The Influence of Plant Functional Groups on Ecosystem Functions in a Grassland in Northern Canada

by

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ABSTRACT

Human development, climate change, diseases and habitat degradation and loss are resulting in species extinction rates up to a thousand times faster than pre-human levels. Biodiversity-ecosystem functioning research examines how this loss of species and changes in the composition of plant communities are likely to influence numerous ecosystem functions. The effects of biodiversity loss on ecosystem properties may also be highly dependent on the identity of the organisms lost. I investigated the influence of plant functional group identity in determining ecosystem properties. I established a removal experiment in a grassland in northern Canada in 2003 with four treatments: a no-removal control and independent removal of forbs, graminoids and legumes. As biodiversity loss is occurring in concert with environmental change, I crossed removals with a fertilizer and a mycorrhizal reduction (fungicide) treatment to determine the context dependency of effects. I showed that graminoids have the largest influence on ecosystem properties in this community, despite not being the most abundant group. Short-term (4 years) biomass compensation for the removals showed no compensation for graminoid removal, but after 7 years there was full biomass compensation for this treatment. Light interception, soil moisture, and soil nutrients were all largely determined by the presence of graminoids in the plant community, and surprisingly legumes had very few effects on any ecosystem property. Graminoids also showed plant-driven environmental effects on leaf decomposition, although no removal treatment resulted in changes in the decomposition of roots. Graminoids promoted decomposition of leaf litter through 2 mechanisms: influence on the decomposition microenvironment and changes in the litter composition. Finally, I have demonstrated that very few of the effects of functional group identity were context dependent on either fertilization or fungicide treatments. These results highlight the importance of considering plant functional group identity when predicting the effects of species loss, and indicate that plant identity, more so than dominance, determines effects on ecosystem properties.
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CHAPTER ONE

Introduction: Links between Biodiversity Loss and Ecosystem Functioning

The dramatic influence of humans on ecosystems this past century has resulted in changes to the biotic structure of ecological communities including a loss of biodiversity (Hooper et al. 2005). Estimates suggest that the rates of species loss are currently 100 to 1000 times their pre-human levels (Pimm et al. 1995). In addition to species loss, many communities have gone through dramatic compositional changes due to human influences such as habitat transformation (Jefferies & Maron 1997), habitat degradation and species introductions (Sax & Gaines 2003). Although this change in biodiversity has raised a number of concerns, perhaps the most important is the potential for a loss in species to have negative effects on the functioning of ecosystems and the services they provide (Duffy 2009). These concerns have led to a proliferation of research on the effects of biodiversity loss on ecosystem functioning (BDEF research).

Biodiversity Effects on Ecosystem Functioning Research

Although BDEF research has only proliferated in the past few decades, it has a long history. Darwin (1859) noted that increased diversity leads to greater productivity and this basic ecological principle has long been used in intercropping studies by agriculturists (Vandermeer 1989). However, more recent interest is commonly attributed to a meeting in Mitwitz, Germany in 1991, the results of which were published in 1994 (Schulze & Mooney 1994; Mooney 2002). In addition to a large number of smaller manipulative experiments, interest in BDEF has also resulted in a number of long-term large-scale experiments, primarily in temperate grasslands, including nearly decade old experiments by Tilman at Cedar Creek, MN (Tilman et al. 2001), BioCON (Reich 2009) and BIODEPTH, a trans-European series of BDEF experiments (Hector et al. 1999).
The wealth of studies over the past two decades have resulted in a number of recent meta-analyses and reviews (Hooper et al. 2005; Srivastava & Vellend 2005; Balvanera et al. 2006; Cardinale et al. 2006; Duffy 2009). The result of these meta-analyses has been a general consensus, although perhaps not overwhelming, that there is a positive relationship between biodiversity and ecosystem functioning. Both Cardinale et al. (2006) and Balvanera et al. (2006) provide clear evidence that biodiversity has significant positive effects on ecosystem functioning in a range of ecosystem types and at various trophic levels. Cardinale et al. (2006) also report, however, that the levels of productivity achieved by mixtures tends to be no different from that achieved by the single most productive species.

This conclusion illustrates one of the most contentious issues in the history of BDEF research—what is the mechanism that drives the described relationships between biodiversity and ecosystem functioning? Mechanisms driving the relationship between ecosystem functioning and biodiversity have traditionally been divided into two classes: sampling effects and complementarity effects. Positive BDEF relationships are often attributed to sampling effects in which one, or only a few species, has a significant effect on ecosystem functioning (Aarssen 1997; Huston 1997). There is ongoing debate as to whether the sampling effect is a biological mechanism relevant in nature, or simply an artefact of experimental design (Huston 1997; Hooper et al. 2005; Cardinale et al. 2006). A second mechanism, however, complementarity effects, can also produce positive BDEF relationships. These effects result from positive interactions between species due to niche differentiation and facilitation (Loreau & Hector 2001) and are generally accepted as genuine biodiversity effects (Lanta & Lepš 2006) with real biological relevance (Loreau 2000). Statistical methods known as additive partitioning have been developed to partition the effects due to the sampling effect and complementarity (Loreau & Hector 2001; Fox 2005). In a subsequent meta-analysis Cardinale et al. (2007) use the additive partitioning (Loreau & Hector 2001) method to show that patterns used to attribute the effects of diversity to sampling effects in their previous analysis (Cardinale et al. 2006) are produced by a combination of complementarity and
sampling effects, and that the contributions of sampling effects are equalled or exceeded by species complementarity (Cardinale et al. 2007).

In addition to the focus on sampling effects and complementarity as the mechanisms driving BDEF relationships, there has been interest in direct effects of species composition. The argument here focuses on the idea that the type of species in a community may have as much impact as the number of species. Early BDEF experiments often showed a significant effect of composition in addition to effects of diversity (Symstad et al. 1998; Hector et al. 1999; Fridley 2003). Tests of the mass ratio hypothesis (Grime 1998), which predicts that the influence of a species or group of species on ecosystem functioning is proportional to their input to primary production, have shown a particular focus on species identity as the driver of ecosystem properties.

These effects of species identity may be particularly important when predicting the effects of species loss in natural extinction scenarios. Certain traits of organisms are likely to predispose some species to extinction across a variety of taxa (reviewed in Duffy et al. 2009) resulting in non-random patterns of extinction. When extinctions are not random, models predict that the effects of extinction on ecosystem processes are likely to depend heavily on the extinction scenario used (Solan et al. 2004; Bunker et al. 2005). Consequently, knowing the effects of species identity on ecosystem functions is crucial in predicting the effects of non-random species loss (Balvanera et al. 2005; Ball et al. 2008). In this thesis I examine some of the various links between plant identity and ecosystem functions in a natural grassland in northern Canada.

**Biodiversity Loss in a Changing Environment**

In addition to the anthropogenically caused biodiversity loss and species compositional change described above, humans also directly influence the habitats or environmental conditions in which these species live. For example, nitrogen input into the terrestrial nitrogen cycle is estimated to have at least doubled as a result of human activities (Vitousek et al. 1997). Anthropogenic impacts on the global
nitrogen cycle are occurring via activities such as combustion of fossil fuels, production of nitrogen fertilizers and cultivation of nitrogen fixing legumes (Galloway et al. 1995; Köchy & Wilson 2001). Increases in nitrogen have especially become a concern in northern ecosystems with the onset of global warming; increasing temperatures due to global warming are expected to increase levels of both nitrogen and phosphorus in the soil due to increased rates of mineralization (Chapin et al. 1995; Shaver et al. 2000).

Changes in environmental conditions could influence the effect that species have on ecosystem properties in two ways. Firstly, changes in environmental conditions could directly influence the diversity or the composition of the plant species in an ecosystem. Different species have different sensitivities to environmental change, resulting in different risks of extinction or increasing in abundance. The extinction risk for a species depends both on intrinsic traits, such as growth form, but also on extrinsic factors, such as environmental conditions (Duffy et al. 2009). For example, nitrogen deposition or fertilization can change species dominance (Vitousek et al. 1997) because different growth forms and functional types show different responses to the additional nutrients (Chapin et al. 1995; Gough & Hobbie 2003). With increasing nitrogen, there are predicted to be decreases in legumes (Suding et al. 2003) and forbs (Zavaleta & Hulvey 2004) with increases in graminoids (Gough & Hobbie 2003; Zavaleta & Hulvey 2004).

Secondly, changes in environmental conditions could influence the effects of species on ecosystem properties if this relationship is context dependent, i.e., the influence of a species on an ecosystem may depend on the environment in which it is found. Ecologists, however, rarely look at BDEF relationships in an environmental context (Cardinale et al. 2000) and the majority of BDEF studies have been done in a single environment. Of the studies that have tested for an interaction between species diversity and environmental conditions, most have detected context-dependence. Various authors have reported that the response of community biomass (Craine et al. 2003) and decomposition (Dijkstra et al.
to fertilization have both been dependent on species composition. Further, diversity effects are frequently reported to be stronger at higher levels of fertility (Fridley 2002; Lanta & Lepš 2006; Dijkstra et al. 2007; Gross et al. 2007). A few studies have reported a lack of dependence on environmental conditions. For example, Craine et al. (2003) reported that effects of elevated CO₂ were consistent across different community compositions. As biodiversity is changing both in concert with, and as a result of, environmental changes, our knowledge of the degree of context dependence is going to greatly affect our ability to predict the effects of biodiversity loss. Some of the experiments described in this thesis test if the effects of different plant functional groups on determining some ecosystem functions are context dependent.

**What is Ecosystem Functioning and Which Ecosystem Properties Are Important?**

Ecosystem function can be defined as “the activities, processes or properties of ecosystems that are influenced by its biota” (Naeem et al. 2002). The nature of the relationship, or the existence of a relationship, between biodiversity and ecosystem functioning depends on the ecosystem property measured (Balvanera et al. 2006). Some researchers use the terms “ecosystem functioning” and “ecosystem properties” synonymously (Hooper et al. 2005), as I have done throughout this thesis. Most BDEF studies have primarily examined effects of biodiversity on primary productivity (Hooper et al. 2005; Balvanera et al. 2006), which is both influenced by, and influences, a wide range of ecosystem properties. In order to increase the generality of these relationships, however, we must examine a broader range of ecosystem properties. A number of studies have expanded the ecosystem properties examined by exploring the effects of biodiversity on nutrient cycling (Hooper & Vitousek 1997, 1998; Symstad 2000; Mulder et al. 2002; Suding et al. 2006) and a meta-analysis found positive biodiversity effects for most ecosystem properties associated with nutrient cycling (Balvanera et al. 2006). The effects of biodiversity on a wider range of ecosystem properties have been examined including decomposition (Stephan et al. 2000; Ruesink & Srivastava 2001), soil microbial communities (Stephan et
Hedlund et al. 2003), invasibility (van Ruijven et al. 2003; Fargione & Tilman 2005) and the composition and diversity of herbivores (Symstad et al. 2000). Generally, plant biodiversity is thought to play a larger role in determining above-ground ecosystem properties, such as primary productivity, than below-ground, such as microbial biomass (Loreau et al. 2001; Porazinska et al. 2003) with belowground or soil processes being primarily influence by the characteristics of the dominant species, rather than diversity itself (Hooper et al. 2005). Balvanera et al. (2006), however, examined the effects on numerous ecosystem properties in a meta-analysis and detected no differences between biotic and abiotic ecosystem properties, stocks and rates, nor between those more related to carbon, nutrient or water cycles.

Different ecosystem functions will respond to changes in biodiversity in different ways. Thus, it is becoming increasingly evident that the importance of species in determining ecosystem functioning may depend on the number of ecosystem properties considered together. Commonly BDEF studies examine only a single ecosystem function at a time, and a saturating relationship between this function and diversity suggests that some species are functionally redundant with respect to that particular ecosystem property (Hector & Bagchi 2007). However, when multiple ecosystem functions are considered simultaneously, or multifunctionality, a greater number of species is required to maintain ecosystem functions (Hector & Bagchi 2007). Different sets of species will influence different functions, so as more functions are considered, more species will be required to maintain these functions (Hector & Bagchi 2007). Consequently, studies that focus on only a single ecosystem property will underestimate the number of species required to maintain multifunctionality (Hector & Bagchi 2007). In this thesis I examine the influence of plant identity on a range of ecosystem properties to examine the consistency of functional group effects across the different ecosystem properties.
Why Study Functional Groups Rather Than The Species Directly?

Plant functional groups are groups of species that show similar responses to the environment and have similar effects on ecosystem function (Diaz & Cabido 2001). Functional groups are based on a set of common biological attributes and do not imply any phylogenetic relationship (Lavorel et al. 1997) although species within a functional group are likely to be closely related (Blondel 2003). The origin of functional groups is likely Root’s (1967) ecological guild concept, where species are grouped together based on similarities of what they do in communities. Subsequently, Cummins (1974) proposed functional groupings of organisms that were independent of taxonomy to address important process-oriented ecological questions. There is a renewed interest in classifying species into groups that are related directly to function, rather than phylogeny (Lavorel et al. 1997).

This renewed interest has been particularly useful in BDEF research because this grouping of species allows simplification in the understanding of complex, ecosystem-level processes. As we examine the influence of the biota on ecosystem properties, functional groups are a convenient method to deal with the practical limitations of collecting and analyzing data on all species (Epstein et al. 2001). Grouping species simplifies the variation between them while still providing predictive power (Reich et al. 2001) and has the advantage of arriving at a more general conclusion (Diekmann & Falkengren-Grerup 2002).

Plant functional groups are purpose-dependent—the classification system will depend on the aim of the study, its scale and the ecosystem process of interest (Woodward & Cramer 1996; Diaz & Cabido 2001). These factors determine whether the functional groups created will be functional response groups or functional effect groups (Diaz & Cabido 2001). Functional response groups are groups of species that respond to the environment in a similar way (e.g. grazing tolerant vs. non-tolerant plants) whereas a functional effect group is a group of species that have similar effects on ecosystem processes (e.g. N-fixers, vs. non-N-fixers) (Diaz & Cabido 2001).
Simplifying species diversity into functional groups for biodiversity research has raised some concerns. Functional groups are often defined *a priori* based on morphology, physiology and phenology. Generally these groupings are made on the assumption that the characteristics that separate these groups are related to the ecosystem function of interest (Tilman et al. 1997; Symstad & Tilman 2001) and numerous studies have converged in their definition of functional groups, at least for grassland studies, separating C_3 and C_4 grasses, legumes and forbs, with additional groups sometimes being based on phenology (Hooper & Vitousek 1997; Wardle *et al.* 1999; Reich *et al.* 2001; Lanta & Lepš 2006; Fornara & Tilman 2008). This approach has been criticized as being subjective, and a more objective grouping of species has been suggested based on a multivariate grouping of plant traits that reflect the function of interest (Fonseca & Ganade 2001). Although this multivariate method has been promoted as being less arbitrary, functional groups defined on traits are often very similar to those defined *a priori* (Chapin *et al.* 1996; Reich *et al.* 2001; Milcu *et al.* 2008). Further, how the species are grouped largely depends on which traits are chosen. Often the traits chosen are those that are easily measured, rather than those which may be more explicitly linked to the function of interest, and it is assumed that these traits can be used as a surrogate of that function (McIntyre *et al.* 1999). Traits may change with the environment in a manner that may not be consistent for all species, and thus the definition of a functional group may depend on growing conditions (Woodward & Cramer 1996; Dyer *et al.* 2001).

Exemplifying these concerns, Wright *et al.* (2006) recently compared the predictive power of functional group richness on ecosystem functioning when functional groups were based on the traditional *a priori* method versus when species were randomly assigned to groups. They found that the traditional scheme didn’t predict ecosystem function any better than groupings of species done at random. Regardless of these concerns, a large number of BDEF experiments have used functional groups to simplify examinations of species diversity both in the past (Hooper & Vitousek 1998; Wardle *et al.* 1999; Tilman *et al.* 2001) and in more recent studies (Gross *et al.* 2007; De Deyn *et al.* 2009; Fornara *et al.*
In this thesis I have created functional effect groups, grouping species \textit{a priori} based on traits (e.g. C:N, stature, N-fixation ability) that were potentially relevant to the ecosystem properties of interest.

\textbf{Methods for Studying Relationships Between Biodiversity and Ecosystem Functioning}

A variety of methods have been used to manipulate diversity in biodiversity ecosystem functioning experiments. Some early experiments used diversity gradients created by varying resource availability (Tilman & Downing 1994), although this method has been frequently criticized (Givnish 1994; Tilman et al. 1994). The majority of experiments have focused on synthetic assemblages of species, also called random assembly experiments (e.g. Tilman et al. 1997; Hector et al. 1999; Fridley 2003; Hooper et al. 2005). These experiments are typically planted communities on common substrate composed of differently randomly assembled combinations of species to represent different diversity levels. Although random assembly experiments are essential for determining a causal relationship between the number of species or functional groups and ecosystem properties, they may be less useful in determining the effect that these groups have in a natural community (Huston 1997; Loreau et al. 2001). As the species combinations are randomly assembled, species combinations in these experiments usually do not mimic real extinction scenarios, and have been criticised as trading off realism for precision in interpretation (Duffy et al. 2009).

Although the majority of BDEF studies are still random assembly experiments, there has been a more recent shift in emphasis to use experiments in which species or functional groups are removed from established natural communities (Diaz et al. 2003). Removal experiments have long been used to study both competition and facilitation in a variety of ecosystems including grasslands (Fowler 1981), salt-mashes (Emery et al. 2001), forest understories (Graham & Turkington 2000) and alpine communities (Choler et al. 2001). These experiments traditionally removed all neighbours surrounding a ‘target species’ (Gurevitch & Unnasch 1989). More recently, removal experiments in which the ‘target
organism’ is removed from the intact community have been used to examine the effects of non-random species loss on ecosystem functioning (e.g. Symstad & Tilman 2001; Smith & Knapp 2003; Wardle & Zackrisson 2005; Wardle et al. 2008).

Removal experiments have advantages over random assembly experiments for studying diversity. Firstly, they use communities that have undergone natural assembly processes and contain species at natural levels of abundance (Diaz et al. 2003). In random assembly experiments consideration is rarely given to natural levels of relative abundance (Diaz & Cabido 2001) and the abundance of some groups (especially legumes) may be unnaturally high (Diaz et al. 2003). Removal experiments may be especially useful in ecosystems where artificial communities are difficult to create, such as arctic ecosystems dominated by long-lived perennials. Removal experiments may be better suited to determining indirect effects of loss of particular species; it is difficult to predict emergent properties of assemblages based on the traits of individuals, as predicted from monocultures, because the complex interactions between species are difficult to predict (Rosenfeld 2002).

There are challenges to using removal experiments. There is generally higher variability between replicates than in random assembly experiments, which makes statistical tests less powerful (Diaz et al. 2003). The intensive nature of the experimental set-up means that the number of plots that can be maintained is limited (Diaz & Chapin 2000). Total removal is difficult to achieve, and re-growth often occurs, especially when working in communities with clonal species (Hils & Vankat 1982). Different removal treatments may result in the removal of different amounts of biomass. Previous removal studies have either not adjusted for differences in biomass (e.g. Buonopane et al. 2005) or used different methods to adjust for the different amounts of biomass removed such as random biomass removals (Symstad & Tilman 2001) or incorporating the biomass removed as a covariate in the analysis (e.g. Wardle & Zackrisson 2005). The primary criticism of this type of experiment is that, when only the above-ground biomass is removed, roots are left in the soil. Decaying roots may result in the release of
nutrients, as well as carbohydrates to soil microbes (Campbell et al. 1991; McLellan et al. 1995). The alternative, however, removing the roots, has numerous adverse effects including disruption of mycorrhizal networks and soil disturbance and these effects are likely enormous compared to root decay (McLellan et al. 1995). Despite these challenges, removal experiments hold a lot of promise in advancing ecological theory in the biodiversity ecosystem functioning field, but also in predicting the effects of extinction in realistic scenarios.

**Thesis Overview**

The primary objective of this research was to examine the importance of functional group identity in determining ecosystem properties. The basis of this research was a functional group removal experiment conducted in a northern grassland in the Yukon Territory, Canada. The experimental design was termed a ‘functional group knock-out’ experiment, in that the role of a functional group in an intact ecosystem was determined by removing a target functional group. In this way, the role of this group of species in an intact community can be determined by observing how the community functions with a full complement of species compared with a community with that group of species removed. With these methods I could determine both the direct influence of the functional group on ecosystem properties through its presence and abundance, but also its indirect effects through interactions with the other members of the community.

The secondary objective was to determine if the roles of functional group identity in determining ecosystem properties were context-dependent. To this extent, the functional group removals were conducted across two environmental treatments, fertilizer and fungicide. By comparing the effects of removals between environments I could determine whether the effect of a functional group on a particular ecosystem property was likely to change if the environmental conditions changed.

This removal experiment provides the basis for all the chapters in this thesis. The effect of functional groups, and the possible context-dependence of these effects, was examined across a range
of ecosystem properties that are discussed in Chapters 2 to 5. In Chapter 2, *Ecosystem properties determined by plant functional group identity*, I explored effects of functional group identity on both biomass compensation and soil properties. I report the short-term (4 years) effects of both the identity of the removed functional group, and also the functional groups remaining, on biomass compensation after plant removal. I further examine whether the effects of the three functional groups, forbs, graminoids and legumes, on soil moisture, soil nutrients and light conform to the predictions of the mass ratio theory (Grime 1998). The mass ratio theory predicts that the properties of an ecosystem will be determined by the components that make up the largest proportion of the biomass, or that ecosystem functioning will be determined by the traits of the dominant plants (Grime 1998). In this chapter I discuss whether forbs, the dominant functional group in our ecosystem, are the primary determinants of ecosystem properties in this northern grassland.

In Chapter 3, *Biomass compensation and plant responses to plant functional group removal*, I examine the longer-term (7 years) effects of plant functional group removals on plant community structure. After briefly exploring the short-term effects of functional group on biomass compensation in Chapter 2, I describe these effects, both on a longer time scale, but also in more detail examining both functional group and species-specific responses to removals. I specifically investigate the responses of dominant, sub-dominant and rare species to removals, as well as effects on species richness and diversity, to examine how functional group identity determines the degree of biomass compensation.

Chapter 4, *Plant identity differentially affects leaf and root decomposition*, focuses on the effects of plant functional group identity on a particular ecosystem property, litter decomposition. Litter decomposition is an important driver of both nutrient and carbon dynamics (Chapin et al. 2009), and as a result it is an important determinant of other ecosystem functions such as productivity. I examined the effects of the composition of the living community on decomposition of both leaf and litter tissue from a common litter source. This study is one of only a few to examine plant-driven environmental effects on
decomposition, and the inclusion of effects on root decomposition makes it unique. The length of this study (5 years) also makes it unique: most decomposition studies typically run for a single year and it is often assumed that short-term and long-term decomposition will follow the same patterns (Aerts 1997), although it is rarely tested.

Chapter 5, *Biodiversity loss influences decomposition through more than one mechanism*, further examines the potential mechanisms through which the identities of the living plant community may influence litter decomposition. Changing the composition of the living community has the potential to affect decomposition through two different mechanisms. First, changing the living community may change the decomposition micro-environment resulting in plant-driven environmental effects on decomposition, as described in Chapter 4. Secondly changing the living plant community necessarily changes the composition of the litter, as the litter community is derived from the previous living plant community. A change in the composition of the litter can change decomposition if the different species decompose at different rates, or if there is an effect of litter mixing between particular species. Finally, these two mechanisms may interact if the decomposition of particular species depends on the environment created by the living community. I tested both mechanisms by decomposing a series of litter mixtures, made up of all possible combinations of the dominant member of each functional group, across the different removal treatments. Finally, within these mixtures I could also examine species-specific decomposition as a cause for non-additive effects in mixtures as I was able to distinguish species within mixtures after decomposition.

Chapter 6 summarizes the results and interpretations of these four studies. I discuss some of the implications of the methods used in the experiments, and also the broader conclusions about the importance and context-dependence of plant identity. Finally, I explore the future implications of my examination of the role of functional group identity in this northern grassland.
References


Duffy, J.E., Srivastava, D.S., McLaren, J.R., Sankaran, M., Solan, M., Griffin, J., Emmerson, M., & Jones, K.E. (2009). Forecasting decline in ecosystem services under realistic scenarios of extinction. In...


CHAPTER TWO

Ecosystem Properties Determined by Plant Functional Group Identity

Introduction

The effect that organisms have on their abiotic and biotic environments is a central research topic in ecology. Over the past decade one focus of this research has been an examination of the relationship between the number of species or functional groups in an ecosystem and the properties or the functioning of that ecosystem, or ‘biodiversity–ecosystem functioning’ research. This research was prompted by the massive current and predicted future loss in biodiversity, and the concern that this loss of species will have negative effects on ecosystem functions such as productivity and carbon storage (Hooper et al. 2005). Two meta-analyses (Balvanera et al. 2006; Cardinale et al. 2006) have shown that most studies support the hypothesis that decreases in species richness cause a decrease in ecosystem functioning. Early biodiversity–ecosystem functioning experiments often showed a significant effect of composition in addition to effects of species richness, promoting the idea that the type of species in a community may have as much impact as the number of species. The mass ratio hypothesis (Grime 1998) predicts that the influence of a species or group of species on ecosystem functioning is proportional to their input to primary production, i.e. ecosystem functioning is determined by the traits of the dominant plants. There have been few direct tests of this hypothesis, and experimental tests have both supported (Vile et al. 2006; Mokany et al. 2008) and rejected (Spehn et al. 2002; Wardle et al. 2008; Peltzer et al. 2009) this hypothesis.

Few experiments have examined the effects of diversity and composition on ecosystem properties in more than one environment, despite the knowledge that the processes that transform

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ecosystems, such as nitrogen deposition, may also result in loss or changes in the types of species present in a community (Hooper et al. 2005). It is imperative to simultaneously examine multiple environments, because changes in conditions may alter communities in ways that are difficult to anticipate (Doak et al. 2008). In those few experiments where biodiversity effects were examined in different environments or contexts, the nature of the relationship between diversity (Reich et al. 2001a; Fridley 2002; Reich et al. 2004; Dijkstra et al. 2007) or composition (Craine et al. 2003) and ecosystem properties often differed.

The majority of studies examining the impacts of diversity and composition on ecosystem functioning have been conducted in artificially created communities using random assemblages of species. These types of experiments are essential for determining a causal relationship between the number of species or functional groups and ecosystem properties, but may be less useful in determining the role of these groups in a natural community (Huston 1997; Loreau et al. 2001). More recently, removal experiments in natural communities have been promoted for biodiversity–ecosystem functioning studies (Diaz et al. 2003) because they use communities that have been formed through natural assembly processes, contain species at their natural abundance and also allow compensatory growth by the remaining species (Diaz et al. 2003). The role of a particular group of species in an intact community can be determined by observing how a community functions with a full complement of species compared with a community with that group of species removed. This method allows us to determine the direct influence of a group of species on ecosystem properties through its presence and abundance, and also its indirect effects on ecosystem properties through interactions with other members of the community.

The nature of the relationship between biodiversity and ecosystem functioning depends on the ecosystem property that is measured (Balvanera et al. 2006). Most studies have focused on the effects on primary productivity (Hooper et al. 2005; Balvanera et al. 2006). Although this is an essential
component of a wide range of ecosystem properties, a broader range of properties must be examined to establish the generality of these results. Diversity and composition of the plant community have been reported to influence numerous other ecosystem properties including soil nutrient availability (Hooper & Vitousek 1998), invasibility (Emery & Gross 2006), soil C accumulation (Fornara & Tilman 2008) and soil moisture (Hooper & Vitousek 1998). Therefore, we chose to examine the impact of different plant functional groups on a fairly wide range of ecosystem properties.

In this study we report results from a functional group removal experiment in which single functional groups (graminoids, legumes and non-leguminous forbs (hereafter called forbs)) were experimentally removed from a series of plots in a grassland in northern Canada. By comparing these plots from which species were removed to plots containing the entire suite of species, we examined the role of identity of the removed functional group in determining a suite of ecosystem properties in an intact community. Secondly, we tested the hypothesis that the dominant functional group, the forbs, would have the largest effect on ecosystem properties, as predicted by the mass ratio hypothesis (Grime 1998). The mass ratio hypothesis has been used to describe the effects of both species (Vile et al. 2006; Mokany et al. 2008) and functional groups (Wardle et al. 2008; Peltzer et al. 2009) based on their proportional abundance in a community. We examined impacts of functional group removal on the remaining members of the plant community through changes in biomass, and also on the potentially limiting soil nutrients, light and soil moisture. Thirdly, we examined whether the influence of a functional group in determining ecosystem function was dependent on environmental context, by using different fertilization and mycorrhizal environments. These environments were chosen because both are relevant to future environmental change. Global warming is expected to cause an increase in soil nutrient levels, especially in northern latitudes, because higher temperatures increase mineralization rates of both nitrogen and phosphorus (Chapin et al. 1995; Shaver et al. 2000). Additionally, the presence of mycorrhizal fungi may change a plant’s response to changes in nutrient status. Mycorrhizae
are affected by soil nitrogen levels, both in terms of their functioning and the type of relationship with plants they exhibit on the parasitic–mutualistic continuum (Johnson 1993). With these three questions, we investigate the importance of functional group identity in determining ecosystem functioning in order to better predict the effects of their loss.

**Methods and Materials**

**Site Description**

The study area is a relatively dry grassland near Kluane Lake in the south-western Yukon in northern Canada (61°4’13” N 138°23’1” W). The area is in the rainshadow of the St. Elias Mountains and receives a mean annual precipitation of c. 230 mm, about half of which falls as rain during the summer, but also includes an average annual snowfall of c. 1 m. The grassland is surrounded by a closed to relatively open spruce forest community dominated by *Picea glauca* (Moench) Voss. The grassland itself is dominated by *Poa glauca* Vahl and *Carex stenophylla* Wahlenb. ssp. *eleocharis* (Bailey) Hultén, and also contains many non-leguminous forbs (dominated by *Erigeron caespitosus* Nutt., *Artemisia frigida* Willd., *Penstemon gormanii* Greene, and *Pulsatilla ludoviciana* (Nutt.) Heller) and legumes (dominated by *Oxytropis campestris* (L.) DC.) (all nomenclature follows Cody (2000)). Grassland species were divided into three functional groups, namely graminoids (grasses and sedges), forbs and legumes.

**Experimental Plant Communities**

Experimental plots were established in May 2003 and removal treatments took place annually for 4 years until the end of the 2006 growing season. The experiment was a 4 x 2 x 2 fully crossed factorial design (4 removal treatments, +/0 fertilizer, +/0 fungicide). Each of the 16 treatments was replicated 5 times and randomly assigned, resulting in a total of 80 plots. The locations of 1 x 1m plots were selected on a constrained random basis, ensuring that all plots contained representatives from each functional group, with a minimum distance of 0.5 m between plots, contained within an area of approximately 0.5
ha. As most of the plant species in the community are clonal, at the beginning of each growing season plots were spaded 10 cm outside the plot boundary to a depth of 25 cm to sever below-ground connections between plants inside and outside the plots.

There were four removal treatments: independent removal of each of the three functional groups (graminoids, forbs and legumes) and a no-removal control. Functional groups were chosen based on traits that were potentially relevant to the ecosystem properties of interest (e.g. C:N, stature, N-fixation ability). Different grassland studies often converge in their definitions of functional groups, separating out C$_3$ and C$_4$ grasses, legumes and forbs, with additional groups sometimes being created based on phenology (e.g. Hooper & Vitousek 1998; Wardle et al. 1999; Symstad & Tilman 2001). In our study additional functional groups were not created based on photosynthetic pathway, as all plants in this ecosystem are C$_3$, or phenology, as the growing season is very short (c. 12-16 weeks). In 2003, plants were removed from the plots and from the buffer zone (the 10-cm strip between the plot boundary and the spade line) using Round-up™ glyphosate, a non-selective herbicide. Herbicide was painted precisely onto the leaves and stems of selected plants, thus having minimal non-target effects on neighbours. Glyphosate strongly bonds to soil particles, which limits its phytotoxicity in soil (Ahrens 1994), and is eventually broken down by soil microorganisms (WHO1994). Herbicide application was repeated every 4-7 days until visible leaf yellowing occurred and plants were then clipped at soil level and removed from the plots. Removal treatments were maintained in 2004 using herbicide application and clipping, and in the subsequent two years the very minimal regrowth of target plants was clipped at ground level early in the growing season. Other functional groups were allowed to invade the newly available space created by the removals.

Fertilizer and fungicide treatments were applied upon completion of the removals, which took place on 20 July in 2003 and in early June of each subsequent year. Fertilizer was applied each year to half the plots in granular form at a rate of 17.5 g N m$^{-2}$, 5.8 g P m$^{-2}$ and 5.8 g K m$^{-2}$. This application rate
was used to be consistent with many other studies being done in the area (e.g. John & Turkington 1997; Turkington et al. 2002). The fungicide Benlate™ (active ingredient benomyl) was applied to half of the plots as a soil drench (2 L m\(^{-2}\) plot) every two weeks from early June to mid-August at a rate of 2.5 g benomyl m\(^{-2}\) per application. Plots that did not receive fungicide received an equivalent amount of water. Several studies have used benomyl to effectively suppress arbuscular mycorrhizal (AM) fungal root colonization in the field (e.g. Newsham et al. 1995; Hartnett & Wilson 1999; Cahill et al. 2008), and it is thought to be a better choice than other fungicides (Paul et al. 1989). Benomyl applications reduced mycorrhizal colonization rates from 50% to less than 10% of root intersections (J. McLaren, unpublished data). It has been suggested that benomyl causes a number of unintended effects, such as effects on bacterial densities (Smith et al. 2000), and it can be difficult to separate intended effects caused by the reduction in AM colonization from unintended direct effects of benomyl. In the most comprehensive test of benomyl effects, Smith et al. (2000) reported that the principal effect of benomyl was suppression of mycorrhizal fungi, that there were mixed or small effects on other soil properties, and that “benomyl applications remain the most useful tool for experimentally manipulating mycorrhizal symbiosis in the field”.

**Response Measurements**

We collected data on above-ground biomass using non-destructive point-intercept sampling (Bret-Harte, 2004). Total leaf hits of all species was determined in July of each year at 100 points arranged in a 10 x 10 grid, each separated by 10 cm in a 1-m\(^2\) quadrat. In a separate set of plots, a series of regression equations was determined that equate the biomass of each species with the total number of leaf hits for a 1 x 1 m plot. For all species, the total number of intercepts was closely correlated with above-ground biomass, with \(r^2\) values consistently above 0.80 (Table 2-1). Five species were too rare to construct a reliable regression equation, and for each of these we used the equation from the species that most closely resembled that species morphologically. Biomass of all species was determined for each plot and
summed to determine total above-ground biomass. Additionally, species were divided into their respective functional groups, and above-ground biomass for each functional group was calculated separately.

### Table 2-1 Regression equations relating biomass to point interception.

<table>
<thead>
<tr>
<th>Species</th>
<th>Biomass regression</th>
<th>r²</th>
<th>Regression p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Androsace septentrionalis</em></td>
<td>Biomass = 0.735 + 1.120 TotalHits</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Antennaria rosea</em></td>
<td>Biomass = 0.293 + 1.800 TotalHits</td>
<td>0.98</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Arabis hollobi</em></td>
<td>Biomass = -0.762 + 2.083 TotalHits</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Artemesia frigida</em></td>
<td>Biomass = -1.928 + 1.603 TotalHits</td>
<td>0.90</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Aster alpinus</em></td>
<td>Biomass = 0.759 + 0.865 TotalHits</td>
<td>0.90</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Astragalus alpinus</em></td>
<td>Biomass = 1.878 + 0.534 TotalHits</td>
<td>0.90</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Astragalus williamsii</em></td>
<td>Biomass = 0.369 + 0.903 TotalHits</td>
<td>0.98</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Calamagrostis purpurascens</em></td>
<td>Biomass = 1.468 + 0.518 TotalHits</td>
<td>0.83</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Carex spp</em></td>
<td>Biomass = 0.917 + 0.548 TotalHits</td>
<td>0.83</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Carex stenophylla ssp. eleocharis</em></td>
<td>Biomass = 0.917 + 0.548 TotalHits</td>
<td>0.83</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Castilleja hyperborean</em></td>
<td>Biomass = -0.762 + 2.083 TotalHits</td>
<td>0.92</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Elymus caldera</em></td>
<td>Biomass = 2.375 + 0.420 TotalHits</td>
<td>0.80</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Elymus trachycaulus</em></td>
<td>Biomass = -0.045 + 0.644 TotalHits</td>
<td>0.99</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Erigeron caepitosus</em></td>
<td>Biomass = 1.614 + 0.909 TotalHits</td>
<td>0.86</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Festuca brachyphyla</em></td>
<td>Biomass = 1.977 + 0.520 TotalHits</td>
<td>0.81</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Oxytropis campestris</em></td>
<td>Biomass = -0.960 + 1.668 TotalHits</td>
<td>0.98</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Penstemon gormanii</em></td>
<td>Biomass = 1.638 + 1.609 TotalHits</td>
<td>0.95</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Plantago canescens</em></td>
<td>Biomass = 0.055 + 0.991 TotalHits</td>
<td>0.99</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Poa glauca</em></td>
<td>Biomass = 1.009 + 0.479 TotalHits</td>
<td>0.81</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Potentilla prostrata</em></td>
<td>Biomass = 0.735 + 1.120 TotalHits</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pulsatilla ludoviciana</em></td>
<td>Biomass = 2.651 + 1.00 TotalHits</td>
<td>0.95</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><em>Silene involucrata</em></td>
<td>Biomass = -0.762 + 2.083 TotalHits</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Zygadeuns elegans</em></td>
<td>Biomass = 1.468 + 0.518 TotalHits</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Very rare species do not have a biomass prediction equation. Instead, we chose the species that was most similar morphologically, and used the regression equation for that species as a substitute. Substituted species are as follows: ¹ *Potentilla prostrata* ² *Castilleja hyperborean* ³ *Carex stenophylla ssp. eleocharis* ⁴ *Calamagrostis purpurascens*
Percentage light interception was determined at approximately solar noon using a quantum meter with 6 evenly spaced sensors on a 50-cm wand (Apogee Instruments Inc., Utah, USA) at 1 m above the soil surface (above all vegetation) and also at the soil surface, with a single measurement for each taken in the middle of the plot. In 2003, light interception was measured on 11 August, and in subsequent years (2004-2006) in mid-July.

Soil moisture (%) was measured using a water content sensor (Hydrosense Water content measurement system, Campbell Scientific, Australia) at a depth of 10 cm. Two measurements were taken in each plot, and the average of these measurements was used in analysis. Soil moisture was measured each year at the peak flowering (mid-July) except in the first year of the experiment, when measurements were not taken until early August.

Nutrient supply rates were estimated using ion exchange membranes (Plant Root Simulator (PRS)™-probes; Western Ag Innovations Inc., Saskatoon, Canada) using separate cation- and anion-exchange resin membranes. Two probes of each type were used in each plot and pooled during analysis to account for soil heterogeneity. In 2003, the PRS™-probes were inserted into the soil in mid-July after the plant removals; in 2004-2006 the probes were inserted in late May, prior to fertilization and fungicide treatment application. The probes were left in place until the end of the growing season (mid-August) to measure in situ nutrient supply rates. Probes were analysed by Western Ag Innovations Inc., Saskatoon, Canada, for NO₃⁻, NH₄⁺, P, K, S, Ca, Mg, Mn, Fe, Cu, Zn, B, Al and Pb.

**Analysis**

We used a four-way repeated-measures ANOVA on each response, except soil nutrients, with the three main plot factors—functional group removal, fertilizer and fungicide—and year as the within-plot repeated-measures factor. Soil nutrient analyses were conducted using a four-way MANOVA on all soil nutrients followed by a four-way ANOVA on each soil nutrient, with the p-value adjusted using a Bonferroni correction. When there was a significant effect of removals, removals were compared using a
Tukey’s comparison of all means (Quinn & Keough 2002). When there was a significant interaction between removals and environment, analyses were run independently for each environment level.

Results

Biomass Compensation

Different amounts of biomass were removed in the initial removal treatments in 2003, with most biomass being removed when the forbs were removed (40.76g m\(^{-2}\) ± 3.83 SE), followed by the graminoids (30.55g m\(^{-2}\) ± 2.08 SE), and the legumes (10.08 g m\(^{-2}\) ± 1.23 SE) \(F_{2,59}=35.77, p<0.001\), Tukey). This resulted in different plots beginning the experiment with different amounts of living biomass. After four growing seasons ‘recovery’ by remaining functional groups, differences still remained between removals in total plot above-ground biomass (Fig. 2-1).

Above-ground biomass was significantly affected by removal treatments, and these effects were dependent on year since removal (Table 2-2, Fig. 2-1). In most cases, all removal treatments had less biomass than the no-removal control, with the removal of forbs resulting in less biomass than either legume or graminoid removal, although in 2005, when biomass was highest, only forb removal plots had less biomass than controls. Effects of fertilizer on above-ground biomass were also dependent on year (Table 2-2), with fertilizer only significantly increasing biomass in 2005, when biomass was higher than in the other 3 years (in which fertilizer was non-significant).
Fig. 2-1 Mean total above-ground biomass (±SE) in different plant functional group removal treatments in 2003-2006. Treatments with the same letter (with subscripts indicating removal treatment) for a given year are not significantly different (p<0.05) using Tukey’s comparison of all means. Legend: ● no removals; □ forbs removed; Δ graminoids removed; ▽ legumes removed.

The different functional group components of total biomass were also affected by removal treatments; differential regrowth by the functional groups was apparent. Neither legume biomass (Table 2-2, Fig. 2-2c) nor forb biomass (Table 2-2, Fig. 2-2d) was influenced by removal treatments. Graminoid biomass, in contrast, increased when either forbs or legumes were removed in plots without fungicide (Removal F_{2,120}=16.73, p<0.001; Fig. 2-2a), although when fungicide was applied to these plots neither differed from the no-removal control (Removal F_{2,120}=5.51, p=0.005; Fig. 2-2b).
Table 2-2 Summary of four-way repeated-measures ANOVA on total biomass (2003-2006) and for independent biomass of each functional group (2003-2006) in a 4-year functional group removal experiment, with additional factorial treatments of fertilizer and fungicide. Forb, graminoid and legume biomass all have the same d.f. Bold values are significant at p < 0.05

<table>
<thead>
<tr>
<th>Source</th>
<th>Total Biomass</th>
<th></th>
<th>Forb Biomass</th>
<th></th>
<th>Graminoid Biomass</th>
<th></th>
<th>Legume Biomass</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f.</td>
<td>F</td>
<td>P</td>
<td>d.f.</td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Removal</td>
<td>3,16</td>
<td>99.36</td>
<td>&lt;0.001</td>
<td>2,12</td>
<td>2.32</td>
<td>0.141</td>
<td>30.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>1,16</td>
<td>28.53</td>
<td>&lt;0.001</td>
<td>1,12</td>
<td>43.54</td>
<td>&lt;0.001</td>
<td>37.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fungicide</td>
<td>1,16</td>
<td>3.56</td>
<td>0.08</td>
<td>1,12</td>
<td>2.30</td>
<td>0.155</td>
<td>23.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Year</td>
<td>1,272</td>
<td>220.72</td>
<td>&lt;0.001</td>
<td>1,204</td>
<td>201.29</td>
<td>&lt;0.001</td>
<td>54.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Removal x Fertilizer</td>
<td>3,16</td>
<td>2.21</td>
<td>0.127</td>
<td>2,12</td>
<td>0.67</td>
<td>0.532</td>
<td>0.54</td>
<td>0.598</td>
</tr>
<tr>
<td>Removal x Fungicide</td>
<td>3,16</td>
<td>0.61</td>
<td>0.620</td>
<td>2,12</td>
<td>0.58</td>
<td>0.573</td>
<td>10.92</td>
<td>0.002</td>
</tr>
<tr>
<td>Removal x Year</td>
<td>3,272</td>
<td>4.18</td>
<td>0.007</td>
<td>2,204</td>
<td>2.35</td>
<td>0.098</td>
<td>2.52</td>
<td>0.082</td>
</tr>
<tr>
<td>Fertilizer x Fungicide</td>
<td>1,16</td>
<td>0.00</td>
<td>0.970</td>
<td>1,12</td>
<td>0.25</td>
<td>0.630</td>
<td>3.36</td>
<td>0.092</td>
</tr>
<tr>
<td>Fertilizer x Year</td>
<td>1,272</td>
<td>8.03</td>
<td>0.005</td>
<td>1,204</td>
<td>10.71</td>
<td>0.001</td>
<td>11.12</td>
<td>0.001</td>
</tr>
<tr>
<td>Fungicide x Year</td>
<td>1,272</td>
<td>1.11</td>
<td>0.293</td>
<td>1,204</td>
<td>0.00</td>
<td>0.960</td>
<td>4.22</td>
<td>0.040</td>
</tr>
<tr>
<td>Removal x Fertilizer x Fungicide</td>
<td>3,16</td>
<td>2.56</td>
<td>0.092</td>
<td>2,12</td>
<td>3.33</td>
<td>0.071</td>
<td>0.29</td>
<td>0.752</td>
</tr>
<tr>
<td>Removal x Fertilizer x Year</td>
<td>3,272</td>
<td>1.14</td>
<td>0.333</td>
<td>2,204</td>
<td>0.01</td>
<td>0.992</td>
<td>0.72</td>
<td>0.487</td>
</tr>
<tr>
<td>Removal x Fungicide x Year</td>
<td>3,272</td>
<td>0.15</td>
<td>0.930</td>
<td>2,204</td>
<td>0.50</td>
<td>0.610</td>
<td>0.69</td>
<td>0.504</td>
</tr>
<tr>
<td>Fungicide x Fertilizer x Year</td>
<td>1,272</td>
<td>0.26</td>
<td>0.608</td>
<td>1,204</td>
<td>0.23</td>
<td>0.631</td>
<td>0.53</td>
<td>0.467</td>
</tr>
<tr>
<td>Removal x Fungicide x Fertilizer x Year</td>
<td>3,272</td>
<td>1.37</td>
<td>0.253</td>
<td>2,204</td>
<td>0.74</td>
<td>0.477</td>
<td>0.13</td>
<td>0.882</td>
</tr>
</tbody>
</table>
Fig. 2-2 Mean functional group above-ground biomass (±SE) in different plant functional group removal treatments in 2003-2006. Results are presented across fertilizer or fungicide treatments unless otherwise specified. Treatments with the same letter (with subscripts indicating removal treatment) for a given year are not significantly different (p<0.05) using Tukey’s comparison of all means; NS is not significantly different at p>0.05. There were no significant Year × Removal interactions, and Tukey’s comparisons are across years. Legend: ● no removals; □ forbs removed; △ graminoids removed; ▽ legumes removed. Note that the forb biomass axis is presented on a different scale than that of graminoids and legumes.
**Ecosystem Properties Responses**

Light interception was significantly reduced by the removal of any functional group, regardless of identity (Table 2-3, Fig. 2-3). The levels of light interception varied among years and between fertilizer treatments, but removal treatment effects remained constant (Table 2-3). Fertilizer and year interacted significantly (Table 2-3) because fertilizer directly increased percentage light interception only in 2005 (all other years were non-significant), when biomass was highest.

**Table 2-3 Summary of four-way repeated-measures ANOVA for light interception (2003-2006) and for soil moisture (2003-2006) in a 4-year functional group removal experiment, with additional factorial treatments of fertilizer and fungicide** Bold values are significant at p < 0.05

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>Light Interception</th>
<th>Soil Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Removal</td>
<td>3,16</td>
<td>9.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>1,16</td>
<td>16.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fungicide</td>
<td>1,16</td>
<td>0.57</td>
<td>0.463</td>
</tr>
<tr>
<td>Year</td>
<td>1,272</td>
<td>21.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Removal x Fertilizer</td>
<td>3,16</td>
<td>0.92</td>
<td>0.455</td>
</tr>
<tr>
<td>Removal x Fungicide</td>
<td>3,16</td>
<td>0.99</td>
<td>0.424</td>
</tr>
<tr>
<td>Removal x Year</td>
<td>3,272</td>
<td>1.84</td>
<td>0.140</td>
</tr>
<tr>
<td>Fertilizer x Fungicide</td>
<td>1,16</td>
<td>0.13</td>
<td>0.723</td>
</tr>
<tr>
<td>Fertilizer x Year</td>
<td>1,272</td>
<td>4.07</td>
<td>0.045</td>
</tr>
<tr>
<td>Fungicide x Year</td>
<td>1,272</td>
<td>1.52</td>
<td>0.219</td>
</tr>
<tr>
<td>Removal x Fertilizer x Fungicide</td>
<td>3,16</td>
<td>0.41</td>
<td>0.745</td>
</tr>
<tr>
<td>Removal x Fertilizer x Year</td>
<td>3,272</td>
<td>0.15</td>
<td>0.931</td>
</tr>
<tr>
<td>Removal x Fungicide x Year</td>
<td>3,272</td>
<td>0.11</td>
<td>0.954</td>
</tr>
<tr>
<td>Fungicide x Fertilizer x Year</td>
<td>1,272</td>
<td>0.30</td>
<td>0.585</td>
</tr>
<tr>
<td>Removal x Fungicide x Fertilizer x Year</td>
<td>3,272</td>
<td>0.72</td>
<td>0.538</td>
</tr>
</tbody>
</table>
Fig. 2-3 Mean percent light interception (±SE) in different plant functional group removal treatments in 2003-2006. Treatments with the same letter (with subscripts indicating removal treatment) for a given year are not significantly different (p<0.05) using Tukey’s comparison of all means. There was no significant Year × Removal interaction and Tukey’s comparison is across years. Legend: ● no removals; □ forbs removed; Δ graminoids removed; ∇ legumes removed.

Soil moisture, in general, was higher in plots with removal of forbs and graminoids than in controls. Removals affected soil moisture, with the effect of removal depending on year (Table 2-3; Yearly Removal effects: 2003 $F_{3,79}=15.17$, p<0.001; 2004 $F_{3,79}=7.07$, p<0.001; 2005 $F_{3,79}=2.27$, p=0.09; 2006 $F_{3,79}=4.24$, p=0.008). Soil moisture was higher in all years in plots where forbs were removed than in controls (except 2005, when summer precipitation was higher than in the other 3 years and no removal treatment affected soil moisture). It was also higher when graminoids were removed in 2003 and 2004 (Fig. 2-4) and when legumes were removed only in 2003 (Fig. 2-4). Soil moisture levels were also affected by environmental context (fertilizer treatments) directly (Table 2-3); across all years fertilizer treatments resulted in lower soil moisture than when plots remained unfertilized.
Fig. 2-4 Mean percent soil moisture (±SE) in different plant functional group removal treatments in 2003-2006. Treatments with the same letter (with subscripts indicating removal treatment) for a given year are not significantly different (p<0.05) using Tukey’s comparison of all means. Legend: ●no removals; □ forbs removed; Δ graminoids removed; ▽ legumes removed.

The MANOVA on soil nutrients showed significant effects of all three treatments (Table 4) and soil nutrients were subsequently examined independently. Removal treatments had different effects on different soil nutrients and these were consistent across years. The effects of removal on total N, NO\textsubscript{3} and Fe compared with values in no-removal controls were consistent across fertilizer and fungicide treatments, but effects on P interacted with fertilizer. Both forb and graminoid removal caused increases in total N (F\textsubscript{3,319}=9.61, p<0.001, Fig. 2-5a) and NO\textsubscript{3} (F\textsubscript{3,319}=7.56, p<0.001, Fig. 2-5b) and a decrease in P, although the P effect was not apparent when plots were not fertilized (fertilized only: F\textsubscript{3,159}=3.89, p=0.01, Fig. 2-5c). Legume removal had no significant effect on any soil nutrient except Fe, which decreased (F\textsubscript{3,319}=5.68, p<0.001, Fig. 2-5d). Removal of any functional group, be it graminoids,
forbs or legumes, had no effect in any year on many of the soil nutrients measured, including NH₄⁺ (F₃,319=0.39, p=0.76; 2006 levels < minimum detectable levels), Mg (F₃,319=0.27, p=0.86), K (F₃,319=3.24, p=0.02), S (F₃,16=1.59, p=0.19), Ca (F₃,319=0.69, p=0.57), Zn (F₃,16=1.75, p=0.16), B (F₃,16=0.94, p=0.42), Al (F₃,16=0.78, p=0.50) and Mn (F₃,319=0.83, p=0.48) and Cu (F₃,319=0.48, p=0.70).

Soil nutrients were also affected by environmental context. Predictably, fertilization increased total N, NO₃⁻, NH₄⁺, K and P (although effects on P interacted significantly with removals, as described above (Table 2-5), but also increased Mn, Fe and Mg and decreased S (Table 2-4). Fungicide application increased levels of total N, NO₃⁻, and S but had no effect on any other measured nutrient (Table 2-5).

Table 2-4 Summary of four-way MANOVA for soil nutrients (2003-2006) in a 4-year functional group removal experiment, with additional factorial treatments of fertilizer and fungicide. Bold values are significant at p<0.05

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Removal</td>
<td>42,342</td>
<td>1.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>14,115</td>
<td>92.55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fungicide</td>
<td>14,115</td>
<td>3.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Year</td>
<td>14,115</td>
<td>54.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Removal x Fertilizer</td>
<td>42,342</td>
<td>1.43</td>
<td>0.046</td>
</tr>
<tr>
<td>Removal x Fungicide</td>
<td>42,342</td>
<td>1.19</td>
<td>0.206</td>
</tr>
<tr>
<td>Removal x Year</td>
<td>42,342</td>
<td>0.68</td>
<td>0.935</td>
</tr>
<tr>
<td>Fertilizer x Fungicide</td>
<td>14,115</td>
<td>1.43</td>
<td>0.159</td>
</tr>
<tr>
<td>Fertilizer x Year</td>
<td>14,115</td>
<td>14.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fungicide x Year</td>
<td>14,115</td>
<td>0.59</td>
<td>0.871</td>
</tr>
<tr>
<td>Removal x Fertilizer x Year</td>
<td>42,342</td>
<td>0.78</td>
<td>0.837</td>
</tr>
<tr>
<td>Removal x Fungicide x Year</td>
<td>42,342</td>
<td>0.91</td>
<td>0.628</td>
</tr>
<tr>
<td>Removal x Fertilizer x Fungicide</td>
<td>42,342</td>
<td>0.87</td>
<td>0.702</td>
</tr>
<tr>
<td>Fungicide x Fertilizer x Year</td>
<td>14,115</td>
<td>1.08</td>
<td>0.379</td>
</tr>
<tr>
<td>Removal x Fungicide x Fertilizer x Year</td>
<td>42,342</td>
<td>0.70</td>
<td>0.918</td>
</tr>
</tbody>
</table>
Table 2-5 Summary of fertilizer and fungicide effects on mean nutrient supply rate in a 4-year functional group removal experiment. Fertilizer effects are presented across both removal and fungicide treatments, when there are no significant interactions. Fungicide effects are presented across both removal and fertilizer treatments, when there are no significant interactions. Only years and nutrients for which there was a significant fertilizer or fungicide effect, respectively, are presented.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Type of Effect</th>
<th>Direction of Effect</th>
<th>d.f.</th>
<th>F</th>
<th>P</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N</td>
<td>Fertilizer</td>
<td>+</td>
<td>319</td>
<td>1815.08</td>
<td>&lt;0.001</td>
<td>2003-6</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>Fertilizer</td>
<td>+</td>
<td>319</td>
<td>1279.14</td>
<td>&lt;0.001</td>
<td>2003-6</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>Fertilizer</td>
<td>+</td>
<td>239</td>
<td>107.48</td>
<td>&lt;0.001</td>
<td>2003-4     *</td>
</tr>
<tr>
<td>K</td>
<td>Fertilizer</td>
<td>+</td>
<td>319</td>
<td>72.84</td>
<td>&lt;0.001</td>
<td>2003-6</td>
</tr>
<tr>
<td>P</td>
<td>Fertilizer</td>
<td>+</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A        **</td>
</tr>
<tr>
<td>Mn</td>
<td>Fertilizer</td>
<td>+</td>
<td>319</td>
<td>55.60</td>
<td>&lt;0.001</td>
<td>2004, 2006</td>
</tr>
<tr>
<td>Fe</td>
<td>Fertilizer</td>
<td>+</td>
<td>319</td>
<td>24.60</td>
<td>&lt;0.001</td>
<td>2004, 2006</td>
</tr>
<tr>
<td>Mg</td>
<td>Fertilizer</td>
<td>+</td>
<td>79</td>
<td>4.34</td>
<td>0.04</td>
<td>2003</td>
</tr>
<tr>
<td></td>
<td>Fertilizer</td>
<td>-</td>
<td>79</td>
<td>4.86</td>
<td>0.03</td>
<td>2005</td>
</tr>
<tr>
<td>S</td>
<td>Fertilizer</td>
<td>-</td>
<td>319</td>
<td>33.07</td>
<td>&lt;0.001</td>
<td>2003-2006</td>
</tr>
<tr>
<td>Cu</td>
<td>Fertilizer</td>
<td>+</td>
<td>159</td>
<td>6.40</td>
<td>0.01</td>
<td>2005-2006  *</td>
</tr>
<tr>
<td>Total N</td>
<td>Fungicide</td>
<td>+</td>
<td>159</td>
<td>44.70</td>
<td>&lt;0.001</td>
<td>2003-2006  ***</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>Fungicide</td>
<td>+</td>
<td>159</td>
<td>641.08</td>
<td>&lt;0.001</td>
<td>2003-2006  ***</td>
</tr>
<tr>
<td>S</td>
<td>Fungicide</td>
<td>+</td>
<td>319</td>
<td>7.84</td>
<td>0.01</td>
<td>2003-6</td>
</tr>
</tbody>
</table>

* Below minimum detectable levels for NH₄⁺ in 2006 and Cu in 2003
** Nutrient levels interacted with removal treatments as indicated in text.
*** Effect present only in unfertilized plots
Fig. 2-5 Mean nutrient supply rate (±SE) in different plant functional group removal treatments. Bars with the same letter are not significantly different using Tukey’s comparison of all means.
Discussion

This study explored the influence of different plant functional groups on ecosystem properties and examined the ability of the mass ratio hypothesis (Grime 1998) to predict these effects. The mass ratio hypothesis predicts that species that make up the greatest proportion of a community will also have the greatest impact on ecosystem functioning (Grime 1998). For the northern grasslands studied here, the mass ratio hypothesis predicts that forbs, which make up 50% of the biomass, should have a greater impact on ecosystem properties than either graminoids (38%) or legumes (12%). However, for most ecosystem properties, there was no difference in the effect of losing either graminoids or forbs in the first 4 years of the experiment, meaning that graminoids had a greater impact in this community than would be expected based on their biomass alone. Our results indicate that the identity of the functional group, and not just the proportion of the biomass it represents in the community, determines the effect of losing that functional group, and thus does not provide support for the mass ratio hypothesis.

Biomass Compensation

Four years after initial removals, plants remaining in the plots had not fully compensated for the loss of biomass; all removal plots still had less biomass than the no-removal controls. Other removal experiments have reported total biomass compensation after 3 years in a New Zealand perennial grassland (Wardle et al. 1999), and after just 1 year (Symstad & Tilman 2001) or 2 years (Smith & Knapp 2003) in a North American prairie. However, our northern grassland has a shorter growing season and lower productivity than grasslands where many other removal experiments have been conducted. In an Alaskan tundra, Bret-Harte et al. (2008) reported that it took up to 6 years for full biomass recovery. As such, the potential for recovery from functional group loss is lower, but given more time, a full recovery of the biomass is likely.

Biomass compensation was dependent on the identity of the functional group removed from the community. If there were no identity effect in this experiment, one would expect the least total
biomass in the forb removal plots, followed by graminoid and legume removal plots, respectively. Both graminoid and legume removal treatments, however, resulted in similar biomass, as plots without legumes recovered more slowly than expected based on the initial amount of biomass removed. Removal of forbs, the dominant group in this community, did result in the largest effect on above-ground biomass, consistent with the mass ratio hypothesis (Grime 1998).

Wardle et al. (1999) and Symstad & Tilman (2001) predict that compensation for biomass loss likely depends more on the traits of the plants remaining than of those removed. We found that in addition to the identity of the removed group, biomass compensation was also dependent on the identity of the functional groups remaining after removals. Neither forbs nor legumes exhibited biomass compensation, regardless of the functional group removed. Graminoids, in contrast, exhibited an increase in biomass with the loss of other functional groups, and the amount of regrowth depended on both the identity of the removed group and also on fungicide treatments. With natural mycorrhizal levels, biomass compensation by graminoids was greatest when forbs were removed, consistent with Grime’s (1988) mass ratio hypothesis. Graminoids showed a lesser degree of biomass compensation for loss of legumes, possibly because of a smaller colonizable area than when forbs are removed, or because extra nitrogen provided by legumes is beneficial to graminoid regrowth (although the latter is unlikely as fertilization had no effect). When mycorrhizal colonization was reduced, however, graminoids did not show biomass compensation for the loss of any functional group. Most grasses benefit from mycorrhizal colonization (Read et al. 1976), and the ability of these groups to expand their niche to colonize the newly available area may be dependent on the mutualistic benefit of mycorrhiza.

The differential recovery patterns of the functional groups explain differences in total biomass between removal treatments after 4 years of recovery. Although graminoids compensated for loss of both forbs and legumes, neither forbs nor legumes compensated for the loss of graminoids. Therefore, based on the short-term response to removals in this experiment, the effect of losing graminoids might
be more detrimental to above-ground biomass than the effect of losing either of the other functional groups. Despite the strong effect of forb removal on total biomass, we predict full biomass compensation in these plots, as graminoids show a tendency to increase in biomass with the loss of forbs. This would also result in effects of removals on total biomass to less closely follow the mass ratio hypothesis over time, as the dominant group removed is also the most likely to have its loss compensated.

**Ecosystem Properties Responses**

There was no difference among functional groups in the amount of light they intercepted, which does not support the mass ratio hypothesis (Grime 1998). Reports of effects of plant richness and composition on light interception have been mixed, with significant effects being reported by some (Smith & Knapp 2003; Spehn et al. 2005; Wacker et al. 2009) but not all (Symstad 2000). In our community graminoids intercept proportionately more light than forbs. We had predicted the opposite—that graminoids would intercept less light, as reported by Tremmel & Bazzaz (1993), because erect leaves have lower K-values (lower possible light interception) than more horizontal leaves (Loomis 1971). However, the high latitude of our site and the low angle of the sun may allow erect leaves such as the graminoids to better intercept light.

Likewise, the effects of functional group identity on soil moisture did not support the mass ratio hypothesis (Grime 1998). Experiments with monocultures have previously shown no effect of identity on soil moisture (Symstad 2000; Reich et al. 2001b), but in our experiment, where the functional groups contributed different proportions to the total community, we found a greater impact of graminoids on soil moisture. Graminoids have previously been found to have a relatively large effect on soil moisture compared with other functional groups, such as woody plants (Köchy & Wilson 2000; McLaren et al. 2004), which was attributed to greater total root lengths of grasses (Köchy & Wilson, 2000).
Graminoids also had a greater impact on soil nutrients than expected. There are various reasons why plants may vary in their effects on soil nutrients, such as differences in litter quality and exudates (Hobbie 1996; Porazinska et al. 2003) or temporal variability in resource inputs and uptake (Porazinska et al. 2003; McLaren et al. 2004). Previous studies have reported effects of species or functional group identity on soil nutrients (Reich et al. 2001b; Scherer-Lorenzen et al. 2003; Aerts et al. 1999; Spehn et al. 2005), although other studies detected no differences (Symstad & Tilman 2001; Van der Krift & Berendse 2001; Porazinska et al. 2003). Removing plant biomass, regardless of identity, is likely to increase nutrient availability because of a decrease in uptake and because decomposing roots left in the soil may mineralize (although immobilization is just as likely) (Bret-Harte et al. 2004). Nitrogen, and nitrate in particular, was the only nutrient for which we detected an increased availability with removal treatments. However, P and Fe both decreased in availability with plant removals. Perhaps these nutrients in particular were required for active growth into bare areas created by the removals. Additionally, a decrease in plant biomass may have resulted in a decrease in the exudates required to mobilize nutrients. For example, several legume species release citrate, which increases the mobilization of Fe (Guerinot 1991), which may explain the decrease in Fe with removal of legumes.

In support of the mass ratio hypothesis, but in contrast to most other biodiversity studies, legumes had very little effect on any ecosystem function we measured. It has been suggested that many of the positive effects of diversity in random assembly biodiversity experiments are due simply to the higher likelihood of legumes being present in higher diversity plots (Huston et al. 2000) and at higher than natural abundances (Diaz et al. 2003). Legumes are often predicted and found to have a large effect on ecosystem functioning due to their ability to fix nitrogen; legumes often increase soil N pools and leaching (Hooper & Vitousek 1998; Spehn et al. 2002; Scherer-Lorenzen et al. 2003). In contrast we found that no soil nutrient tested, including N, was affected by the presence of legumes, except Fe. Although low temperatures in arctic and sub-arctic environments can reduce nodulation and nitrogen
fixation, both rhizobia and legumes have been found to adapt to arctic conditions and fix nitrogen at rates comparable to legumes in temperate climates (Bordeleau & Prevost 1994). Thus, in this system, legumes at their natural abundance do not appear to have any significant influence on many ecosystem functions.

One concern with using removal experiments to examine identity effects is that the removal effect may be due to the amount of biomass removed more so than to the identity of the removed group. Previous removal studies have either not adjusted for differences in biomass (e.g. Buonopane et al. 2005) or accounted for differences in biomass using different methods such as random biomass removals (Symstad & Tilman 2001; O’Connor & Crowe 2005) or incorporating the biomass removed as a covariate in the analysis (e.g. Wardle & Zackrisson 2005). An analysis of covariance, however, assumes that the covariate has the same distribution for all groups, and therefore should not be used as a correction for different values of the covariate for the different treatments (Quinn & Keough 2002). We chose to do a more qualitative comparison of the effects of the functional groups, by comparing the ranking of the biomass removal treatments to the ranking of the effects of removals (Wardle et al. 2008).

We found that for most ecosystem functions examined, the role of the different functional groups was not context-dependent, for either a fertilized or fungicide-treated environment. These results contrast with numerous studies using artificially created communities (Reich et al. 2001a; Fridley 2002; Craine et al. 2003; Reich et al. 2004). Removal experiments have reported mixed results for interactions between removal treatments and environmental conditions, with many (Shevtsova et al. 1997; Klanderud 2005; Wardle et al. 2008) but not all (Hobbie et al. 1999) showing interactive effects.

In conclusion, functional group identity plays a critical role in determining the effect of species loss on ecosystem properties. These effects are not always dependent on the relative abundance of the group of species removed from the community, and as such do not consistently provide support for
Grime’s (1998) mass ratio hypothesis. Secondly, this experiment provides an example of an ecosystem where changing environmental context rarely affects the impact of functional groups on ecosystem properties. We show that this ecosystem may be less vulnerable to altered nutrient conditions associated with changing climate with respect to changing impacts of species within an ecosystem. This does not imply that a changing climate will not have an impact on ecosystem properties; a changing community composition in response to climate will almost certainly drive changes in ecosystem functioning. Thirdly, in this northern ecosystem, graminoids influence ecosystem properties beyond what is expected based on their biomass contribution to the plant community. One of the most important drivers of future change in arctic vegetation is likely to be increased nutrient availability (Dormann & Woodin 2002), and grasses are particularly responsive to fertilization in a longer-term experiment in our region (Turkington et al. 2002) and in general (Dormann & Woodin 2002; Gough & Hobbie 2003). Knowing that graminoids are both more likely to change in abundance in future climates, and play a particularly important role in determining ecosystem function, suggests that the impacts of climate change may be more severe than when predicted without respect to changing plant identities.
References


CHAPTER THREE

Biomass Compensation and Plant Responses to Plant Functional Group Removal

Introduction

Changes in community structure can be caused by climate conditions (Montana 1992), disturbance (Chapin & Shaver 1981), resource availability (Turkington et al. 2002) and biotic interactions such as competition (McKane et al. 2002). Currently, some of the biggest changes in plant community structure are anthropogenically-induced losses of biodiversity (Sala et al. 2000). Changes in plant diversity and composition caused by human activities have been shown to influence ecosystem functioning (reviewed in Hooper et al. 2005; Cardinale et al. 2006; Duffy 2009; Balvanera 2006). To predict how this species loss may impact ecosystem functioning is likely to require a knowledge of both the effects of the species that are lost, and also the species that are likely to replace them.

A variety of biotic factors have been suggested to determine plant community composition, and the relative importance of these factors may differ between northern ecosystems and the more commonly studied temperate ecosystems. Competition is often discussed as the major biotic factor determining the structure of plant communities (Connell 1983; Schoener 1983; Fowler 1986; Goldberg & Barton 1992) even in the nutrient poor and stressful environments found in northern ecosystems (McKane et al. 2002; Storm & Suss 2008). Conversely, Grime (1977) predicts that competition is unimportant in structuring low productivity northern plant communities. More recently, positive interactions (facilitation) have been reported to have stronger influences on plant community structure as environmental conditions become more stressful (Callaway et al. 2002; Brooker et al. 2008), such as

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2 A version of this chapter has been submitted for publication. McLaren, J.R. and Turkington, R. Biomass compensation and plant responses to plant functional group removal.
in northern ecosystems. Alternatively, plant abundance and distribution in a tussock tundra may be directly limited by environmental conditions more so than by species interactions (Hobbie et al. 1999).

Removal experiments have long been used to test the importance of competition in structuring plant communities (e.g. Fowler 1981; Köchy & Wilson 2000). Traditionally, removal experiments examining competitive interactions have removed all neighbours surrounding a ‘target species’ (Gurevitch & Unnasch 1989). These experiments were often conducted in old fields and were relatively short in duration (Aksenova et al. 1998). More recently, experiments removing a target organism from an intact community have been used to examine the effects of local non-random species loss both on ecosystem functioning and on the remaining plant community (Diaz & Chapin 2000). Removal experiments are especially useful in ecosystems where artificial communities are difficult to create, such as arctic ecosystems dominated by long-lived perennial species. In arctic or alpine ecosystems, the remaining plant community has shown positive (Herben et al. 1997; Aksenova et al. 1998), negative (Shevtsova et al. 1997; Aksenova et al. 1998) and no response (Hobbie et al. 1999; Bret-Harte et al. 2004) to removals. Few experimental removals in these ecosystems, however, have been maintained over the long-term (Bret-Harte et al. 2008).

Removal treatments necessarily result in a reduction in plant biomass and provides newly available bare space for colonization by new, or by growing individuals. If the removals result in an increase in growth by the remaining plants sufficient to return plant biomass to pre-removal levels, the community is considered to have shown full biomass compensation (Bret-Harte et al. 2008). Whether full or partial biomass compensation occurs depends on the reproductive output, recruitment and, especially in northern perennial communities, vegetative growth of the plants remaining in the community (Bret-Harte et al. 2008). The degree of compensation is dependent on both the type of species removed and on the different components of the community that remain to re-colonize the bare spaces. There have been numerous studies examining the response of dominant and subdominant
species to removals (Hobbie et al. 1999; Smith et al. 1999; Munson & Lauenroth 2009) but less is known about the response of rare species even though they often contribute the most to community richness (Munson & Lauenroth 2009). The ecology of common and rare species can be quite different (Kunin & Gaston 1997) and they may not respond to removals in the same direction.

Continued global warming will cause an increase in decomposition rates in the arctic leading to increased soil fertility (Chapin et al. 1995). Because the plants in these communities are adapted to low soil nitrogen, they may be extremely vulnerable to increases in nitrogen (Bowman et al. 2006; Manninen et al. 2009). These nutrient-limited systems will have increased primary productivity with simultaneous changes in species richness and composition (Theodore & Bowman 1997; Thomas et al. 1999; Turkington et al. 2002) as different species respond differently to the increased nutrients. Typically, fertilization mainly benefits graminoids over other functional groups in boreal ecosystems (Manninen et al. 2009) because they have higher photosynthetic rates, and N and water use efficiencies, and as a result have a potentially higher reserve of carbon to invest in growth (Bowman et al. 1995).

The colonization of plants by mycorrhizae may also affect the distribution and abundance of species. Mycorrhizal fungi are essential for the growth and competitive ability of some plants (Janos 1980; Hartnett et al. 1993) because of the numerous benefits they provide including improved plant uptake of phosphorus and other nutrients (Sanders & Fitter 1992; Hartnett & Wilson 1999). In this way mycorrhizae can mediate direct plant-plant interactions by modifying plant traits, which in turn influence competitive interactions.

In addition to the direct effects that fertilization and mycorrhizae have on plant community structure, the effects of species loss may interact with these environmental variables. For example, the competitive ability of a species can change along gradients of resource availability (Tilman 1984), and the ability of species to colonize after plant removals is likely to depend on environmental conditions. The consequences of species loss on ecosystem properties, including community composition, may vary
among ecosystems, depending on both the abiotic and biotic properties of those ecosystems (Wardle & Zackrisson 2005). Changes in environmental conditions, however, do not always alter species interactions after removals (Hobbie et al. 1999).

It is critical to have both transient and long-term measurements to fully understand the effects of functional group loss on the remaining members of the community (Bret-Harte et al. 2004). In this study, single functional groups (graminoids, legumes and non-leguminous forbs (hereafter called forbs)) were experimentally removed from a series of plots in a grassland in northern Canada. In the short-term results (Chapter 2) we reported a lack of full biomass compensation for any of the removal treatments four years after functional group removals. After four years, although grasses showed some compensatory growth following the removal of forbs or legumes, neither forbs nor legumes showed any compensatory growth following removals. Here we report the medium term results of plant community responses 7 years after functional groups were removed. Because of the lack of response to removals on the short-term, we predicted that given on the medium term graminoid removal treatments would show the lowest degree of biomass compensation. In these medium-term results, we also look more specifically on the responses of dominant, sub-dominant, and rare species to removals, as well as effects on richness and diversity, to further investigate the causes of the degree of biomass compensation for the different removals. We also examined whether these community responses were dependent on environmental context, by conducting removals under different fertilization and mycorrhizal environments.

**Methods and Materials**

This removal experiment was part of a larger experiment examining the role of plant functional group identity in determining various ecosystem functions. Details of the methods are described in Chapter 2 and are summarized below.
Site Description

The study area is a dry grassland near Kluane Lake in the south-western Yukon in northern Canada (61°04.218 N 138°23.018 W). The area is in the rainshadow of the St. Elias Mountains and receives a mean annual precipitation of ca. 230 mm, about half of which falls as rain during the summer, and an average annual snowfall of about 100 cm. The grassland is surrounded by a closed to relatively open spruce forest community dominated by Picea glauca (Moench) Voss. The grassland is dominated by Poa glauca Vahl and Carex stenophylla Wahlenb. ssp. eleocharis (Bailey) Hultén, and also contains many non-leguminous forbs (dominated by Artemisia frigida Willd., Penstemon gormanii Greene and Pulsatilla ludoviciana (Nutt.) Heller and legumes (dominated by Oxytropis campestris (L.) DC.) (all nomenclature follows Cody (2000)). Grassland species were divided into three functional groups, namely, graminoids (grasses and sedges), forbs, and legumes.

Experimental Plant Communities

Experimental plots were established in May 2003 and maintained annually for 7 years through the 2009 growing season. The experiment was a 4 x 2 x 2 fully crossed factorial design (4 removal treatments, +/0 fertilizer, +/0 fungicide), replicated 5 times, for a total of 80 1m x 1m plots.

There were four removal treatments: independent removal of each of the three functional groups (graminoids, forbs and legumes) and a no-removal control. In 2003, individual plants were removed from the plots using Round-up™ glyphosate, a non-selective herbicide, painted precisely to the leaves and stems of selected plants. Herbicide application was repeated every 4-7 days until visible leaf yellowing occurred and plants were then clipped at soil level and removed from the plots. Removal treatments were maintained in 2004 using herbicide application and clipping, and in the subsequent five years the very minimal regrowth was clipped at ground level early in the growing season.

Fertilizer and fungicide treatments were applied immediately after the removals (July 20) in 2003 and in early June of each subsequent year. Granular fertilizer was applied to half the plots at a rate
of 17.5 g N m⁻², 5.8 g P m⁻² and 5.8 g K m⁻². This rate was used to be consistent with many other studies being done in the area (e.g. Turkington et al. 1998; Turkington et al. 2002). The fungicide Benlate™ (active ingredient benomyl) was applied to half of the plots as a soil drench (2 L m⁻² plot⁻¹) every two weeks from early-June to mid-August at a rate of 2.5 g benomyl m⁻² per application. Plots that did not receive fungicide received an equivalent amount of water. Benomyl applications reduced mycorrhizal colonization rates from 50% to less than 10% of root intersections (J. McLaren, unpublished data).

Percent cover of all species was determined each July 2003-2007, and in 2009, using 100 points arranged in a 10 x 10 grid, each separated by 10 cm in a 1 m² quadrat; data were not collected in 2008. In an earlier experiment, a series of regression equations was determined that equated the biomass of each species with the total number of leaf hits for a 1 m x 1 m plot (Chapter 2). Using these equations we calculated the biomass of each species for each plot for each year.

Biomass of all of the species in each plot was summed to determine total above-ground biomass, and also separated into their respective functional groups to determine biomass of each functional group individually. In addition to their classification into functional groups, seven species were designated as common, and twelve species were classified as rare (<5% of the average total biomass). Common species included: four forbs (Artemisia frigida, Erigeron caespitosus Nutt., Penstemon gormanii, Pulsatilla ludoviciana), two graminoids (Poa glauca, Carex stenophylla) and a single legume (Oxytropis campestris). Species will hereafter be referred to by the genus name only. Biomass of rare species were summed within their respective functional groups to determine the biomass of rare forbs, graminoids and legumes independently and all were summed to determine rare species biomass. Biomass data for common and rare species was transformed by analyzing the amount of change from the initial biomass at the beginning of the experiment (2003), which was measured almost immediately after the removals were established, to conform to assumptions of normality. All other data did not require transformation. We calculated species richness and species diversity (using
the Shannon-Weiner diversity index) for graminoids and forbs in each plot, but did not do so for the legumes because of their low numbers of species.

**Analysis**

We used a 4-way repeated measures ANOVA on the measured plant community response variables including: total and functional group biomass; change in biomass for each of the seven common species; change in total rare species biomass and rare species biomass in each individual functional group; and species richness and diversity, with the three main plot factors - functional group removal, fertilizer and fungicide - and year as the within-plot repeated measures factor. When there was a significant effect of removals, removals were compared using a Tukey’s comparison of all means (Quinn & Keough 2002). When there was a significant interaction between removals and environment, analyses were run independently for each environment level.

**Results**

Total above-ground biomass was affected by removal treatments and this effect was dependent on both fertilization and year (Table 3-1). The effect of removals depended on fertilization; legume removal resulted in a significant decrease in above-ground biomass only when plots were not fertilized (Total Biomass: Removal x Fertilizer, Table 3-1). The effect of removals also changed with time (Removal x Year, Table 3-1); early in the experiment the removal treatment plots had only partial compensatory regrowth, and it was only after the fifth year that full biomass compensation occurred in plots where forbs or graminoids had been removed (Total Biomass: Removal x Year, Table 3-1, Fig. 3-1a).
Table 3-1 Summary of the major responses to treatments from a 4-way repeated measures ANOVA on total biomass and for the individual biomass of each functional group in a 7-year functional group removal experiment (2003-2009), with additional factorial treatments of fertilizer and fungicide. Forb, graminoid and legume biomass all have the same df. Bold values are significant at p < 0.05.

<table>
<thead>
<tr>
<th>Source</th>
<th>Total Biomass</th>
<th>Forb biomass</th>
<th>Graminoid biomass</th>
<th>Legume biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>P</td>
<td>df</td>
</tr>
<tr>
<td>Removal</td>
<td>3.64</td>
<td>46.37</td>
<td>&lt;0.001</td>
<td>2.48</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>1.64</td>
<td>21.91</td>
<td>&lt;0.001</td>
<td>1.48</td>
</tr>
<tr>
<td>Fungicide</td>
<td>1.64</td>
<td>5.79</td>
<td>0.019</td>
<td>1.48</td>
</tr>
<tr>
<td>Removal x Year</td>
<td>3.384</td>
<td>4.50</td>
<td>0.004</td>
<td>2.288</td>
</tr>
<tr>
<td>Removal x Fertilizer</td>
<td>3.64</td>
<td>3.31</td>
<td>0.026</td>
<td>2.48</td>
</tr>
</tbody>
</table>

The response of total biomass to the removal treatments was complex because the change in biomass of each functional group was specific to the functional group being removed. Forb biomass was higher when graminoids were removed, but this effect didn’t appear until the fifth year of the experiment (Forb biomass: Removal x Year, Table 3-1, Fig. 3-1b). Conversely, graminoid biomass was higher when forbs were removed throughout the duration of the experiment (Graminoid biomass: Removal effect, Table 3-1, Fig. 3-1c). Effects of removals on legume biomass were intermittent and inconsistent (Legume Biomass: Removal x Year, Table 3-1, Fig. 3-1d).

Fertilization caused an increase in total biomass and in the biomass of forbs and graminoids, but lead to a significant decline in legumes (Table 3-1, Fig. 3-2a). Fungicide also caused an increase in total biomass but only affected graminoids and not the forbs or legumes (Table 3-1, Fig. 3-2b). The effect of fertilization on both graminoids and legumes interacted with year; graminoids increased with fertilization each year, beginning in the second year of the experiment (Fertilizer x Year Interaction $F_{1,288}=10.30$, $p=0.002$ ), and legumes decreased with fertilization beginning in the third year of the experiment (Fertilizer x Year Interaction $F_{1,288}=20.20$, $p<0.001$) (Fig. 3-2).
Fig. 3-1 Total above-ground biomass (±SE) (a) and biomass of the individual functional groups, forbs (b), graminoids (c) and legumes (d) in different plant functional group removal treatments from 2003-2009. Treatments with the same letter for a given year are not significantly different (p<0.05) using Tukey’s comparison of all means. Legend: ● no removals; □ forbs removed; Δ graminoids removed; ∇ legumes removed.

Fig. 3-2 Above-ground biomass (+1SE) by a) fertilizer and b) fungicide treatments for total biomass and biomass of the individual functional groups forbs, graminoids and legumes. Fertilizer effects interacted with year and are presented only for the fourth year of a 7-year plant functional group removal experiment. Fungicide effects are averaged across years. Dark bars are plots with a) fertilizer and b) fungicide, and grey bars are untreated plots. * indicates significance at p<0.05
The biomass of some of the dominant species was also affected by removal treatments, and this effect was often context-dependent. Of the forbs, *Artemisia* biomass was not affected by removals, and *Erigeron* was affected only inconsistently and intermittently (Table 3-2, Fig. 3-3a,b). The removal of graminoids resulted in a larger increase in *Penstemon* biomass (Fig. 3-3c) but a smaller increase in *Pulsatilla* biomass compared to other removal treatments (Table 3, Fig. 3-3d), although effects on *Penstemon* did not appear until the fourth year of the experiment, and only when plots were both fertilized and had fungicide applied (Removal x Fertilizer x Fungicide x Year Interaction $F_{2,228}=3.51$, p=0.032). Effects on the abundance of the dominant legume, *Oxytropis*, were rare (Fig. 3-3e). For the graminoids, *Poa* biomass was not affected by removals (Table 3-3, Fig. 3-3f), but in the latter years of the experiment, *Carex* biomass increased with the removal of forbs when plots were fertilized (Removal x Fertilizer x Year Interaction $F_{2,228}=5.90$, p=0.003, Fig. 3-3g).

**Table 3-2** Summary from a 4-way repeated measures ANOVA on change of biomass between 2003-2006 for dominant species in a 4-year functional group removal experiment. Removal df = 2,48 and Removal x Year df = 2,228 for all species. Bold values are significant at p < 0.05.

<table>
<thead>
<tr>
<th>Species</th>
<th>Removal F</th>
<th>Removal P</th>
<th>Removal x Year F</th>
<th>Removal x Year P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Artemisia frigida</em></td>
<td>2.75</td>
<td>0.074</td>
<td>0.04</td>
<td>0.965</td>
</tr>
<tr>
<td><em>Erigeron caepitosus</em></td>
<td>1.06</td>
<td>0.355</td>
<td>6.58</td>
<td>0.002</td>
</tr>
<tr>
<td><em>Penstemon gormanii</em></td>
<td>2.86</td>
<td>0.067</td>
<td>9.36</td>
<td>0.000</td>
</tr>
<tr>
<td><em>Pulsatilla ludoviciana</em></td>
<td>5.76</td>
<td>0.006</td>
<td>0.10</td>
<td>0.901</td>
</tr>
<tr>
<td><em>Oxytropis campestris</em></td>
<td>1.18</td>
<td>0.315</td>
<td>12.68</td>
<td>0.000</td>
</tr>
<tr>
<td><em>Poa glauca</em></td>
<td>1.90</td>
<td>0.160</td>
<td>0.45</td>
<td>0.639</td>
</tr>
<tr>
<td><em>Carex stenophylla</em></td>
<td>7.42</td>
<td>0.002</td>
<td>7.42</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Fig. 3-3 Change in biomass (±1SE) from the initial condition in year 1 for dominant species in a 7-year plant functional group removal experiment from 2004-2009. For some species, removals interacted with fertilizer or fungicide treatments; *Penstemon gormanii* biomass (c) is presented only for plots with fertilizer and fungicide, *Oxytropis campestris* biomass (e) only for plots without fertilizer or fungicide, and *Carex stenophylla* biomass only for plots with fertilizer (pooled across fungicide treatments). Treatments with the same letter for a given year are not significantly different (p<0.05) using Tukey’s comparison of all means. For *Pulsatilla ludoviciana* removals did not interact with year, and Tukey’s comparison is across all years. Values above 0 indicate that biomass has increased since the experiment was established in year 1, and values below 0 indicate a decrease in biomass. Legend: ● no removals; □ forbs removed; Δ graminoids removed; ∇ legumes removed.
Table 3-3 Summary of main treatment effects from a 4-way repeated measures ANOVA on richness and diversity of forbs and graminoids (2003-2009) in a 7-year functional group removal experiment, with additional factorial treatments of fertilizer and fungicide. Bold values are significant at p < 0.05.

<table>
<thead>
<tr>
<th>Source</th>
<th>Forbs Richness</th>
<th>Forbs Diversity</th>
<th>Graminoids Richness</th>
<th>Graminoids Diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>P</td>
<td>df</td>
</tr>
<tr>
<td>Removal</td>
<td>2,48</td>
<td>1.89</td>
<td>0.162</td>
<td>4.61</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>1,48</td>
<td>0.88</td>
<td>0.354</td>
<td>0.00</td>
</tr>
<tr>
<td>Fungicide</td>
<td>1,48</td>
<td>0.00</td>
<td>0.966</td>
<td>3.22</td>
</tr>
</tbody>
</table>

The biomass of rare species was affected by removal treatments. The removal of either forbs or graminoids caused an increase in the total biomass of rare species, but this effect was not apparent until the fifth year of the experiment (Fig. 3-4a, Removal x Year Interaction F3,304=9.72, p<0.001). The biomass of rare forbs increased with graminoid removal after the fourth year (Fig. 3-4b, Removal x Year Interaction F2,228=9.72, p<0.001) but removals had no effect on the biomass of rare graminoids (Fig. 3-4c, Removal F2,48=2.40, p=0.102). Finally, the biomass of rare legumes was seldom affected (Fig. 3-4d). Fertilization directly increased rare species biomass, but the effect was transient and disappeared by the third year (Fig. 3-5a, Fertilizer x Year Interaction F1,304=10.96, p=0.001). The application of fungicide decreased rare species biomass but this effect did not appear until the fifth year of the experiment (Fig. 3-5b, Fungicide x Year Interaction F1,304=4.01, p=0.046).

Species richness and diversity were both affected by removal treatments. Removals had no effect on forb species richness, but the richness of graminoids was higher when forbs were removed compared with intact control plots (Table 3-3). Conversely, removal treatments affected forb diversity, but not graminoid diversity (Table 3-3). There were also direct effects of fungicide, with fungicide resulting in a decrease in both the richness and diversity of graminoids (Table 3-3). Fertilization had no direct effects on either richness or diversity (Table 3-3).
Fig. 3-4 Change in biomass (±1SE) from the initial condition in year 1 for a) rare species, and rare species separated into their respective functional groups b) forbs, c) graminoids and c) legumes in different plant functional group removal treatments from 2004-2009. Treatments with the same letter for a given year are not significantly different (p<0.05) using Tukey's comparison of all means. Values above 0 indicate that biomass has increased since the experiment was established and values below 0 indicate a decrease in biomass. Legend: ● no removals; □ forbs removed; Δ graminoids removed; ∇ legumes removed.
Fig. 3-5 Change in biomass (±1SE) from the initial condition in year 1 by a) fertilizer and b) fungicide treatments for rare species in a plant functional group removal experiment from 2004-2009. Values above 0 indicate that biomass has increased since the experiment was established and values below 0 indicate a decrease in biomass. Filled circles are treated plots and open circles are untreated plots. * indicates significance at p<0.05

Discussion

Plots that had graminoids or legumes removed showed full biomass compensation after five years. Plots that had forbs removed (the most abundant functional group), still only showed partial biomass compensation after 7 years. Relatively rapid biomass compensation has been observed in experiments in more temperate climates (Wardle et al. 1999; Polley et al. 2006) including complete recovery in as little as a single year (Fowler 1981). In an old field community, Gurevitch and Unnasch (1989) reported only partial biomass compensation 2 years after the removal of dominant species. In northern ecosystems we predict that biomass compensation would happen more slowly than in temperate ecosystems due to the shorter growing season and slower growth rates, although in an alpine tundra, total biomass compensation for removals occurred within 2 years of removal of dominant species (Suding et al. 2006). Hobbie et al. (1999) observed full biomass recovery after 4 years when a dominant species was removed from an arctic tundra. Bret-Harte et al. (2004) reported no compensation for biomass loss 4 years after removal of dominant shrubs, but full biomass compensation after 6 years for
most removal treatments (Bret-Harte et al. 2008). Similarly, we showed only partial biomass compensation after 4 years (Chapter 2) but full biomass recovery beginning the following year in plots where graminoids and legumes were removed.

The degree of compensation also depended on the identity of the species colonizing the space made available by removals. Beginning in the fifth year of the experiment, forb biomass was higher in plots where graminoids had been removed than in the intact communities, indicating partial biomass compensation for the graminoid loss by the remaining forbs in the plots. That same year, there was full biomass compensation for graminoid removal in these plots. The slow response of forbs was also reported by Bret-Harte et al (2008) who found that forbs had no response to removals, at least for the first 6 years of the experiment, although other experiments have shown early increases in forbs after grass removal (Pinder 1975; Polley et al. 2006). Graminoids, in contrast, began compensating for the loss of forbs in the second year of the experiment. Despite the early increase in graminoid growth, forb removal plots still only exhibited partial biomass compensation by the seventh year of the experiment, likely because of the large amount of biomass removed (ca. 50% of total biomass; Chapter 2) with this removal treatment.

In addition to effects of removals, we found that fertilization caused an increase in both forb and graminoid biomass, and therefore total biomass. Graminoids are often found to respond more strongly to fertilization than other growth forms (Bret-Harte et al. 2004; Bowman et al. 2006), especially in boreal regions (Manninen et al. 2009). Forbs respond to fertilizer less consistently, and have been both shown both to increase and not respond to fertilizer in arctic tundra experiments depending on habitat type (Theodore & Bowman 1997). Legumes were slower to respond to fertilizer, but after 4 years legume biomass decreased in fertilized plots. Legumes are commonly reported to decline with added nutrients when legumes are in competition with species that are not N-fixing (Gurevitch & Unnasch 1989). This decrease in legumes biomass, however, was insufficient to offset the increase in
biomass of the other functional groups, resulting in an overall positive effect of fertilization on total community biomass.

We found differing responses of the abundant species in this community to removals. The response of particular species is likely to depend on both the species removed and on the identity of the colonizer (Armesto & Pickett 1985, 1986; Warren et al. 2002). The growth of each colonizing species in particular will depend on the ability of that species to invade bare space, and also the ability of their neighbours to resist invasion (Herben et al. 1997). None of the most abundant species from each of the three functional groups, *Artemisia, Poa* and *Oxytropis*, responded to any of the removal treatments. Hobbie et al. (1999) also found little effect of removals in an arctic tundra, with only 3 out of 13 species responding to the removal of dominant species. They concluded that direct limitation by the environment may be more important than species interactions in the tundra in determining plant abundance (Hobbie et al. 1999).

Although it has been suggested that no relationship exists between the abundance of a species and the magnitude of its response to removals (Fowler 1981), we found that responses to removals by the entire community were being driven by the subdominant species; there were differing responses to removals between the dominant and subdominant species. The subdominant forb and graminoid responded positively to removal treatments. *Penstemon* showed a larger increase in biomass with the removal of graminoids, and *Carex* increased with the removal of forbs. Much of the research examining the response of subdominants to removals has explored the effects of removing dominant species, not entire functional groups. With the removal of dominant species, subdominants have been found to increase (Smith et al. 1999; Munson & Lauenroth 2009) or not respond (Gurevitch & Unnasch 1989; Hobbie et al. 1999). Aksenova (1998) found that removal generally increased the abundance of dominant species, but decreased the subdominants that may have required facilitation from the
dominant to survive in the community. Although the first subdominant for both forb and graminoid functional groups increased, there was no consistent response to removal by other subdominants.

Even though rare species were the largest contributors to species richness, less is known about the effect of removals on rare species, which may respond differently than dominant or other abundant species (Munson & Lauenroth 2009). We found that the removal of graminoids, and sometimes forbs, increased the biomass of rare species, although these effects were not evident for the first 5 years of the experiment. Munson (2009) also reported that while subdominant species responded on the short-term to removal of dominant grasses, a longer-term effect was required to influence the rare species, whose dynamics are not as strongly determined by competition. Although rare forbs increased with the removal of graminoids, rare graminoids showed no effect of removals, even when relatively large amounts of (forb) biomass were removed, resulting in a much larger response to graminoid than forb removal in rare species biomass. Similar to effects on dominant species, the response to removals of rare species depended both on the identity of the group removed and of those remaining. Rare species also responded directly to our environmental treatments, although these responses were highly dependent on year. Rare species responded positively to both fertilizer and fungicide, but fertilizer effects were short lived and disappeared by the fourth year of the experiment, whereas fungicide effects didn’t appear until the fifth year. When examined, rare species often did not respond to fertilizer in the same manner as dominants (Gurevitch & Unnasch 1989; Bret-Harte et al. 2004), which may again be a result of the lack of a strong influence of competition on these species.

The effects of removals on richness and diversity was dependent on functional group identity, both of the group removed and of the group responding to the removals. Graminoid richness increased with the removal of forbs; forbs were the most abundant functional group in this community, and their removal exposed additional niches for new graminoid species. Wardle et al. (1999) reported no decrease in richness with functional group removals, as richness was compensated by the remaining
functional groups. Despite the increase in graminoid richness, graminoid diversity was not affected by removals. The increase in richness may have been offset by a decrease in evenness resulting from an increase in the biomass of more abundant species (Carex) without a concomitant increase in the biomass of rare graminoids. Forb richness was not reciprocally increased by the removal of graminoids. Removal of dominants often (Gurevitch & Unnasch 1989; Smith et al. 1999; Suding et al. 2006) but not always (Suding et al. 2006; Munson & Lauenroth 2009) increases richness or diversity by releasing subordinate species from competition, or by opening niches for new species. Richness and diversity of forbs in this system may be more limited by harsh environmental conditions than by competition.

We also found no direct effect of fertilizer on richness or diversity. Diversity has been found to both increase and decrease following fertilization, depending on the nutrient status of the ecosystem (Theodore & Bowman 1997). Diversity is often expected to decrease following fertilization due to competitive displacement of smaller species resulting from light limitation (Goldberg & Miller 1990). In contrast, in nutrient poor systems, diversity may be expected to increase following fertilization due to release of subordinate species from nutrient limitation (Tilman 1984). Factors other than nitrogen may be the primary factor in determining competitive outcomes in this system, resulting in no change after fertilization. A decrease in mycorrhizal colonization, however, resulted in a decrease in both the richness and diversity of graminoids. Smith et al. (1999) reported the opposite effect, that diversity increased with fungicide, although they concluded that these effects were indirect influences on the plant species in their tallgrass prairie by decreasing the abundance of the dominant grass species. We found that both total graminoid biomass and Poa (the most abundant graminoid) in particular increased with fungicide. If the other graminoid species showed high mycorrhizal dependence, a reduction in mycorrhiza may have decreased their competitive ability, allowing them to be competitively excluded by Poa.

Some, but not all, of the dominant species showed a context-dependent response to functional group removals. For example, Penstemon and Carex only responded to removal treatments in plots that
had been fertilized. In a removal experiment in an arctic tundra, Bret-Harte et al. (2004) also found a removal x fertilizer interaction with graminoids showing a greater response to fertilizer in removal plots than in the intact community. These species-specific responses, however, did not translate into a context-dependent effect of either forb or graminoid removal on total biomass, or the biomass of any of the functional groups. A lack of context-dependence for the effect of removals is much more common than the context-dependence we found for specific species. Removals have shown no context-dependence on warming in an arctic tundra (Hobbie et al. 1999) or fertilization in a boreal forest understory (Manninen et al. 2009), although another removal experiment does show context dependency on island size for total plant cover (Wardle & Zackrisson 2005), and invasibility (Wardle et al. 2008). Although the context-dependence of sub-dominant species do not currently translate into effects on total biomass, the sub-dominant species are the primary species responding to functional group loss, and in the long-term these environmental interactions may become more important.

Many of the removal effects did not appear until many years after the treatments were initiated, showing the importance of longer-term experiments, especially in northern ecosystems. Many studies in the arctic tundra have reported no effects of removals on remaining biomass. It has often been suggested that this lack of response may have been due to the short-term nature of the experiments and that species interactions may become more important over a longer time period (Hobbie et al. 1999; Bret-Harte et al. 2004). In our experiment, there was no compensatory growth by the remaining functional groups for graminoid removal after 4 years and this suggested that full biomass compensation would be unlikely (Chapter 2). In the following year, however, we found full biomass compensation after graminoid removal; forbs did not begin to respond to removals until the fifth year resulting in this delay in biomass compensation. This delay is similar to that reported by Bret-Harte et al. (2004; 2008) in a removal experiment in arctic tundra. They also reported no biomass compensation for removals in the short term (Bret-Harte et al. 2004) but after 6 years there was full biomass
compensation for nearly all removal treatments (Bret-Harte et al. 2008). We also found a slow response to environmental treatments directly for some functional groups; legumes showed a significant decrease in biomass with fertilization, but only after 4 years of treatments. In northern ecosystems, where growing seasons are short and growth rates are slow, predictions based on short-term responses may lead to inaccurate conclusions about the impacts of both species loss and environmental change.
References


CHAPTER FOUR

Plant Functional Group Identity Differentially Affects Leaf and Root Decomposition

Introduction

Biodiversity loss is one of the largest impacts humans have had on ecosystems during this past century and there is a growing concern on the potential effects of this loss. This concern has led to numerous studies examining whether current losses in biodiversity will negatively affect the functioning of ecosystems (Hooper et al. 2005; Balvanera et al. 2006; Cardinale et al. 2006; Cardinale et al. 2007; Duffy 2009). Loss of species or functional groups necessarily affects the composition of communities and it has often been reported that the effects of community composition can be just as important as those of changing species diversity (see Hooper & Vitousek 1998; Scherer-Lorenzen et al. 2003). Studies are rare that directly examine the role of the type, rather than the number, of species on ecosystem functioning through influences on both the abiotic and biotic environment.

The majority of studies examining the effects of biodiversity on ecosystem functioning have focused on the impacts of changing plant diversity on primary productivity, whereas other ecosystem functions such as soil nutrient availability and decomposer activity have been less frequently studied (Hooper et al. 2005; Balvanera et al. 2006). Although there is consistent evidence for a positive relationship between plant diversity and primary productivity (Balvanera et al. 2006; Cardinale et al. 2006), reported effects of plant diversity on litter decomposition processes are less clear (Hector et al. 2000; Knops et al. 2001; Wardle et al. 2006). Soils hold more than twice as much carbon as vegetation or the atmosphere (Jobbagy & Jackson 2000), and influence over decomposition processes not only

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affects the incorporation of vegetation carbon into the soils, but also the early stages of its release back into the atmosphere. Between 50 and 90% of all primary productivity will enter detrital food webs (McNaughton et al. 1989) and decomposition of this material provides a major source of carbon and nutrients to the soils, and is thus an important determinant of other ecosystem functions such as productivity and nutrient cycling. Most previous studies have used artificially constructed communities, such as diversity gradients (Hector et al. 2000; Milcu et al. 2008; Scherer-Lorenzen 2008) or plant monocultures (Hobbie et al. 2006), to examine plant-driven environmental effects on decomposition. However, Diaz et al. (2003) argue that it is preferable to remove species or functional groups from established natural communities because they take place in communities that have undergone natural assembly processes and have natural species abundances (Diaz et al. 2003).

Large scale variation in climate is the most important determinant of the rate of litter decomposition (Aerts 1997) but the rate is also controlled by local conditions such as variation in soil moisture (Bryant et al. 1998), soil nutrients (Knorr et al. 2005) and the decomposer community (Dang et al. 2005). The identity and composition of the plant community itself can influence these same local conditions, such as soil moisture and nutrients (Chapter 2, Hooper & Vitousek 1997), thus potentially influencing decomposition through changes in the local decomposition environment. Although numerous studies have examined environmental influences on decomposition (e.g. Hobbie 1996; Prescott et al. 1999), only a few have examined plant-driven environmental influences and these often report weak (Hector et al. 2000) or no significant effects (Knops et al. 2001); (Although see Ashton et al. 2005; Hobbie et al. 2006). Further, the effects of plant composition on decomposition may be context-dependent on environmental conditions. The processes that are currently causing a shift in the environmental properties of ecosystems, such as nitrogen deposition, can also result in the loss or changes in the types of species present in a community (Hooper et al. 2005). Few experiments,
however, examine the effects of diversity on ecosystem properties in more than one environmental context.

Most litter decomposition studies are relatively short-term, often only occurring for a single growing season. In tropical regions most dead plant material will completely decompose in a single season, but in more temperate regions up to 70% of leaf tissue can still remain after the first year (Aerts 1997) and longer-term experiments are necessary. Although climatic influences on longer-term litter decomposition are often assumed to be similar to those on first year litter decomposition (Aerts 1997), the phase of the decomposition process has been shown to determine both the chemical control of decomposition (Berg et al. 1996) and element loss from litter (Rustad 1994). Both litter quality and quantity change through time with the easily decomposable material disappearing first resulting in the accumulation of more recalcitrant material (Harmon et al. 2009). Thus, it may be inappropriate to extrapolate plant-driven control over short-term litter decomposition patterns to patterns of longer-term decomposition, especially in northern ecosystems where litter decomposes very slowly.

A large proportion of the plant biomass available for decomposition is root tissue, especially in grasslands (Seastedt 1988). Soil organic matter in grasslands originates primarily from root death and decomposition (Gill & Burke 2002) and root decomposition is a major source of carbon and nutrient turn-over in most systems (Dornbush et al. 2002). Despite the important influence on ecosystem carbon dynamics, few general principles have been defined for the factors that affect root decomposition rates (Silver & Miya 2001). Decomposing roots are buried and thus experience different moisture conditions, microbial communities and nutrient availability than leaves (Ostertag & Hobbie 1999) and the extremes in these environmental variables may be buffered in the soil (Silver & Miya 2001).

In this study we examined the influence of plant functional group identity on decomposition rates of leaf and root material over both short-term (single growing season) and long-term (up to 5 years) decomposition processes. We removed single functional groups (graminoids, legumes and non-
leguminous forbs) from a series of plots in a northern grassland. We examined whether the influence of a functional group on decomposition rates was dependent on environmental context, by conducting these removals under different fertilization and mycorrhizal environments. These environments were chosen to represent different ‘environmental contexts’, and both are relevant to future environmental change.

**Methods and Materials**

This removal experiment was part of a larger experiment examining the role of plant functional group identity in determining various ecosystem functions. Chapter 2 describes the methods in detail, and they are in described briefly below.

**Site Description**

The study area is a dry grassland near Kluane Lake in the south-western Yukon in northern Canada (61°04.218 N 138°23.018 W). The area receives a mean annual precipitation of ca. 230 mm, about half of which falls as rain during the summer, but also includes an average annual snowfall of about 100 cm. The grassland is surrounded by a closed to relatively open spruce forest community dominated by *Picea glauca* (Moench) Voss. Grassland species were divided into three functional groups, namely, graminoids (grasses and sedges; dominated by *Poa glauca* Vahl and *Carex stenophylla* Wahlenb. ssp. *eleocharis* (Bailey) Hultén), forbs (dominated by *Erigeron caespitosus* Nutt., *Artemisia frigida* Willd.), and legumes (dominated by *Oxytropis campestris* (L.) DC.); all nomenclature follows Cody (2000).

**Experimental Plant Communities**

Experimental plots were established in May 2003 and maintained annually for 5 years through the 2007 growing season. The experiment was a 4 x 2 x 2 fully crossed factorial design (4 removal treatments, +/-0 fertilizer, +/-0 fungicide). Each of the 16 treatments was replicated 5 times, for a total of 80 plots.
There were four removal treatments: independent removal of each of the three functional
groups (graminoids, forbs and legumes) and a no-removal control. In 2003, plants were removed from
the plots using Round-up™ glyphosate, a non-selective herbicide. Herbicide was painted precisely to the
leaves and once plants had visibly yellowed, stems of selected plants were clipped at soil level and
removed from the plots. Removal treatments were maintained in 2004 using herbicide application and
clipping, and in the subsequent three years the very minimal regrowth was clipped at ground level early
in the growing season.

Fertilizer and fungicide treatments were applied upon completion of the removals (July 20) in
2003 and in early June of each subsequent year. Fertilizer was applied each year to half the plots in
granular form at a rate of 17.5 g N.m⁻², 5.8 g P.m⁻² and 5.8 g K.m⁻². This application rate was used to be
consistent with many other studies being done in the area (e.g. John & Turkington 1997; Turkington et
al. 2002). Half of the plots received the fungicide Benlate™ (active ingredient benomyl) as a soil drench
(2 L.m⁻² plot) every two weeks from early-June to mid-August at a rate of 2.5 g benomyl.m⁻² per
application, and the other half of the plots received an equivalent amount of water. Benomyl
applications reduced mycorrhizal colonization rates from 50% to less than 10% of root intersections (J.
McLaren, unpublished data).

*Decomposition Experiment*

Fresh leaf and root material from *Elymus trachycaulus* (Link) Gould ex Shinners collected near Kluane
Lake, YT, was dried at 40°C for 48 hours and placed separately in 10 x 5 cm litter bags made from 1 mm
mesh nylon screening. *Elymus trachycaulus* was chosen because sufficient leaf and root material was
available, and it was present, but not dominant, in the experimental area.

Above-ground litter bags contained 0.5 g of dried leaf material; leaves were collected and cut
into 8 cm lengths so as to fit into the litter bags. Below-ground litter bags contained 0.25 g of dried root
material; roots were washed free of soil and the 0.5-2 mm diameter size class was separated out.
Above-ground bags were placed into gaps in the vegetation, in contact with the litter layer, and secured to the surface. Below-ground bags were sprayed with water prior to being inserted into the soil to a depth of 7 cm (the majority of the roots in this system are within this depth) at an angle of 45°, and secured to the surface with a nylon tether.

We examined both single-season (short-term) and multiple-year (long-term) decomposition for leaf and root litter. For long-term decomposition, five above-ground and below-ground bags were placed into each plot in mid-July 2003, once the experimental communities had been established. One of each type of litter bag was collected in mid-August of each year, after the plants in the surrounding community had senesced, from 2003-2007. In the 4th year of the study (2006) onward, only above-ground bags were processed due to our inability to distinguish between root litter and root growth penetration of the below-ground bags. To measure short-term decomposition, an additional replicate for both above- and below-ground bags was placed into the plots in early-June of each year between 2004 and 2006 and collected at the end of that growing season (i.e., mid-August of the same year); thus single growing season decomposition rates were collected for each year. We also determined winter decomposition rates for a single season: bags of each type were placed into plots in mid-August 2005, and removed in early-June 2006. After removal from plots, decomposed leaf and root litter was removed from the bags, dried at 60 °C for 48 hours and weighed. “Loss bags” were created for each year and litter bag type; bags were carried into the field, and returned to the laboratory to determine handling loss, and initial litter bag weights were corrected depending on handling loss.

Fresh plant material was used for both the above and below-ground decomposition experiments. Although senesced material is often preferable for decomposition studies, fresh plant material was used as a standard substrate to assess the effects of our treatments on decomposition through effects on the decomposition microenvironment and decomposer activity because it was easy to collect, and sufficient green material was available for the number of replicates required. We chose to
use dried green plant material as a standard substrate, rather than cotton strips or filter paper, as the plant material more closely resembles natural sources of decomposition. However, to determine whether effects of functional group removals would differ for fresh and senesced plant material, in the 2006 growing season, an additional set of above-ground litter bags was created using freshly senesced litter collected in August 2005. No below-ground bags were created for these replicates; freshly-senesced root material is difficult to distinguish from older root material, and many root decomposition studies use live roots (Ostertag & Hobbie 1999).

**Analysis**

Decomposition is expressed as a percent loss of mass based on oven-dry mass of litter pre- and post-collection. Single-season decomposition rates were analyzed using a 4-way ANOVA on percent decomposition with the main effects being functional group removal, fertilizer, fungicide, and year (considered a nominal variable). Multi-year decomposition rates were analyzed using a 4-way ANOVA on percent decomposition with the main effects being functional group removal, fertilizer, fungicide and number of years since establishment (considered a continuous variable). When there was a significant interaction with year, or between environments, analyses were run independently for each year or environment level. Winter decomposition bags and bags containing senesced litter (both single year analyses) were analyzed using a 3-way ANOVA with the main effects being functional group removal, fertilizer, and fungicide. For all analyses, when removal treatments were significantly different, they were compared using a Tukey’s post-hoc comparison of means. All data fit the assumptions of the ANOVA and no transformations were required. All analyses were conducted using JMP statistical software (2003 SAS Institute, USA).
Results

**Above-ground Decomposition**

Above-ground decomposition was affected by plant functional group identity (Table 4-1); specifically, removal of grass and forb functional groups significantly decreased single-season leaf decomposition rates (Fig. 4-1a). The effect of plant functional group removal did not interact with any other environmental variable (fertilizer or fungicide) (Table 4-1). Fertilization also had no direct effect on the single-season decomposition rate (Table 4-1). Fungicide, however, affected decomposition directly, resulting in a decrease in the single-season leaf decomposition rate (Table 4-1, Mean proportion litter remaining 0.638 ± 0.005 no fungicide vs. 0.656 ± 0.005 fungicide). The relative pattern of above-ground decomposition remained the same in the winter (Removal: $F_{3,80}=5.36$ $p=0.002$; Fig. 4-2a) although only grass, not forb, removal resulted in a significant response. Likewise, in winter, above-ground decomposition but was not affected by fertilizer (Fertilizer: $F_{1,80}=1.79$ $p=0.19$) but decreased with fungicide application (Fungicide: $F_{1,80}=8.00$ $p=0.006$; Mean proportion litter remaining 0.655 ± 0.008 no fungicide vs. 0.690 ± 0.010 fungicide).

Table 4-1 *Summary of 4-way ANOVA for single-season leaf decomposition (2003-2006) and root decomposition (2003-2006).* Bold values are significant at P <0.05

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>$F_{\text{leaf}}$</th>
<th>Prob(_{\text{leaf}})</th>
<th>$F_{\text{root}}$</th>
<th>Prob(_{\text{root}})</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.611</td>
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<td>7.934</td>
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<td>0.011</td>
<td>0.916</td>
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<td>0.148</td>
</tr>
<tr>
<td>Year</td>
<td>3</td>
<td><strong>29.927</strong></td>
<td>&lt;0.001</td>
<td><strong>46.104</strong></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Removal*Fungicide</td>
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<td>1.511</td>
<td>0.212</td>
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<tr>
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<td>0.200</td>
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</tr>
<tr>
<td>Removal*Year</td>
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<td>Fungicide*Fertilizer</td>
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<td>0.795</td>
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<td>Fungicide * Year</td>
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<td>1.295</td>
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<tr>
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<tr>
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</tr>
<tr>
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<td>0.217</td>
</tr>
<tr>
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<td>0.888</td>
<td>0.536</td>
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<td>0.580</td>
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Fig. 4-1 Mean (+ 1SE) proportion material remaining in different plant functional group removal treatments for a) single season leaf decomposition 2003-2006 b) single season root decomposition 2004-2006 c) multiple season leaf decomposition 2003-2007 and d) multiple season root decomposition 2003-2005. Different letters indicate significant differences between removal treatments (Tukey’s comparison of all means). Removal treatments did not interact with year for multiple season decomposition (c and d), and thus Tukey’s comparison of all means is pooled across years.
Fig. 4-2 Mean (±SE) proportion material remaining in different plant functional group removal treatments for winter decomposition in a) leaves, b) roots in unfertilized plots and c) roots in fertilized plots. Different letters indicate significant differences between removal treatments (Tukey’s comparison of all means).
Long-term above-ground decomposition was also affected by functional group identity (Table 4-2); specifically, removal of the grass functional group significantly decreased the multiple-year leaf decomposition rates (Fig. 4-1c). The effect of removals did not vary across years decomposed, fertilizer or fungicide treatments (no interactions with Removal, Table 4-2). There was a significant Fungicide x Fertilizer interaction (Table 4-2), although when analyzed independently across fungicide levels, fertilizer did not affect decomposition either with \( F_{1,200}=0.32 \) p=0.57 or without \( F_{1,200}=2.84 \) p=0.09 fungicide.

**Table 4-2 Summary of 4-way ANOVA for multi-year growing season leaf decomposition (2003-2006) and root decomposition (2003-2005).** Bold values are significant at P <0.05

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>F_leaf</th>
<th>Prob_leaf</th>
<th>F_root</th>
<th>Prob_root</th>
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<tr>
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**Below-ground Decomposition**

Functional group removals did not affect single-season decomposition rates (Fig. 4-1b). The effect of removals on single-season below-ground decomposition depended on year (Table 4-1) being significant only in 2003 (2003 Removal: \( F_{3,80}=4.56 \) p=0.006), when removal of legumes decreased decomposition rates below the no-removal plots. Neither environmental variable, fungicide nor fertilizer, had any effect on single-season below-ground root decomposition (Table 4-1). The effect of functional group removals on winter below-ground decomposition rates depended on fertilizer (Removal x Fertilizer: \( F_{3,80}=6.28 \) p=0.006).
p=0.001). In non-fertilized plots, functional group removal had a significant effect on decomposition rate (Removal: $F_{3,80}=5.09$ p=0.005) with loss of forbs resulting in slower decomposition than loss of grasses (Fig. 4-2b). In fertilized plots, removal had no effect on decomposition rate (Removal: $F_{3,80}=2.60$ p=0.07, Fig. 4-2c). There was no effect of removal on long-term below-ground decomposition rate (Table 4-2, Fig. 4-1d). There was a significant interaction between fertilizer and years since establishment (Table 4-2), but in independent yearly analyses there was no significant effect of fertilizer in any year.

**Fresh vs. Senesced Leaves**

The effect of functional group removals on decomposition for senesced leaves was similar to that of fresh leaves, although only grass, not forb, removal resulted in a significant response (Removal: $F_{3,80}=5.95$ p<0.001; Fig. 4-3). The direct effects of environmental treatments on decomposition differed between green and senesced leaves. Fertilizer increased decomposition of the senesced leaves (Fertilizer: $F_{1,80}=4.70$ p=0.03; Mean proportion litter remaining 0.906 ± 0.007 no fertilizer vs. 0.888 ± 0.006 fertilizer) whereas fungicide application had no effect (Fungicide: $F_{1,80}=3.31$ p=0.07).

![Functional group removal](image)

**Fig. 4-3** Mean (±SE) proportion of leaf material remaining in different plant functional group removal treatments for senesced leaves. Different letters indicate significant differences between removal treatments (Tukey’s comparison of all means).
Discussion

In this study we show that plant functional group identity has a direct influence on above-ground decomposition rates by changing the decomposition micro-environment and that the importance of functional group identity differed between above-ground and below-ground decomposition. For above-ground decomposition, both grasses and forbs create a microenvironment that increases the rate of decomposition, but the effects of identity on below-ground decomposition are less frequent and transient. Further, we show that functional group identity affects both short-term and long-term decomposition rates in the same way, despite the nature of the decomposing material changing through time.

Above-ground Decomposition

This is one of only a few studies to report significant effects of the living plant community composition on decomposition rates; plant functional groups affect short-term above-ground decomposition differentially, with the removal of grasses and forbs both slowing decomposition. Earlier work on environmental effects on decomposition focused on large-scale environment-driven decomposition (Hobbie 1996; Prescott et al. 1999), but predicted losses of plant diversity and changes in community composition have resulted in recent interest in the potential for plant-driven effects on decomposition. Effects of the surrounding plant community diversity on decomposition are rare (Knops et al. 2001; Milcu et al. 2008; but see Scherer-Lorenzen 2008), but effects through changing plant composition are more common (Hobbie et al. 2006; Vivanco & Austin 2008). Wardle (1999) had to remove all of the vegetation before he detected an effect on the decomposer food chain, and in later studies he showed that removal of different shrub species slowed the rate of decomposition (Wardle & Zackrisson 2005; Jonsson & Wardle 2008).

Removals had similar effects on decomposition in winter as in summer, with removal of grasses slowing decomposition. During winter, microclimatic differences between vegetation types may be
minimized by snow cover (Moore 1984). The maintenance of functional group differences in these conditions provides evidence that the grasses may increase the rate of decomposition through effects on the biotic, rather than abiotic, decomposition environment. In addition to differences between functional groups, similar amounts of leaf material were lost over winter as during the growing season. Although perhaps unexpected, in northern ecosystems much of the litter decomposition occurs over the winter months, when the soil is mainly frozen (Moore 1984; Hobbie & Chapin 1996). Our over-winter leaf decomposition was measured during the non-growing season and includes the fall freeze-up and spring melt and substantial decomposition occurs during these seasons. Litter mass loss may be due to physical processes associated with freezing and-thawing (Hobbie & Chapin 1996) or possibly due to microbial decomposition of the litter, as soil respiration has been measured at temperatures below 0°C (Brooks et al. 1996).

Decomposition studies rarely examine longer-term processes, with many studies being a single year and three years the maximum for most (Aerts et al. 2006; Harmon et al. 2009). The mechanisms behind short-term decomposition likely differ from longer-term decomposition, where the more recalcitrant material remains, and long-term decomposition may be less influenced by differences in environmental conditions (Harmon et al. 2009). In our study, the removal of grasses consistently decreased decomposition rates through the first five years of decomposition, and functional group removals had the same effects on both short-term and long-term decomposition rates. Although the material decomposing changes over time, and thus the decomposition processes also change, the influences of the different plant functional group remained consistent.

The ability of grasses and forbs to increase the rate of decomposition may be through changes in the abiotic or biotic (decomposer community) environment. Few of the previous studies reporting plant-driven effects on decomposition identified a mechanism for these effects but they have suggested both changes in the abiotic environment through temperature effects (Hobbie et al. 2006) and in the
biotic environment with changes in the decomposer community occurring with plant invasion (Ashton et al. 2005). In a concurrent study we characterized the influence of these functional groups on numerous soil properties (Chapter 2). Abiotic photo-degradation may be an important component of decomposition in dry grasslands (Parton et al. 2007), but functional groups showed no difference in their effects on light interception (Chapter 2). Further, plants may compete with saprobes for nutrients (Moorhead et al. 1998) and this may be affected by the different competitive abilities of the functional groups. Although we earlier reported an effect of functional group identity on soil nutrients (Chapter 2), there was no effect of fertilization on decomposition in this study, suggesting that limitation of nutrients by plant competition would not produce the observed effects. Finally, the presence of forbs and grasses decreased soil moisture (Chapter 2) which we predict would have a negative effect on decomposition in this dry ecosystem, not the positive influence of these groups as observed. Finally, in this same community, Marshall (2008) was unable to detect any effect of plant functional group identity on the soil microbial community as measured by changes in substrate induced respiration or PLFA profiles. The methods used to examine the microbial community, however, may not be adequately sensitive. Because grass leaves were used as the decomposition substrate, there is a possibility that by removing grasses as a treatment we may have lost their associated microbiota that may be required for grass decomposition – however, this would not explain the negative effect we similarly detected when we removed forbs. Thus, none of the biotic or abiotic variables we measured (light interception, soil nutrients and soil moisture) can account for our observed effects of functional groups on decomposition rates. Clearly there are other effects of functional groups on the decomposition environment, either abiotic or biotic, that we did not measure which are responsible for functional group identity effects on decomposition. For example, Hobbie et al. (2006) reported that the effects of trees on soil temperature may affect litter decomposition.
A concern with using removal experiments to examine effects of plant composition on decomposition rates is that the removal effects may be confounded by the differing amounts of above-ground biomass removed with each treatment, or to differing amounts of belowground biomass remaining (and decomposing) after removals. The functional groups removed in this experiment all have significantly different above-ground biomass, with the removal of the most biomass in the forb removal treatment and the least with legume removals (Chapter 2). Although we did not directly account for differing amounts of biomass removed we conclude that the effects of removals on decomposition are not due simply to differences in biomass between removal treatments because grasses were not the dominant functional group in this community yet they had the most consistent positive effect on decomposition. Differing amounts and qualities of roots decomposing in the soils are more difficult to account for, and potential effects of these are often ignored in removal experiments. One would predict that the effects of these roots would decrease through time, as the biomass becomes incorporated into the soil. The effects of removals on short-term decomposition, however, are consistent across all 5 years for above-ground decomposition, and after the 1st year for belowground effects, and we are confident that the effects of decomposing roots resulting from removals play, at most, a minor role in the effects of functional group composition on litter decomposition.

The effect of plant functional group identity on above-ground decomposition was not context-dependent for either fertilization or fungicide treatments. These treatments were chosen to represent different ‘environmental contexts’ and both are relevant to future environmental change. Global warming is expected to cause an increase in soil nutrient levels, especially in northern latitudes, because higher temperatures increase mineralization rates of both nitrogen and phosphorus (Chapin et al. 1995; Shaver et al. 2000). Additionally, the presence of mycorrhizal fungi may change a plant’s response to changes in nutrient status. Soil nitrogen levels influence both the functioning of mycorrhizae and also their degree of mutualism with plants (Johnson 1993). In addition to not having an interaction with the
removal treatments, fertilization had no effect on above-ground decomposition directly during either the growing season or during the winter. The effect of fertilization is variable in different systems and has been reported to accelerate the rate of decomposition (Madritch & Hunter 2003), have no effect (Hobbie 2000) or slow the rate of decomposition (Prescott et al. 1999) and may be highly dependent on the quality of the litter (Prescott et al. 1999; Hobbie 2000; Knorr et al. 2005). The direct effect of fungicide, in contrast, was a slowing of leaf decomposition during both seasons. We expected that fungicide treatment would decrease mycorrhizal colonization rates and thereby increase decomposition because mycorrhizal infected plants may better be able to compete with soil saprobes for nutrients (Christensen & Jakobsen 1993). The decrease in decomposition rate may be the result of a few different mechanisms. The saprophytic fungi may have been negatively affected by benomyl application resulting in a decreased decomposition rate. A related study in the fifth season of this project, however, found that fungicide did not affect PLFA profiles or total fungal biomass of the soil flora (Marshall 2008). As such, any effect of fungicide on soil saprophytic fungi would likely be positive (compensating for the loss of mycorrhizae), resulting in a positive effect on decomposition. Alternatively, arbuscular mycorrhizae have been reported to have direct saprotrophic capabilities (Hodge et al. 2001), and may have had a direct role in decomposing the leaves.

We used fresh leaf material, rather than senesced litter, as the standard decomposition source. Although decomposition rates of fresh litter were faster than senesced litter, the effect of functional group removals was similar for the two litter types, with removal of grasses decreasing decomposition rate in both fresh and senesced litter. Additionally, the effect of identity on litter decomposition was not context dependent for either decomposition material. Thus the fresh litter used in this experiment provides an appropriate approximation for the effect of functional group removals on litter decomposition. The direct effects of the environmental context (fungicide and fertilizer) did differ between the two materials. Although fungicide slowed decomposition of fresh material, there was no
effect on the senesced litter. In contrast, fertilizer had no effect on fresh material, yet accelerated the decomposition of senesced litter. We would have predicted the opposite result, as we considered the fresh litter to be a higher quality litter (both because of its lower C:N and faster decomposition) which is expected to be more limited by fertility levels whereas low quality litter is more limited by carbon (Prescott et al. 1999; Hobbie 2000; Knorr et al. 2005).

**Below-ground Decomposition**

Neither short-term nor long-term root decomposition were influenced by functional group removals. In contrast with leaves, root decay rates are often much more closely linked with root quality as opposed to environmental parameters (Silver & Miya 2001). This is likely because roots occur in the soil and both they, and the community of decomposers, are well buffered from environmental extremes (Silver & Miya 2001). Therefore the plant community may have little effect on root decomposition rates unless they affect the quality of the root tissue itself, through compositional changes or changes in soil fertility levels - an effect that was not be measured in this study.

The only effects of functional group removals on below-ground decomposition were transient effects on short-term decomposition, and did not parallel those for leaf decomposition. During the first growing season after removal treatments were established, removal of legumes caused a decrease in decomposition rate. Fertilization had no effect on root decomposition so it is not likely that the effects of legume removal are due to the loss of their nitrogen fixation abilities from the community. Although the effects were transient, it is also unlikely to be a direct effect of the removals as the legumes had the least biomass removed of all three removal treatments (Chapter 2). The identity of the surrounding plant community could positively affect root decomposition through a variety of mechanisms. Living roots, depending on species, exude a variety of different rhizodeposits (Clarholm 1985), easily metabolizable carbon compounds that may enhance microbial degradation. Different functional groups
may dry out the soil at different rates (McLaren et al. 2004) and drying and rewetting of soils can both increase and decrease the rate of root decomposition (van der Krift et al. 2002).

In contrast to winter leaf decomposition, effects of removals on winter root decomposition differed from those during the growing season, and were also dependent on fertilizer application. Without fertilizer, removal of forbs slowed decomposition rate while removal of grasses increased the rate. Although the type of roots used in the litter bags were the same in the different removal treatments, the general decomposition environment may be altered by the quality of the other associated roots of the living species. Decomposition rates have been reported to be negatively correlated with root C:N (Silver & Miya 2001) and grasses are often characterized by their high C:N and low decomposability (Wardle et al. 1997). Thus, removing grasses from a community may decrease the overall C:N of the root material, improving resource conditions for decomposers. Conversely, removal of forbs should increase the proportion of high C:N grass roots in the community, creating poorer conditions for decomposition. The effect of removal disappeared with fertilization—the additional nutrients would likely swamp any differences in resource availability due to changes in C:N in the roots.

In conclusion, we show that the composition of the living plant community influences above-ground decomposition rates through effects on the decomposition micro-environment. Grasses and forbs both promote decomposition, thus changes in plant community composition may result in shifts in the carbon dynamics of this northern ecosystem. These effects of the surrounding plant community may be tempered, in part, by the lack of influence on root decomposition, which is buffered by the soil environment. Although both fertilizer and fungicide influenced overall decomposition rates, there were few interactions between removal treatments and these environmental variables. Species change is currently happening in concert with environmental change, and therefore to predict the micro-environment effects of species loss, we must understand the role that different plant species will play in this new environment. We have shown that the roles of different plant functional groups remain
constant within an ecosystem, even if the environmental variables differ, and this provides us with much greater predictive power in determining the effects of species loss on ecosystem functioning.


References


CHAPTER FIVE

Biodiversity Loss Influences Decomposition Through More Than One Mechanism

Introduction

Human impacts on ecosystems through land-use, habitat fragmentation and climate change have led to large losses of species and changes in the composition of communities (Chapin et al. 2000) and there is growing concern that these changes will have negative effects on ecosystem properties and the goods and services they provide (Hooper et al. 2005). This has prompted many studies focusing on the effects of loss of species and functional groups on ecosystem functioning (reviewed in Hooper et al. 2005; Balvanera et al. 2006). There have been substantially fewer studies directly examining the effects of changing community composition, although analysis of biodiversity-ecosystem functioning experiments have shown that plant composition plays a major role (see Hooper & Vitousek 1998; Scherer-Lorenzen et al. 2003). Much of the biodiversity-ecosystem functioning research has examined the impacts of changing plant biodiversity on primary productivity (Hector et al. 1999; Hooper et al. 2005) and there has been far less emphasis on other ecosystem functions that may respond differently. Plant biodiversity shows different effects on above-ground (such as plant productivity) and below-ground (such as soil nutrient availability and soil microbial biomass) processes in terrestrial ecosystems (Loreau et al. 2001; Hooper et al. 2005). Studies examining the impacts of changing plant composition and richness on decomposition are particularly important because the rate of decomposition is an important determinant of numerous other ecosystem functions such as productivity, nutrient cycling, and carbon flux (Knops et al. 2001).

4 A version of this paper has been submitted for publication. McLaren, J.R. and Turkington, R. Biodiversity loss influences decomposition through more than one mechanism.
Changing the types or number of plant species in a community can affect decomposition rates through at least two mechanisms (Fig. 5-1). By mechanism, in this context, we refer to a process or pathway that affects decomposition rates, and not to two other widely discussed biodiversity mechanisms -- complementarity and selection effects (Loreau & Hector 2001).

Fig. 5-1 Possible mechanisms by which plant composition may affect litter decomposition rates

First, changes in plant composition may affect litter decomposition through influences on the decomposition microenvironment. Different species or functional groups of plants have varying effects on many ecosystem properties (Chapter 2; Hooper & Vitousek 1997) and consequently changes in the plant community may affect litter decomposition through influences on the local microenvironment such as soil temperature (Hobbie et al. 2006), the decomposer community (Wardle et al. 1999) and competition for nutrients between the vegetation and the saprotrophic community (Moorhead et al. 1998). Many studies have examined direct environmental effects, such as effects of soil nutrient status,
on decomposition (Hobbie 1996; Prescott et al. 1999), but only a few have examined plant-species driven environmental effects on decomposition (Hector et al. 2000; Knops et al. 2001; Hobbie et al. 2006), including one in this system (Chapter 4).

Second, changing the members of the living plant community necessarily changes their contributions to the composition and quality of the litter community5. Examination of species-specific decomposition and litter mixing studies have been used to link litter composition to decomposability. Individual species vary in their decomposition rates (Cornelissen 1996) because of leaf characteristics such as leaf nitrogen and lignin contents (Aerts 1997), carbon quality (Hobbie 1996) and secondary chemicals (Cornelissen & Thompson 1997; Madritch & Hunter 2004). Litter is rarely composed of a single species, and the combination of litter from multiple species may also affect decomposition rate. Although litter mixing studies have produced no consistent patterns relating litter diversity to decomposition rates (reviewed by Hättenschwiler et al. 2005), numerous studies have reported non-additive effects of mixing different litter types, where litters decompose at different rates in mixture than they do in monoculture (Wardle et al. 1997; Gartner & Cardon 2004; Moore & Fairweather 2006).

Finally, interactions between these two mechanisms also may affect decomposition rate and experiments that compare their relative impacts are rare. A few studies have examined these two mechanisms independently, including examination of the effects of tree species identity (Hobbie et al. 2006), and plant species diversity (Hector et al. 2000; Knops et al. 2001; Scherer-Lorenzen 2008). However, their experimental designs did not allow a test of interactions between them which may account for the inconsistent results (Hobbie et al. 2006) and lack of strong diversity effects (Hector et al. 2000). More recently, three studies placed different litter combinations into plant communities having different levels of richness or species composition and this allowed a direct examination of interactions

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5 Hereafter, we will refer to the growing plant community as “plants” and the decomposing plant community as “litter”.

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(Jonsson & Wardle 2008; Milcu et al. 2008; Vivanco & Austin 2008); all three studies reported interactions between the two mechanisms.

Further, the effects of plant composition on decomposition, through both of these mechanisms, may be context-dependent on environmental conditions. Changes in environmental factors that may influence decomposition, soil nutrient status and mycorrhizal presence, may result in a change, or even a loss, in the types of plant species present in a community (Hooper et al. 2005). Few studies have examined the effects of plant richness or composition on ecosystem functioning, and litter decomposition in particular, in more than one environmental context. We examine effects of plant functional group identity on decomposition within three different environmental contexts to examine constancy of effects across soil fertility levels, across changes in abundance of mycorrhizae, and across years within the same experiment.

Although wide-scale climatic conditions, such as actual evapotranspiration levels, have the greatest influence on decomposition rates (Aerts 1997), local environmental conditions such as soil nutrient content (Carreiro et al. 2000; Hobbie 2000) are also important. Global warming is expected to cause an increase in soil nutrient levels, especially in northern latitudes, because higher temperatures increase mineralization rates of both nitrogen and phosphorus (Chapin et al. 1995; Shaver et al. 2000). Rates of litter decomposition are generally thought to be limited by the availability of nitrogen because of the inverse relationship between C:N and decomposition and because N accumulates in litter during early decay (Prescott et al. 1999) but not all studies support this conclusion -- even in N-limited systems (Hobbie 2000). Whether fertilization has a significant effects on decomposition or not may depend on other factors such as the fertilization rate and the quality of the litter (Knorr et al. 2005).

Our second environmental context, mycorrhizal fungi in the soil, also may directly affect litter decomposition rates because they may have saprotrophic functions (Hodge et al. 2001). They may also act indirectly, because a decrease in mycorrhizal colonization may reduce the ability of plants to
compete with saprophytic soil microflora (Christensen & Jakobsen 1993). Finally, mycorrhizae are affected by soil nitrogen levels, both in terms of their functioning and also the type of relationship they exhibit with plants on the parasitic-mutualistic continuum (Johnson 1993).

In 1983 John L. Harper commented that “[t]he concentration of [research] effort within one small field and on small quadrats within it, gives the studies high precision and high relevance, but with an absolute sacrifice of generality. We do not know whether most of what we have observed in the field can be generalized to other fields, or, indeed, to less intensively studied parts of the same field.” (Harper 1983) For this reason, we chose to repeat this experiment in two separate years, 2004 and 2006, to examine the consistency of the results. Many field experiments are run in only a single location, or for a single growing-season, and a significant result gives the expectation that, were the experiment to be repeated in a different location or year, the results would be the same. Of the seven studies already described that examined effects of richness or composition on litter decomposition through both mechanisms, only Scherer-Lorenzen (2008) repeated the test of either mechanism in more than 1 year, and none repeated their examination of the effects of litter composition on short-term decomposition rates. It may indeed be more likely to expect inconsistency between years in localized and highly controlled experiments; long-term experiments show changes in the mechanisms driving effects of species composition after 10 years at Cedar Creek (Fargione et al. 2007) or in species composition itself after 100 years in the Park Grass experiments at Rothamsted (Silvertown et al. 2006). In boreal-forest understory experiments in our region, effects of fertilization differed between the short-term and longer-term (10 year) patterns (Turkington et al. 2002) and the authors speculate that it is quite likely that ecosystems with slow-growing long-lived plants never attain equilibrium.

In this study we examined the effects of plant functional group identity on decomposition through both changes in the decomposition microenvironment, and changes in the species composition of the litter. We removed a single plant functional group from a series of plots in a grassland community
in northern Canada. By comparing plots that had functional groups removed with control plots having an entire community of species, we could determine the role of functional group identity in the intact community. Because changes in plant composition are expected to happen in concert with environmental changes, we chose to examine the impacts of losing different plant functional groups in different environments by crossing the removal treatments with a fertilizer and a fungicide treatment used to decrease mycorrhizal colonization rates, and by repeating the experiment in 2 separate years. To examine litter composition effects, we created a series of litter bags with all possible combinations of leaves from the dominant species of each of the three functional groups and these bags were placed in all three environments (removals, fertilization and fungicide). This experimental design has two advantages over other studies. First, by placing all litter combinations in all environments, our design permitted us to investigate the interactions between changes in the environment and changes in litter composition. More uniquely, because we were able to distinguish species within mixtures after decomposition, we could test changes in species-specific decomposition as causes for non-additive effects in mixtures.

**Methods and Materials**

This removal experiment was part of a larger experiment examining the role of plant functional group identity in determining various ecosystem functions. Chapter 2 describe the methods in detail, and they are in described briefly below.

**Site Description**

The study area is a dry grassland near Kluane Lake in the south-western Yukon in northern Canada (61°04.218 N 138°23.018 W). The area receives a mean annual precipitation of ca. 230 mm, about half of which falls as rain during the summer, but also includes an average annual snowfall of about 100 cm. The grassland is surrounded by a closed to relatively open spruce forest community dominated by *Picea*
The grassland is dominated by *Poa glauca* Vahl and *Carex stenophylla* Wahlenb. ssp. *eleocharis* (Bailey) Hultén, and also contains many non-leguminous forbs (dominated by *Erigeron caespitosus* Nutt., *Artemisia frigida* Willd.), and legumes (dominated by *Oxytropis campestris* (L.) DC.); all nomenclature follows Cody (2000). Grassland species were divided into three functional groups, namely, graminoids (grasses and sedges), forbs, and legumes.

**Experimental Plant Communities**

Experimental plots were established in May 2003 and maintained annually for 4 years through the 2006 growing season. The experiment was a 4 x 2 x 2 fully crossed factorial design (4 removal treatments, +/-0 fertilizer, +/-0 fungicide). Each of the 16 treatments was replicated 5 times, resulting in a total of 80 plots.

There were four removal treatments: independent removal of each of the three functional groups (graminoids, forbs and legumes) and a no-removal control. Functional groups were chosen based on traits that were potentially relevant to the ecosystem properties of interest (e.g. C:N, stature, N-fixation ability). In 2003, plants were removed from the plots using Round-up™ glyphosate, a non-selective herbicide. Herbicide was painted precisely to the leaves and once plants had visibly yellowed, stems of selected plants were clipped at soil level and removed from the plots. Removal treatments were maintained in 2004 using herbicide application and clipping, and in the subsequent two years the very minimal regrowth was clipped at ground level early in the growing season, but other functional groups were allowed to invade the newly available space created by the removals.

Fertilizer and fungicide treatments were applied upon completion of the removals (July 20) in 2003 and in early June of each subsequent year. Fertilizer was applied each year to half the plots in granular form at a rate of 17.5 g N m⁻², 5.8 g P m⁻² and 5.8 g K m⁻². This application rate was used to be consistent with many other studies being done in the area (e.g. John & Turkington 1997; Turkington et al. 2002). The fungicide Benlate™ (active ingredient benomyl) was applied to half of the plots as a soil
drench (2 L m\(^{-2}\) plot) every two weeks from early-June to mid-August at a rate of 2.5 g benomyl m\(^{-2}\) per application. Plots that did not receive fungicide received an equivalent amount of water. Benomyl applications reduced mycorrhizal colonization rates from 50% to less than 10% of root intersections (J. McLaren, unpublished data).

**Decomposition Experiment**

Fresh leaf material from the dominant species from each functional group - *Poa glauca* (graminoid), *Artemisia frigida* (forb), *Oxytropis campestris* (legume) – was dried at 40 C for 48 hours and placed in 10 x 5cm litter bags made from 1mm mesh size nylon screening. To preserve the leaf structural properties, leaves were not ground or cut, except *P. glauca*, which was cut into 8cm lengths to fit into the litterbags. All possible combinations of 1, 2 and 3 species were created using a replacement series design i.e., total leaf biomass per litter bag was held constant at 0.6 g and mixtures were made up of 0.6g (monocultures), 0.3g (2 species mixtures) or 0.2g (3 species mixtures) of each of the component species.

The decomposition experiment was done in 2004 and repeated in 2006. In mid-June each year (shortly after the growing season began), one replicate bag of each of the seven possible species combinations was placed into each plot, and these were collected in early August, after approx. 50 days, when the plants in the surrounding community had senesced. Decomposed leaves were removed from bags, dried at 60 °C for a minimum of 48 hours and weighed. We were still able to differentiate between species post-decomposition, and thus for bags with multiple species, species material was separated and dry mass recorded independently for each species.

Although senesced material is often preferable for decomposition studies, we decided to use fresh material for both experiments as a standard substrate to assess the effects of our treatments, as it was easy to collect and sufficient material was available for the number of replicates required. Although this material is not ‘litter’ in the strictest sense, for ease of reading we refer to this process as ‘litter decomposition’ throughout the paper.
Analysis

Decomposition is expressed as a proportion of dry mass loss occurring during the single growing season in the field. Individual species masses within species combinations were pooled (creating a single decomposition value per bag) for all except species-specific analyses. The proportion decomposed was standardized as:

\[
\frac{\text{Initial Mass} - \text{Final Mass}}{\text{Initial Mass}}
\]

We used a 4-way ANOVA on proportion decomposed with the three environments examined within each year (functional group removal, fertilizer and fungicide) and litter species composition (hereafter termed litter composition) used as main effects. When there was a significant Environment X Litter Composition interaction, analyses were run independently for each environment level.

To examine the independent effect of Environment (functional group removal, fertilizer, and fungicide), we examined changes in decomposition of a standard source (each of the seven species combinations) in the different environments using a 4-way ANOVA. When environments interacted, separate ANOVA’s were run within each environment level to dissect differences in decomposition. When functional group removals were significantly different, they were analyzed using a Tukey’s post-hoc comparison of means. To examine the independent effects of litter species mixture, proportion decomposed of different species mixtures were compared using an ANOVA. When the effect of composition was significant, species mixtures were analyzed using a Tukey’s post-hoc comparison of means.

We calculated expected decomposition based on monoculture decomposition rates. When monoculture rates differed between environments, expected decomposition was calculated based on environment-specific monoculture rates. Observed to expected comparisons were standardized as:

\[
\frac{\text{Observed decomposition} - \text{Expected decomposition}}{\text{Expected decomposition}}
\]
A positive value indicates positive non-additive effects of species mixing on decomposition, and a negative value indicates negative non-additive effects. The mean value of each composition treatment was compared against zero using a t-test.

We analyzed species-specific decomposition within species combinations using a nested ANOVA, with species nested within composition. A Tukey’s post-hoc comparison of all means was used to examine species decomposition rates within and between species mixtures.

Results

Effects of Decomposition Microenvironment

The effects of plant functional group removals on decomposition varied by year. In 2004 removals had no effect on decomposition (Table 5-1; Fig. 5-2a) whereas in 2006 both the removal of graminoids and forbs slowed decomposition (Fig. 5-2b). There was a significant fertilizer x fungicide interaction in both years (Table 1). Fertilizer effects, however, were not significant either with or without fungicide in 2004 (with fungicide $t_{278} = -1.15\ p=0.25$; without fungicide $t_{278} = 1.76\ p=0.08$) or 2006 (with fungicide $t_{278} = 1.87\ p=0.06$; without fungicide $t_{278} = -0.87\ p=0.39$).

Fig. 5-2 Standardized litter decomposition ($\frac{(\text{Initial} - \text{Final Mass})}{\text{Initial Mass}}$) (pooled mean for all litter compositions, ±SE) in different functional group removal treatments in a) 2004 and b) 2006. Different letters indicate significant differences between removal treatments (p<0.05, Tukey’s comparison of all means).
Table 5-1 Results of a 4-way ANOVA for the litter decomposition experiment in 2004 and 2006

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>F</th>
<th>p</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Removal</td>
<td>3</td>
<td>0.64</td>
<td>0.589</td>
<td>13.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fungicide</td>
<td>1</td>
<td><strong>6.49</strong></td>
<td><strong>0.011</strong></td>
<td>0.99</td>
<td>0.321</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>1</td>
<td>0.38</td>
<td>0.536</td>
<td>0.40</td>
<td>0.530</td>
</tr>
<tr>
<td>Litter Composition</td>
<td>6</td>
<td><strong>53.86</strong></td>
<td><strong>&lt;0.001</strong></td>
<td>33.65</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>Removal*Fungicide</td>
<td>3</td>
<td>1.38</td>
<td>0.247</td>
<td>0.78</td>
<td>0.507</td>
</tr>
<tr>
<td>Removal*Fertilizer</td>
<td>3</td>
<td>1.92</td>
<td>0.126</td>
<td>1.48</td>
<td>0.219</td>
</tr>
<tr>
<td>Removal*Litter Composition</td>
<td>18</td>
<td>0.78</td>
<td>0.722</td>
<td>0.49</td>
<td>0.963</td>
</tr>
<tr>
<td>Fungicide*Fertilizer</td>
<td>1</td>
<td><strong>6.69</strong></td>
<td><strong>0.010</strong></td>
<td>4.79</td>
<td><strong>0.029</strong></td>
</tr>
<tr>
<td>Fungicide*Litter Composition</td>
<td>6</td>
<td>0.75</td>
<td>0.608</td>
<td>2.27</td>
<td><strong>0.036</strong></td>
</tr>
<tr>
<td>Fertilizer*Litter Composition</td>
<td>6</td>
<td>0.53</td>
<td>0.788</td>
<td>0.49</td>
<td>0.812</td>
</tr>
<tr>
<td>Removal<em>Fungicide</em>Fertilizer</td>
<td>3</td>
<td>1.10</td>
<td>0.347</td>
<td>2.46</td>
<td>0.062</td>
</tr>
<tr>
<td>Removal<em>Fungicide</em>Litter Composition</td>
<td>18</td>
<td>1.30</td>
<td>0.180</td>
<td>0.53</td>
<td>0.946</td>
</tr>
<tr>
<td>Removal<em>Fertilizer</em>Litter Composition</td>
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<td>0.65</td>
<td>0.860</td>
<td>0.39</td>
<td>0.989</td>
</tr>
<tr>
<td>Fungicide<em>Fertilizer</em>Litter Composition</td>
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<td>1.07</td>
<td>0.381</td>
<td>0.40</td>
<td>0.877</td>
</tr>
<tr>
<td>Removal<em>Fungicide</em>Fertilizer*Litter Composition</td>
<td>18</td>
<td>1.15</td>
<td>0.305</td>
<td>0.89</td>
<td>0.591</td>
</tr>
</tbody>
</table>

**Effects of Litter Composition**

In 2004, the effect of litter composition, pooled across all environments, was significant (Table 5-1).

Species monocultures decomposed at different rates: the forb \(A. frigida\) decomposed more slowly than either the grass \(P. glauca\) or the legume \(O. campestris\) (Fig. 5-3a). Species mixtures also decomposed at different rates: the grass-legume combination decomposed fastest, while the legume-forb combination decomposed more slowly than all others (Fig. 5-3a).

Species mixtures displayed positive non-additive effects on decomposition; every combination that contained grass decomposed significantly faster than expected (GL: \(t_{1,79}=13.57\) \(p<0.001\), GF: \(t_{1,79}=6.58\) \(p<0.001\), LF: \(t_{1,79}=-1.67\) \(p=0.10\), GLF: \(t_{1,79}=7.15\) \(p<0.001\); Fig. 5-3b). These non-additive effects can be further explored by examining species-independent responses within each mixture (Litter Composition (Species): \(F_{3,899}=21.70, p<0.001\)). Within the grass-legume combination, both species had faster decomposition than their respective monocultures (Fig. 5-3c). In the other two combinations containing grass, the positive non-additive effects were due to an acceleration of the decomposition of
the species accompanying the grass in both cases (grass decomposition rates did not differ from monoculture)(Fig. 5-3c). The legume-forb combination decomposed marginally slower than expected (Fig. 5-3b), but decomposition rates of neither species within the mixture differed from monoculture (Fig. 5-3c).

In 2006, the effect of litter composition differed between fungicide treatments (Table 5-1) and thus litter composition was examined independently within each fungicide treatment (with Fungicide: $F_{3,279}=15.02$, $p<0.001$; without Fungicide: $F_{3,279}=19.63$, $p<0.001$). In both non-fungicide and fungicide plots, forbs decomposed more slowly than the other species (Fig. 5-4a, b). Again, species mixtures decomposed at different rates; the grass-legume combination had the fastest decomposition, and the legume-forb combination the slowest in both non-fungicide and fungicide plots (Fig. 5-4a, b).

In contrast to 2004, 2006 species mixtures displayed negative non-additive effects on decomposition; every combination that contained forbs decomposed significantly slower than expected, in both non-fungicide (GL: $t_{1,39}=1.22$ $p=0.22$, GF: $t_{1,39}=-6.01$ $p<0.001$, LF: $t_{1,39}=-5.61$ $p=0<0.001$, GLF: $t_{1,39}=-2.32$ $p=0.03$; Fig. 5-4c) and fungicide plots (GL: $t_{1,39}=-1.91$ $p=0.06$, GF: $t_{1,39}=-5.22$ $p<0.001$, LF: $t_{1,39}=-6.71$ $p<0<0.001$, GLF: $t_{1,39}=-4.47$ $p=0.03$; Fig. 5-4d). Again, within the mixtures, species differed in their decomposition rates, in both plots without fungicide (Litter Composition (Species): $F_{3,479}=16.90$, $p<0.001$) and with fungicide(Litter Composition (Species): $F_{3,479}=13.55$, $p<0.001$). In contrast to 2004, there were no clear patterns in species-specific decomposition within mixtures producing these patterns in either non-fungicide (Fig. 5-4e) or fungicide (Fig. 5-4f) plots.
Fig. 5-3 a) Standardized 2004 litter decomposition (Initial - Final Mass)/Initial Mass) (pooled mean across environments, ±SE) of seven different combinations of species. b) Standardized 2004 deviation from expected decomposition ((Proportion Decomposition Observed - Proportion Expected)/Proportion Expected) (pooled mean across environments, ±SE) of four species combinations. Significant values indicate presence of non-additive effects and an * indicates that a value is significantly different from 0 (t-test, p<0.05). c) Standardized 2004 litter decomposition (Initial - Final Mass)/Initial Mass) (pooled mean across all environments, ±SE) for each species within seven species combinations. For all panels, different letters indicate significant differences between litter compositions (Tukey’s comparison of all means); G = graminoid, L = legume F = forb.
Fig. 5-4  a, b) Standardized 2006 litter decomposition ( (Initial - Final Mass)/Initial Mass) (pooled mean across environments, ±SE) of seven different combinations of species.  c, d) Standardized 2006 deviation from expected decomposition ((Proportion Decomposition Observed - Proportion Expected)/ Proportion Expected) (pooled mean across environments, ±SE) of four species combinations. Significant values indicate presence of non-additive effects and an * indicates that a value is significantly different from 0 (t-test, p<0.05).  e, f) Standardized 2006 litter decomposition ( (Initial - Final Mass)/Initial Mass) (pooled mean across all environments, ±SE) for each species within seven species combinations. Non-fungicide treated plots (a,c,e) and fungicide-treated plots are separated (b,d,f). For all panels, different letters indicate significant differences between litter compositions (Tukey’s comparison of all means); G = graminoid, L = legume F = forb.
Discussion

Our results show that the removal of plant functional groups affects litter decomposition rates both through effects on the decomposition microenvironment and also through effects on the species composition of the litter. These effects were highly dependent on the year of the study; the presence, direction and species responsible for each of these effects were different in the two years.

Effects of Decomposition Microenvironment

Removal of both graminoids and forbs slowed decomposition. This is one of only a few studies to demonstrate significant effects of changing plant community composition on decomposition through changes in the decomposition microenvironment. Previous studies on the effects of the living plant community have produced mixed results with changes in plant diversity having no strong effect on decomposition (Hector et al. 2000; Knops et al. 2001; Milcu et al. 2008), but increases in functional group diversity having positive effects (Scherer-Lorenzen 2008). Effects of plant composition on decomposition are more common than the effects of diversity. Effects of species identity on decomposition have been reported in both artificial (Hobbie et al. 2006) and natural (Vivanco & Austin 2008) monocultures of trees and Jonsson (2008) reported that removal of shrubs slowed decomposition. In an earlier study using only a single litter type we also found that removal of both graminoids and forbs slowed decomposition, and that this effect was maintained over long-term decomposition processes (Chapter 4). Two large-scale plant richness manipulations, BIODEPTH (Scherer-Lorenzen 2008) and the Jena Experiment (Milcu et al. 2008), both reported that the presence of legumes in a community accelerated decomposition. In contrast, in our removal experiment, where legumes occur at low natural densities, we detected no effects of legumes on decomposition, and graminoids and forbs were the only functional groups to show significant effects.

Effects of removing functional groups on decomposition were always negative. Living plants are often thought to negatively influence decomposition by competing with saprobes for limiting nutrients
(Moorhead et al. 1998) or preventing abiotic photo-degradation (Parton et al. 2007) by shading plant litter. Functional groups may also have positive influences on decomposition, however, as exemplified by the effects of legumes in diversity manipulations (Milcu et al. 2008; Scherer-Lorenzen 2008).

Effects of functional group removals on decomposition were only detected in 2006, and not in 2004. Effects of plant community composition on decomposition rates are rare, and this may be due to the age of the plant community. Of the seven similar studies discussed in the introduction, four were done in young communities (<4 years old) and only one of these (Scherer-Lorenzen, 2008) showed a significant effect on the decomposition micro-environment. The remaining 3 studies were done in older communities (> 10 years old) and all of these reported effects on the decomposition microenvironment. We detected no effects in 2004 when removals had only taken place the previous summer, but did detect effects in 2006, 3 years after the plots had been established.

Few studies have been able to characterize changes in the decomposition micro-environment resulting from changing plant composition that affects decomposition. Vivanco and Austin (2008) measured numerous soil variables, but found nothing that mirrored effects on decomposition rate. Hobbie et al. (2006) showed tree identity influenced soil temperature, which they speculated influenced decomposition.

A variety of soil properties in our removal plots have previously been measured (Chapter 2), although none of the variables measured are likely to result in the observed decomposition patterns. Plots where either legumes or graminoids were removed had similar above-ground biomass, suggesting that biomass effects alone would not drive decomposition effects. Although removal of forbs and graminoids resulted in similar increases in soil N, and decreases in P (Chapter 2), as decomposition was not affected by fertilization, we do not believe decomposition patterns are driven by these differences in soil nutrients. Finally, removal of both graminoids and forbs resulted in higher soil moisture in plots but in this dry ecosystem, soil moisture is more likely to encourage rather than retard decomposition.
(Bryant et al. 1998; Aerts 2006). None of the ecosystem properties we examined correspond to changes in decomposition rates, and we suggest plant identity influences on a further ecosystem property not measured here may be responsible, such as changes in soil temperature as reported by Hobbie et al. (2006).

There were no direct effects of any of our other main environmental manipulations (fertilizer and fungicide) on decomposition rates. A meta-analysis concluded that nitrogen enrichment, when averaged across all studies, has no statistically significant effect on litter decomposition rates but there was wide variation in direction of responses (Knorr et al. 2005). Whether fertilization depresses or accelerates decomposition may be determined by the quality of the litter, where high quality litter is usually stimulated by fertilization, and for slow decomposing litter, additional N may depress the decomposition rates even further (Carreiro et al. 2000; Knorr et al. 2005). Secondary metabolites and alkaloids are common in other members of both the Artemisia (Talley et al. 2002) and Oxytropis (Pfister et al. 2001) genera, and the litter species used in this experiment may be of such ‘poor quality’ that additional nitrogen might not accelerate decomposition rates. Finally, this system may be more limited by water rather than by soil nutrient levels and in a separate study we showed that fertilizer had no effect on productivity in either year of this experiment (Chapter 2).

Further, we had predicted that fungicide application may affect decomposition by decreasing mycorrhizal colonization and thus reducing the ability of plants to compete with saprophytic soil microflora (Christensen & Jakobsen 1993). Although mycorrhiza are common in this system (approx. 50% root colonization), the mycorrhizae may not be playing a large role in determining ecosystem functions, including decomposition. In a related study, we found few ecosystem properties were affected by fungicide application (Chapter 2). The main effect of mycorrhizal fungi in a community is often thought to be increasing plant access to scarce or immobile soil nutrients (Bever et al. 2001). If this ecosystem is limited by water rather than nutrients, as we hypothesize, the mycorrhizae may not have a
large influence in this community, although a number of studies demonstrate that mycorrhizal colonization can protect host plants against drought (Auge 2001).

In addition to no direct effect of either fertilizer or fungicide on decomposition rates there was no interaction between the removal treatments and fertilizer or fungicide, indicating that the effects of functional group identity on decomposition are not context dependent. Of studies that have examined the context dependency of species richness on ecosystem functioning, those done in artificially created communities generally showed context dependency (Reich et al. 2001; Fridley 2002; Craine et al. 2003; Reich et al. 2004; De Deyn et al. 2009), whereas removal experiments in natural communities have shown context dependence of effects with some (Shevtsova et al. 1997; Klanderud 2005; Wardle et al. 2008) but not all (Hobbie et al. 1999) studies. Our lack of context dependence i.e. the effect of identity on decomposition was not altered by fertilizer and fungicide treatments, is not surprising given that there was no context dependence for a variety of other ecosystem properties measured in this system during the same period (Chapter 2).

Effects of Litter Composition

Functional group identity also affected decomposition via changes in the composition of the litter in both years. Effects of identity were partially due to differences in decomposition rates between species monocultures, with the dominant grass and legume both decomposing faster than the forb. The faster decomposition of the grass was surprising; grasses are often reported to have slower decomposition because of their higher C:N (Wardle et al. 1997; Hector et al. 2000). In this ecosystem, the dominant grass (Poa C (44.9%): N(1.7%)) had a higher C:N than both the forb (Artemisia C(45.9%): N(2.4%)) and the legume (Oxytropis C (45.2%): N(3.3%)). Other leaf quality factors have been argued to be more important than N in predicting decomposition, such as P (Hoorens et al. 2003), C quality (Hobbie 1996) and water soluble content (Wardle et al. 2003), even in systems that are otherwise N-limited (Hobbie 1996). We did not measure any other litter quality index, so it is possible that the decomposition rates
of these species were determined by another unmeasured trait. For example, the secondary metabolites and anti-herbivory alkaloids in *Artemisia* (Talley et al. 2002) and *Oxytropis* (Pfister et al. 2001), as discussed above, may slow decomposition.

In addition to differences between decomposition rates of the monocultures, litter combinations showed positive non-additive effects of mixing on decomposition in 2004, and negative non-additive effects in 2006. Positive effects of litter mixing are common and reviews report that more than half of all mixtures result in accelerated decay (Gartner & Cardon 2004; Hättenschwiler et al. 2005). Our study is unique in that we examined species-specific decomposition rates within mixtures. The acceleration in decomposition in 2004 due to the presence of grass was primarily due to an increase in the decomposition of the species associated with the grass, rather than any change in the decomposition of the grass itself. If one uses monoculture decomposition rate as an index of litter quality, the grass litter was of higher quality than either of the associated species, and thus these results support Seastedt’s (1984) hypothesis that high quality litter could be expected to increase the decomposition rate of associated litter. Numerous mechanisms have been proposed for such an effect including passive and active (by fungi) translocation of nutrients between litter types (McTiernan et al. 1997), altering microenvironment characteristics such as water retention within the litter layer (Wardle et al. 2003), and increases in habitat heterogeneity for decomposers (Hättenschwiler et al. 2005).

Negative non-additive effects have been reported much less frequently than positive effects; of the 162 mixtures from approximately 30 studies reviewed by Hättenschwiler et al. (2005), only 20% reported negative non-additive effects compared with 50% reporting positive effects. In our study in 2006 all effects were negative and decomposition of the mixtures was always slower than expected. The direction of non-additive effects, when present, in litter mixing studies have been reported to vary with time (Moore & Fairweather 2006) and litter composition (Wardle et al. 1997; Wardle et al. 2002).
In addition to the change in direction of the non-additive mixing effects, the species responsible for the effects also changed. Graminoids were central to the positive effects in 2004, but forbs were central to the negative effects in 2006. All mixtures that contained forbs, and only mixtures that contained forbs decomposed slower than expected. Although effects of graminoids on mixtures in 2004 were due to a change in the decomposition rate of associated species, such clear species-specific patterns were not present in 2006. Again using monoculture decomposition rates as an index of litter quality, the forb has the poorest quality. These negative non-additive effects support the corollary of the Seastedt (1984) hypothesis, i.e., poor quality litter decreases the decomposition rates of mixtures. Mechanisms proposed for such an effect include high amounts of secondary compounds, such as phenolics, in one of the litters (Hoorens et al. 2003), or the increased heterogeneity of litter mixtures may prevent the establishment of the subset of decomposers that do best on each litter type individually (Smith & Bradford 2003). In 2004, litter composed of the highest quality species produced positive non-additive effects, and in 2006, litter composed of the lowest quality species produced negative non-additive effects.

We do not know the reasons why both the direction of, and the species responsible for, the non-additive effects of litter composition differed between 2004 and 2006. We believe this to be a real effect because our sample sizes were large and our p-values extremely small. Of the seven studies we discuss in the introduction that examined effects of richness or composition on litter decomposition, only Scherer-Lorenzen (2008) repeated the test in more than 1 year, and none repeated their test of the effects of litter composition on short-term decomposition rates. Thus, we don’t know if such a change in the direction of effects between years is an unexpected or unusual result. Environmental context has previously been reported to affect not only decomposition rate, but also the direction of non-additive effects (Jonsson & Wardle 2008), and differences in environmental conditions between the two years may have resulted in the switch in the direction of non-additive effects. In a related study at these sites,
we measured numerous ecosystem properties in each of the plots, including plant biomass, soil moisture, light interception, and 14 different soil nutrients, and although there are small differences in these variables between years (higher soil moisture, Fe and Zn and lower Mn, B, S, and Al in 2004 compared with 2006) none of these variables intuitively relate to differences in decomposition between the two years (Chapter 2). Although the importance of plant functional group identity in determining the effects of litter mixing is evident, we can only speculate as to what factor(s) caused the switch in the direction of non-additive effects.

Few previous studies have examined the influence of plant species composition on decomposition through both mechanisms, i.e. changes in the decomposition microenvironment and changes in the composition of the decomposing material (e.g. Hector et al. 2000; Knops et al. 2001; Hobbie et al. 2006), and fewer had designs enabling them to examine the interaction between these variables. Our experimental design, placing a replicate of each species mixture in all environments, allowed us to examine these interactions and is one of only four studies we know of to do so (Jonsson & Wardle 2008; Milcu et al. 2008; Vivanco & Austin 2008). Litter mixing effects did not depend on the identity of living plants present in the community. Two of the previous studies reported significant interactions between the two mechanisms, with legume decomposition increasing with increasing diversity (Milcu et al. 2008) and affinity effects, enhanced decomposition of the species found in the living plant community (Vivanco & Austin 2008). Affinity effects, or home advantage, have also been reported by Ayres (2009) for three tree species. We were surprised by the lack of interaction in our study as we hypothesized that if there were strong independent effects of both removals and substrate composition, which we found, then an interaction would also occur. For example, we predicted affinity effects, or that ecosystems without graminoids in their biota would decompose grass leaf material more slowly than ecosystems containing graminoids. Certain groups of decomposer organisms may be primarily responsible for the decay of particular plant functional groups (Wardle et al. 2006), and the decomposers in an ecosystem
may be adapted to the litter present (Hunt et al. 1988). However, distances between our treatment plots were not large (minimum 0.5m) and decomposer fauna may quite easily disperse across these distances. Additionally, numerous decomposer fauna are also considered to be generalists in both habitat and feeding preferences (Wardle et al. 2006), so all plots are likely to contain the decomposers necessary for decomposing each litter type.

Although, we did not detect direct interactions between the effects of decomposition microenvironment and the composition of the decomposing material, we did find indirect interactions between them, which, although less predictable, may be just as important. When we consider multiple-mechanism effects on the same ecosystem property, we have shown that the sum of these effects may produce unexpected increased effects. For example, the loss of graminoids from this ecosystem caused decreases in decomposition both through changes in the decomposition environment, and through the loss of the positive effects of graminoids in litter mixtures. Thus, the effects of losing graminoids from this community would be greater than we might predict based on either mechanism alone. In contrast, the presence of forbs in the living community also had positive effects on decomposition through changes in the microenvironment, but their presence in litter mixtures slowed decomposition, and consequently the effects of losing forbs may be less than we might predict based on each mechanism independently. When considering multiple mechanisms, the effects of species loss may be additive, as in the case of the graminoids, or even change from positive or negative to neutral, as in the forbs, despite a lack of direct interactions between the mechanisms.

In conclusion, biodiversity ecosystem functioning studies have been primarily focused on the effects of losing plant diversity on plant productivity. Responses to biodiversity loss will vary for different ecosystems, different ecosystem processes and different compartments in the same ecosystem (Hooper et al. 2005), and as a consequence there has recently been an increase in the number of studies that examine ecosystem functions other than productivity. We have shown that not only is it important to
investigate multiple ecosystem processes, but it is important to consider the variety of mechanisms through which biodiversity may affect a single ecosystem process, and to consider these mechanisms in multiple years. Interactions between these different mechanisms may produce results greater than those predicted by any single pathway alone and therefore examining the different pathways through which species loss may affect ecosystem properties is essential for predicting future consequences of biodiversity loss.
References


CHAPTER SIX

Conclusions

Summary of Thesis
I used a functional group removal experiment to examine the importance of plant functional group identity in influencing ecosystem properties. I conducted these removals in multiple fertilizer and fungicide environments to determine whether the influence of functional groups on ecosystem properties was context dependent.

Chapter 2 discusses the effects of functional group identity on both biomass compensation and soil properties after 4 years of treatments and whether the mass ratio theory could be used to predict the effects of removals. I showed that graminoids have the largest influence on ecosystem properties in this community, despite not making up the dominant proportion of the biomass. Biomass compensation was influenced by the identity of the functional group removed and also the identity of those species remaining; the effect of graminoid removal was particularly severe as none of the remaining functional groups compensated for their loss. Light interception, soil moisture and soil nutrients were all largely determined by the presence of grasses in the plant community, and surprisingly, legumes had little effect on any ecosystem property. Finally, I found that these effects of functional group identity were not context-dependent, and were consistent across the multiple environments under which the experiments were conducted. The results do not support the mass ratio theory, and that plant identity, more so than dominance, determines the influence over ecosystem properties.

Chapter 3 discusses the longer-term (7 years) effects of plant functional group removals on biomass compensation, and responses of both functional groups and plant species to removals. My results on biomass compensation contrast with the shorter-term effects reported in the previous chapter. There was full biomass compensation for both graminoid and legume removal after 5 years,
although forb removal still only resulted in partial compensation. The dominant species in each functional group showed little response to removals, and the functional group responses were driven primarily by the subdominant species. Again, the majority of these effects showed no context dependence.

Chapter 4 discusses the effect of plant functional group identity on both the short- and long-term decomposition of leaf and root tissue. Graminoid removals had strong effects on leaf decomposition, making this one of only a few studies to find plant-identity driven environmental control over decomposition, and in combination with the previous 2 chapters, emphasizes the importance of graminoids in this system. This was the first study to look for plant-driven environmental control over root decomposition, and in contrast with the above-ground effects, showed no effect on decomposition of roots. Once again, these effects were consistent regardless of the different fertilization and fungicide treatments.

Chapter 5 discusses the effect of plant functional group identity on decomposition through two mechanisms: effects on the decomposition microenvironment, and effects on the composition of the litter. The experimental design made this one of only a few recent studies that was able to test for an interaction between these two mechanisms. I found that graminoid removal affected decomposition through both mechanisms independently, and that indirect interactions between these mechanisms had the potential to amplify the effects of graminoid loss. Consequently, this shows that it is not only important to examine the effects of species loss on multiple ecosystem functions, but also to consider effects on a single ecosystem function through multiple mechanisms in order the accurately predict the effects of biodiversity declines.
Lessons Learned From This Functional Group Removal Experiment

Were the Methods Appropriate?

Defining the functional groups

In Chapter 3 I examined the response of plant species to the removal treatments, and to the environmental treatments independent of their functional groups. Not all species within a functional group responded to treatments in the same way. For example, for both the forb and graminoid functional groups, the dominant species did not respond to removal treatments whereas the subdominant species showed a positive response to removals in both cases. The lack of a consistent response among species within functional groups questions whether the functional groups, defined and chosen a priori, were representative of the species in this community.

I propose that this is an illustration of the differences between functional effect groups and functional response groups (sensu Diaz & Cabido 2001). Functional effect groups are a group of plant species that have the same effect on ecosystem processes whereas a functional response group is a group of plant species that responds to the abiotic and biotic environment in similar ways (Diaz & Cabido 2001). As I was interested in the effect of functional group identity on ecosystem processes, my grouping of species into functional groups for the removal treatments were intended to be functional effect groups.

The functional groups were chosen a priori based on traits that I believed would be critical for the ecosystem processes of interest, with traits including the C:N, stature and N-fixation ability of the species. The functional groups I chose for these experiments paralleled the functional effect groups used in many other experiments, including both those that had done a priori (Hooper 1998; Wardle et al. 1999; De Deyn et al. 2009) and post-hoc (Chapin et al. 1996; Craine et al. 2001; Reich et al. 2001b) methods for grouping species. Although I did not verify my species groupings by examining the
independent effect of each species on ecosystem properties, I am confident that the groupings represent true functional effect groups.

The groupings of species indicated by the responses of the different species to removals and environmental treatments in Chapter 3, in contrast, would be functional response groups. Although functional effect groups and functional response groups may often be very similar, they may also be separate groupings of species, depending on both the traits used to define the group and the environmental condition of interest (Diaz & Cabido 2001). There may be little overlap in the traits used to define these two groups. Traits used to define a response group are those that determine the potential of that group to increase or decrease in abundance under particular conditions, and are likely to include traits representing the reproductive or growth (in the case of perennial plants) strategies. Functional effect groups, on the other hand, would be defined by traits more likely to have an effect on their surrounding environment, including their C:N ratio and specific leaf area.

The importance of compensating for biomass removal in removal experiments

One of the main drawbacks of removal experiments is the concern that observed effects will be due to the act of the removal itself, rather than the identity of the removed group. The removal treatment removes biomass and nutrients, alters the spatial structure of the community, and causes disturbance in the litter layer and the soil (Diaz & Chapin 2000). The physical disturbance of the removal itself may initially predominate over the effects of the absence of the removed plants, particularly in established communities (Diaz et al. 2003). I tried to minimize this disturbance by precisely painting herbicide onto the leaves of the target group and then clipping these plants at the soil surface (Chapters 2-5). Although above-ground physical disturbance may be minimized in this instance, the roots of the plants remain in place to decompose, which may result in a pulse of nutrients to the soil, as discussed in Chapter 1. These roots often account for a small amount of the organic matter in the soil, especially in northern ecosystems (Hobbie 1992) and this removal technique seemed like the least invasive method. Other
removal experiments have controlled for the disturbance effect by creating plots that were subjected to “a mild physical disturbance simulating the effects of removal” (Bret-Harte *et al*. 2008) or by simply tugging on plants in control plots without removals (Hobbie *et al*. 1999).

Perhaps a more substantial concern is that the ecosystem effect caused by a removal treatment may be simply a function of the amount of biomass removed, rather than the identity of the removed group (Diaz *et al*. 2003). Removal experiments have dealt with this concern in different ways. In some experiments, the amount of biomass removed is not a problem when, for example, all removal treatments have the same amount of biomass removed (Smith & Knapp 2003; Suding *et al*. 2006), or, in cases where all plants were initially removed from the plots and treatments were imposed on re-growing seedlings alone (Wardle *et al*. 1999). In a number of studies where different removal treatments did remove differing amounts of biomass, the issue was not discussed or dealt with in the papers directly (Shevtsova *et al*. 1997; Aksenova *et al*. 1998; Smith *et al*. 1999; Buonopane *et al*. 2005; Klanderud 2005).

A direct approach is to include a biomass removal control in the experimental design, and to randomly remove biomass from plots at levels equivalent to that removed by the various removal treatments. This approach has been used by Symstad (2000) and Symstad and Tilman (2001) in a temperate grassland, O’Conner and Crowe (2005) with grazing gastropods, and Lyons and Schwartz (2001) in an old-field. This approach is desirable because it creates a direct control for both the amount of biomass removed and the disturbance caused by the removal itself, but it substantially adds to the number of replicates required to perform the experiment. In my experiment, this approach would have nearly doubled the number of plots, to a size that would have been unmanageable.

An alternative is a *post-hoc* approach where the differing levels of biomass between the removal treatments is accounted for statistically after the experiment. In a few studies the amount of removed biomass in the various treatments was used as a covariate in the final analysis (Herben *et al*. 1997;
Wardle & Zackrisson 2005). Wardle and Zackrisson (2005) tested the extent to which results obtained at the end of the experiment were a reflection of the amount of vegetation removed at the beginning by using the biomass of the removed vegetation as a covariate. They found that the covariate was rarely significant and attributed their removal results to identity, not biomass, of the removed group (Wardle & Zackrisson 2005). With this method, however, the covariate is being used as a correction for different values of biomass removed in the different treatments, which breaks the assumption of an ANCOVA that the covariate has the same distribution for all groups (Quinn & Keough 2002). Bret-Harte et al. (2004) acknowledged that because their biomass was not randomly distributed it could not be used as a covariate, and instead used a multiple regression technique to account for differences in biomass removed. They regressed their response variables (such as nutrient availability) against the amount of biomass removed, including their removal treatments as categorical factors in the analysis (Bret-Harte et al. 2004). After generating models with all possible combinations of these factors, they used AIC to select the models that best predicted their response variables, and the selection of these models indicated that the biomass removed was not the most important factor in the prediction (Bret-Harte et al. 2004).

Rather than attempt to account for biomass removal statistically, I chose to do a more qualitative comparison of the effects of the functional groups, as done by Wardle et al. (2008). I accounted for the different amounts of biomass removal by comparing the ranking of the biomass removal treatments to the ranking of the effects of the removals, especially in our consideration of the mass ratio theory (Grime 1998) in Chapter 2. Further, as I was interested in the role of plant functional group identity in influencing ecosystem function, the dominance of a particular species or group of species in a community may be considered a ‘trait’ of that species, and consequently is an important component of its effects on an ecosystem.
Plant Functional Group Identity is Important

I detected effects of plant functional group identity on nearly every ecosystem property examined. In particular, the role of graminoids in determining these ecosystem properties was especially influential. The effect of removing graminoids and forbs was similar on both soil moisture and soil nutrients, but because of their differences in abundance, graminoids played a proportionally greater influence on these ecosystem properties. Graminoids were especially important in determining decomposition processes. They promoted decomposition both through plant-identity driven effects on decomposition micro-environment, and also promoted the decomposition rates of other species when included in litter mixtures. Graminoids were also the first group to respond to removals by increasing in abundance, whereas other groups showed substantial delays before exhibiting compensatory growth for vegetation removal.

I was surprised by the strong role of graminoids, especially their influence over such a broad range of ecosystem properties. Although I refer to this ecosystem as a ‘northern grassland’, grasses are not the dominant species (measured as proportional biomass) in this plant community. As discussed in Chapter 2, the mass ratio theory would predict that ecosystem functioning would primarily be determined by forbs, the dominant functional group in this ecosystem (Grime 1998). I attributed differences in light interception to the stature of the grasses (Chapter 2), and previous studies have attributed the greater effects of grasses on soil moisture to their large root mass (Köchy & Wilson 2000), which may also determine their influence on soil nutrients. I partially attributed their effects on decomposition to their lack of anti-herbivore compounds in comparison with the other dominant plants (Chapter 5). Although I suspect different traits are responsible for the graminoid functional group’s effects on the different ecosystem properties, the graminoids appear to have a suite of traits that make them the primary determinants of many ecosystem properties.
I also expected that legumes would have had a greater influence on ecosystem properties than they did, especially those linked to soil nutrients. The presence of legumes in experimental plant communities has been a contentious part of the history of biodiversity-ecosystem function research (e.g. Huston et al. 2000). The sampling effect is sometimes attributed to the higher likelihood of legumes being present in higher diversity plots (Huston et al. 2000; Mulder et al. 2002) and legumes are often planted at higher than natural abundances in experimental communities (Diaz et al. 2003). Regardless, legumes are often found to have a large effect on ecosystem properties in these experiments (Hooper & Vitousek 1998; Spehn et al. 2002; Scherer-Lorenzen et al. 2003), leading to a set of BDEF experiments being done explicitly without legumes (van Ruijven & Berendse 2003, 2009). The lack of effects caused by legume removal in this community with naturally (low) abundance of legumes supports the mass ratio hypothesis, but is contrary to earlier evidence of the importance of legumes in plant communities.

**Context Dependence is Less Important**

One of the primary goals of this research was to determine whether the role of functional group identity in determining ecosystem properties was context-dependent. I tested this using two types of environmental change: increased nutrient supply (fertilizer) and suppressed mycorrhizal colonization (fungicide). As both species composition and environmental conditions are likely to change simultaneously, if the effects of plant identity on ecosystem properties do show context dependence, knowing their effects in a single environment would provide little predictive power for more natural scenarios.

I found little evidence of any context-dependence among the variety of ecosystem properties I examined. There was no context-dependence on either environmental treatment for light interception, soil moisture, and most soil nutrients (Chapter 2) as well as the variety of decomposition measures I examined (Chapter 4 and 5). The importance of context-dependence in an ecosystem could be
dependent on a variety of factors. It may be dependent on the type of community examined. While experiments in artificial communities generally show context dependency of species richness on ecosystem functioning (Reich et al. 2001a; Fridley 2002; Craine et al. 2003; Reich et al. 2004; Dijkstra et al. 2007; De Deyn et al. 2009), the presence of context dependency in removal experiments in natural environments is more mixed. Although the effect of removals in some experiments are context dependent (Shevtsova et al. 1997; Klanderud 2005; Wardle et al. 2008), other removal experiments show no interactive effects (Hobbie et al. 1999; Wardle & Zackrisson 2005; Manninen et al. 2009). The importance of context dependence may depend on the response variable examined, as displayed by our context dependence for species, but not functional group biomass. Finally, it may be dependent on the environmental context chosen. For example, I did not detect any context dependence on mycorrhizal abundance, and found few direct effects of mycorrhizae, indicating that mycorrhiza may simply not play an important role in the plant-soil relationship in this system, despite their abundance.

**It’s Important To Do These Experiments Long-term**

One of the key challenges to studying natural ecosystems is that they show natural variability through time. Long term experiments following changes in species composition show that species abundances are still changing after 10 years at Cedar Creek (Fargione et al. 2007), after 20 years in the boreal forest at Kluane (Turkington et al. 2002; Turkington unpublished data) and after 100 years in the Park Grass experiments at Rothamsted (Silvertown et al. 2006). Conducting ecological experiments in the context of these fluctuations provides challenges. A common conclusion in ecological studies is that results may have been different or more pronounced if the experiments had run over a longer time period (e.g. Hector et al. 2000; Vivanco & Austin 2008; Ayres et al. 2009) especially in systems such as arctic tundra with longer-lived plants (Hobbie et al. 1999; Bret-Harte et al. 2004).

For example, decomposition processes are rarely examined over the long-term. The litter decomposition study described in Chapter 4 is one of the few examinations of long-term decomposition
processes. I chose to examine both short- and long-term decomposition because decomposition rates in northern ecosystems are very slow, but also because the mechanisms behind short-and long-term decomposition patterns were likely to differ. For example, long-term decomposition has been predicted to be less influenced by differences in environmental conditions (Harmon et al. 2009) and may therefore be less influenced by plant-driven environmental changes. Little is known about long-term decomposition patterns, however, because most decomposition studies follow the litter for only 1 to 2 years (Aerts et al. 2006; Harmon et al. 2009). I found little support for predicted differences in short- and long-term decomposition patterns. Both types of decomposition responded to plant removals in the same way, with the removal of grasses consistently decreasing above-ground decomposition across all time scales.

As the discipline of biodiversity-ecosystem functioning research matures, the importance of long-term research in this area also becomes evident. Much of the earlier evidence came from short-term experiments in synthetic communities and extrapolation of the conclusions from these experiments to longer time scales and more natural ecosystems was difficult (Symstad et al. 2003). Now that some manipulative BDEF experiments have been running for close to a decade, some of the earlier conclusions on the importance of biodiversity are becoming stronger. Overyielding responses to biodiversity became more frequent with time in an 8 year experiment in a serpentine grassland (Hooper & Dukes 2004). The mechanism driving the BDEF relationship has been reported to shift from sampling effects to complementarity effects with time both in Cedar Creek after 7 years (Tilman et al. 2001) and 10 years (Fargione et al. 2007) and in another 8-year grassland experiment without legumes (van Ruijven & Berendse 2009). In a recent review of biodiversity-ecosystem functioning studies, Duffy (2009) concludes that some of the criticisms of BDEF studies, such as the prevalence of the sampling effect, may be because the experiments have generally not run for enough time. Finally, Cardinale et al.
reported in their meta-analysis a general trend of increases in overyielding and transgressive overyielding through time in BDEF experiments.

In the first four years of this experiment, removal effects on ecosystem properties were seldom dependent on length of time since the experimental communities were established. Effects of removals on neither light interception nor soil nutrients varied with time. Effects of removals on soil moisture did vary with year, but there was no consistent trend in soil moisture as the age of the experiment increased. In contrast, I found large differences in biomass compensation by the different functional groups in our short- and long-term measurements. By the fourth year of the experiment there was still no evidence of compensatory growth for graminoid removal and I predicted, based on these short-term results, that full biomass compensation in graminoid removal plots was unlikely (Chapter 2). My conclusions changed, however, after the mid-term (7 years) analysis, as there had been full biomass compensation for graminoid removal after 5 years (Chapter 3). Forbs had responded slowly to removal treatments, resulting in this delay in biomass compensation, and the differing conclusions on the two time scales in the experiment.

**Future Implications**

One of the most important conclusions from this research is that the influence of functional groups on ecosystem properties cannot be predicted based on their relative abundance in the community; the species’ identity must also be considered. More specifically, in this ecosystem the graminoids had more influence on ecosystem properties than I would have predicted based on their abundance alone. That the graminoids have a suite of traits that make them important in determining ecosystem properties is of particular concern because of their predicted responses to global change. Future change in arctic vegetation is likely to be driven by increased nutrient availability (Dormann & Woodin 2002), and graminoids are known to increase in abundance with fertilization both in general (Jonasson 1992;
Theodose & Bowman 1997; Dormann & Woodin 2002; Gough & Hobbie 2003) and in our region (Turkington et al. 2002).

Thus, in this northern grassland, the graminoids are both more likely to be at increased abundances in future communities but have also proved to be functionally important in these plant communities. This link highlights one of the likely future focuses of biodiversity-ecosystem functioning research; examining the correspondence between the traits that influence ecosystem functioning and the traits that influence the risk of extinction or decline in response to environmental drivers (Duffy 2009). Focusing on these two sets of traits and the covariance between them is one way to make biodiversity-ecosystem functioning research both more realistic and applicable to conservation scenarios (Gross & Cardinale 2005). In this northern grassland, knowing that graminoids are likely to increase in future climates, and that they play a particularly important role in determining the processes in this ecosystem suggests that the impact of future climate change in the arctic is going to be closely tied to this plant functional group.
References


