ratio mass spectrometry (IRMS, Dennis Leigh technology, UK). The standard deviation of $\delta^{13}C$ of duplicate samples was no more than 0.33‰ for microbial biomass and 0.88‰ for moss biomass.

$\delta^{13}C$ notation represents the ratios (R) of ^{13}C:^{12}C relative to the VPDB (Vienna Pee Dee Belemnite) standard. For microbial biomass samples $\delta^{13}C$ was given by:

$$\text{Microbial biomass } \delta^{13}\text{C} = \frac{\left(\delta^{13}\text{C}_{\text{fum}} \times \text{mgC}_{\text{fum}}\right) - \left(\delta^{13}\text{C}_{\text{unfum}} \times \text{mgC}_{\text{unfum}}\right)}{\left(\text{mgC}_{\text{fum}} - \text{mgC}_{\text{unfum}}\right)}$$

where mgC_{fum} and $\text{mgC}_{\text{unfum}}$ represent mg of C measured in fumigated and unfumigated samples respectively. δ^{13}C values were converted to isotopic ratio (^{13}C/^{12}C) for each sample (R_{sample}):

$$R_{\text{sample}} = \left(\frac{\delta^{13}\text{C}}{1000} \times R_{\text{PDB}}\right) \times R_{\text{PDB}}$$

where $R_{\text{PDB}} = 0.011237$ is the ratio for Pee Dee Belemnite. R_{sample} was converted to $^{13}\text{C}_{\text{atom \%}}$:

$$^{13}\text{C}_{\text{atom \%}} = \left(\frac{R_{\text{sample}}}{R_{\text{sample}} + 1}\right) \times 100$$

The enrichment relative to natural abundance was calculated as $^{13}\text{C}_{\text{atom \% excess}}$:

$$^{13}\text{C}_{\text{atom \% excess}} = \ ^{13}\text{C}_{\text{atom \% of sample}} - \ ^{13}\text{C}_{\text{atom \% of natural abundance}}$$

5.3.4 Calculating Carbon Use Efficiency

This study used two methods to estimate moss CUE. The first has previously been used to measure CUE in arctic mosses, and in this thesis (Chapter 4), and uses the proportional retention of ^{13}C in moss biomass over time (Street *et al.*, 2013), and in this study is termed 'proportional retention CUE'. The second method adapts the 'proportional retention CUE' measure to take into account that in pulse labelling experiments mosses may assimilate ^{13}C through recently respired ^{13}C-CO_2 after initial uptake. Estimates of CUE taking into account this indirect uptake of ^{13}C-CO_2 is termed 'adapted uptake CUE'.

The 'proportional retention CUE' estimate was calculated as:

$$\text{Proportional retention CUE} = \frac{^{13}\text{C Moss biomass}_{\text{Day 14 intact sward}}}{^{13}\text{C Moss biomass}_{\text{Day 1 intact sward}}} \times 100$$

where ^{13}C moss biomass is the mg ^{13}C in moss biomass at a given time point. Vascular plant CUE was also estimated using the 'proportional retention CUE' equation. The 'adapted uptake CUE' estimate was calculated as:

$$\text{Adapted uptake CUE} = \frac{^{13}\text{C Moss biomass}_{\text{Day 14 intact sward}} \times (1 - \hat{p}_{\text{indirect uptake}})}{^{13}\text{C Moss biomass}_{\text{Day 1 intact sward}}} \times 100$$

where $\hat{p}_{\text{indirect uptake}}$ is the proportion of ^{13}C in moss biomass which was not assimilated during pulse labelling but instead from later uptake through photosynthesis of recently respired ^{13}C-CO_2. $\hat{p}_{\text{indirect uptake}}$ is given by:

$$\hat{p}_{\text{indirect uptake}} = \frac{^{13}\text{C Moss biomass}_{\text{Day 14 transplant}}}{^{13}\text{C Moss biomass}_{\text{Day 14 } \textit{in situ}}}$$

5.3.5 Statistical analysis

The effect of nutrient addition and drought on the fate of recently assimilated ^{13}C in moss and soil microbial biomass was tested using linear mixed effects (LME) models. The fixed effects were nutrient addition, drought and the two-way interaction. The random effect was block to take account of the blocked experimental design. For all LME models assumptions of normality and equal variances were checked graphically and when necessary response variables logged to improve normality. Weight functions were used to account for unequal variances following Zuur *et al.* (2009). We determined the significance of fixed effects by comparing models with and without the factor of interest using likelihood ratio tests (LRT). All statistical analysis was carried out in the R programming language 3.3.1 (R Core Team, 2016) using the additional packages *nlme* (Pinheiro & Bates, 2013) and *plyr* (Wickham, 2011).

5.4 Results

Moss biomass averaged 62.5g m^2 but did not differ between nutrient addition or drought treatments (Fig 5.2a). Aboveground vascular plant biomass was increased from 231.7g m^2 to 491.2g m^2 in plots with nutrient addition (LRT=25.02, P<0.0001, Fig 5.2b), for which there was a 2% reduction in soil moisture (LRT=4.80, P=0.0284, Fig 5.2c). The drought treatment significantly reduced soil moisture from an average of 47.6% to 28.0% (LRT=62.31, P<0.0001, Fig 5.2c) although drought did not alter aboveground vascular plant biomass (LRT=0.96, P=0.3267, Fig 5.2b).

The uptake and incorporation of ^{13}C in moss biomass was significantly reduced in plots with nutrient addition (Fig 5.3). Reduced ^{13}C incorporation was found one day after pulse-labelling in moss in the intact grassland sward (LRT=28.30, P<0.0001, Fig 5.3a), 14 days after pulse labelling in the intact grassland sward (LRT=17.40, P<0.0001, Fig 5.3b) and 14 days after pulse labelling in transplanted moss communities (LRT=11.61, P=0.0007, Fig 5.3c). Drought did not alter incorporation of ^{13}C in moss biomass although the generally lower reduction in enrichment one day after pulse labelling was marginally non-significant (LRT=3.66, P=0.0556, Fig 5.3a).

The ^{13}C incorporation in soil microbial biomass, sampled from intact grassland soil, was temporarily reduced in nutrient addition plots: one day after pulse labelling, nutrient addition had no effect on ^{13}C in soil microbial biomass (Fig 5.4a), however after 14 days ^{13}C in soil microbial biomass was lower in plots with nutrient addition (LRT=4.37, P=0.0366, Fig 5.4b). Drought had a longer lasting effect on ^{13}C incorporation in soil microbial biomass, increasing enrichment one day (LRT=22.83, P<0.0001, Fig 5.4a) and 14 days after pulse labelling (LRT=8.66, P=0.0032, Fig 5.4b).

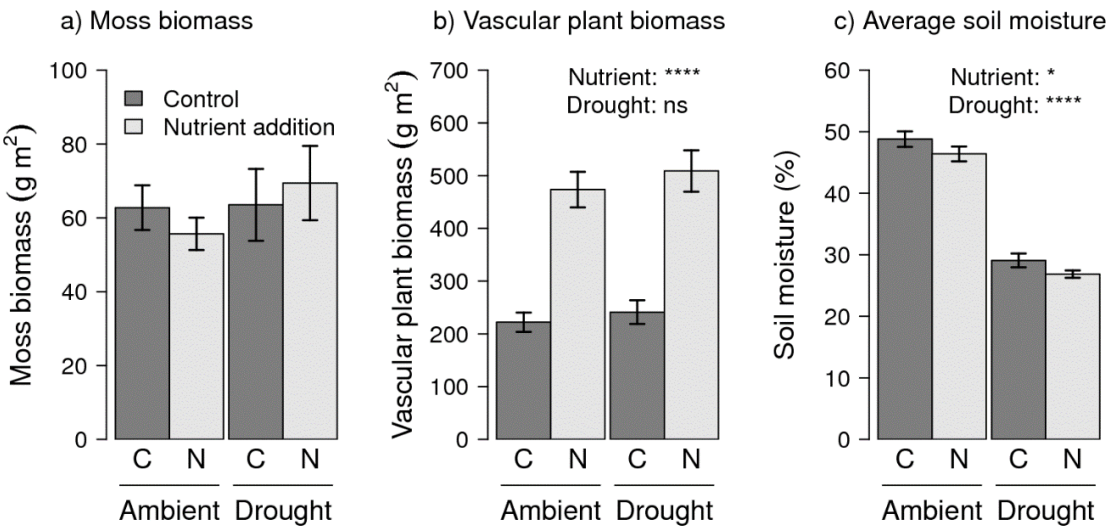


Figure 5.2 Grassland characteristics in response to drought and nutrient addition on, a) moss biomass, b) vascular plant biomass, c) average field plot soil moisture over the duration of the experiment. Significance of nutrient and drought treatments assessed using likelihood ratio tests where: P<0.0001****, P<0.001***, P<0.01**, P<0.05*, not significant=ns.

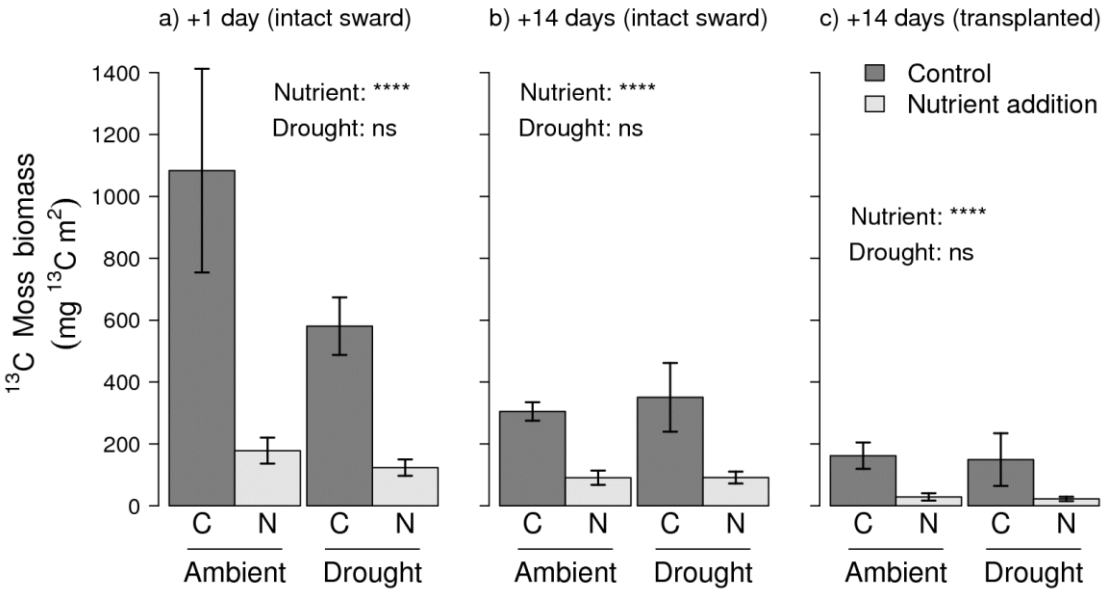


Figure 5.3 Enrichment of ^{13}C in moss biomass measured in samples: a) 1 day after pulse labelling from the intact sward, b) 14 days after pulse labelling from the intact sward, c) 14 days after pulse labelling from transplanted moss communities. Significance of nutrient and drought treatments assessed using likelihood ratio tests where: P<0.0001****, P<0.001***, P<0.01**, P<0.05*, not significant=ns.

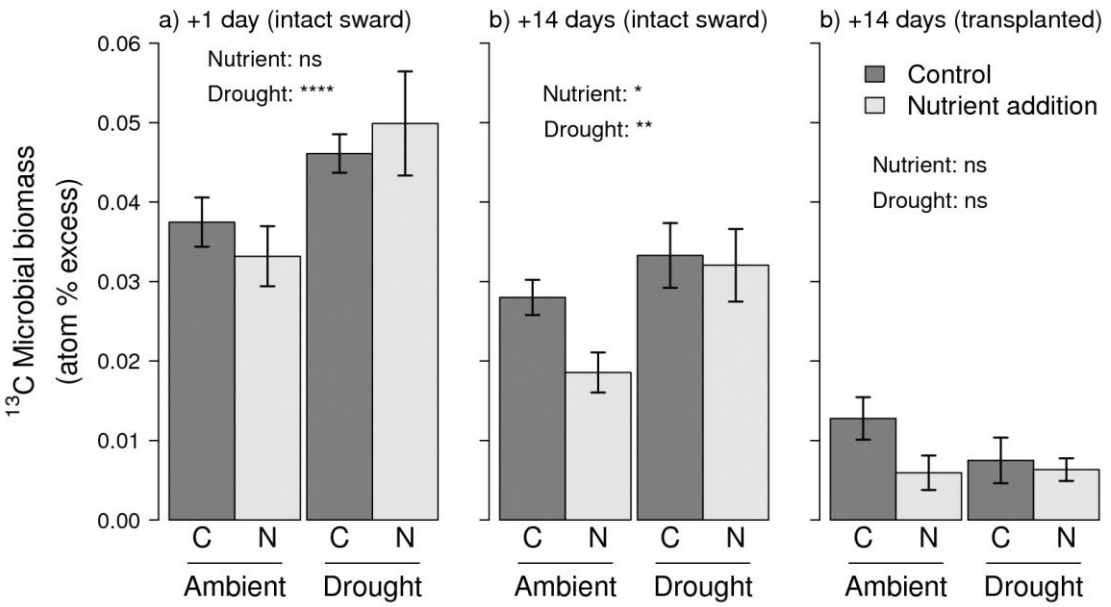


Figure 5.4 Enrichment of ^{13}C in microbial biomass measured in ^{13}C atom % excess from samples: a) 1 day after pulse labelling, b) in situ soil cores 14 days after pulse labelling, c) transplanted soil cores 14 days after pulse labelling. Significance of nutrient and drought treatments assessed using likelihood ratio tests where: P<0.0001****, P<0.001***, P<0.01**, P<0.05*, not significant=ns.

5.4.1 Estimates of Carbon Use Efficiency

Carbon use efficiency in mosses was estimated using two methods: proportional retention CUE and adapted uptake CUE. The proportional retention CUE estimate for mosses, using samples taken from the intact grassland sward, was significantly increased from 46.4% under ambient conditions to 70.2% under drought (LRT=4.36, P=0.0369, Fig 5.5b), however the general increase with nutrient addition was not significant (LRT=3.14, P=0.0766). Adapted uptake CUE was on average 20.7 percentage points lower than proportional retention CUE and was increased independently in plots with nutrient addition (LRT=5.65, P=0.0174) and drought (LRT=4.42, P=0.0356, Fig 5.5c). The adapted uptake CUE estimate takes into account that on average 38.7% of the ^{13}C in moss biomass did not come directly from the initial pulse labelling but from later photosynthesis of recently respired $^{13}C\text{-}CO_2$ from surrounding vegetation and soils (Fig 5.5a). Vascular plant CUE, estimated using proportional retention CUE, was decreased from 53.2% to 38.2% with nutrient addition, averaged across five species (LRT=4.22, P=0.0399, Fig 5.6).

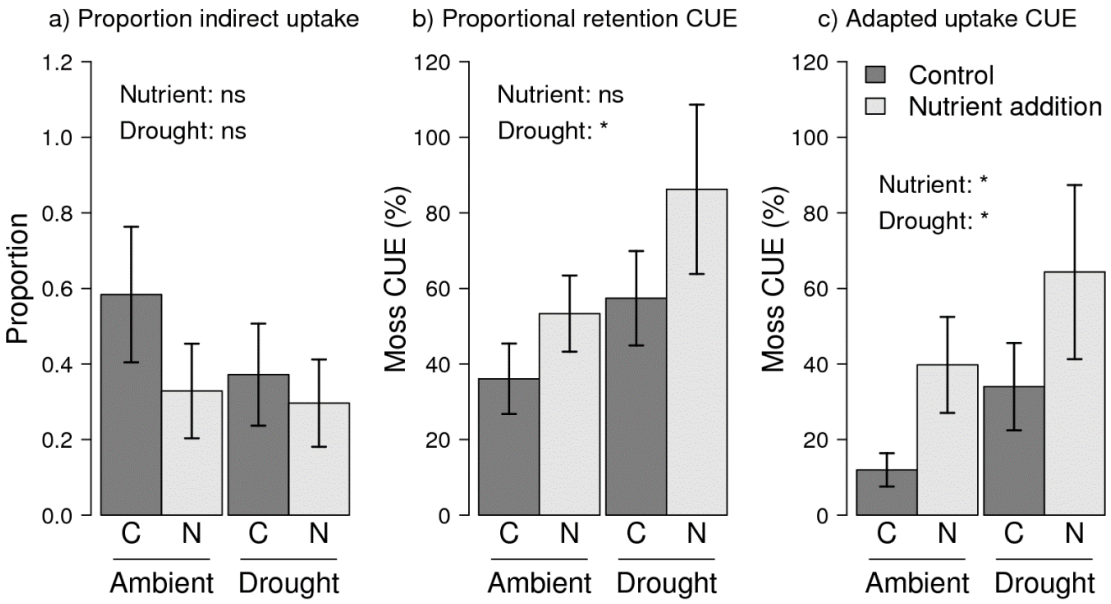


Figure 5.5 Proportion of C indirectly taken up, and estimates of CUE: a) percentage of ^{13}C in transplanted moss biomass relative to moss biomass from the intact sward, showing uptake of ^{13}C not from initial pulse labelling but from ^{13}C-CO_2 recently respired from surrounding plants and soil, b) Percentage of ^{13}C remaining in moss biomass in the intact sward over 14 days, termed 'proportional decline CUE', c) percentage of ^{13}C remaining in moss biomass over 14 days, but correcting for the indirect uptake of ^{13}C estimated in panel a, termed 'adapted uptake CUE'. Significance of nutrient and drought treatments assessed using likelihood ratio tests where: P<0.0001****, P<0.001***, P<0.01**, P<0.05*, not significant=ns.

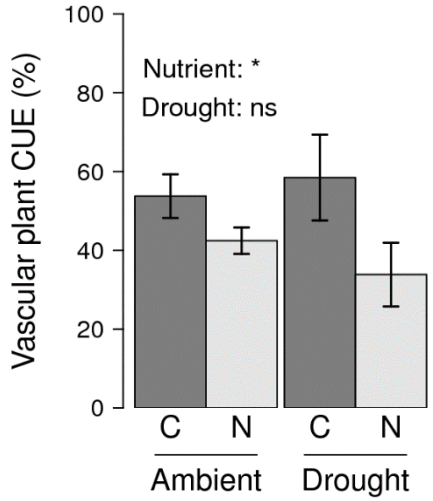


Figure 5.6 Vascular plant CUE in response to drought and nutrient addition, estimated using the proportional decline CUE estimate. C=control plots, N=nutrient addition. Significance of nutrient and drought treatments assessed using likelihood ratio tests where: P<0.0001****, P<0.001***, P<0.01**, P<0.05*, not significant=ns.

5.5 Discussion

The overall aim of this study was to investigate the effect of drought and nutrient addition on C cycling in mosses and whether it differed to C cycling in vascular plants. Estimates of moss CUE depended on CUE methodology (36-86% and 12-64%) and overlapped estimates of vascular plant CUE (34-58%). However the response of mosses to drought and nutrient addition was different to vascular plants: moss CUE was increased by drought and nutrient addition, while vascular plant CUE was not changed by drought but decreased by nutrient addition (Fig 5.5 and 5.6). Drought and nutrient addition had independent effects on moss C cycling, with no evidence of an interaction.

5.5.1 Estimates of CUE

It has been suggested that mosses may retain a greater proportion of photosynthesised C in biomass than vascular plants and therefore have higher CUE (De Deyn *et al.*, 2011a; Street *et al.*, 2013). In this study it was hypothesised that these high moss CUE values may be overestimates because in ^{13}C-CO_2 pulse-labelling experiments, mosses photosynthesise ^{13}C indirectly after initial pulse-labelling from recently respired ^{13}C-CO_2 from enriched vegetation and soils. This leads to the appearance of a greater retention of initial ^{13}C uptake during pulse-labelling experiments which is not accounted for in commonly used CUE estimates. In this study 38.7% of the ^{13}C in moss biomass at the end of the experiment did not come from the initial pulse-labelling, but from later uptake (Fig 5.5a). This was estimated using the proportion of ^{13}C in transplanted moss biomass relative to ^{13}C in moss biomass which had remained intact in the grassland sward. As such, estimates of moss CUE using proportional decline were 20.7 percentage points higher than adapted uptake CUE estimates. The proportional decline method has previously been used to estimate CUE in arctic mosses at 62-81% over 19 days, which was greater than that for a community with vascular plants (Street *et al.*, 2013). However, the results from this study suggest that estimates of moss CUE in pulse-labelling experiments should account for uptake of ^{13}C after the initial pulse labelling.

Both calculations of moss CUE in this study fall within those of vascular plants when considered across the range of experimental treatments (Fig 5.6). However in control plots moss CUE was 18 to 42 percentage points lower than vascular plant CUE. For plants, it is often thought that 50% of photosynthetic C is lost through respiration, and that the ratio of respiration to photosynthesis is invariant, even under changes in temperature and CO_2 concentrations (Gifford, 1995; Dewar *et al.*, 1998; Cheng *et al.*, 2000; Van Oijen *et al.*, 2010). Although this ratio has also been found to increase with forest age (DeLucia *et al.*, 2007), depend on crop species (Albrizio & Steduto, 2003) and decrease with size of plants (Van Iersel, 2003). Estimating plant CUE using the retention of ^{13}C differs to the methods used in most previous studies, and will require further investigation across a range of plant species to investigate how results may differ to previous estimates of plant CUE. The results in this study suggest that vascular plant CUE is sensitive to changes in nutrient addition, but that the effect of drought and nutrient addition on CUE can differ between mosses and vascular plants.

This study focusses on C cycling in mosses and vascular plants from one to 14 days after pulse labelling. Previous research suggests moss ^{13}C enrichment often peaks one day after pulse labelling, while after two weeks most of the labile C is either respired or allocated to growth (De Deyn *et al.*, 2011a; Street *et al.*, 2013). Not including the initial 24 hours after pulse-labelling will result in higher estimates of CUE as the initial rapid decline in enrichment is not included, however this method therefore investigates longer-term C cycling, rather than being heavily dependent on C cycling over the first 24 hours. The comparisons made in this study between mosses and vascular plants therefore all use the same time scale.

5.5.2 C cycling responses to drought

In this study it was hypothesised that moss C uptake and CUE would be reduced by drought due to their sensitivity to water availability. While drought did not alter ^{13}C uptake, moss CUE was higher under drought conditions for both CUE estimates (Fig 5.5b,c). Although moss CUE has not previously been measured in response to drought, these results are in contrast to previous

research which shows respiration in mosses is maintained at lower water potentials than photosynthesis, suggesting drought could increase respiratory losses relative to photosynthesis and therefore lower CUE (Proctor *et al.*, 2007). Other research shows that drought tends to have a negative effect on moss growth, for example during dry conditions mosses in peatlands switch from net C uptake to emission (Nijp *et al.*, 2014), while drought tends to reduce moss percentage cover (Bates *et al.*, 2005) with similar responses to inter-annual rainfall variation (Ingerpuu & Kupper, 2007).

Despite the general negative effect of drought on moss growth in previous studies, there are several factors which may influence moss CUE and explain our result that drought increased moss adapted uptake CUE. Nine years of drought in a Mediterranean forest increased C content in moss biomass, potentially due to changes in sclerophylly (Sardans & Peñuelas, 2008). This suggests the increase in moss CUE in this study could be due to increased overall C content in response to drought. However summer drought events, as used in this study, may have less of an effect on moss growth than expected. First, it has been hypothesised that mosses may get much of their moisture from dewfall (Bates *et al.*, 2005), such that the drought manipulation used in this study may have had minimal effects on water availability for moss growth. Second, the primary growth period for mosses may be during wetter periods, outside the growth period for many vascular plants, and therefore outside the time period of summer drought events (Bates *et al.*, 2005). Even if mosses did rely on dewfall and were not in their primary growth period during this study, neither consideration would explain why moss CUE increased in drought plots. This suggests there may be an important mechanism controlling moss growth when water availability is low which increases the retention of C over time and which could be related to increases in overall C content.

Water stress has previously been found to reduce ^{13}C incorporation in mosses (Rice & Giles, 1996; Williams & Flanagan, 1996; Rice, 2000). The increase in C isotope discrimination as water content decreases, has allowed $\delta^{13}C$ to be used as a measure of the water availability for

mosses at a given site or season (Deane-Coe *et al.*, 2015). This potential change in moss ^{13}C discrimination under drought is unlikely to alter the results in this study. Firstly, measures of natural abundance $\delta^{13}C$ revealed no difference between control (mean=-28.04‰, SD=0.71) and drought treatments (mean=-28.06‰, SD=0.67). Secondly, conclusions regarding CUE depend on the proportional retention of ^{13}C in moss biomass, so that any difference in discrimination will not alter estimates of CUE as it controls for differences in initial ^{13}C uptake.

Transfer of ^{13}C to the microbial community was increased under drought conditions, suggesting greater transfer of ^{13}C to roots which subsequently becomes accessible to soil microbes (Fig 5.4). ^{13}C in the microbial biomass from the intact grassland sward could come from root exudation from all plant species. The very low enrichment in transplanted cores, which were not directly pulse-labelled with ^{13}C-CO_2, suggests some ^{13}C from other indirect sources, which could either be from mosses or lateral flow of C belowground from surrounding soil into the transplanted soil. Drought has generally been found to increase the proportion of recently assimilated C which is allocated to roots (Sanaullah *et al.*, 2012a; Burri *et al.*, 2014), or that the proportion transferred is maintained under drought (Hasibeder *et al.*, 2014). The role of mosses transferring C belowground is not fully understood, although rewetting mosses after desiccation can leach sufficient carbohydrates to support mycorrhizal growth (Turetsky, 2003). In this study it is unclear whether mosses released ^{13}C with subsequent uptake by the soil microbial community, or if the ^{13}C was from lateral movement of C in the soil.

5.5.3 C cycling responses to nutrient addition

Contrary to expectations, C uptake in mosses was reduced in nutrient addition plots (Fig 5.3), however this may have been brought about by changes in vascular plant biomass. Nutrient addition doubled aboveground vegetation (Fig 5.2b), and likely increased shading of low stature mosses, potentially leading to lower moss photosynthesis and the observed lower C uptake. A negative effect of nutrient addition on moss growth is supported by research in grassland communities where mosses have been found to decline following increased N deposition or

fertilisation as they are likely unable to compete with vascular plants (Carroll *et al.*, 2000; Virtanen *et al.*, 2000; Arróniz-Crespo *et al.*, 2008).

Although nutrient addition decreased moss C uptake, it was associated with increased CUE (Fig 5.5). Some moss species exposed to a pulse of nutrients can have efficient nutrient uptake and fast growth (Bates, 1994), suggesting moss CUE could be increased, by nutrient addition, and therefore supporting the results in this study. However, in a neighbouring field site ^{13}C retention in moss biomass, an estimate of CUE, did not differ with cessation of fertiliser addition (De Deyn *et al.*, 2011a). This suggests that any effect of nutrient addition on moss CUE may differ between initial application and long-term annual application or cessation. More broadly, these results follow findings in a range of systems that removing nutrient limitation increases CUE (Sinsabaugh *et al.*, 2008; Manzoni *et al.*, 2012b; Vicca *et al.*, 2012). Overall, this study found that with nutrient addition less C is cycled through the moss community, however the C that is cycled is done so more efficiently with a greater proportion retained in moss biomass over time.

5.5.4 Conclusions

This study suggests that the unique physiology and morphology of mosses results in differences in C cycling in response to nutrient addition and drought events, and that the moss CUE responses to these environmental drivers differ to vascular plant CUE responses. Increasing nutrient addition was associated with higher moss CUE but lower vascular plant CUE, while drought increased moss CUE, but had no effect on vascular plant CUE. The overall level of moss CUE depended on the method of estimation as, in a pulse-labelling experiment, 38.7% of C in moss biomass can come from enriched ^{13}C-CO_2 which has been recently respired by plants or soil. Using a new measure of CUE, which takes this into account, moss CUE varied from 12% to 64% across treatments, overlapping the range in vascular plants (35-60%). Under climate change, mosses therefore have the potential to alter C cycling in grasslands, particularly by increasing C retention in response to drought, in contrast to vascular plants, and especially when mosses account for a large proportion of aboveground biomass.

6 General Discussion

It is predicted that with climate change, precipitation regimes will become more extreme, and for the UK this may include summer drought where rainfall is up to 60% below average (Met Office, 2015). How plants and soils respond to such water stress will determine the ability of grassland communities to maintain agricultural production, continue to sequester carbon (C), and retain nutrients. This thesis has focussed on understanding how C cycling in plants and soil microbial communities responds to drought and subsequent rewetting, but also vary with changes in nutrient availability, as a result of changes in agricultural practices.

Four key findings emerged from these studies. First, plant productivity was resistant to drought in field studies, but not in the glasshouse study. Second, drought reduced CO_2 fluxes, but only occasionally changed the balance of respiration relative to C uptake. Third, the effect of drought and nutrient addition on plant carbon use efficiency (CUE) was species specific. Fourth, both soil microbial community function and structure were altered by drought and changes in nutrient availability. These overarching findings are discussed in the context of previous research, followed by a discussion of the potential for further research.

6.1 Plant productivity was resistant to summer drought in field experiments

This thesis found that plant productivity was resistant to drought events in two field experiments (Chapter 2, 4 and 5). In contrast, in a glasshouse experiment, the reduced soil moisture treatment caused a decline in plant biomass in both monocultures and four-species mixtures (Chapter 3). It is known that plant productivity increases with precipitation; globally, aboveground net primary productivity (ANPP) increases as mean annual precipitation (MAP) increases, while at a given site ANPP will also increase in years with greater precipitation (Estiarte *et al.*, 2016). Despite this, plant productivity responses to experimental drought are mixed. Drought has both reduced plant productivity (Wang *et al.*, 2007; Vogel *et al.*, 2012; Isbell *et al.*, 2015), and had no effect (Mirzaei *et al.*, 2008; Jentsch *et al.*, 2011; Carter & Blair, 2012). Given the results in

this thesis, it may be predicted that the reduced soil moisture treatment in the mesocosms experiment brought about greater water stress for plants than the field drought manipulations. Yet, this thesis showed that plant productivity in species-rich temperate grasslands was consistently resistant to two summer droughts of contrasting number of days.

This thesis has investigated drought events by using rain-out shelters to experimentally manipulate precipitation in the field (Chapter 2, 4 and 5). Rain-out shelters are a widely used technique, which can effectively intercept rainfall (Beier *et al.*, 2012), although shelters can alter radiation reaching the plants and may have small effects on air and soil temperatures (Vogel *et al.*, 2013). The use of rain-out shelters, designed and built for this thesis, included a roofed control treatment to check for unanticipated artifacts (Chapter 2). These roofed control shelters had no detectable effect on air and soil temperatures, plant biomass or CO_2 fluxes, suggesting they are effective for studying drought events (Chapter 2). The severity of drought brought about by the rainout shelters likely differed between field experiments. Rain-out shelters intercepted rainfall for 35 days (Chapter 2) and 48 days (Chapter 4 and 5), which were both longer than predicted 100-year recurrence drought events, based on long-term data from nearby weather stations. However, to avoid disturbing the plant community, drought plots were not trenched to isolate them hydrologically from surrounding soil. This means the drought severity will likely have been less severe than if trenching had been carried out. However the field drought manipulations were severe enough to change plant C:N, CO_2 fluxes, soil microbial community structure, microbial biomass C and nitrogen (N), and plant and soil microbial CUE (Chapter 2, 4 and 5). In contrast, the reduced soil moisture treatment in the glasshouse experiment, may have imposed greater water stress on plants, compared with the field drought experiments. This is supported by the results that plant biomass was reduced in the glasshouse experiment (Chapter 3), but not in the field experiments (Chapter 2, 4 and 5).

It has been suggested that resistance of plant productivity to drought increases with species richness (Isbell *et al.*, 2015), decreases with increasing pre-drought plant biomass (Wang *et al.*,

2007), and is altered by management intensity (Vogel *et al.*, 2012). In the glasshouse mesocosm experiment, where reduced soil moisture did lead to a reduction in plant biomass, the size of the reduction in plant biomass did not depend on the level of nitrogen addition or shade conditioning and with only a very marginal difference between species (Chapter 3). The relative importance of initial plant biomass, species diversity or species identity in determining resistance of plant productivity to drought is still unclear for grasslands with real-world levels of species diversity and agricultural management.

6.2 Drought reduced CO_2 fluxes, but only occasionally changed the balance of respiration relative to C uptake

Drought reduced ecosystem respiration across all field and mesocosm studies (Chapter 2, 3 and 4), and also reduced net ecosystem exchange (NEE) in the mesocosm study (Chapter 3). In the mesocosm experiment, reductions in NEE were greater than reductions in ecosystem respiration, suggesting greater reductions in photosynthesis than ecosystem respiration (Chapter 3). In contrast experimental drought in the field experiment led to no change in NEE, despite reductions in ecosystem respiration, suggesting similar declines in both respiration and photosynthesis (Chapter 2). These ratios of respiration to photosynthesis are particularly important as they can determine the amount of C not lost in respiration which is then available for longer term C storage in either plants or soil (Bradford & Crowther, 2013). Previous research of drought has shown both an overall balancing of reductions in photosynthesis and respiration (Fry *et al.*, 2013), but, more commonly, greater reductions in NEE compared with respiration, suggesting proportionally more C is lost through respiration (Wu *et al.*, 2011; Bloor & Bardgett, 2012; Li *et al.*, 2016). It is likely that as drought and water stress became more severe, as may have been the case in the glasshouse mesocosm experiment (Chapter 3), the balance shifted so that more C was respired by plants relative to C uptake. This is because respiration tends to continue, or even increase under water stress, while photosynthetic machinery is more sensitive to water availability (Flexas *et al.*, 2006). The results in this thesis suggest that as water stress

becomes more severe, proportionally more C will be lost in respiration, from plants and soils, relative to C uptake.

The potential feedbacks to climate, through changing the ratio of C uptake to respiration, were also investigated through estimating ecosystem CUE. This is a measure of the CUE in both plants and soil microbes, and indicates how efficiently carbon is used in new biomass relative to total C uptake (Chapter 4). Assessed over 24 hours and 14 days, ecosystem CUE was not changed by drought. In contrast to results from CO_2 fluxes in chapters 2 and 3, this suggests that the effect of drought may not regularly change the proportion of C lost in respiration, and therefore have less chance of C losses having a positive feedback to climate change. Few studies have investigated ecosystem CUE, although in forests increased nutrient availability has been found to increase ecosystem CUE, although assessed using very different methods (Fernández-Martínez *et al.*, 2014). This thesis is the first time ecosystem CUE has been calculated using ^{13}C-CO_2 stable isotope techniques, but suggests a promising approach to understand whole community responses to environmental change and potential feedbacks to C cycling and climate.

6.3 Plant CUE responses to drought and nutrient addition were species specific

The proportional retention of ^{13}C in plant biomass was used to estimate plant CUE, which showed species-specific responses to drought and nutrient addition (Chapter 4 and 5). Previous research on a neighbouring long-term grassland restoration experiment showed that while ^{13}C retention did not depend on grassland restoration practices, there were species-specific differences in retention of C in plant biomass (De Deyn *et al.*, 2011a). This thesis confirms such species-specific differences, but in contrast to previous research also shows variability in plant CUE in response to both drought and nutrient addition.

Estimating plant CUE using the proportional retention of ^{13}C, follows work in the arctic dwarf shrub community (Bradford & Crowther, 2013; Street *et al.*, 2013). In this thesis, estimates of

plant CUE over two weeks tended to be lower (typically under <40%; Chapter 4) than arctic dwarf shrub community (58-74%; Street *et al.* 2013), despite this study measuring CUE over a shorter length of time. Using the proportional retention of ^{13}C to estimate CUE, relies on the assumption that no additional ^{13}C-CO_2 is photosynthesised after initial pulse-labelling. However, this thesis shows that in mosses this later photosynthesis of ^{13}C-CO_2 led to overestimates of moss CUE (Chapter 5). As it was not possible in this thesis to account for later uptake of enriched respiration in vascular plants (Chapter 4 and 5), CUE estimates for vascular plants may be overestimates. However any overestimation of CUE is most likely for low stature plants, such as mosses, as ^{13}C-CO_2 enrichment from plants and soil is likely to be greater near the soil surface. This thesis is the first time the proportional retention of ^{13}C in plant biomass has been used to investigate the effect of climate change on plant CUE, and shows that plant CUE responses to drought vary between species. However this thesis also shows assumptions in plant CUE calculations must be taken into account.

6.4 Both soil microbial community function and structure were altered by drought and changes in nutrient availability

Soil microbial community structure was sensitive to drought and long term-restoration treatments (Chapter 2), but less sensitive to short-term nutrient addition (Chapter 4). For example, drought increased fungal PLFA concentrations but did not change bacterial PLFA concentrations in response to long-term cessation of fertiliser addition, used to restore plant species diversity (Chapter 2). Additionally upon rewetting microbial biomass C and N recovered more rapidly than did community structure, suggesting elements of C and N cycling can recover before the microbial community structure has fully recovered (Chapter 2). In contrast, short-term nutrient addition, applied at a higher rate than in Chapter 2, had no effect on overall soil microbial structure (Chapter 4). This supports the general findings that fungi are more resistant than bacteria to drought, but that there can be a large range of responses to water stress (Yuste *et al.*, 2011; De Vries *et al.*, 2012a).

The primary measure used for microbial community function was microbial CUE, which was primarily increased by drought in combination with nutrient addition. Previous research suggests microbial CUE only changes in very dry soils (Herron *et al.*, 2009; Tiemann & Billings, 2011), and increases after rewetting (Zeglin *et al.*, 2013a). In contrast this thesis shows microbial CUE was sensitive to relatively small changes in soil moisture, and then decreases upon rewetting (Chapter 4). The changes in microbial CUE were likely to be due osmoregulation in response to water stress rather than changes in overall community composition (Chapter 4). However, the results did suggest that differences in solutes used for osmoregulation between bacteria and fungi may have been important to determining the interactive effect between drought and nutrient addition without an overall change in soil microbial community structure (Chapter 4). This thesis confirms the challenge in understanding how microbial community structure influences community function (Thiele-Bruhn *et al.*, 2012; You *et al.*, 2014), yet suggests differences in microbial physiology and morphology are important for determining responses to drought and nutrient addition even when no overall change in community composition is detected.

Changes in microbial biomass and microbial CUE are particularly important for potential feedbacks to climate change. Microbial biomass was reduced by drought, although this response was dependent on plant community composition (Chapter 2), while microbial CUE was reduced by drought without nutrient addition, and increased with nutrient addition (Chapter 4). Evidence is growing for the key role of soil microbes in processing plant inputs and ultimately synthesising soil organic matter (SOM; Schmidt *et al.* 2011; Kallenbach, Grandy & Frey 2016). Both the size of the microbial biomass and the efficiency with which it produces new biomass are likely to be important (Manzoni *et al.*, 2012b; Miltner *et al.*, 2012; Lange *et al.*, 2015; Kallenbach *et al.*, 2016). This thesis suggests that the effect of drought on microbial biomass and activity and its potential influence on SOM formation is likely to depend on the complex interplay between plant community composition and level of nutrient availability.

6.5 Conclusion

Drought events can have widespread impacts on grassland plants and soils. This thesis found that although plant productivity was generally resistant to drought, there were changes in grassland biogeochemical cycles and changes in soil microbial community structure and function. Many of the changes brought about by drought differed depending on grassland management, with an important role for long-term grassland restoration treatments, which altered the effect of drought on CO_2 fluxes, microbial biomass C and microbial community composition, while short-term nutrient addition altered the effect of drought on microbial CUE and species-specific plant CUE. Plant C cycling responses to drought differed depending on plant functional group, species identity and level intraspecific trait variation, showing that the response of grassland biogeochemical cycles to drought can be modulated by differences within and between species. Changes in microbial community function in response to drought and nutrient addition were likely related to differences in morphology and physiology between bacteria and fungi potentially through differences in osmoregulation. Overall, this thesis shows the mechanisms by which drought may alter C cycling and feedbacks to climate are complex, but at least in part, depend on nutrient availability.

6.6 Further work

This thesis has investigated the effect of drought and changes in nutrient availability on plants and soil microbial communities in species-rich temperature grasslands. The research raises several questions and challenges which would benefit from further research.

First, climate change is predicted to not only change precipitation regimes, but also temperature and CO_2. There may be important interactions between these climatic variables such that the effect of drought and temperature, for example, are non-additive. Through focussing just on drought it has been possible for this thesis to investigate the effect of drought in detail, however

future research will need to consider the multiple climate drivers in addition grassland properties, such as nutrient availability.

Second, this thesis uses a new methodology for labelling C in soil microbial communities to measure microbial CUE, and study in combination with plant CUE. Further research can use the methodology to investigate how microbial and plant CUE may relate to each other in different ecosystems and respond to different environmental drivers. For example the methodology would allow looking at how increasing plant diversity may alter microbial CUE through utilising the range of root exudates from the plant community. However it will be important to test and take into account the assumptions made in calculating plant CUE.

Third, in this thesis it was only possible to study intraspecific trait variation in four species. However given the importance of intraspecific trait variation on grassland C and N, further research could extend the number of species studied. This would allow representatives from a greater range of plant functional groups and species with differing growth strategies.

Finally, this thesis has focussed on the effect of single drought events on plant and soil communities. As drought events become more frequent with climate change, research needs to consider how grassland resistance and recovery to a single drought may impact resilience to future drought events.

References

Albert, C.H., Thuiller, W., Yoccoz, N.G., Soudant, A., Boucher, F., Saccone, P. & Lavorel, S. (2010) Intraspecific functional variability: Extent, structure and sources of variation. *Journal of Ecology*, **98**, 604–613.

Albrizio, R. & Steduto, P. (2003) Photosynthesis, respiration and conservative carbon use efficiency of four field grown crops. *Agricultural and Forest Meteorology*, **116**, 19–36.

Allison, S.D., Wallenstein, M.D. & Bradford, M. a. (2010) Soil-carbon response to warming dependent on microbial physiology. *Nature Geoscience*, **3**, 336–340.

Arróniz-Crespo, M., Leake, J.R., Horton, P. & Phoenix, G.K. (2008) Bryophyte physiological responses to, and recovery from, long-term nitrogen deposition and phosphorus fertilisation in acidic grassland. *New Phytologist*, **180**, 864–874.

Ayres, E., van der Wal, R., Sommerkorn, M. & Bardgett, R.D. (2006) Direct uptake of soil nitrogen by mosses. *Biology letters*, **2**, 286–288.

Backhaus, S., Kreyling, J., Grant, K., Beierkuhnlein, C., Walter, J. & Jentsch, A. (2014) Recurrent Mild Drought Events Increase Resistance Toward Extreme Drought Stress. *Ecosystems*, **17**, 1068–1081.

Badri, D. V. & Vivanco, J.M. (2009) Regulation and function of root exudates. *Plant, Cell and Environment*, **32**, 666–681.

Bahn, M., Lattanzi, F. a, Hasibeder, R., Wild, B., Koranda, M., Danese, V., Brüggemann, N., Schmitt, M., Siegwolf, R. & Richter, A. (2013) Responses of belowground carbon allocation dynamics to extended shading in mountain grassland. *New Phytologist*, **198**, 116–26.

Bapiri, A., Bååth, E. & Rousk, J. (2010) Drying-rewetting cycles affect fungal and bacterial growth differently in an arable soil. *Microbial ecology*, **60**, 419–28.

Bardgett, R.D., Hobbs, P.J. & Frostegard, A. (1996) Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. *Biology and Fertility of Soils*, **22**, 261–264.

Bardgett, R.D., Manning, P., Morriën, E. & De Vries, F.T. (2013) Hierarchical responses of plant-soil interactions to climate change: Consequences for the global carbon cycle. *Journal of Ecology*, **101**, 334–343.

Bardgett, R.D. & McAlister, E. (1999) The measurement of soil fungal:bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. *Biology and Fertility of Soils*, **29**, 282–290.

Bardgett, R.D., Smith, R.S., Shiel, R.S., Peacock, S., Simkin, J.M., Quirk, H. & Hobbs, P.J. (2006) Parasitic plants indirectly regulate below-ground properties in grassland ecosystems. *Nature*, **439**, 969–72.

Bardgett, R. D., & Wardle, D. A. (2010). *Aboveground-belowground linkages: biotic interactions, ecosystem processes, and global change*. Oxford University Press.

Bates, J.W. (1994) Responses of the Mosses Brachythecium rutabulum and Pseudoscleropodium purum to a Mineral Nutrient Pulse. *Functional Ecology*, **8**, 686–693.

Bates, J.W., Thompson, K. & Grime, P.J. (2005) Effects of simulated long-term climatic change on the bryophytes of a limestone grassland community. *Global Change Biology*, **11**, 757–769.

Baxendale, C.L., Orwin, K.H., Poly, F., Pommier, T. & Bardgett, R.D. (2014) Are plant-soil feedback responses explained by plant traits? *New Phytologist*, **204**, 408–23.

Beier, C., Beierkuhnlein, C., Wohlgemuth, T., Penuelas, J., Emmett, B., Körner, C., De Boeck, H.J., Christensen, J.H., Leuzinger, S., Janssens, I. a & Hansen, K. (2012) Precipitation manipulation experiments--challenges and recommendations for the future. *Ecology Letters*, **15**, 899–911.

Beierkuhnlein, C., Thiel, D., Jentsch, A., Willner, E. & Kreyling, J. (2011) Ecotypes of European grass species respond differently to warming and extreme drought. *Journal of Ecology*, **99**, 703–713.

Bisbee, K.E., Gower, S.T., Norman, J.M. & Nordheim, E. V. (2001) Environmental controls on ground cover species composition and productivity in a boreal black spruce forest. *Oecologia*, **129**, 261–270.

Blankinship, J.C., Niklaus, P. a & Hungate, B. a (2011) A meta-analysis of responses of soil biota to global change. *Oecologia*, **165**, 553–65.

Bloor, J.M.G. & Bardgett, R.D. (2012) Stability of above-ground and below-ground processes to extreme drought in model grassland ecosystems: Interactions with plant species diversity and soil nitrogen availability. *Perspectives in Plant Ecology, Evolution and Systematics*, **14**, 193–204.

Borken, W. & Matzner, E. (2009) Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Global Change Biology*, **15**, 808–824.

Bradford, M.A. & Crowther, T.W. (2013) Commentary Carbon use efficiency and storage in terrestrial ecosystems. *New Phytologist*, **199**, 7–9.

Bradford, M.A., Wieder, W.R., Bonan, G.B., Fierer, N., Raymond, P.A. & Crowther, T.W. (2016) Managing uncertainty in soil carbon feedbacks to climate change. *Nature Climate Change*, **6**, 751–758.

Brookes, P.C., Landman, A., Pruden, G. & Jenkinson, D.S. (1985) Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry*, **17**, 837–842.

Bullock, J.M., Pywell, R.F., Burke, M.J.W. & Walker, K.J. (2001) Restoration of biodiversity enhances agricultural production. *Ecology Letters*, **4**, 185–189.

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

Burri, S., Sturm, P., Prechsl, U.E., Knohl, A. & Buchmann, N. (2014) The impact of extreme summer drought on the short-term carbon coupling of photosynthesis to soil CO2 efflux in a temperate grassland. *Biogeosciences*, **11**, 961–975.

Carroll, J.A., Johnson, D., Morecroft, M., Taylor, A., Caporn, S.J. & Lee, J.A. (2000) The effect of long-term nitrogen additions on the bryophyte cover of upland acidic grasslands. *Journal of Bryology*, **22**, 83–89.

Carter, D.L. & Blair, J.M. (2012) High richness and dense seeding enhance grassland restoration establishment but have little effect on drought response. *Ecological Applications*, **22**, 1308–1319.

Chang, J., Ciais, P., Viovy, N., Vuichard, N., Herrero, M., Havlík, P., Wang, X., Sultan, B. & Soussana, J.F. (2016) Effect of climate change, CO2 trends, nitrogen addition, and land-cover and management intensity changes on the carbon balance of European grasslands. *Global Change Biology*, **22**, 338–350.

Chang, J., Ciais, P., Viovy, N., Vuichard, N., Sultan, B. & Soussana, J.-F. (2015) The greenhouse gas balance of European grasslands. *Global Change Biology*, **21**, 3748–3761.

Chaves, M.M. (2002) How Plants Cope with Water Stress in the Field? Photosynthesis and Growth. *Annals of Botany*, **89**, 907–916.

Chaves, M.M., Maroco, J.P. & Pereira, J.S. (2003) Review: Understanding plant responses to drought — from genes to the whole plant. *Functional Plant Biology*, **30**, 239–264.

Chen, S., Lin, G., Huang, J. & Jenerette, G.D. (2009a) Dependence of carbon sequestration on the differential responses of ecosystem photosynthesis and respiration to rain pulses in a semiarid steppe. *Global Change Biology*, **15**, 2450–2461.

Chen, S., Lin, G., Huang, J. & Jenerette, G.D. (2009b) Dependence of carbon sequestration on the differential responses of ecosystem photosynthesis and respiration to rain pulses in a semiarid steppe. *Global Change Biology*, **15**, 2450–2461.

Cheng, W., Sims, D.A., Luo, Y., Coleman, J.S. & Johnson, D.W. (2000) Photosynthesis, respiration, and net primary production of sunflower stands in ambient and elevated atmospheric CO2 concentrations: an invariant NPP:GPP ratio? *Global Change Biology*, **6**, 931–941.

Cleveland, C.C. & Liptzin, D. (2007) C:N:P stoichiometry in soil: Is there a "Redfield ratio" for the microbial biomass? *Biogeochemistry*, **85**, 235–252.

Cornelissen, J.H.C., Lang, S.I., Soudzilovskaia, N.A. & During, H.J. (2007) Comparative cryptogam ecology: A review of bryophyte and lichen traits that drive biogeochemistry. *Annals of Botany*, **99**, 987–1001.

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K. & Paul, E. (2013) The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? *Global Change Biology*, **19**, 988–995.

Craine, J.M., Ocheltree, T.W., Nippert, J.B., Towne, E.G., Skibbe, A.M., Kembel, S.W. & Fargione, J.E. (2012) Global diversity of drought tolerance and grassland climate-change resilience. *Nature Climate Change*, **3**, 63–67.

Craven, D., Isbell, F., Manning, P., Connolly, J., Bruelheide, H., Ebeling, A., Roscher, C., van Ruijven, J., Weigelt, A., Wilsey, B., Beierkuhnlein, C., de Luca, E., Griffin, J.N., Hautier, Y., Hector, A., Jentsch, A., Kreyling, J., Lanta, V., Loreau, M., Meyer, S.T., Mori, A.S., Naeem, S., Palmborg, C., Polley, H.W., Reich, P.B., Schmid, B., Siebenkäs, A., Seabloom, E., Thakur, M.P., Tilman, D., Vogel, A. & Eisenhauer, N. (2016) Plant diversity effects on grassland productivity are robust to both nutrient enrichment and

drought. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **371**, 20150277.

Csonka, L.N. (1989) Physiological and Genetic Responses of Bacteria to Osmotic Stress. *Microbiological Reviews*, **53**, 121–147.

De Boeck, H.J., Dreesen, F.E., Janssens, I.A. & Nijs, I. (2011) Whole-system responses of experimental plant communities to climate extremes imposed in different seasons. *New Phytologist*, **189**, 806–17.

De Deyn, G.B., Quirk, H., Oakley, S., Ostle, N.J. & Bardgett, R.D. (2011a) Rapid transfer of photosynthetic carbon through the plant-soil system in differently managed species-rich grasslands. *Biogeosciences*, **8**, 1131–1139.

De Deyn, G.B., Shiel, R.S., Ostle, N.J., McNamara, N.P., Oakley, S., Young, I., Freeman, C., Fenner, N., Quirk, H. & Bardgett, R.D. (2011b) Additional carbon sequestration benefits of grassland diversity restoration. *Journal of Applied Ecology*, **48**, 600–608.

De Lucia, E.H., Drake, J.E., Thomas, R.B. & Gonzalez-Meler, M. (2007) Forest carbon use efficiency: Is respiration a constant fraction of gross primary production? *Global Change Biology*, **13**, 1157–1167.

Deane-Coe, K.K., Mauritz, M., Celis, G., Salmon, V., Crummer, K.G., Natali, S.M. & Schuur, E.A.G. (2015) Experimental Warming Alters Productivity and Isotopic Signatures of Tundra Mosses. *Ecosystems*, **18**, 1070–1082.

De Vries, F.T. & Bardgett, R.D. (2016) Plant community controls on short-term ecosystem nitrogen retention. *New Phytologist*, **210**, 861–874.

De Vries, F.T., Bracht Jørgensen, H., Hedlund, K. & Bardgett, R.D. (2015) Disentangling plant and soil microbial controls on carbon and nitrogen loss in grassland mesocosms. *Journal of Ecology*, **103**, 629–640.

De Vries, F.T., Brown, C. & Stevens, C.J. (2016) Grassland species root response to drought: consequences for soil carbon and nitrogen availability. *Plant and Soil*, 1–16.

De Vries, F.T., Hoffland, E., van Eekeren, N., Brussaard, L. & Bloem, J. (2006) Fungal/bacterial ratios in grasslands with contrasting nitrogen management. *Soil Biology and Biochemistry*, **38**, 2092–2103.

De Vries, F.T., Liiri, M.E., Bjørnlund, L., Bowker, M. a., Christensen, S., Setälä, H.M. & Bardgett, R.D. (2012a) Land use alters the resistance and resilience of soil food webs to drought. *Nature Climate Change*, **2**, 276–280.

De Vries, F.T., Manning, P., Tallowin, J.R.B., Mortimer, S.R., Pilgrim, E.S., Harrison, K.A., Hobbs, P.J., Quirk, H., Shipley, B., Cornelissen, J.H.C., Kattge, J. & Bardgett, R.D. (2012b) Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. *Ecology Letters*, **15**, 1230–1239.

DeLucia, E.H., Drake, J.E., Thomas, R.B. & Gonzalez-Meler, M. (2007) Forest carbon use efficiency: is respiration a constant fraction of gross primary production? *Global Change Biology*, **13**, 1157–1167.

DeMalach, N., Zaady, E. & Kadmon, R. (2017) Light asymmetry explains the effect of nutrient enrichment on grassland diversity. *Ecology Letters*, **20**, 60–69.

Dewar, R.C., Medlyn, B.E. & McMurtrie, R.E. (1998) A mechanistic analysis of light and carbon use efficiencies. *Plant, Cell and Environment*, **21**, 573–588.

Diaz, S., Hodgson, J.G., Thompson, K., Cabido, M., Cornelissen, J.H.C., Jalili, A., Montserrat-Marti, G., Grime, P.J., Zarrinkamar, F., Asri, Y., Band, S.R., Basconcelo, S., Castro-Diez, P., Funes, G., Hamzehee, B., Khoshnevi, M., Perez-Harguindeguy, N., Perez-Rontome, M.C., Shirvany, F.A., Vendramini, F., Yazdani, S., Abbas-Azimi, R., Bogaard, A., Boustani, S., Charles, M., Dehghan, M., de Torres-Espuny, L., Falczuk, V., Guerrero-Campo, J., Hynd, A., Jones, G., Kowsary, E., Kazemi-Saeed, F., Maestro-Martinez, M., Romo-Diez, A., Shaw, S., Siavash, B., Villar-Salvador, P. & Zak, M.R.

(2004) The plant traits that drive ecosystems: Evidence from three continents. *Journal of Vegetation Science*, **15**, 295–304.

Díaz, S., Lavorel, S., de Bello, F., Quétier, F., Grigulis, K. & Robson, T.M. (2007) Incorporating plant functional diversity effects in ecosystem service assessments. *Proceedings of the National Academy of Sciences*, **104**, 20684–9.

Dijkstra, P., Thomas, S.C., Heinrich, P.L., Koch, G.W., Schwartz, E. & Hungate, B. a. (2011) Effect of temperature on metabolic activity of intact microbial communities: Evidence for altered metabolic pathway activity but not for increased maintenance respiration and reduced carbon use efficiency. *Soil Biology and Biochemistry*, **43**, 2023–2031.

Eisenhauer, N., Cesarz, S., Koller, R., Worm, K. & Reich, P.B. (2012) Global change belowground: impacts of elevated CO2, nitrogen, and summer drought on soil food webs and biodiversity. *Global Change Biology*, **18**, 435–447.

Estiarte, M., Vicca, S., Peñuelas, J., Bahn, M., Beier, C., Emmett, B.A., Fay, P.A., Hanson, P.J., Hasibeder, R., Kigel, J., Kröel-Dulay, G., Larsen, K.S., Lellei-Kovács, E., Limousin, J.-M., Ogaya, R., Ourcival, J.-M., Reinsch, S., Sala, O.E., Schmidt, I.K., Sternberg, M., Tielbörger, K., Tietema, A. & Janssens, I.A. (2016) Few multiyear precipitation-reduction experiments find a shift in the productivity-precipitation relationship. *Global Change Biology*, **22**, 2570–2581.

Evans, S.E., Byrne, K.M., Lauenroth, W.K. & Burke, I.C. (2011) Defining the limit to resistance in a drought-tolerant grassland: long-term severe drought significantly reduces the dominant species and increases ruderals. *Journal of Ecology*, **99**, 1500–1507.

Everwand, G., Fry, E.L., Eggers, T. & Manning, P. (2014) Seasonal Variation in the Capacity for Plant Trait Measures to Predict Grassland Carbon and Water Fluxes. *Ecosystems*, **17**, 1095–1108.

FAO (2005) *Grasslands of the World*, (ed. by C. Suttie, J.M., Reynolds, S.G., Batello) Food and Agriculture Organization of the United Nations, Rome.

Farrer, E.C. & Suding, K.N. (2016) Teasing apart plant community responses to N enrichment: the roles of resource limitation, competition and soil microbes. *Ecology Letters*, **19**, 1287–1296.

Fernández-Martínez, M., Vicca, S., Janssens, I. a., Sardans, J., Luyssaert, S., Campioli, M., Chapin III, F.S., Ciais, P., Malhi, Y., Obersteiner, M., Papale, D., Piao, S.L., Reichstein, M., Rodà, F. & Peñuelas, J. (2014) Nutrient availability as the key regulator of global forest carbon balance. *Nature Climate Change*, **4**, 471–476.

Flexas, J., Bota, J., Galmes, J., Medrano, H. & Ribas-Carbo, M. (2006) Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. *Physiologia Plantarum*, **127**, 343–352.

Foley, J.A., Defries, R., Asner, G., Barford, C., Bonan, G., Carpenter, S., Chapin, F., Coe, M., Daily, G., Gibbs, H., Helkowski, H., Holloway, T., Howard, E., Kucharik, C., Monfreda, C., Patz, J., Prentice, I., Ramankutty, N. & Snyder, P. (2005) Global Consequences of Land Use. *Science*, **309**, 570–574.

Fornara, D.A., Banin, L. & Crawley, M.J. (2013) Multi-nutrient vs. nitrogen-only effects on carbon sequestration in grassland soils. *Global change biology*, **19**, 3848–3857.

Frey, S.D., Lee, J., Melillo, J.M. & Six, J. (2013) The temperature response of soil microbial efficiency and its feedback to climate. *Nature Climate Change*, **3**, 1–4.

Fry, E.L., Manning, P., Allen, D.G.P., Hurst, A., Everwand, G., Rimmler, M. & Power, S. a. (2013) Plant Functional Group Composition Modifies the Effects of Precipitation Change on Grassland Ecosystem Function. *PLoS ONE*, **8**, e57027.

Fry, E.L., Power, S. a. & Manning, P. (2014) Trait-based classification and manipulation of plant functional groups for biodiversity-ecosystem function experiments. *Journal of Vegetation Science*, **25**, 248–261.

Fuchslueger, L., Bahn, M., Fritz, K., Hasibeder, R. & Richter, A. (2014) Experimental drought reduces the transfer of recently fixed plant carbon to soil microbes and alters the

bacterial community composition in a mountain meadow. *New Phytologist*, **201**, 916–927.

Fuchslueger, L., Bahn, M., Hasibeder, R., Kienzl, S., Fritz, K., Schmitt, M., Watzka, M., Richter, A. & Austin, A. (2016) Drought history affects grassland plant and microbial carbon turnover during and after a subsequent drought event. *Journal of Ecology*, **104**, 1453–1465.

Geyer, K.M., Kyker-snowman, E., Grandy, A.S. & Frey, S.D. (2016) Microbial carbon use efficiency: accounting for population, community, and ecosystem-scale controls over the fate of metabolized organic matter. *Biogeochemistry*, **127**, 173–188.

Gifford, R.M. (1995) Whole plant respiration and photosynthesis of wheat under increased CO2 concentration and temperature: Long-term vs . short-term distinctions for modelling. *Global Change Biology*, **1**, 385–396.

Gordon, C., Woodin, S.J., Alexander, J.J. & Mullins, C.E. (1999) Effects of increased temperature, drought and nitrogen supply on two upland perennials of contrasting functional type: *Calluna vulgaris* and *Pteridium aquilinum*. *New Phytologist*, **142**, 243–258.

Gross, N., Robson, T.M., Lavorel, S., Albert, C., Le Bagousse-Pinguet, Y. & Guillemin, R. (2008) Plant response traits mediate the effects of subalpine grasslands on soil moisture. *New Phytologist*, **180**, 652–62.

Hafner, S., Unteregelsbacher, S., Seeber, E., Lena, B., Xu, X., Li, X., Guggenberger, G., Miehe, G. & Kuzyakov, Y. (2012) Effect of grazing on carbon stocks and assimilate partitioning in a Tibetan montane pasture revealed by 13CO 2 pulse labeling. *Global Change Biology*, **18**, 528–538.

Hagedorn, F. & Joos, O. (2014) Experimental summer drought reduces soil CO2 effluxes and DOC leaching in Swiss grassland soils along an elevational gradient. *Biogeochemistry*, **117**, 395–412.

Hagerty, S.B., van Groenigen, K.J., Allison, S.D., Hungate, B. a., Schwartz, E., Koch, G.W., Kolka, R.K. & Dijkstra, P. (2014) Accelerated microbial turnover but constant growth efficiency with warming in soil. *Nature Climate Change*, **4**, 3–6.

Harper, C.W., Blair, J.M., Fay, P.A., Knapp, A.K. & Carlisle, J.D. (2005) Increased rainfall variability and reduced rainfall amount decreases soil CO2 flux in a grassland ecosystem. *Global Change Biology*, **11**, 322–334.

Harpole, W.S., Sullivan, L.L., Lind, E.M., Firn, J., Adler, P.B., Borer, E.T., Chase, J., Fay, P.A., Hautier, Y., Hillebrand, H., MacDougall, A.S., Seabloom, E.W., Williams, R., Bakker, J.D., Cadotte, M.W., Chaneton, E.J., Chu, C., Cleland, E.E., D'Antonio, C., Davies, K.F., Gruner, D.S., Hagenah, N., Kirkman, K., Knops, J.M.H., La Pierre, K.J., McCulley, R.L., Moore, J.L., Morgan, J.W., Prober, S.M., Risch, A.C., Schuetz, M., Stevens, C.J. & Wragg, P.D. (2016) Addition of multiple limiting resources reduces grassland diversity. *Nature*, **537**, 1–9.

Hasibeder, R., Fuchslueger, L., Fritz, K., Richter, A. & Bahn, M. (2014) Summer drought alters dynamics of carbon allocation to roots and root respiration in mountain grassland. *New Phytologist*, **16**, 12991.

Henry, F., Nguyen, C., Paterson, E., Sim, A. & Robin, C. (2005) How does nitrogen availability alter rhizodeposition in Lolium multiflorum Lam. during vegetative growth? *Plant and Soil*, **269**, 181–191.

Herron, P.M., Stark, J.M., Holt, C., Hooker, T. & Cardon, Z.G. (2009) Microbial growth efficiencies across a soil moisture gradient assessed using 13C-acetic acid vapor and 15N-ammonia gas. *Soil Biology and Biochemistry*, **41**, 1262–1269.

Holland, E., Robertson, G., Greenberg, J., Groffman, P., Boone, R. & Gosz, J. (1999) *Soil CO2, N2O, and CH4 exchange. Standard soil methods for long-term ecological research* (ed. by P. Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins), pp. 185–201. Oxford University Press., Oxford.

Ingerpuu, N. & Kupper, T. (2007) Response of calcareous grassland vegetation to mowing and fluctuating weather conditions. *Journal of Vegetation Science*, **18**, 141–146.

IPCC (2014) Climate Change 2014 Synthesis Report Summary Chapter for Policymakers. *Ipcc*, 31.

Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W.S., Reich, P.B., Scherer-Lorenzen, M., Schmid, B., Tilman, D., van Ruijven, J., Weigelt, A., Wilsey, B.J., Zavaleta, E.S. & Loreau, M. (2011) High plant diversity is needed to maintain ecosystem services. *Nature*, **477**, 199–202.

Isbell, F., Craven, D., Connolly, J., Loreau, M., Schmid, B., Beierkuhnlein, C., Bezemer, T.M., Bonin, C., Bruelheide, H., de Luca, E., Ebeling, A., Griffin, J.N., Guo, Q., Hautier, Y., Hector, A., Jentsch, A., Kreyling, J., Lanta, V., Manning, P., Meyer, S.T., Mori, A.S., Naeem, S., Niklaus, P.A., Polley, H.W., Reich, P.B., Roscher, C., Seabloom, E.W., Smith, M.D., Thakur, M.P., Tilman, D., Tracy, B.F., van der Putten, W.H., van Ruijven, J., Weigelt, A., Weisser, W.W., Wilsey, B. & Eisenhauer, N. (2015) Biodiversity increases the resistance of ecosystem productivity to climate extremes. *Nature*, **526**, 574–577.

Isbell, F., Reich, P.B., Tilman, D., Hobbie, S.E., Polasky, S. & Binder, S. (2013) Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity. *Proceedings of the National Academy of Sciences*, **110**, 11911–6.

Jentsch, A., Kreyling, J. & Beierhkuhnlein, C. (2007) A new generation of climate change experiments: events , not trends. *Frontiers in Ecology and the Environment*, **5**, 315–324.

Jentsch, A., Kreyling, J., Elmer, M., Gellesch, E., Glaser, B., Grant, K., Hein, R., Lara, M., Mirzaei, H., Nadler, S.E., Nagy, L., Otieno, D., Pritsch, K., Rascher, U., Schädler, M., Schloter, M., Singh, B.K., Stadler, J., Walter, J., Wellstein, C., Wöllecke, J. &

Beierkuhnlein, C. (2011) Climate extremes initiate ecosystem-regulating functions while maintaining productivity. *Journal of Ecology*, **99**, 689–702.

Jobbagy, E.G. & Jackson, R.B. (2000) The Vertical Distribution of Soil Organic Carbon and Its Relation to Climate and Vegetation. *Ecological Applications*, **10**, 423–436.

Johnson, D., Leake, J.R., Ostle, N.J., Ineson, P. & Read, D.J. (2002) In situ 13 CO 2 pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. *New Phytologist*, **153**, 327–334.

Jung, V., Albert, C.H., Violle, C., Kunstler, G., Loucougaray, G. & Spiegelberger, T. (2014) Intraspecific trait variability mediates the response of subalpine grassland communities to extreme drought events. *Journal of Ecology*, **102**, 45–53.

Kahmen, A., Perner, J. & Buchmann, N. (2005) Diversity-dependent productivity in semi-natural grasslands following climate perturbations. *Functional Ecology*, **19**, 594–601.

Kalapos, T., Boogaard, R. & Lambers, H. (1996) Effect of soil drying on growth, biomass allocation and leaf gas exchange of two annual grass species. *Plant and Soil*, **185**, 137–149.

Kalbitz, K., Solinger, S., Park, J.-H., Michalzik, B. & Matzner, E. (2000) Controls on the dynamics of dissolved organic matter in soils: A review. *Soil Science*, **165**, 277–304.

Kallenbach, C.M., Grandy, A. & Frey, S.D. (2016) Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nature Communications*, **7**, 13630.

Kaufman, L.H. (1982) Stream Aufwuchs Accumulation: Disturbance frequency and stress Resistance and resilience. *Oecologia*, **52**, 57–63.

Kharin, V. V., Zwiers, F.W., Zhang, X. & Hegerl, G.C. (2007) Changes in temperature and precipitation extremes in the IPCC ensemble of global coupled model simulations. *Journal of Climate*, **20**, 1419–1444.

Killham, K. & Firestone, M.K. (1984) Proline transport increases growth efficiency in salt-stressed Streptomyces griseus. *Applied and Environmental Microbiology*, **48**, 239–241.

Kirkham, F.W., Tallowin, J.R.B., Dunn, R.M., Bhogal, A., Chambers, B.J. & Bardgett, R.D. (2014) Ecologically sustainable fertility management for the maintenance of species-rich hay meadows: a 12-year fertilizer and lime experiment. *Journal of Applied Ecology*, **51**, 152–161.

Kirkham, F.W., Tallowin, J.R.B., Sanderson, R. a., Bhogal, A., Chambers, B.J. & Stevens, D.P. (2008) The impact of organic and inorganic fertilizers and lime on the species-richness and plant functional characteristics of hay meadow communities. *Biological Conservation*, **141**, 1411–1427.

Knapp, A.K., Fay, P. a, Blair, J.M., Collins, S.L., Smith, M.D., Carlisle, J.D., Harper, C.W., Danner, B.T., Lett, M.S. & McCarron, J.K. (2002) Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science*, **298**, 2202–5.

Koujima, I., Hayashi, H., Tomochika, K., Okabe, A. & Kanemasa, Y. (1978) Adaptational change in proline and water content of *Staphylococcus aureus* after salt concentration. *Applied and environmental microbiology*, **35**, 467–470.

Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I., Mellado-Vázquez, P.G., Malik, A.A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B.C., Trumbore, S.E. & Gleixner, G. (2015) Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications*, **6**, 6707.

Lemaire, G., Wilkins, R. & Hodgson, J. (2005) Challenges for grassland science: Managing research priorities. *Agriculture, Ecosystems and Environment*, **108**, 99–108.

Lennon, J.T. & Jones, S.E. (2011) Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nature Reviews Microbiology*, **9**, 119–130.

Lepš, J., de Bello, F., Šmilauer, P. & Doležal, J. (2011) Community trait response to environment: Disentangling species turnover vs intraspecific trait variability effects. *Ecography*, **34**, 856–863.

Li, L., Fan, W., Kang, X., Wang, Y., Cui, X., Xu, C., Griffin, K.L. & Hao, Y. (2016) Responses of greenhouse gas fluxes to climate extremes in a semiarid grassland. *Atmospheric Environment*, **142**, 32–42.

Lundquist, E.J., Jackson, L.E. & Scow, K.M. (1999) Wet-dry cycles affect dissolved organic carbon in two California agricultural soils. *Soil Biology and Biochemistry*, **31**, 1031–1038.

Macgillivray, C.W. & Grime, P.J. (1995) Testing predictions of the resistance and resilience of vegetation subjected to extreme events. *Functional Ecology*, **9**, 640–649.

Manzoni, S., Schimel, J.P. & Porporato, A. (2012a) Responses of soil microbial communities to water stress : results from a meta-analysis. *Ecology*, **93**, 930–938.

Manzoni, S., Taylor, P., Richter, A., Porporato, A. & Agren, G.I. (2012b) Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytologist*, **196**, 79–91.

Mariotte, P., Vandenberghe, C., Kardol, P., Hagedorn, F. & Buttler, A. (2013) Subordinate plant species enhance community resistance against drought in semi-natural grasslands. *Journal of Ecology*, **101**, 763–773.

Maron, J.L. & Jefferies, R.L. (2001) Restoring enriched grasslands: effects of mowing on species richness, productivity and nitrogen retention. *Ecological Applications*, **11**, 1088–1100.

Martin-Olmedo, P., Rees, R.M. & Grace, J. (2002) The influence of plants grown under elevated CO2 and N fertilization on soil nitrogen dynamics. *Global Change Biology*, **8**, 643–657.

Messier, J., McGill, B.J. & Lechowicz, M.J. (2010) How do traits vary across ecological scales? A case for trait-based ecology. *Ecology Letters*, **13**, 838–848.

Met Office (2015) *Developing H++ climate change scenarios for heatwaves, droughts, floods, windstorms and cold snaps,* Adaptation Sub-Committee of the Climate Chnage Committee.

Metcalfe, D.B., Meir, P., Aragão, L.E.O.C., Lobo-do-Vale, R., Galbraith, D., Fisher, R.A., Chaves, M.M., Maroco, J.P., da Costa, A.C.L., de Almeida, S.S., Braga, A.P., Gonçalves, P.H.L., de Athaydes, J., da Costa, M., Portela, T.T.B., de Oliveira, A.A.R., Malhi, Y. & Williams, M. (2010) Shifts in plant respiration and carbon use efficiency at a large-scale drought experiment in the eastern Amazon. *New Phytologist*, **187**, 608–621.

Milcu, A., Roscher, C., Gessler, A., Bachmann, D., Gockele, A., Guderle, M., Landais, D., Piel, C., Escape, C., Devidal, S., Ravel, O., Buchmann, N., Gleixner, G., Hildebrandt, A. & Roy, J. (2014) Functional diversity of leaf nitrogen concentrations drives grassland carbon fluxes. *Ecology Letters*, **17**, 435–444.

Miltner, A., Bombach, P., Schmidt-Brücken, B. & Kästner, M. (2012) SOM genesis: Microbial biomass as a significant source. *Biogeochemistry*, **111**, 41–55.

Mirzaei, H., Kreyling, J., Hussain, M.Z., Li, Y., Tenhunen, J., Beierkuhnlein, C. & Jentsch, A. (2008) A single drought event of 100-year recurrence enhances subsequent carbon uptake and changes carbon allocation in experimental grassland communities. *Journal of Plant Nutrition and Soil Science*, **171**, 681–689.

Moyano, F.E., Manzoni, S. & Chenu, C. (2013) Responses of soil heterotrophic respiration to moisture availability: An exploration of processes and models. *Soil Biology and Biochemistry*, **59**, 72–85.

Muhr, J., Franke, J. & Borken, W. (2010) Drying-rewetting events reduce C and N losses from a Norway spruce forest floor. *Soil Biology and Biochemistry*, **42**, 1303–1312.

Nakagawa, S. & Schielzeth, H. (2013) A general and simple method for obtaining R 2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, **4**, 133–142.

NEA (2011) *UK National Ecosystem Assessment Synthesis of the Key Findings*,.

Nijp, J.J., Limpens, J., Metselaar, K., van der Zee, S.E.A.T.M., Berendse, F. & Robroek, B.J.M. (2014) Can frequent precipitation moderate the impact of drought on peatmoss carbon uptake in northern peatlands? *New Phytologist*, **203**, 70–80.

O'Gorman, P.A. & Schneider, T. (2009) The physical basis for increases in precipitation extremes in simulations of 21st-century climate change. *Proceedings of the National Academy of Sciences*, **106**, 14773–14777.

Oksanen, A.J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., Hara, R.B.O., Simpson, G.L., Solymos, P., Stevens, M.H.H. & Wagner, H. (2013) Package "vegan." *Community Ecology Package*, **2**, 0–10.

Orwin, K.H. & Ostle, N.J. (2012) Moss species effects on peatland carbon cycling after fire. *Functional Ecology*, **26**, 829–836.

Orwin, K.H. & Wardle, D.A. (2004) New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances. *Soil Biology and Biochemistry*, **36**, 1907–1912.

Ostle, N.J., Whiteley, A.S., Bailey, M.J., Sleep, D., Ineson, P. & Manefield, M. (2003) Active microbial RNA turnover in a grassland soil estimated using a 13CO2 spike. *Soil Biology and Biochemistry*, **35**, 877–885.

Payne, W.J. & Wiebe, W.J. (1978) Growth yield and efficiency in chemosynthetic microorganisms. *Annual Review of Microbiology*, **32**, 155–183.

Paz-Ferreiro, J., Medina-Roldán, E., Ostle, N.J., McNamara, N.P. & Bardgett, R.D. (2012) Grazing increases the temperature sensitivity of soil organic matter decomposition in a temperate grassland. *Environmental Research Letters*, **7**, 14027.

La Pierre, K.J., Blumenthal, D.M., Brown, C.S., Klein, J.A. & Smith, M.D. (2016) Drivers of Variation in Aboveground Net Primary Productivity and Plant Community Composition Differ Across a Broad Precipitation Gradient. *Ecosystems*, **19**, 521–533.

Pimentel, D. & Burgess, M. (2013) Soil Erosion Threatens Food Production. *Agriculture*, **3**, 443–463.

Pimm, S.L. (1984) The complexity and stability of ecosystems. *Nature*, **307**, 321–326.

Pinheiro, J. & Bates, D. (2013) Package "nlme." *Linear and nonlinear mixed effects models*, **3**, 1–113.

Plassart, P., Terrat, S., Thomson, B.C., Griffiths, R.I., Dequiedt, S., Lelievre, M., Regnier, T., Nowak, V., Bailey, M., Lemanceau, P., Bispo, A., Chabbi, A., Maron, P.-A., Mougel, C. & Ranjard, L. (2012) Evaluation of the ISO standard 11063 DNA extraction procedure for assessing soil microbial abundance and community structure. *PloS one*, **7**, e44279.

Poirier, M., Durand, J.L. & Volaire, F. (2012) Persistence and production of perennial grasses under water deficits and extreme temperatures: Importance of intraspecific vs. interspecific variability. *Global Change Biology*, **18**, 3632–3646.

Poorter, H., Jagodzinski, A.M., Ruiz-Peinado, R., Kuyah, S., Luo, Y., Oleksyn, J., Usoltsev, V. a., Buckley, T.N., Reich, P.B. & Sack, L. (2015) How does biomass distribution change with size and differ among species? An analysis for 1200 plant species from five continents. *New Phytologist*, **208**, 736–749.

Poorter, H., Niklas, K.J., Reich, P.B., Oleksyn, J., Poot, P. & Mommer, L. (2012) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist*, **193**, 30–50.

Proctor, M.C.F., Oliver, M.J., Wood, A.J., Alpert, P., Stark, L.R., Cleavitt, N.L., Mishler, B.D., Building, G.P., Road, S., Vegas, L. & Herbarium, J. (2007) Desiccation-tolerance in bryophytes: a review. *The Bryologist*, **110**, 595–621.

Proctor, M.C.F. & Smirnoff, N. (2000) Rapid recovery of photosystems on rewetting desiccation-tolerant mosses: chlorophyll fluorescence and inhibitor experiments. *Journal of experimental botany*, **51**, 1695–704.

Pywell, R.F., Bullock, J.M., Hopkins, A., Walker, K.J., Sparks, T.H., Burke, M.J.W. & Peel, S. (2002) Restoration of species-rich grassland on arable land: assessing the limiting processes using a multi-site experiment. *Journal of Applied Ecology*, **39**, 294–309.

R Core Development Team (2016). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ravenscroft, C.H., Fridley, J.D. & Grime, P.J. (2014) Intraspecific functional differentiation suggests local adaptation to long-term climate change in a calcareous grassland. *Journal of Ecology*, **102**, 65–73.

Reich, P.B., Ellsworth, D.S., Walters, M.B., Vose, J.M., Gresham, C., Volin, J.C. & Bowman, W.D. (1999) Generality of Leaf Trait Relationships : A Test across Six Biomes. *Ecology*, **80**, 1955–1969.

Rice, S.K. (2000) Variation in carbon isotope discrimination within and among Sphagnum species in a temperate wetland. *Oecologia*, **123**, 1–8.

Rice, S.K. & Giles, L. (1996) The influence of water content and leaf anatomy on carbon isotope discrimination and photosynthesis in Sphagnum. *Plant, Cell and Environment*, **19**, 118–124.

Rinnan, R. & Baath, E. (2009) Differential Utilization of Carbon Substrates by Bacteria and Fungi in Tundra Soil. *Applied and Environmental Microbiology*, **75**, 3611–3620.

Rodwell, J. S. (1992). *British plant communities, Volume 3: Grasslands and montane communities*. Cambridge University Press.

Sanaullah, M., Chabbi, A., Rumpel, C. & Kuzyakov, Y. (2012a) Carbon allocation in grassland communities under drought stress followed by 14C pulse labeling. *Soil Biology and Biochemistry*, **55**, 132–139.

Sanaullah, M., Chabbi, A., Rumpel, C. & Kuzyakov, Y. (2012b) Soil Biology & Biochemistry C pulse labeling. *Soil Biology and Biochemistry*, **55**, 132–139.

Sanderman, J., Baldock, J.A. & Amundson, R. (2008) Dissolved organic carbon chemistry and dynamics in contrasting forest and grassland soils. *Biogeochemistry*, **89**, 181–198.

Sardans, J. & Peñuelas, J. (2008) Drought changes nutrient sources, content and stoichiometry in the bryophyte Hypnum cupressiforme Hedw. growing in a Mediterranean forest. *Journal of Bryology*, **30**, 59–65.

Sardans, J., Rivas-Ubach, A. & Peñuelas, J. (2012) The C:N:P stoichiometry of organisms and ecosystems in a changing world: A review and perspectives. *Perspectives in Plant Ecology, Evolution and Systematics*, **14**, 33–47.

Schimel, J.P., Balser, T.C. & Wallenstein, M.D. (2007) Microbial Stress-Response Physiology and Its Implications for Ecosystem. *Ecology*, **88**, 1386–1394.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. a., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D. a. C., Nannipieri, P., Rasse, D.P., Weiner, S. & Trumbore, S.E. (2011) Persistence of soil organic matter as an ecosystem property. *Nature*, **478**, 49–56.

Schnyder, H., Isselstein, J., Taube, F., Auerswald, K., Schellberg, J., Wachendorf, M., Herrmann, A., Gierus, M., Wrage, N. & Hopkins, A. (2010) *Grassland in a changing world*,.

Shantz, H.L. (1954) The Place of Grasslands in the Earth's Cover. *Ecology*, **35**, 143–145.

Shi, Z., Thomey, M.L., Mowll, W., Litvak, M., Brunsell, N. a., Collins, S.L., Pockman, W.T., Smith, M.D., Knapp, A.K. & Luo, Y. (2014) Differential effects of extreme drought on

production and respiration: synthesis and modeling analysis. *Biogeosciences*, **11**, 621–633.

Siefert, A., Violle, C., Chalmandrier, L., Albert, C.H., Taudiere, A., Fajardo, A., Aarssen, L.W., Baraloto, C., Carlucci, M.B., Cianciaruso, M. V., de L. Dantas, V., de Bello, F., Duarte, L.D.S., Fonseca, C.R., Freschet, G.T., Gaucherand, S., Gross, N., Hikosaka, K., Jackson, B., Jung, V., Kamiyama, C., Katabuchi, M., Kembel, S.W., Kichenin, E., Kraft, N.J.B., Lagerström, A., Bagousse-Pinguet, Y. Le, Li, Y., Mason, N., Messier, J., Nakashizuka, T., Overton, J.M., Peltzer, D.A., Pérez-Ramos, I.M., Pillar, V.D., Prentice, H.C., Richardson, S., Sasaki, T., Schamp, B.S., Schöb, C., Shipley, B., Sundqvist, M., Sykes, M.T., Vandewalle, M. & Wardle, D.A. (2015) A global meta-analysis of the relative extent of intraspecific trait variation in plant communities. *Ecology Letters*, **18**, 1406–1419.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S.D., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R. & Zeglin, L.H. (2008) Stoichiometry of soil enzyme activity at global scale. *Ecology Letters*, **11**, 1252–1264.

Sinsabaugh, R.L., Manzoni, S., Moorhead, D.L. & Richter, A. (2013) Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. *Ecology Letters*, **16**, 930–9.

Sinsabaugh, R.L., Turner, J.M., Talbot, J., Waring, B.G., Powers, J.S., Kuske, C.R., Moorhead, D.L. & Follstad Shah, J.J. (2016) Stoichiometry of microbial carbin use efficiency in soils. *Ecological Monographs*, **86**, 172–189.

Smart, S.M., Bunce, R.G.H., Marrs, R., LeDuc, M., Firbank, L.G., Maskell, L.C., Scott, W.A., Thompson, K. & Walker, K.J. (2005) Large-scale changes in the abundance of common higher plant species across Britain between 1978, 1990 and 1998 as a consequence of

human activity: Tests of hypothesised changes in trait representation. *Biological Conservation*, **124**, 355–371.

Smith, M.D. (2011) The ecological role of climate extremes: Current understanding and future prospects. *Journal of Ecology*, **99**, 651–655.

Smith, R.S., Shiel, R.S., Bardgett, R.D., Millward, D., Corkhill, P., Evans, P., Quirk, H., Hobbs, P.J. & Kometa, S.T. (2008) Long-term change in vegetation and soil microbial communities during the phased restoration of traditional meadow grassland. *Journal of Applied Ecology*, **45**, 670–679.

Smith, R.S., Shiel, R.S., Bardgett, R.D., Millward, D., Corkhill, P., Rolph, G., Hobbs, P.J. & Peacock, S. (2003) Soil microbial community, fertility, vegetation and diversity as targets in the restoration management of a meadow grassland. *Journal of Applied Ecology*, **40**, 51–64.

Smith, R.S., Shiel, R.S., Bardgett, R.D., Millward, D., Evans, P., Harrison, K., Quirk, H. & Hobbs, P.J. (2009) DEFRA Project BD1451: Diversification of Grassland.

Smith, R.S., Shiel, R.S., Millward, D. & Corkhill, P. (2000) The interactive effects of management on the productivity and plant community structure of an upland meadow : an 8-year field trial. *Journal of Applied Ecology*, **37**, 1029–1043.

Spohn, M., Klaus, K., Wanek, W. & Richter, A. (2016a) Microbial carbon use efficiency and biomass turnover times depending on soil depth - Implications for carbon cycling. *Soil Biology and Biochemistry*, **96**, 74–81.

Spohn, M., Pötsch, E.M., Eichorst, S.A., Woebken, D., Wanek, W. & Richter, A. (2016b) Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland. *Soil Biology and Biochemistry*, **97**, 168–175.

Stark, J. & Firestone, M.K. (1995) Mechanisms for soil moisture effects on activity of nitrifying bacteria. *Applied and Environmental Microbiology*, **61**, 218–221.

Steinweg, J.M., Plante, A.F., Conant, R.T., Paul, E. a. & Tanaka, D.L. (2008) Patterns of substrate utilization during long-term incubations at different temperatures. *Soil Biology and Biochemistry*, **40**, 2722–2728.

Stevens, C.J., Dise, N.B., Mountford, J.O. & Gowing, D.J. (2004) Impact of nitrogen deposition on the species richness of grasslands. *Science*, **303**, 1876–9.

Street, L.E., Subke, J.-A., Sommerkorn, M., Sloan, V., Ducrotoy, H., Phoenix, G.K. & Williams, M. (2013) The role of mosses in carbon uptake and partitioning in arctic vegetation. *New Phytologist*, **199**, 163–75.

Tavi, N.M., Martikainen, P.J., Lokko, K., Kontro, M., Wild, B., Richter, A. & Biasi, C. (2013) Linking microbial community structure and allocation of plant-derived carbon in an organic agricultural soil using 13CO2 pulse-chase labelling combined with 13C-PLFA profiling. *Soil Biology and Biochemistry*, **58**, 207–215.

Thakur, M.P., Milcu, A., Manning, P., Niklaus, P.A., Roscher, C., Power, S., Reich, P.B., Scheu, S., Tilman, D., Ai, F., Guo, H., Ji, R., Pierce, S., Ramirez, N.G., Richter, A.N., Steinauer, K., Strecker, T., Vogel, A. & Eisenhauer, N. (2015) Plant diversity drives soil microbial biomass carbon in grasslands irrespective of global environmental change factors. *Global Change Biology*, **21**, 4076–4085.

Thiele-Bruhn, S., Bloem, J., De Vries, F.T., Kalbitz, K. & Wagg, C. (2012) Linking soil biodiversity and agricultural soil management. *Current Opinion in Environmental Sustainability*, **4**, 523–528.

Tibbett, M., Sanders, F.E. & Cairney, J.W.G. (2002) Low-temperature-induced changes in trehalose, mannitol and arabitol associated with enhanced tolerance to freezing in ectomycorrhizal basidiomycetes (Hebeloma spp.). *Mycorrhiza*, **12**, 249–255.

Tiemann, L.K. & Billings, S. a. (2011) Changes in variability of soil moisture alter microbial community C and N resource use. *Soil Biology and Biochemistry*, **43**, 1837–1847.

Tilman, D. (1990) Constraints and tradeoffs : toward a predictive theory of Constraints competition and succession. *Oikos*, **58**, 3–15.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R. & Polasky, S. (2002) Agricultural sustainability and intensive production practices. *Nature*, **418**, 671–7.

Tilman, D. & Downing, J.A. (1994) Biodiversity and stability in grasslands. *Nature*, **367**, 363–365.

Tilman, D., Reich, P.B. & Knops, J.M.H. (2006) Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature*, **441**, 629–32.

Tsiafouli, M.A., Thébault, E., Sgardelis, S.P., de Ruiter, P.C., van der Putten, W.H., Birkhofer, K., Hemerik, L., de Vries, F.T., Bardgett, R.D., Brady, M.V., Bjornlund, L., Jørgensen, H.B., Christensen, S., Hertefeldt, T.D., Hotes, S., Gera Hol, W.H., Frouz, J., Liiri, M., Mortimer, S.R., Setälä, H., Tzanopoulos, J., Uteseny, K., Pižl, V., Stary, J., Wolters, V. & Hedlund, K. (2015) Intensive agriculture reduces soil biodiversity across Europe. *Global Change Biology*, **21**, 973–985.

Turetsky, M.R. (2003) The Role of Bryophytes in Carbon and Nitrogen Cycling. *The Bryologist*, **106**, 395–409.

Tylianakis, J.M., Didham, R.K., Bascompte, J. & Wardle, D.A. (2008) Global change and species interactions in terrestrial ecosystems. *Ecology Letters*, **11**, 1351–1363.

Van Iersel, M.W. (2003) Carbon use efficiency depends on growth respiration, maintenance respiration, and relative growth rate. A case study with lettuce. *Plant, Cell and Environment*, **26**, 1441–1449.

Van Oijen, M., Schapendonk, A. & Höglind, M. (2010) On the relative magnitudes of photosynthesis, respiration, growth and carbon storage in vegetation. *Annals of botany*, **105**, 793–797.

Vicca, S., Luyssaert, S., Peñuelas, J., Campioli, M., Chapin, F.S., Ciais, P., Heinemeyer, A., Högberg, P., Kutsch, W.L., Law, B.E., Malhi, Y., Papale, D., Piao, S.L., Reichstein, M., Schulze, E.D. & Janssens, I.A. (2012) Fertile forests produce biomass more efficiently. *Ecology Letters*, **15**, 520–6.

Virtanen, R., Johnston, A.E., Crawley, M.J. & Edwards, G.R. (2000) Bryophyte biomass and species richness on the park grass experiment, Rothamsted, UK. *Plant Ecology*, **151**, 129–141.

Vogel, A., Fester, T., Eisenhauer, N., Scherer-Lorenzen, M., Schmid, B., Weisser, W.W. & Weigelt, A. (2013) Separating drought effects from roof artifacts on ecosystem processes in a grassland drought experiment. *PloS one*, **8**, e70997.

Vogel, A., Scherer-lorenzen, M. & Weigelt, A. (2012) Grassland Resistance and Resilience after Drought Depends on Management Intensity and Species Richness. *PloS one*, **7**, e36992.

Walter, J., Grant, K., Beierkuhnlein, C., Kreyling, J., Weber, M. & Jentsch, A. (2012) Increased rainfall variability reduces biomass and forage quality of temperate grassland largely independent of mowing frequency. *Agriculture, Ecosystems and Environment*, **148**, 1–10.

Wang, Y., Yu, S. & Wang, J. (2007) Biomass-dependent susceptibility to drought in experimental grassland communities. *Ecology Letters*, **10**, 401–410.

Ward, S.E., Bardgett, R.D., McNamara, N.P., Adamson, J.K. & Ostle, N.J. (2007) Long-Term Consequences of Grazing and Burning on Northern Peatland Carbon Dynamics. *Ecosystems*, **10**, 1069–1083.

Ward, S.E., Bardgett, R.D., McNamara, N.P. & Ostle, N.J. (2009) Plant functional group identity influences short-term peatland ecosystem carbon flux: evidence from a plant removal experiment. *Functional Ecology*, **23**, 454–462.

Ward, S.E., Ostle, N.J., Oakley, S., Quirk, H., Henrys, P. a. & Bardgett, R.D. (2013) Warming effects on greenhouse gas fluxes in peatlands are modulated by vegetation composition. *Ecology Letters*, **16**, 1285–1293.

Ward, S.E., Smart, S.M., Quirk, H., Tallowin, J.R.B., Mortimer, S.R., Shiel, R.S., Wilby, A. & Bardgett, R.D. (2016) Legacy effects of grassland management on soil carbon to depth. *Global Change Biology*, **22**, 2929–2938.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H.M., van der Putten, W.H. & Wall, D.H. (2004) Ecological linkages between aboveground and belowground biota. *Science*, **304**, 1629–33.

Wesche, K., Krause, B., Culmsee, H. & Leuschner, C. (2012) Fifty years of change in Central European grassland vegetation: Large losses in species richness and animal-pollinated plants. *Biological Conservation*, **150**, 76–85.

White, D.C., Davies, J.S., Nickels, J.S., King, J.D. & Bobbie, R.J. (1979) Determination of the sedimentary microbial biomass by extractible lipid phosphate. *Oecologia*, **40**, 51–62.

Wickham, H. (2011) The Split-Apply-Combine Strategy for Data. *Journal of Statistical Software*, **40**, 1–29.

Williams, T.G. & Flanagan, L.B. (1996) Effect of change in water content on photosynthesis, transpiration and discrimination against 13CO2 and C18O16O in Pleurozium and Sphagnum. *Oecologia*, **108**, 38–46.

Wu, Z., Dijkstra, P., Koch, G.W., Peñuelas, J. & Hungate, B.A. (2011) Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. *Global Change Biology*, **17**, 927–942.

Xu, Z., Ren, H., Cai, J., Wang, R., Li, M.-H., Wan, S., Han, X., Lewis, B.J. & Jiang, Y. (2014) Effects of experimentally-enhanced precipitation and nitrogen on resistance, recovery and resilience of a semi-arid grassland after drought. *Oecologia*, **176**, 1187–1197.

You, Y., Wang, J., Huang, X., Tang, Z., Liu, S. & Sun, O.J. (2014) Relating microbial community structure to functioning in forest soil organic carbon transformation and turnover. *Ecology and Evolution*, **4**, 633–647.

Yuste, J.C., Peñuelas, J., Estiarte, M., Garcia-Mas, J., Mattana, S., Ogaya, R., Pujol, M. & Sardans, J. (2011) Drought-resistant fungi control soil organic matter decomposition and its response to temperature. *Global Change Biology*, **17**, 1475–1486.

Zeglin, L.H., Bottomley, P.J., Jumpponen, A., Rice, C.W., Arango, M., Lindsley, A., McGowen, A., Mfombep, P. & Myrold, D.D. (2013a) Altered precipitation regime affects the function and composition of soil microbial communities on multiple time scales. *Ecology*.

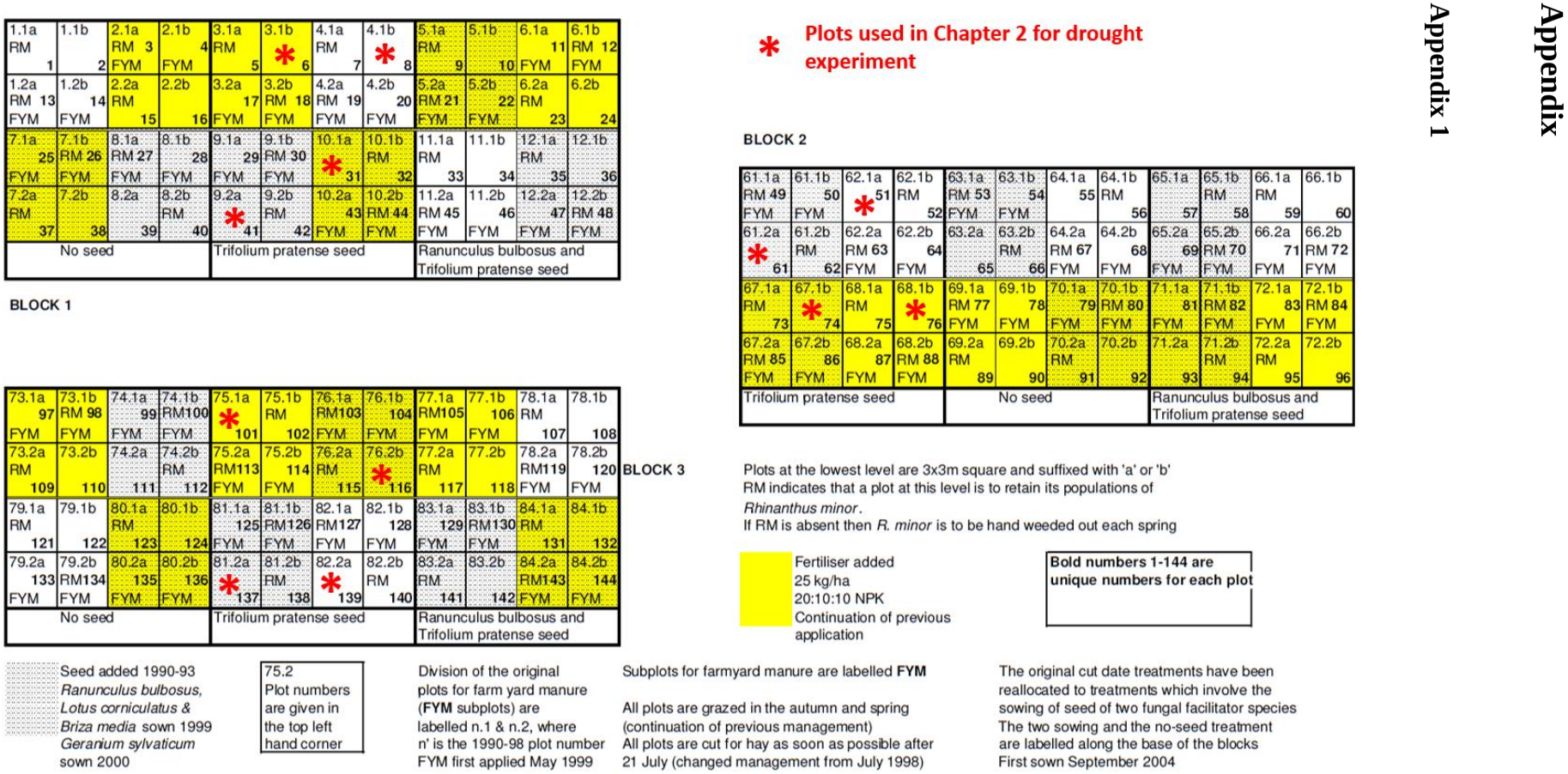
Zeglin, L.H., Ottomley, P.J.B., Umpponen, A.J., Ice, C.W.R., Rango, M.A., Indsley, A.L. & Owan, A.M.C.G. (2013b) Altered precipitation regime affects the function and composition of soil microbial communities on multiple time scales. *Ecology*, **94**, 2334–2345.

Zhao, M. & Running, S.W. (2010) Drought-induced reduction in global terrestrial net primary production from 2000 through 2009. *Science*, **329**, 940–943.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009) *Mixed effects models and extensions in ecology with R*. Springer.

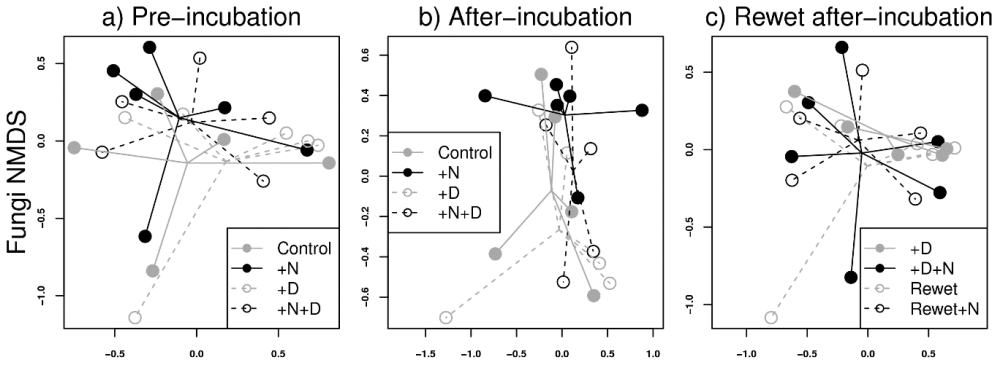
Zwicke, M., Picon-Cochard, C., Morvan-Bertrand, A., Prud'homme, M.P. & Volaire, F. (2015) What functional strategies drive drought survival and recovery of perennial species from upland grassland? *Annals of Botany*, **116**, 1001–1015.

Appendix

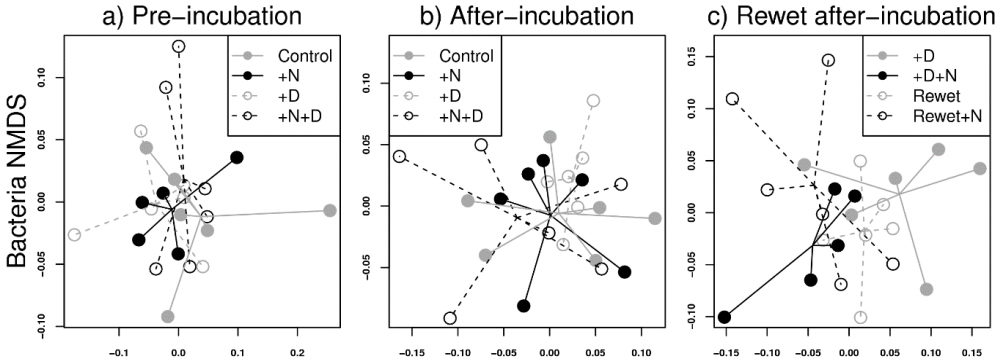


Appendix Figure A1 Plot details of the Colt Park long-term grassland restoration experiment. Plots used in chapter 2 are highlighted by red asterisks. Figure adapted from Smith *et al.* (2009).

Appendix 2



Appendix Figure A2.1 The effect of nutrient addition, drought and subsequent re-wetting on the soil fungi community structure, a) in soil before the laboratory incubation, b) after the laboratory incubation and c) after the laboratory incubation comparing soils from field drought plots with the same soils which have been re-wetted.



Appendix Figure A2.2 The effect of nutrient addition, drought and subsequent re-wetting on the soil bacteria community structure, a) in soil before the laboratory incubation, b) after the laboratory incubation and c) after the laboratory incubation comparing soils from field drought plots with the same soils which have been re-wetted.

Appendix Table A2.1 The effect of drought, nutrient addition and rewetting on fungi and bacteria community structure. Permutational Multivariate Analysis of Variance using T-RFLP data.

	a) Pre-incubation		***b) After-incubation***			***c) Rewet: After-incubation***	
Fungi T-RFLP	F (d.f.)	*P*	F (d.f.)	*P*		F (d.f.)	*P*
Drought	0.807 (1,21)	0.4915	1.194 (1,21)	0.2877	Rewet	0.332 (1,21)	0.9620
Nutrient	0.840 (1,21)	0.5005	0.991 (1,21)	0.4016	Nutrient	1.034 (1,21)	0.3437
Block	1.448 (1,21)	0.2298	1.270 (1,21)	0.2647	Block	5.200 (1,21)	**0.0050**
Drought x Nutrient	0.341 (1,21)	0.9331	0.460 (1,21)	0.8951	Rewet : Nutrient	0.297 (1,21)	0.9760
	a) Pre-incubation		***b) After-incubation***			***c) Rewet: After-incubation***	
Bacteria T-RFLP	F (d.f.)	*P*	F (d.f.)	*P*		F (d.f.)	*P*
Drought	0.859 (1,22)	0.5654	0.509 (1,22)	0.9141	Rewet	1.104 (1,22)	0.3547
Nutrient	1.153 (1,22)	0.3337	1.043 (1,22)	0.3796	Nutrient	2.565 (1,22)	**0.0110**
Block	1.359 (1,22)	0.2098	1.075 (1,22)	0.3586	Block	1.491 (1,22)	0.1389
Drought x Nutrient	1.485 (1,22)	0.1459	0.801 (1,22)	0.6503	Rewet : Nutrient	1.211 (1,22)	0.2937