EFFECTS OF PLANT FUNCTIONAL GROUP REMOVAL ON THE SOIL MICROBIAL COMMUNITY DIVERSITY AND COMPOSITION

by

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ABSTRACT

A major objective of biodiversity-ecosystem functioning (BDEF) research is to determine the consequences of species loss, caused both naturally and anthropogenically, on the functioning of ecosystems. The impact of plant species loss on the soil microbial community has not received much attention even though soil microbes influence many important ecosystem functions such as decomposition and nutrient cycling. The objective of this research was to investigate how the functional group composition of the aboveground plant community influenced the belowground microbial community. Plant functional groups (graminoids, legumes and non-leguminous forbs) were removed from a northern grassland system in the Yukon Territory, Canada. One metre square plots had one of the three functional groups removed or left intact as a control and this was crossed with a fertilizer treatment and a fungicide treatment that targeted mycorrhizal fungi. After five seasons (2003-07) of implementing treatments the soil microbial community was analyzed using substrate-induced respiration (SIR, a measure of metabolic diversity) and phospholipid fatty acid analysis (PLFA, a measure of community composition). Plant functional group removal had almost no effect on the soil microbial community. The only response detected was an increase in stress (indicated by the PLFA stress ratio of cy19:0 to 18:1ω7c) which occurred when legumes were removed and fertilizer was not added, indicating that legumes had a positive effect on the nutrient status of microbes. Likewise, soil properties (total carbon, pH, moisture and nutrients) showed limited response to plant removals. Fertilization decreased the metabolic diversity of the soil microbial community. We detected no soil microbial or plant biomass response to the fungicide indicating that mycorrhizae had little influence in this system. Based on the low-productivity of the grassland, and the lack of response in both the soil properties and the microbial community, we hypothesize that the main

determinants of the microbial community may be litter input. When litter decomposition rates are slow, such as in this northern system, five growing seasons may not be sufficient to detect the impact of a changing plant community on the soil microbes.

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CO-AUTHORSHIP STATEMENT

Chapter 2 was co-authored by Roy Turkington and Jennie McLaren. The main experimental design was created by Jennie who also carried out the experiment for the first four seasons. I carried out the experiment in the fifth season, decided on the microbial sampling methods and performed all laboratory analysis. I analyzed all data in this manuscript and wrote all drafts. Roy gave critical feedback and advice on the content and organization of the manuscript and assisted with revisions.

1 INTRODUCTION

High biodiversity is often viewed as a sign of a healthy ecosystem, and ia therefore desirable. Because of this, the current increasing rate of species extinctions is causing concern in both the scientific and the public communities. Human alterations to ecosystems and increased instances of invasive species have eliminated many native species or drastically changed their abundance (Chapin III *et al*. 2000, Olden *et al*. 2004, Hooper *et al*. 2005). As of April 2007 there were 361 species in Canada listed as endangered or threatened, of which about 50% are vascular plants, with 136 more species listed as of special concern (COSEWIC 2007). Much research has examined the consequences of decreased diversity on ecosystems (see reviews by Hooper *et al*. 2005 Balvanera *et al*. 2006, Cardinale *et al*. 2006,) but there is an on-going debate over the interpretation of the results and conclusions (Mooney 2002); many aspects of the influence of species loss on ecosystem function are poorly understood. Studies investigating how future species loss will affect complex ecosystems provide valuable information regarding what direction management should take in order to maintain a sustainable planet.

1.1 Biodiversity-Ecosystem Functioning

The need for information on the effects of species loss on ecosystems is one of the reasons behind the increase in Biodiversity-ecosystem functioning (BDEF) research. BDEF research investigates the central question as to how a change in biodiversity will affect ecosystem functions (Tilman 2000, Srivastava and Vellend 2005, Balvanera *et al.* 2006). The definition of an ecosystem function varies depending on the researcher. Here

we will refer to an ecosystem function as any property of an ecosystem that is influenced by the biota of that system (Naeem *et al*. 2002). These can include broad processes such as productivity, decomposition, nutrient cycling, and stability from disturbance, and more specific properties such as nitrogen leaching or supporting pollinators (Costanza *et al*. 1998, de Groot *et al*. 2002, Srivastava and Vellend 2005). Some of these functions have been directly linked to 'ecosystem services' or the resources and benefits gained by humans, such as water purification, materials for manufacturing and even aesthetic pleasure (de Groot *et al*. 2002).

Two important meta-analyses of BDEF research agree that there is generally a positive effect of biodiversity on ecosystem functioning (Balvanera *et al*. 2006, Cardinale *et al*. 2006) and this applies across different trophic groups (Cardinale *et al*. 2006). There is a lot of variation, however, in results and Balvanera *et al*. (2006) demonstrated that the positive relationship between biodiversity and ecosystem function was weaker in natural system experiments than in greenhouse experiments, and highly-studied systems (grasslands, freshwater, marine and forests) showed weaker relationships than more poorly-studied systems (salt marshes, bacterial, and crop systems).

Several hypotheses have been proposed to describe the potential relationship between biodiversity and ecosystem functioning (Naeem *et al*. 2002). First, the "redundancy hypothesis" states that some species perform similar roles in an ecosystem and losing a few has no significant impact on function. Second, the "singular hypothesis" considers the role of each species to be unique, causing the loss of each species to result in a decrease in ecosystem function. There are variations of these two hypotheses, for example if considering singularity of species functional groups, redundancy with functional groups is also being considered. Third the "idiosyncratic hypothesis" considers the result of species loss to be unpredictable because the effect of losing a species depends on the identity of the species and the conditions of the system,

such as nutrient availability or co-occurring species. Naeem *et al*. (2002) point out that idiosyncrasy is not the same as no effect. The loss of species does have an effect on the system, but the magnitude and direction of this effect is unpredictable. This is of great concern to ecological managers because losing a species could have drastic impacts on a system but there is no way of predicting which species are high impact, and thereby managing to prevent their loss.

One of the first studies to test for the patterns predicted by these hypotheses was Naeem *et al.*'s (1994) microcosm experiment, which manipulated diversity within 4 trophic levels. Almost all ecosystem functions measured were affected by diversity level: carbon dioxide use and plant productivity increased with diversity while other ecosystem functions responded idiosyncratically to diversity, such as short-term decomposition and nutrient retention. These results support more than one hypothesis in the same system depending on the processes measured (Naeem *et al.* 1994). Balvanera *et al*. (2006) found mostly idiosyncratic relationships in their meta-analysis of BDEF studies, while the meta analysis by Cardinale *et al.* (2006) found the relationship between diversity and ecosystem function to follow the redundancy hypothesis; increases in function decreased once low levels of diversity were reached. However, the Cardinale *et al.* (2006) study only used experiments measuring biomass and resource depletion as ecosystem functions while Naeem *et al*. (1994) and Balvanera *et al.* (2005)used more varied ecosystem functions.

Two main mechanisms have been proposed to explain how diversity impacts ecosystem functions; niche complementarity and the sampling effect. Niche complementarity occurs when species interact to be more productive in polyculture than any one species grown in monoculture (Huston and McBride 2002). This could occur as the result of more efficient resource uptake when species have non-overlapping niche space (Srivastava and Vellend 2005), such as utilizing different forms of nitrogen, or by

facilitation when one species creates a favourable environment for another (Hooper *et al*. 2005). With the sampling effect, it is argued that an increase in productivity may be due to the inclusion of one particular species without synergistic effects of other species (Huston and McBride 2002, Hooper *et al*. 2005). This species could have a larger impact on community productivity due to being a superior competitor and dominating the community (Tilman 1999) or by having a disproportionate effect on the community through certain traits, such as nitrogen fixation (Stephan *et al.* 2000). The more species in a mixture, the more likely such a species, with a strong effect on ecosystem functioning, will be included in the mixture. In Cardinale *et al.*'s (2006) meta-analysis of BDEF studies positive effects on biomass were consistent with the sampling effect. However, in a later analysis of plant diversity studies, Cardinale *et al.* (2007) found complementarity played just as large a role as the sampling effect and that the effect of complementarity increases over time and may not be evident in experiments which run for less than two growing seasons. These two mechanisms are not mutually exclusive and can operate simultaneously in the same ecosystem (Hooper *et al*. 2005, Cardinale *et al.* 2007). Loreau and Hector (2001) have developed a methodology to separate the effect of complementarity and the sampling effect and determine their relative strengths, called the additive partitioning method.

1.2 Measures of Biodiversity

The majority of BDEF studies consider only species richness (Díaz and Cabido 2001, Schmid *et al*. 2002), although there are many components to diversity, such as genetic diversity, species composition, and species relative abundance. The choice of diversity measure could impact the conclusions drawn about a particular system and is an important aspect of experimental design. In addition, even in a relatively species poor system the number of species can make investigating the influence of each species with

sufficient replication logistically unreasonable. In such a case, a measure of diversity that can simplify experimental design is needed.

Some research focus has shifted from the more traditional measures of species diversity to functional group diversity and composition (Tilman *et al*. 1997, Díaz and Cabido 2001, Petchey and Gaston 2006). Functional groups are groups of species that have similar responses to the environment (functional response groups) or similar effects on the ecosystem (functional effect groups), regardless of relatedness (Díaz and Cabido 2001, Petchey and Gaston 2006). This implies that removal of one species from a functional group could have negligible effects on the ecosystem because other species in the same functional group can fill its role (i.e. redundancy). Research has shown that functional group composition can have significant impacts on ecosystem processes. In a review of 26 studies that investigated the effect of species richness and functional diversity (richness and composition) on various measures of ecosystem function, only two reported an effect of species richness when there was no effect of functional composition, and in sixteen cases effects of functional composition were reported while effects of species richness and functional richness were not (Díaz and Cabido 2001). For example, when species and functional diversity were manipulated in a grassland ecosystem functions were not affected by changes in species diversity, but functional composition affected plant productivity, plant total N, soil NO_3 and NH_4, plant percent N and light penetration (Tilman *et al*. 1997).

1.3 Importance of the Belowground Community

While there have been a relatively large number of studies focusing on the effects of biodiversity loss on ecosystem functioning, very little of this research has investigated the effects of plant species loss on the functioning of the belowground system. In a review of 103 papers that included 446 ecosystem-property measurements,

only 6% (28) of the measurements included effects of primary producers on the belowground community (Balvanera *et al*. 2006).This lack of research effort makes it difficult to determine if higher plant diversity results in higher soil microbial diversity and what influence that soil microbial diversity has on the ecosystem. Investigating the effects on the microbial community is important, because of microbial influences on many important ecosystem properties such as decomposition, nitrogen cycling and carbon storage (Zhu and Miller 2003, O'Donnell *et al*. 2005, Wardle 2005). The importance of the soil microbiota in ecosystems is a relatively new concept for many ecologists and it remains understudied (Naeem *et al*. 2002, Wall *et al.* 2005), but is gaining attention.

Estimates of soil microbial diversity are staggering, with one of the first soil bacteria DNA studies giving 1.5×10^{10} bacteria per gram of soil from about 4000 different genomes (Torsvik *et al*. 1990); of these not even 5% are properly described (Wall *et al*. 2005). This can be quite daunting for researchers, because many of the ecological properties traditionally investigated aboveground can be difficult or almost impossible to study belowground. Interactions between soil organisms, food web position, and indirect interactions are difficult to determine from DNA sequences or other molecular methods that make up a large portion of our belowground species knowledge (Wall *et al*. 2005).

While redundancy in microbial function may be expected among such high species numbers, certain processes may be limited to a small fraction of the soil community. Schimel *et al*. (2005) classify microbial processes as broad or narrow. Broad processes, such as glycolysis, are performed by a wide variety of microbes and this is where redundancy in function would most likely be found; losing some organisms that perform these processes is not likely to have a large effect on the system. Narrow processes, such as nitrification or methanogenesis, are only performed by specific

microbes (O'Donnell *et al*. 2005, Schimel *et al*. 2005). For example, white-rot fungi is the only organism capable of decomposing lignin (Prescott 2005). Even different communities carrying out the same narrow process may not be completely redundant; two different communities of denitrifying bacteria taken from fields with differing management practices were reported to have different denitrification rates when exposed to the same environmental conditions (Cavigelli and Robertson 2000). However, one study that manipulated bacterial diversity in microcosms found no increase in decomposition rates or the number of consumers in a soil food web suggesting redundancy, but there were also idiosyncratic effects based on composition of bacteria in each diversity level (Jiang 2007). This suggests that changes in soil community structure have the potential to have significant impacts on ecosystem functioning and are important to consider in biodiversity studies.

In past BDEF studies, choice of methods has been shown to influence the result of biodiversity manipulations. Manipulating species diversity or manipulating functional group diversity can affect whether a response is detected (Díaz and Cabido 2001). The scale of experimental plots can alter results, due to edge effects (Groppe *et al*. 2001) or inclusion of landscape-scale environmental variation (Schmid *et al*. 2002). Choice of microbial community measure can equally influence results. There are many techniques for analyzing the microbial community and different techniques measure different things. For the present study, two methods were chosen that measure different aspects of the microbial community. Substrate-induced respiration (SIR) measures the active community and is a way of looking at the metabolic diversity of soil microbes (Campbell *et al*. 2003, Leckie 2005). Phospholipid fatty acid analysis (PLFA) measures the entire community, active and dormant, and gives a profile of community structure and allows for comparison of abundance of different groups of microbes, such as Gram positive and Gram negative bacteria (Leckie 2005). Using these two complimentary methods will give

a more complete picture of changes in the microbial community and one method may detect changes not picked up by the other. For instance, if diversity manipulations cause active microbes to become dormant this would not be detected by PLFA but would be reflected by a change in SIR.

1.4 Plant and Soil Community Interaction

The soil-plant-microbial system is a complex system with many positive and negative feedbacks between the components – different plant species can have differential impacts upon the soil microbial community, either directly or indirectly via the soil, and changes in the microbes can, in turn, feedback to the soil and to the plant community. Plants are in direct contact with the soil system and individual plant species can have varying effects on the soil microbial community. The litter that falls to the ground and organic compounds that plants add to the soil through root exudation is the basal energy source for the decomposer community. But different plant species produce litter of varying quality, differing amounts of carbon through root exudation, and compete at varying levels with soil biota for nutrients (Wardle 2002). For example, coniferous trees produce low-quality litter compared to deciduous trees, and coniferous forests tend to have lower soil microbial biomass than deciduous forests (Wardle 2002). Different assemblages of plants can therefore be expected to have different impacts on the belowground system.

Several studies have provided evidence that root exudation influences not only microbial biomass, but also the make-up of the soil microbial community. Grayston *et al*. (1998) added sucrose to soil and this resulted in a microbial community that was better at metabolizing sucrose, glucose and fructose compared to control soil. This indicates that compounds in the soil select for microbes able to metabolize those compounds. The authors also reported different carbon utilization patterns in soil grown under *Lolium*

perenne, Agrostis capillaris, Triticum aestivum and *Trifolium repens* (Grayston *et al*. 1998). These differences could be due to the varying composition of root exudates, although more studies are needed to determine if there is a relationship between what plants are putting into the soil and what carbon sources are utilized by the soil community. Other studies have also found evidence for different plant species supporting different soil microbial communities (Griffiths *et al.* 1992, Bever 1994) and there is evidence supporting that this is a result of differences in root exudation (Griffiths *et al*. 1992, Grayston *et al*. 1996).

Plant species also alter soil properties which could in turn affect the soil microbial community. Chen and Stark (2000) reported that soil with wheatgrass (*Agropyron desertorum*) was higher in nitrate and had a lower C:N ratio than soils with big sagebrush (*Artemisia tridentata*), which in turn could select for different groups of microbes. The difference was attributed to variation in substrate quality between plant species (Chen and Stark 2000). It has also been shown that plant species can alter soil pH. Reich *et al.* (2005) found that the calcium content of tree species litter significantly altered soil pH and this resulted in a change in the number and diversity of earthworms. As earthworms are ecosystem engineers, both of these effects of tree species litter have the potential to dramatically change the soil microbial community.

Changes in the environment could alter the effects plants have on the soil microbial community. In a glasshouse experiment, nitrate addition significantly increased the abundance of bacterial-feeding nematodes, which indicates higher numbers of bacteria (Griffiths *et al*. 1992), perhaps due to increased productivity of the plants. However, in a different glasshouse experiment nitrogen addition had no effect on the soil microbial community (Bardgett *et al*. 1999). Soil type has been shown to influence plant effects: Bezemer *et al.* (2006) reported plant species effects on soil microbial composition in chalk soil but not in sandy soil. Some environmental changes are long-

lasting. In a different study, past land management (abandoned for approximately 10 years) had a stronger effect on soil community structure than current plant community composition (Buckley and Schmidt 2001). Care should be taken when extrapolating plant species effects from one system to another, because the above studies show the importance of environment- and site-specific conditions.

The soil microbial community also influences the aboveground community by decomposing material and cycling nutrients back for reuse by plants. Feedbacks can be influenced by which plant species are present. When four different species were grown in soil previously occupied by either the same or different species, three species grew better in conspecific soil, while one species showed reduced growth (Bezemer *et al*. 2006). Two of the positive growth responses and the negative response correlated to the composition of the microbial phospholipid profiles, and not to soil chemical properties. This implies that certain plants select for particular soil microbial communities. A positive feedback could occur by a slow-growing plant producing low-quality litter that is not conducive to rapid decomposition, which would result in out-competing fast-growing plants requiring rapid nutrient cycling (Wardle 2005). Work by Bever (1994, 1997) provides evidence for important negative feedbacks between the soil community and plant species. Survival and growth rates were both lower for four plant species when grown in conspecific soil compared to soil from under the other three species (Bever 1994). This could occur through the actions of root pathogens, which can be very host specific (Bever 1994, van der Putten 2003).

1.5 Plant Diversity Effects on the Soil Community

If different plant species have varying affects on the soil biota, then different assemblages of plants will also be expected to have varying affects. This is the basis for the idea that more diverse assemblages of plants will provide a more diverse resource

base for soil biota and therefore support a more diverse soil microbial community (Hooper *et al*. 2005, Srivastava and Vellend 2005, Balvanera *et al*. 2006, Cardinale *et al*. 2006). Niche complementarity and the sampling effect are most often applied to ecosystem functions such as productivity of the aboveground community but they are also applicable to the belowground community. Multiple species of plants will input a wide range of root exudates into the soil and create a heterogeneous soil supporting a high diversity of soil organisms (i.e. niche complementarity). The sampling effect can be illustrated by one plant species inputting a large quantity of litter or root exudates into the soil compared to other species in the community. A community including this plant species would produce more resources for the soil community and could therefore support larger numbers of soil organisms, but not necessarily a higher diversity.

The general effects of diversity on ecosystem function were discussed earlier. Here I will focus on the effect of aboveground plant species diversity on the soil microbial community. As part of the European-wide BIODEPTH experiment, Stephan *et al*. (2000) reported that increasing grassland plant diversity from a monoculture to a 32-species polyculture caused an increase in metabolization rates of carbon sources by the soil microbes, showing either an increase in microbial biomass or an increase in microbial activity levels. The diversity of soil microbes was also increased as indicated by an increase in diversity of carbon sources metabolized. A large proportion of the variation in this study was explained by the presence of the legume *Trifolium repens* - illustrating the sampling effect (Stephan *et al*. 2000). In a similar field experiment, increasing diversity was shown to increase microbial respiration rates and biomass (as measured by PLFA amounts, Zak *et al*. 2003), due to an increased productivity of the aboveground system that could not be accounted for by any one species - evidence supporting niche complementarity. These results suggest an overall positive effect of plant diversity on the soil microbial community. Other studies have shown less consistent patterns and effects

can vary depending on the variable measured. In a microcosm experiment grassland plant diversity had no effect on soil microbial C biomass but significantly increased microbial N (Niklaus *et al*. 2006). The authors also measured more efficient nitrogen uptake at higher levels of species richness, mainly due to a strong effect by the legumes.

There have also been studies investigating the effects of functional groups on the soil biota. Removal of functional groups (C_3 grasses, C_4 grasses and dicots) in a grassland field experiment had idiosyncratic effects on the bacterial community (Wardle *et al*. 1999). However, removal of C_3 grasses had a disproportionate effect on various ecosystem measures, mostly due to *Lolium perenne*, which had a negative effect on total plant biomass, although this did not translate into negative effects on microbial biomass. A detrimental effect on the soil biota was found only when all plants were removed (Wardle *et al*. 1999). In another study of grassland species, Niklaus *et al.* (2006) reported no effect of any plant functional groups on microbial C or N, but the soil nitrate pool was significantly increased in the presence of legumes. In a glasshouse pot experiment the richness of functional groups (C_3 grass, C_4 grasses, legumes, and non-leguminous dicots) had idiosyncratic effects on different ecosystem properties (e.g. microbial biomass), but of those functions that were positively affected (e.g. biomass) there was evidence of niche complementarity (Wardle *et al*. 2000). Plant identities played a large role in this system, with certain combinations of plants performing better than others (Wardle *et al*. 2000). Environmental changes can influence the effects of plant functional groups on the soil microbial community. Potential nitrification of the soil microbial community was greater in soil of medium fertility compared to low-fertility soil, and legumes had a greater positive effect on potential nitrification in the low-fertility soil (Niklaus *et al*. 2006). The degree to which soil systems are affected by functional groups depends on the system, and again care must be taken when extrapolating results to other systems.

1.6 Conclusions

Research on the effects of aboveground plant diversity on the soil biota show extremely varied responses. The outcomes of losing plant species can change depending on the site, environmental conditions, response measured and plant species lost. This has made finding general patterns and making predictive models difficult. There is not likely to be a straight forward effect of aboveground plant diversity on the soil biota. Despite this, the importance and influence of the soil community on ecosystems demands that they are rigorously investigated in ecosystem studies.

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2. EFFECTS OF PLANT FUNCTIONAL GROUP REMOVAL ON THE SOIL MICROBIAL COMMUNITY[1]

2.1 Introduction

Studying the soil microbial community can be intimidating to traditional 'aboveground' ecologists. It is highly species diverse and it adds a complex and poorly understood aspect to ecosystem studies. However, the importance of soil organisms should not be ignored. Soil organisms control decomposition (Wardle *et al*. 1999), nutrient cycling (Cavigelli and Robertson 2000), and can influence many aboveground processes, such as plant community composition (Wardle 2005, Bezemer *et al*. 2006, van der Heijden *et al*. 2008). This is a two-way process where the nature of the above-ground vegetation also impacts below-ground processes. A major objective of the present study is to investigate how the functional group composition of the plant community influences the belowground microbial community.

Species loss has received much attention in both the scientific community and the general public. High rates of species loss have lead to questions about the overall effect of lowered diversity on ecosystems (Chapin III *et al*. 2000, Hooper *et al*. 2005) – forming the body of research termed biodiversity-ecosystem functioning (BDEF), that investigates how species diversity impacts various aspects of ecosystems (Srivastava and Vellend 2005, Balvanera *et al*. 2006). Only a small portion of this research has considered the belowground community (Balvanera *et al*. 2006) and the results are often varied. Results depend on which microbial process is measured and the methods chosen to analyze the soil microbial community (Wardle *et al*. 1999, Zak *et al*. 2003, Niklaus *et al*. 2006), the experimental design (Balvanera *et al*. 2006), site location

[1] A version of this chapter will be submitted for publication. Marshall, C., J. McLarnen and R. Turkington. Effect of plant functional group removal on the soil microbial community.

(Bezemer *et al*. 2006) and plant species identity (Chen and Stark 2000). There has been little success in detecting consistent patterns that describe plant diversity effects on the soil microbial community.

Plants interact with the soil microbial community in many ways. Nutrients required by soil microbes often come from plant litter or through root exudation (Griffiths *et al.* 1992, Grayston *et al.* 1996,1998). Roots also physically alter the soil structure, creating varying microhabitats suitable for different microbes (Bardgett *et al*. 2005). Mycorrhizal associations with plant roots add another dimension to the plant-soil-microbe system because mycorrhizae can directly and indirectly influence soil microbes (see below). The alteration of any component of the plant-soil-microbe system has the potential to change the intensity and direction of these interactions and to influence soil microbial community composition.

A more diverse plant community could potentially create a more heterogeneous soil environment and support a wider variety of soil organisms. At the Swiss site of the European BIODEPTH experiment, diversity of soil bacteria increased with increasing plant diversity (Stephan *et al*. 2000). Several studies have reported increased microbial biomass with higher plant diversity (Spehn *et al.* 2000, Zak *et al.* 2003), but Wardle *et al.* (2003) and Niklaus *et al*. (2006) detected no change in microbial biomass with plant species or functional group diversity. Other studies detected idiosyncratic relationships between the soil microbial community structure and function and plant functional group diversity and composition (Wardle *et al.* 1999, 2000).

Predicted future environmental changes include not only increased temperatures but also increased nutrient levels as the result of processes such as nitrogen deposition and increased decomposition (Shaver *et al*. 2000). These changes will be especially important in more northern ecosystems where decomposition rates and nutrient cycling are traditionally slowed by the lower temperatures (Jonasson *et al.*1999, Shaver *et al*.

2000). Future conditions could include both decreased biodiversity due to climate change (IPCC 2007) and habitat destruction (Chapin III *et al*. 2000) and increased nutrients and it is therefore important to investigate the effects of plant diversity under different environmental conditions.

There are many ways an increase in nutrients could affect the soil community. It was previously thought that soil microbes were carbon-limited. Recent studies have shown that they may actually be nitrogen-limited (Wagener and Schimel 1998, Chen and Stark 2000). Furthermore, nitrogen requirements appear to vary among soil microorganisms (Schimel *et al*. 2005). If a microbe is nitrogen-limited, the addition of fertilizer could increase its numbers and cause a shift in community composition and structure. For example, soil communities under conditions of high nutrients tend to be bacterial-dominated whereas lower-nutrient soils tend to support fungal-dominated communities (Wardle 2005). Therefore the addition of fertilizer may result in a shift of the soil community towards bacteria and a decrease in the fungal:bacterial ratio (Wallenstein *et al*. 2006).

The nature of the microbial community may also be influenced by mycorrhizal fungi. Mycorrhizae form associations with 80% of land plants (Leake *et al.* 2005). The fungi involved in these associations form an intimate symbiosis with plant roots and therefore have immediate access to plant root exudates. Mycorrhizae can alter the chemical composition and relative abundance of these exudates, which are a source of nutrients for many soil organisms and may also compete with soil organisms for nutrients (Artursson *et al*. 2006). Andrade *et al.* (1997) reported higher numbers of bacteria in areas of the roots not occupied by arbuscular mycorrhizal (AM) fungi, implying a greater release or higher quality of root exudates from those areas. Close physical associations are often made between bacteria and mycorrhizae; the majority of studies show that mostly Gram-positive bacteria form associations with AM fungi

(Artursson *et al*. 2006). The physiological state of the mycorrhizal hyphae has also been shown to allow associations with different bacterial strains (Toljander *et al*. 2005). Because of the many interactions between mycorrhizae and soil organisms it is difficult to predict the effects of removing mycorrhizae on the soil community but these effects could potentially have a major impact on the ecosystem.

The objective of this study was to investigate how the functional group composition of the aboveground plant community influences the belowground microbial community. We describe a removal experiment, where each of three functional groups (graminoids, forbs and legumes) was removed from the system to determine the impact of that functional group. We predicted that removing any plant functional group would decrease the diversity of the soil microbial community because of a reduced base of resources being released to the soil by the plant community (Grayston *et al*. 1998, Stephan *et al*. 2000). The study also incorporates two treatments, a fertilizer treatment and a fungicide treatment, which created different environmental conditions to test if the experimental system would respond differently under different scenarios. Soil microbial community structure was analyzed by phospholipid fatty acid (PLFA) analysis, which targets the entire soil microbial community. Potential function of the microbial community was analyzed by substrate-induced respiration. A less diverse microbial community would be expected to have less ability to metabolize different carbon sources and to have a less diverse fatty acid profile (Leckie 2005).

The primary questions addressed in this thesis are:

1) Does plant functional group composition exert an effect on belowground microbial community structure and function?

2) Do soil nutrient levels have an effect on belowground community structure and function and does the effect of aboveground plant composition on the belowground community change under different nutrient levels?

3) Do mycorrhizae have an effect on belowground community structure and function and do the effects of aboveground composition on the belowground community change when mycorrhizae are removed?

2.2 Methods

2.2.1 Research site

The study was conducted beside Emerald Lake (GPS reference is 61 04 218 N, 138 23 018 W, elevation 829m.a.s.l.) about 10km north of the Arctic Institute of North America Research Station, at Kluane Lake in the south-western Yukon in northern Canada. The community was a relatively dry grassland, dominated by *Poa glauca* and *Carex stenophylla*, and also contained many dicots including legumes. Mean annual precipitation is ca. 230 mm, about half of which falls as rain during the summer months. The surrounding area is a spruce forest community dominated by *Picea glauca*. Plant species present in the community were classified into three functional groups: graminoids, non-leguminous dicots (hereafter called forbs) and legumes (Table 2.1).

Table 2.1. Functional groups and species present at the study site. Species names and authorities are as presented in Cody (1996).

Graminoids	Non-leguminous forbs	Leguminous Forbs
Calamagrostis purpurascens R. Br.	*Androsace septentrionalis* L.	*Astragalus alpinus* L.
Carex stenophylla ssp. Wahlenb. *eleocharis* (Bailey) Hultén	*Antennaria rosea* Greene	*Astragalus williamsii* Rydb.
Elymus calderi Barkworth	*Arabis holboellii* Hornem.	*Oxytropis campestris* (L.) DC.
Elymus trachycaulus (Link) Gould	*Artemisia frigida* L.	
Festuca brachyphyla Schultes & Schultes fil.	*Aster alpinus* L.	
Poa glauca Vahl	*Castilleja hyperborean* Pennell	
	Erigeron caespitosus Nutt	
	Penstemon gormanii Green	
	Plantago canescens Adams	
	Potentilla prostrate Rottb.	
	Pulsatilla ludoviciana (Nutt.) Heller	
	Zygadenus elegans Pursh	

2.2.2 Removal experiment

The plots used in this experiment were originally established by Jennie McLaren[2] in 2003. The experiment was a 4 x 2 x 2 fully crossed factorial design with 4 removal treatments (one each of the three functional groups removed and a no-removal control), 2 fertilizer treatments (+/0), and 2 fungicide treatments (+/0). Each treatment had 5 replicates, for a total of 80 plots. Treatments were applied each summer from 2003 to 2007. All data described in this thesis were collected during the 2007 growing season.

Plots were 1 m x 1 m and were spaded 10 cm outside the edge of the plot to a depth of 25 cm to sever any root connections with nearby plots. This ensured plots would not be influenced by treatments applied to nearby plots. A rope fence was constructed around the perimeter of the study area to prevent grazing by wild horses but did not exclude smaller herbivores.

2.2.3 Implementation of treatments

For each removal treatment the target functional group was removed from the plot and from the 10 cm buffer zone around the plot. Functional group removal was originally conducted by using a paintbrush to coat the plants with the herbicide Round-up™, which is a glyphosate non-selective herbicide. Using a paintbrush ensured limited influence on non-target plants. This method allowed the root system to remain intact and thus caused minimal disturbance to the soil. Glyphosate remaining in the soil bonds to soil particles and is no longer able to kill plants (Ahrens 1994, WHO 1994) and it is eventually broken down by soil microorganisms (WHO 1994). In following years at the

[2] Jennie McLaren is a PhD candidate in Roy Turkington's lab and has been working at the Kluane site since 2003. Jennie has been studying the effects of plant functional group on vegetation dynamics and ecosystem properties and my project ties in closely with questions that Jennie has been addressing.

start of the growing season removal treatments were maintained by physically removing any target functional group plants that had regrown. Any regrowth by species in the other functional groups was left in the plot.

A granular fertilizer treatment was applied to half of 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 was applied annually immediately after the removal treatments.

A fungicide (Benomyl) treatment was applied to half of the plots at the same time as the fertilizer treatment, at a per-plot rate of 2.5 gm^{-2} of the active ingredient mixed with 2 L of water. Plots that were not treated with fungicide were given an equivalent amount of water at this time. The treatment was reapplied every two weeks for the entire summer growing season (10 week duration, 5 applications). Studies have shown Benomyl to be very effective at reducing mycorrhizal root colonization (80% reduction) while having limited non-target effects on other soil biota and soil nutrients (Smith *et al.* 2000).

2.2.4 Aboveground Community Response Measurements

An index of the biomass of each species was determined using the pin drop-method. Using 100 intersection points on a 100 cm x 100 cm quadrat the number of leaf intersections for each species was recorded per pin drop. The number of leaf hits per species per plot has been shown to have a strong correlation to species-specific biomass (r^2 ranged from 0.80 to 0.99, J. McLaren, personal communication), and using previously obtained regressions all leaf intersection values were converted to biomass estimates (gm^{-2}).

2.2.5 Soil Response Measurements

Soil, which is fine, sandy and mostly mineral in content, was collected on June 29th, 2007. Two soil cores, 2 cm diameter and 5-8 cm deep, were taken from each plot, pooled, and mixed well. Test cores taken outside the plots determined that this procedure would sample soil from the majority of the rooting layer. Also, this depth was often the maximum depth of soil before it became too rocky to sample with the soil corer. pH was measured using a Waterproof pH Tester 20 (Eutech Instruments Illinois). Approximately 10 g of soil were taken from each sample, mixed with de-ionized water in a 1:2 soil:water ratio and after allowing equilibration with the air (30 minutes of periodically stirring the mixture followed by 30 minutes with no stirring).

Soil moisture levels were measured using a Hydrosense Water content measurement system (Campbell Scientific, Australia) at two locations in each plot. Measurements were taken at 5-8 cm depth on 26 July, 2007.

In situ soil nutrient supply rates were measured using ion exchange membranes (Plant Root Simulator (PRS)^{TM} probes; Western Ag Innovations Inc., Saskatoon, SK). Four probes were inserted into each plot at the beginning of the growing season; two cation and two anion probes. We attempted to place the probes randomly in the plots but this was confined to areas of the plots that were free from rock and had sufficiently deep soil to allow proper placement of the probes. Probes were inserted in early June and removed mid-August. After removal the probes were analyzed by Western Ag Innovations Inc. (Saskatoon, SK) for NO_3, NH_4, P, K, S, Ca, Mg, Mn, Fe, Cu, Zn, B, Al, and Pb.

2.2.6 Soil Microbial Community Response Measurements

Soil for microbial community analyses was collected on 4 July, 2007. Three 2 cm cores were taken from each plot to a depth of 5-8 cm and composited. The soil was stored in a cooler on ice and flown to Vancouver, BC the following day where it was refrigerated. Soil was homogenized and sieved using a 2mm sieve. All soil was stored in refrigeration at 4°C, but soils designated for PLFA analysis were freeze-dried in batches of approximately 12 samples for about 24 hours until all samples were dried. These were returned to the refrigerated storage.

Soil microbial community structure and function was analyzed using two techniques. The first analyzed the functional potential or catabolic diversity of the community through substrate induced respiration (SIR). Soil samples were analyzed using MicroResp™ plates, following the protocol described by Campbell *et al.* (2003). The MicroResp™ system creates a metabolic profile of the microbial community based on the ability of the community to metabolize a series of ecologically relevant carbon sources. The system consists of a 96 deep-well plate and a CO_2 detection plate sealed together. Eleven different carbon sources were tested (Table 2.2) plus a water control with 8 replicates of each in one plate (one plate per experimental plot). These carbon sources were chosen to represent compounds likely to be found in plant root exudates (Grayston *et al.* 2004). The CO_2 detection plate contains a pH indicator dye, cresol red, and CO_2 produced is measured by the colour change above each well. The equivalent of 0.1 g of dry soil was added to each well and 5 mg of carbon per gram of dry soil was calculated for each carbon source and this was added in a deionised water solution for a total volume of 100µL per well. Colour change was measured after 6 hours of incubation at 20°C using a plate reader at 590 nm. Although this method does not identify different

species of microbes, it gives a picture of the microbial community's metabolic diversity and potential function and allows for relatively quick analysis of multiple whole-soil samples and comparisons among treatments (Campbell *et al.* 2003). Studies show Microresp™ is more sensitive to differences in community structure than other popular methods, such as BIOLOG plates (Campbell *et al.* 2003) and a method described by Degens and Harris (1997) which uses CO_2 evolution in soil slurries with different carbon sources in MacCartney bottles (Lalor *et al.* 2007).

Table 2.2. Carbon sources used for substrate-induced respiration.

Carbon Source	Type of Carbon Source
Glucose	Monosaccharide
Fructose	Monosaccharide
Sucrose	Disaccharide
Maltose	Disaccharide
Lysine	Amino Acid
L-ornithine	Amino Acid
L-phenylalanine	Amino Acid
Tartaric Acid	Organic Acid
Citric Acid	Organic Acid
Caffeic Acid	Phenolic Acid
Ferulic Acid	Phenolic Acid

The second method was phospholipid fatty acid (PLFA) analysis and this technique analyzed the entire microbial community, alive in the soil at the time of sampling (Leckie 2005). PLFA analyzes community structure using the abundance of different membrane lipids, the composition of which varies in different organisms. The procedure used was modified from Bligh and Dyer (1959) and Frostegård *et al*. (1993). Using About 2 g of freeze dried soil, membrane lipids were extracted using citrate buffer, methanol and chloroform. The resulting extract was then split using chloroform into two

phases and the lipid phase was stored in the dark at -20℃. The lipid phase was passed through a solid phase extraction cartridge with acetone to remove neutral lipids and waxes and phospholipids were eluted using methanol. A standard was added to the eluted phospholipids and this was converted to fatty acid methyl esters (FAMEs) by methylation (Kirk *et al.* 2004, Leckie 2005) using methanol, potassium hydroxide, tolulene and hexane. A community profile is obtained by passing the final samples through a gas chromatogram. Different phospholipids can be identified based on retention times and abundance of each fatty acid is determined by area under the curve of the gas chromatogram (Table 2.3). Certain PLFAs are associated with certain groups of organisms, for example, monoenoic unsaturated PLFAs are associated with Gram-negative bacteria. This allows the relative abundance of these groups to be compared among treatments along with community composition as a whole (Leckie 2005). The fungal to bacterial ratio was calculated using i15:0, a15:0, i16:0, a17:0, cy17:0, 18:1ω7 and cy19:0 to represent bacteria and 18:2ω6,9 to represent fungi (Bardgett *et al.* 1996, Bååth and Anderson 2003). Bacteria change membrane lipids in response to stress. One of these changes is an increase in cyclopropyl fatty acids compared to their monoenoic precursors (Iyyemperumal and Shi 2007, Kieft *et al.* 1997). The ratios of cy19:0 and its precursor 18:1ω7c and cy17:0 to its precursor 16:1ω7c were calculated as a measure of stress in this experiment.

PLFA nomenclature follows Steer and Harris (2000). PLFAs are presented as the number of carbon atoms followed by a colon and then the number of double bonds. The position of double bonds follows 'ω' and the number represents the first carbon atom in the double bond from the methyl end of the molecule. A 'c' after the double bond position indicates a cis configuration. Branches are represented by a prefix of 'i' for an iso-branching pattern or 'a' for an anteiso-branching pattern. The prefix 'cy' designates a cyclopropane fatty acid. 10Me indicates a methyl group on the tenth carbon atom from

the carboxyl end of the molecule. For example, 18:1ω5c is a 18 carbon fatty acid with one double bond between the fifth and sixth carbon atoms in the cis configuration.

2.2.7 Statistical Analysis

Treatment effects and interaction effects were analyzed using MANOVAs and ANOVAs. All non-normal variables were transformed as required to satisfy the assumptions of normality and multivariate normality, independence of data and equal variance. Plant species biomass values were rank-normal transformed and analyzed in 3 MANOVAs, with species grouped by functional group. Total functional group biomass was also analyzed. F-tests were used to determine significance between different treatments. Canonical correlation analysis was performed between all sets of variables to identify relationships. All statistical analyses were performed using SAS 9.1 for Windows. Nitrate was not analyzed because it was highly correlated with total nitrogen.

Table 2.3. Phospholipid fatty acids used for analysis of microbial communities and the soil biota grouping they represent.

Gram-positive bacteria	Gram-negative bacteria	Total bacteria	Actino-bacteria	AMF	Saprophytic fungi	Fungi	Common
i15:0	i16:1ω7c	15:0	16:0ω6m/10Me17:0	16:1ω5c	18:2ω6,9	18:1ω9c	16:0
a15:0	i17:1ω8c	17:0	17:0ω7m/10Me18:0				
i16:0	cy17:0		18:0ω8m/ 0Me19:0				
16:1ω9c	18:1ω7						
16:1ω7	18:1ω5c						
i17:0	cy19:0						
a17:0							
18:0							
18:1ω9c							

2.3 Results

2.3.1 Aboveground Variables

Plant functional group removal and fertilization significantly affected total plant biomass (Table 2.4). Biomass was lowest in plots where forbs were removed compared to all other plots and the biomass of plots without legumes had a lower biomass than control plots. The biomass of plots with graminoids removed did not have a significantly decreased biomass compared to control plots (Figure 2.1). Fertilization increased total aboveground biomass (Figure 2.2).

Table 2.4. ANOVA for total plant biomass (gm^{-2}) per plot. Values in bold are significant ($p<0.05$, Wilks' Lambda).

Treatment	df Treatments	df Error	Mean Square	F-value	P-value
Functional group removal (R)	3	76	2928.52	**11.17**	**<0.001**
Fertilizer (F)	1	78	3221.96	**12.29**	**0.001**
Fungicide (B)	1	78	539.71	2.06	0.075
R*F	3	76	858.93	3.38	0.115
R*B	3	76	102.14	0.39	0.761
F*B	1	78	471.85	0.08	0.185
R*F*B	3	76	21.89	0.08	0.968

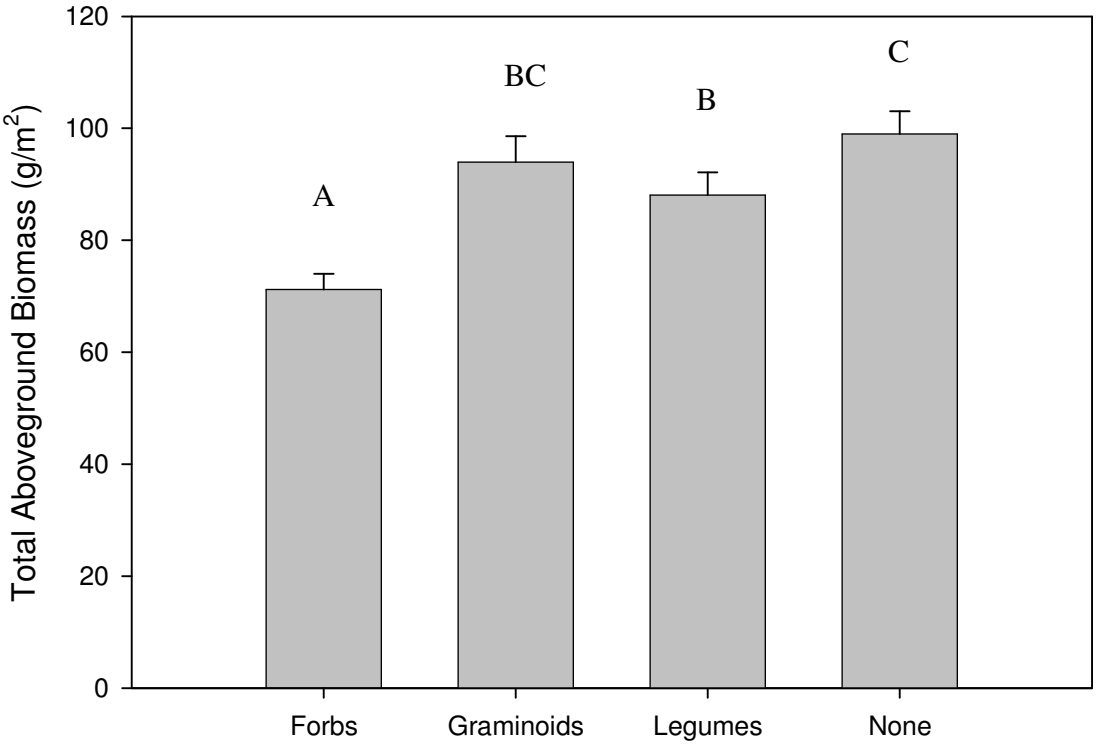

Figure 2.1. Effect of removing different functional groups (forbs, graminoids, legumes or no removal) on mean (+ 1SE) total aboveground biomass (gm^{-2}). Bars with different letters are significantly different ($p<0.05$, F-test).

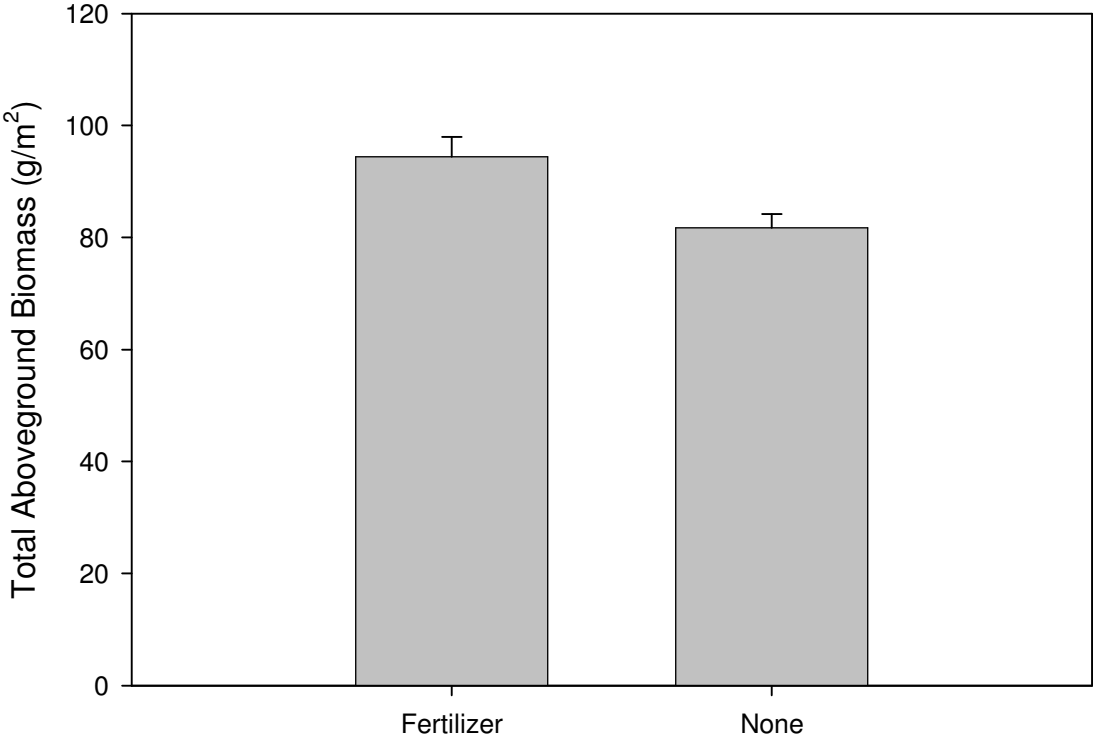

Figure 2.2. Effect of fertilization on mean (+ 1SE) total aboveground biomass (gm^{-2}). Bars are significantly different ($p<0.05$, F-test).

When plant species were separated into functional groups (Table 2.5), removal significantly affected all functional groups. Legumes increased in biomass when either forbs or graminoids were removed. Graminoids increased when forbs were removed and forbs increased when grasses were removed. Fertilization significantly lowered the biomass of legumes and increased the biomass of graminoids. Fungicide application did not affect any functional group biomass.

Table 2.5. ANOVAs for plant functional group biomass. Values in bold are significant ($p<0.05$, Wilks' Lambda).

	Legumes		Graminoids		Forbs	
Treatment	F-value	P-value	F-value	P-value	F-value	P-value
Functional group removal (R)	4.97	0.011	32.39	<.001	7.49	0.006
Fertilizer (F)	35.35	<.001	75.82	<.001	3.71	0.060
Fungicide (B)	0.05	0.817	1.99	0.148	1.03	0.314
R*F	1.55	0.223	1.84	0.181	1.43	0.249
R*B	0.09	0.910	2.69	0.078	0.32	0.726
F*B	0.00	0.976	0.05	0.821	1.90	0.175
R*F*B	0.07	0.930	1.55	0.224	0.34	0.716

Individual legume species biomass was significantly affected by plant functional group removal and fertilization (Table 2.6). *Oxytropis campestris* increased in biomass when either forbs or graminoids were removed (F-value=6.29, p-value=0.004) and it was reduced with fertilization (F-value=35.00, p-value=<0.001). No other legumes significantly responded to treatments.

The MANOVA on individual graminoids species biomass showed significant effects for removal treatment, fertilization, and fungicide (Table 2.6). The removal by fertilization effect also showed significance, however no individual species were significantly affected. This effect is like due to *Elymus caldera,* which was marginally not significant for removal by fertilization (F-value=3.07, p-value=0.056); biomass of *E. caldera* increased when legumes were removed only when fertilizer was added. *Calamagrostis purpurascens* (F-value=7.71, p-value=0.001) and *Poa glauca* (F-value=7.72, p-value=0.001) increased when forbs were removed. *C. purpurascens* (F-value=5.09, p-value=0.029), *Carex stenophylla* (F-value=10.50, p-value=0.002) and *P. glauca* (F-value=16.06, p-value<0.001) all increased with fertilization. *C. purpurascens* (F-value=11.19, p-value=0.002) and *E. calderi* (F-value=4.96, p-value=0.031) decreased with fungicide while *P. glauca* (F-value=9.86, p-value=0.003) increased.

Individual forbs species biomass was significantly affected by removal treatment and fertilization (Table 2.6). *Penstemon gormanii* (F-value=3.41, p-value=0.041) and *Plantago canescens* (F-value=3.22, p-value=0.049) increased in biomass when grasses were removed. *Arabis holboellii* (F-value=4.52, p-value=0.039) and *Artemisia frigida* (F-value=10.23, p-value=0.002) increased with fertilization while *Castilleja hyperborean* (F-value=10.69, p-value=0.002) decreased in biomass.

Table 2.6. MANOVAs for individual plant species biomass. Values in bold are significant ($p<0.05$, Wilks' Lambda).

	Legumes		Graminoids		Forbs	
Treatment	F-value	P-value	F-value	P-value	F-value	P-value
Functional group removal (R)	**3.13**	**0.008**	**4.69**	**<.001**	**1.93**	**0.017**
Fertilizer (F)	**12.84**	**<.001**	**11.31**	**<.001**	**3.03**	**0.005**
Fungicide (B)	0.42	0.737	**3.19**	**0.011**	1.87	0.072
R*F	1.31	0.259	**2.15**	**0.022**	0.68	0.854
R*B	1.40	0.224	1.05	0.411	0.86	0.650
F*B	2.61	0.063	0.35	0.904	0.53	0.833
R*F*B	0.47	0.832	1.35	0.204	1.04	0.432

2.3.2 Soil Variables

Soil variables (soil moisture, pH, nutrients and soil carbon) were significantly affected by functional group removal, fertilization, fungicide, and the fertilization by fungicide interaction (Table 2.7). Removal of legumes decreased ammonium compared to all other removal treatments (Table 2.8). Removal of graminoids decreased potassium compared to the control. When graminoids were removed, soil aluminum increased to be significantly more than plots where forbs were removed (where aluminum decreased), but neither were significantly different from the controls.

Table 2.7. MANOVA for soil variables (soil carbon, mean soil moisture, pH, and nutrients). Values in bold are significant ($p<0.05$, Wilks' Lambda).

Treatment	Num df	Dem df	F-value	P-value
Functional group removal (R)	42	**152.06**	**1.91**	**0.003**
Fertilizer (F)	14	**51**	**69.06**	**<0.001**
Fungicide (B)	14	**51**	**2.11**	**0.027**
R*F	42	152.06	1.11	0.316
R*B	42	152.06	0.87	0.687
F*B	14	**51**	**2.35**	**0.013**
R*F*B	42	152.06	1.12	0.312

Table 2.8. ANOVA for the effects of functional group removals, fertilization, fungicide and fertilizer by fungicide interaction on soil variables (soil carbon, mean soil moisture, pH, and nutrients). Values in bold are significant ($p<0.05$, Wilks' Lambda). There are 3 df for all removal values and 1 df for fertilization, fungicide and their interaction.

	Removal		Fertilizer		Fungicide		Fertilizer by Fungicide	
Species	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
Total C	0.84	0.475	0.06	0.812	0.20	0.659	0.03	0.859
Soil Moisture	0.56	0.641	**33.68**	**<.001**	**7.21**	**0.009**	**5.02**	**0.029**
pH	2.09	0.111	**228.12**	**<.001**	0.00	0.986	0.55	0.462
Total N	0.33	0.800	**274.94**	**<.001**	**20.65**	**<.001**	**18.55**	**<.001**
NH_4^+	**3.09**	**0.033**	**12.00**	**0.001**	3.98	0.050	0.66	0.418
Ca	0.22	0.885	**13.33**	**0.001**	0.82	0.369	0.14	0.714
Mg	0.55	0.648	**17.36**	**<.001**	0.77	0.384	0.12	0.735
K	**3.53**	**0.020**	**62.12**	**<.001**	0.21	0.649	0.10	0.758
P	0.44	0.725	**380.50**	**<.001**	1.10	0.298	2.17	0.146
Fe	2.05	0.110	**9.44**	**0.003**	0.00	0.948	0.59	0.444
Mn	1.10	0.354	**55.94**	**<.001**	0.82	0.367	1.71	0.196
Zn	2.59	0.060	**6.94**	**0.011**	0.91	0.345	0.27	0.605
Bo	1.30	0.281	**8.39**	**0.005**	0.08	0.780	0.00	0.956
Al	**3.48**	**0.021**	0.91	0.345	0.73	0.396	0.45	0.396

All of the nutrients measured except aluminum were affected by fertilization; ammonium, potassium, phosphorus, iron, manganese and zinc increased while calcium, magnesium and boron decreased (Table 2.8). Average soil moisture was affected by the interaction between fertilization and fungicide (Table 2.8). When fungicide was not applied fertilization decreased soil moisture (Figure 2.3). Fungicide application also decreased soil moisture, but only when there was no fertilization. Fertilization interacted significantly with fungicide on total nitrogen, with fertilization increasing nitrogen. Fungicide increased total nitrogen but only when there was no fertilizer applied (Table 2.13, Figure 2.4). There was no effect of any of the treatments on soil carbon content.

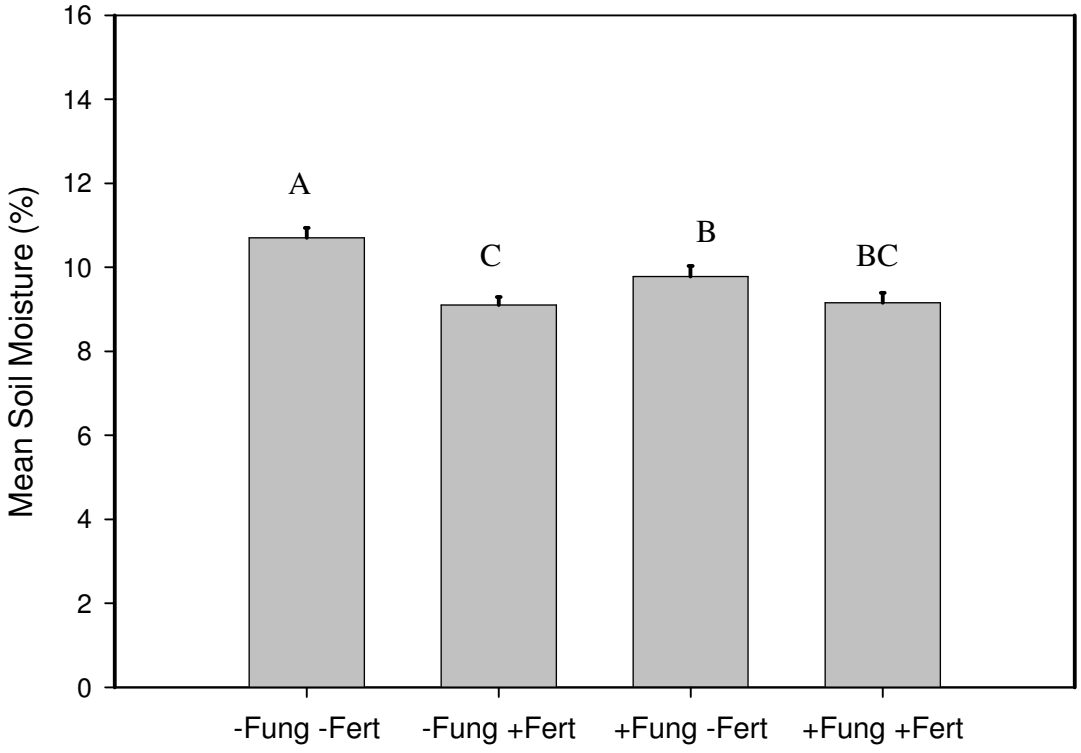

Figure 2.3. Effect of fungicide (fung) and fertilization (fert) application on mean (+ 1SE) soil moisture (%). Bars with different letters are significantly different ($p < 0.05$, F-test).

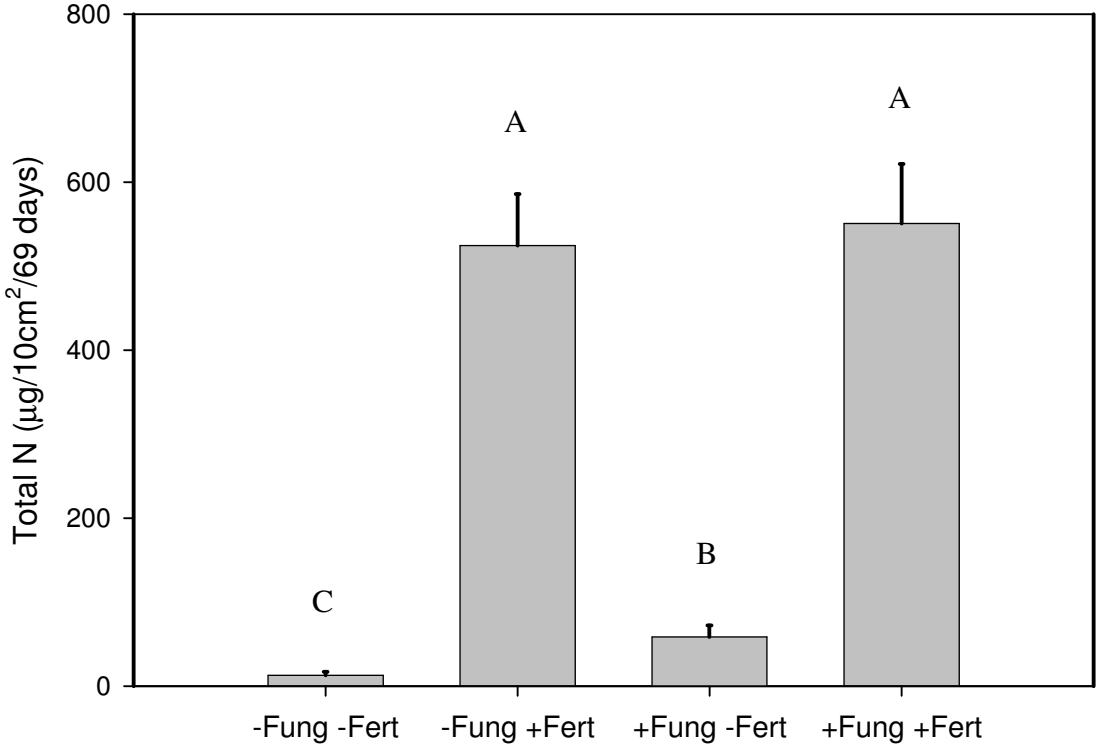

Figure 2.4. Effect of fungicide (fung) and fertilization (fert) application on mean (+ 1SE) total soil nitrogen (µg/10cm^2/69 days). 69 day is the length of time the IEM probes were buried in the plots. Bars with different letters are significantly different ($p < 0.05$; F-test).

Out of 14 canonical correlations between plant species biomass and soil properties, only two were significant (p=0.0010 and p=0.0269, Table A.1 in Appendix 1). These two canonical correlations accounted for 14% of the plant species variation and 33% of the soil properties variation. In turn, the plant species canonical variables explained 22% of the soil properties and the canonical variables of the soil properties explained 9% of variation in plant species (Table 2.9).

Table 2.9. The proportion of variance (for the first two canonical variables) of plant species biomass and soil properties explained by their own canonical variables and those of the other set of variables.

	Plant Species Biomass Canonical Variables	Soil Properties Canonical Variables
Plant Species Biomass	0.14	0.22
Soil Properties	0.09	0.33

Canonical correlation analysis showed one significant canonical correlation (p=0.0042, Table A.2 in Appendix 2) out of a possible three between functional group biomass and the soil properties. This canonical variable explained 34% of the variability in functional group biomass and 11% for the soil properties. The soil canonical variable explained 17% of the biomass variation and the functional group canonical variable explained 5% of the soil properties (Table 2.10). The variation explained was mostly accomplished by a positive relationship between legumes and soil moisture and potassium (Tables 2.11 and 2.12).

Table 2.10. The proportion of variance (for the first canonical variable) of functional group biomass and soil properties explained by their own canonical variables and those of the other set of variables.

	Functional Group Canonical Variables	Soil Properties Canonical Variables
Functional Group Biomass	0.34	0.17
Soil Properties	0.05	0.11

Table 2.11. Correlations between soil properties and their canonical variables (Soil CC) and the canonical variables of functional group (FG CC) biomass.

	Soil CC	FG CC
Total C	0.143	0.099
Soil Moisture	0.450	0.312
pH	-0.167	-0.116
Total N	0.273	0.189
NH4+	-0.232	-0.161
Ca	0.071	0.049
Mg	0.039	0.027
K	0.504	0.349
P	0.328	0.227
Fe	-0.208	-0.144
Mn	0.107	0.074
Zn	0.354	0.245
Bo	-0.144	-0.100
Al	0.143	-0.059

Table 2.12. Correlations between functional group (forbs, graminoids, legumes) biomass and their canonical variables and the canonical variables of the soil properties.

	Functional Group Canonical Variables	Soil Properties Canonical Variables
Forbs	0.137	0.095
Graminoids	0.544	0.377
Legumes	-0.981	-0.680

2.3.3 Microbial Community Structure and Function

2.3.3.1 SIR profiles

Functional group removal had no significant effects on the microbial community's metabolic diversity. Fertilization was the only treatment to affect SIR profiles (Table 2.13). Fertilization decreased the ability of the soil community to metabolize amino acids (lysine, L-ornithine, and L-phenylalanine), organic acids (tartaric acid and citric acid) and caffeic acid, one of the phenolics (Table 2.14, Figure 2.5).

Table 2.13. MANOVA for SIR profiles of the soil microbial community in response to functional group removal, fertilization and fungicide application. Values in bold are significant ($p<0.05$, Wilks' Lambda).

Treatment	Num df	Dem df	F-value	P-value
Functional group removal (R)	33	145.07	1.03	0.435
Fertilizer (F)	11	49.00	**2.99**	**0.004**
Fungicide (B)	11	49.00	1.02	0.445
R*F	33	145.07	0.73	0.849
R*B	33	145.07	0.99	0.485
F*B	11	49.00	0.32	0.978
R*F*B	33	145.07	1.32	0.136

Table 2.14. ANOVA for the effect of fertilizer on the soil microbial community's ability to metabolize each carbon source. Values in bold are significant ($p<0.05$, Wilks' Lambda). There is 1 df in the numerator and 73 df in the denominator in all cases.

Treatment	Type of C Source	Mean Square	F-value	P-value
Glucose	Monosaccharide	0.081	0.07	0.786
Fructose	Monosaccharide	0.674	0.90	0.346
Sucrose	Disaccharide	1.825	3.10	0.083
Maltose	Disaccharide	1.658	3.25	0.076
Lysine	Amino Acid	5.489	**9.25**	**0.004**
L-Ornithine	Amino Acid	9.730	**14.96**	**<.001**
L-Phenylalanine	Amino Acid	5.235	**8.76**	**0.004**
Tartaric acid	Organic Acid	4.734	**5.57**	**0.022**
Citric acid	Organic Acid	4.328	**4.99**	**0.029**
Caffeic acid	Phenolic Acid	8.402	**12.30**	**0.001**
Ferulic acid	Phenolic Acid	2.217	2.73	0.104

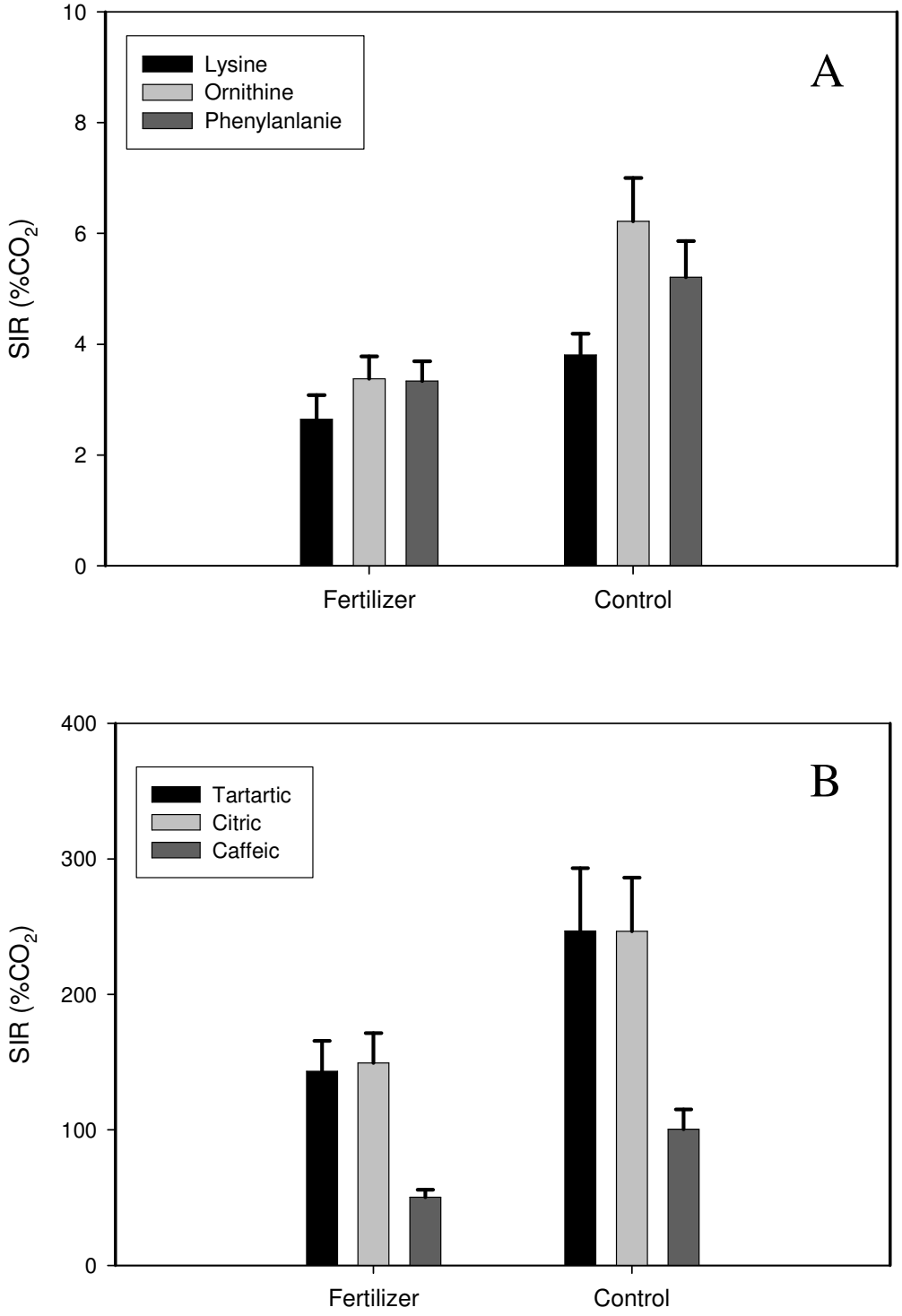

Figure 2.5. Effect of fertilization on mean (+ 1SE) SIR (%CO_2 required to produce a colour change in the plate well). A. Fertilization effect on the three amino acids, lysine, L-ornithine, L-phenylalanine. B. Fertilization effect on the organic acids, tartaric acid and citric acid, and the phenolic acid, caffeic acid.

A canonical correlation analysis of SIR profiles with soil properties showed no significant relationships between the two groups. There were some patterns in correlations between the raw variables, but these were somewhat weak (Table 2.15). The soil community's ability to metabolize recalcitrant carbon sources increased with potassium, which had decreased with the removal of graminoids. However, the removal of graminoids did not significantly affect SIR. Metabolization of amino acids decreased with boron. There were also no significant canonical correlations with the SIR profiles and plant species biomass or functional group biomass.

Table 2.15 Pearson's Correlation Coefficients between the ability of the microbial community to metabolize the various carbon sources (SIR) and the soil properties. Values in bold are significant ($p<0.05$).

	Glucose	Fructose	Sucrose	Maltose	Lysine	L-Ornithine	L-Phenyl-alanine	Tartaric Acid	Citric Acid	Caffeic Acid	Ferulic Acid
Total Carbon	**0.25**	0.20	-0.02	0.16	0.19	0.15	0.19	0.12	0.08	0.08	0.13
Soil Moisture	**-0.23**	**-0.25**	-0.15	-0.22	-0.12	-0.09	-0.03	-0.11	-0.09	-0.09	-0.11
pH	-0.03	0.05	-0.22	-0.03	-0.07	-0.01	-0.03	-0.08	-0.05	-0.04	-0.08
Total N	0.07	-0.02	-0.04	0.07	0.13	0.07	0.07	0.11	0.09	0.09	0.12
NH_4^+	0.12	0.18	-0.14	0.12	0.09	0.10	0.10	0.07	0.03	0.04	0.09
Ca	-0.14	-0.05	-0.18	-0.11	-0.06	-0.03	-0.06	-0.07	-0.08	-0.08	-0.05
Mg	-0.06	0.00	0.17	-0.06	-0.01	0.08	0.04	0.01	0.00	0.00	0.00
K	**0.23**	0.17	0.08	**0.22**	0.20	0.20	0.19	**0.28**	**0.26**	**0.26**	**0.27**
P	0.06	-0.03	-0.03	0.07	0.07	0.04	0.02	0.15	0.12	0.12	0.15
Fe	0.06	-0.09	-0.05	0.00	-0.08	-0.12	-0.14	-0.06	-0.05	-0.06	-0.07
Mn	0.10	0.00	-0.05	0.08	0.00	0.00	-0.02	0.08	0.06	0.05	0.06
Zn	-0.09	-0.10	-0.01	-0.06	-0.06	-0.04	-0.03	-0.03	-0.04	-0.04	-0.02
B	-0.22	**-0.30**	-0.13	-0.16	**-0.23**	**-0.27**	**-0.26**	-0.21	-0.18	-0.19	-0.21
S	-0.10	-0.06	-0.07	-0.05	-0.16	-0.16	-0.19	-0.13	-0.10	-0.10	-0.13
Pb	-0.06	-0.18	0.12	-0.14	-0.17	-0.20	-0.19	-0.14	-0.11	-0.12	-0.15
Al	-0.19	**-0.23**	-0.02	-0.20	-0.05	-0.14	-0.13	-0.11	-0.15	-0.14	-0.06

2.3.3.2 PLFA profiles

Functional group removal did not significantly change PLFA profiles (Table 2.16) but one PLFA was significantly increased by fertilization (F-value=4.41,p-value=0.0380); 18:1ω7c/10Me19:1ω7c, a fatty acid that is used as a representation of total microbial biomass. No other individual PLFA changed with fertilization. The ANOVA on fungal:bacterial ratios gives no significant effect of any treatment. The ratio of the stress PLFA cy19:0 and its precursor 18:1ω7c was increased by the interaction of removal treatment and fertilization (Table 2.17). Removing legumes without adding fertilizer increased the stress ratio (Figure 2.6). The other stress ratio considered (cy17:0 to 16:1ω7c) was unaffected by the treatments.

Table 2.16. MANOVA for PLFA profiles of the soil microbial community in response to functional group removal, fertilization and fungicide application. Values in bold are significant ($p<0.05$, Wilks' Lambda).

Treatment	Num df	Dem df	F-value	P-value
Functional group removal (R)	69	126.33	1.31	0.093
Fertilizer (F)	23	**42**	**4.31**	**<0.001**
Fungicide (B)	23	42	1.69	0.069
R*F	69	126.33	1.23	0.158
R*B	69	126.33	1.04	0.427
F*B	23	42	0.79	0.725
R*F*B	69	126.33	0.85	0.774

Table 2.17. ANOVA for PLFA stress ratio of cy19:0 to its precursor 18:1ω7c. Values in bold are significant (p<0.05, Wilks' Lambda).

Treatment	Num df	Dem df	Mean Square	F-value	P-value
Functional group removal (R)	3	56	0.002	0.74	0.531
Fertilizer (F)	1	58	0.001	0.35	0.559
Fungicide (B)	1	58	0.010	3.23	0.079
R*F	3	56	0.011	**3.50**	**0.023**
R*B	3	56	0.005	1.57	0.210
F*B	1	58	0.008	2.71	0.107
R*F*B	3	56	0.001	0.35	0.793

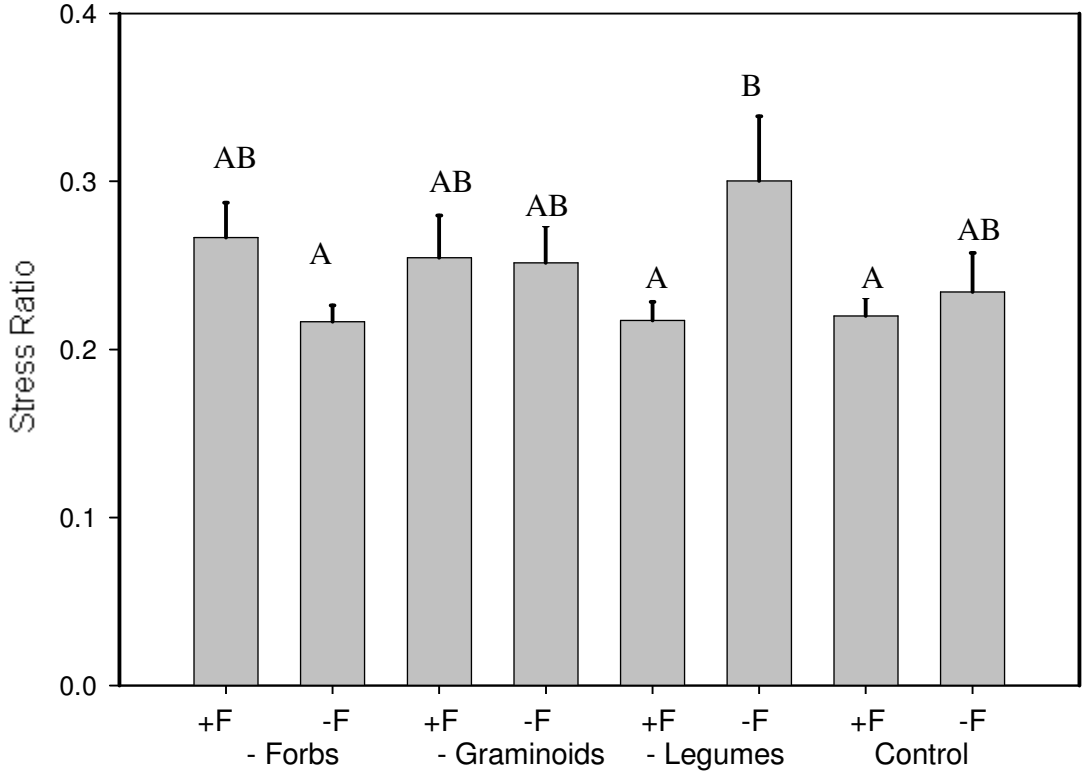

Figure 2.6. Effect of functional group (forbs, graminoids, legumes, or no removal) removal and fertilization on the stress ratio cy19:0: 18:1ω7c (mean ± 1 SE). +F = fertilizer applied, -F = no fertilizer added. Bars with different letters are significantly different (p < 0.05; F-test).

A canonical correlation analysis showed no significant canonical correlations between the SIR and PLFA profiles. However, there were some interesting trends in the correlations between the PLFA profiles and the SIR profiles (Table 2.18). All but one of the 23 PLFAs were negatively correlated with the metabolization of ferulic acid and all but two with caffeic acid, the other phenolic. All but five PLFAs were correlated with fructose, but none were correlated with the similar compound glucose. There were no patterns in which PLFAs correlated to the SIR profiles (i.e. no patterns with those PLFAs indicative of Gram-positive bacteria, etc.).

Table 2.18. Pearson's Correlation Coefficients between PLFA values and the ability of the microbial community to metabolize the various carbon sources. Values in bold are significant ($p<0.05$).

	Glucose	Fructose	Sucrose	Maltose	Lysine	L-Ornithine	L-Phenyl-alanine	Tartaric Acid	Citric Acid	Caffeic Acid	Ferulic Acid
i15:0	-0.16	-0.17	-0.11	-0.08	-0.01	-0.06	-0.03	-0.11	-0.12	-0.19	**-0.29**
a15:0	-0.17	-0.20	-0.14	-0.12	-0.06	-0.12	-0.07	-0.14	-0.16	**-0.24**	**-0.33**
15:O	-0.14	**-0.34**	**-0.30**	**-0.31**	**-0.24**	**-0.32**	**-0.28**	**-0.32**	**-0.35**	**-0.40**	**-0.44**
i16:1ω7c	-0.17	**-0.30**	**-0.27**	**-0.25**	-0.20	**-0.27**	-0.22	-0.22	**-0.25**	**-0.35**	**-0.36**
i16:0	-0.17	-0.22	-0.16	-0.14	-0.08	-0.15	-0.10	-0.15	-0.18	**-0.27**	**-0.35**
16:1ω7c	0.00	-0.14	-0.18	-0.15	-0.11	-0.10	-0.08	-0.02	-0.02	-0.12	-0.08
16:1ω5c	-0.19	**-0.32**	**-0.25**	-0.22	-0.14	-0.21	-0.16	-0.20	-0.23	**-0.30**	**-0.36**
16:O	-0.18	**-0.28**	**-0.23**	-0.20	-0.14	-0.20	-0.15	-0.21	**-0.24**	**-0.32**	**-0.39**
i17:1ω8c	-0.19	**-0.34**	**-0.28**	**-0.27**	-0.18	**-0.26**	-0.20	**-0.24**	**-0.26**	**-0.35**	**-0.40**
16:0ω6m/10Me 17:0	-0.20	**-0.25**	-0.19	-0.16	-0.08	-0.16	-0.12	-0.15	-0.18	**-0.27**	**-0.35**
i17:0	-0.16	-0.21	-0.14	-0.11	-0.03	-0.10	-0.07	-0.13	-0.15	**-0.23**	**-0.33**
a17:0	-0.17	**-0.24**	-0.17	-0.15	-0.08	-0.15	-0.11	-0.17	-0.20	**-0.28**	**-0.36**
cy17:0	-0.18	**-0.25**	-0.18	-0.16	-0.10	-0.17	-0.12	-0.17	-0.21	**-0.30**	**-0.38**
17:O	-0.12	**-0.27**	-0.22	-0.19	-0.11	-0.19	-0.17	-0.18	-0.21	**-0.28**	**-0.35**
17:0ω7m/10Me 18:0	-0.13	**-0.33**	**-0.27**	**-0.29**	-0.21	**-0.30**	**-0.28**	**-0.29**	**-0.33**	**-0.38**	**-0.42**
18:2ω6,9	-0.14	**-0.32**	**-0.25**	**-0.24**	-0.20	**-0.24**	-0.21	**-0.26**	**-0.31**	**-0.34**	**-0.41**
18:1ω9c	-0.17	**-0.34**	**-0.29**	**-0.27**	-0.20	**-0.28**	-0.22	**-0.25**	**-0.29**	**-0.38**	**-0.42**
18:1ω7c	-0.19	**-0.34**	**-0.30**	**-0.27**	-0.21	**-0.29**	-0.22	**-0.25**	**-0.28**	**-0.38**	**-0.41**
18:1ω5c	-0.14	**-0.32**	**-0.27**	**-0.23**	**-0.24**	**-0.25**	**-0.24**	**-0.24**	**-0.29**	**-0.31**	**-0.36**
18:O	-0.18	**-0.27**	-0.20	-0.18	-0.12	-0.19	-0.15	-0.20	**-0.23**	**-0.30**	**-0.37**
18:1ω7c7m/10Me 19:1ω7c	-0.12	**-0.26**	-0.22	**-0.23**	-0.15	**-0.30**	-0.22	**-0.23**	**-0.28**	**-0.37**	**-0.34**
18:0ω8m/10Me 19:0	-0.13	**-0.24**	-0.22	-0.17	-0.17	-0.19	-0.17	-0.15	-0.19	**-0.27**	**-0.32**
cy19:0	-0.19	**-0.28**	**-0.25**	-0.21	-0.16	-0.20	-0.18	-0.22	**-0.26**	**-0.32**	**-0.38**

There were four significant canonical correlations between PLFA profiles and the soil properties (Table 2.19) that explained 37% of variation in the soil variables and 11% of the PLFA profiles. The variables for the soil properties in turn only explained 5% of the variance in PLFA profiles but the PLFA canonical variables explained 26% of the variance in the soil properties (Table 2.20). The correlations between the canonical variables of the PLFA profiles and soil properties formed no clear pattern. There were no significant canonical correlations between plant species biomass and PLFA profiles (Table 2.21). However, it is interesting to note that 14 of the PLFAs had correlations with the forb *Penstemon gormanii* higher than 0.4. There was no trend as to which PLFAs correlated and which did not. There were also no significant canonical correlations with the functional group biomass, but 20 of the 23 PLFAs were weakly correlated with graminoid biomass and 5 with forb biomass (Table 2.22).

Table 2.19. Test of whether the canonical correlations between the PLFA profiles and soil properties are equal to zero. If the canonical correlation is not equal to zero (i.e. reject the null hypothesis), than it is considered significant. Values in bold are significant ($p<0.05$).

Canonical Correlation	Proportion	Cumulative	df	F Value	Pr > F
1	0.343	0.343	322	**1.71**	**<.001**
2	0.151	0.502	286	**1.44**	**<.001**
3	0.100	0.602	252	**1.29**	**0.008**
4	0.087	0.688	220	**1.21**	**0.047**
5	0.075	0.764	190	1.12	0.176
6	0.064	0.827	162	1.02	0.439
7	0.045	0.872	136	0.91	0.737
8	0.037	0.910	112	0.83	0.873
9	0.029	0.939	90	0.75	0.950
10	0.024	0.963	70	0.66	0.980
11	0.018	0.981	52	0.55	0.993
12	0.010	0.991	36	0.42	0.998
13	0.006	0.997	22	0.33	0.998
14	0.003	1.000	10	0.21	0.994

Table 2.20. The proportion of variance (for the four canonical variables) of PLFA profiles and soil properties explained by their own canonical variables and those of the other set of variables.

	PLFA Canonical Variables	Soil Properties Canonical Variables
PLFA Profiles	0.11	0.05
Soil Variables	0.26	0.37

Table 2.21. Pearson's Correlation Coefficients between PLFA values and the biomass of each plant species. Values in bold are significant ($p<0.05$). Species names are given as the first three letters of the genus followed by the first three letters of the species name. Full names can be found in Table 2.1.

	AndSep	*AntRos*	*AraHol*	*ArtFri*	*AstAlp*	*AstrAlp*	*AstrWil*	*CalPur*	*CarSte*	*CasHyp*	*ElyCal*
i15:0	0.11	-0.02	0.00	**-0.30**	0.02	0.06	0.08	0.21	0.04	0.01	0.16
a15:0	0.15	-0.04	0.00	**-0.25**	0.01	0.07	0.04	0.21	0.05	-0.03	0.14
15:O	0.18	-0.03	0.02	**-0.27**	-0.13	0.12	0.16	**0.25**	0.15	0.03	0.10
i16:1ω7c	0.22	0.01	0.05	-0.15	-0.14	0.09	0.13	0.12	0.12	-0.02	-0.01
i16:0	0.14	-0.05	0.00	**-0.29**	-0.01	0.13	0.12	0.21	0.07	-0.01	0.11
16:1ω7c	0.01	0.11	0.15	**-0.23**	-0.15	**0.27**	0.19	-0.11	0.01	-0.19	**-0.34**
16:1ω5c	0.08	0.00	-0.02	**-0.34**	-0.10	0.16	0.10	0.13	0.02	0.11	-0.02
16:O	0.15	-0.03	0.00	**-0.31**	-0.04	0.16	0.11	0.16	0.06	0.03	0.05
i17:1ω8c	0.11	-0.02	0.00	**-0.29**	-0.11	0.14	0.10	0.16	0.06	0.02	0.00
16:0ω6m/10Me 17:0	0.08	-0.02	0.00	**-0.37**	0.00	0.14	0.11	**0.22**	0.04	0.05	0.10
i17:0	0.10	-0.01	-0.01	**-0.36**	-0.03	0.11	0.16	0.22	0.07	0.06	0.13
a17:0	0.15	-0.05	0.00	**-0.31**	-0.03	0.13	0.16	0.21	0.10	0.03	0.13
cy17:0	0.16	-0.04	0.01	**-0.32**	0.00	0.10	0.10	0.21	0.05	0.05	0.09
17:O	0.07	0.03	0.00	**-0.29**	-0.10	0.14	0.17	0.21	0.09	0.05	0.02
17:0ω7m/10Me 18:0	0.21	-0.02	-0.05	**-0.24**	-0.12	0.20	0.14	0.21	0.13	0.02	0.12
18:2ω6,9	0.18	-0.02	-0.05	**-0.26**	-0.07	0.17	0.15	0.07	0.08	0.10	-0.01
18:1ω9c	0.15	-0.02	-0.01	**-0.32**	-0.11	0.18	0.16	0.14	0.08	0.09	-0.05
18:1ω7c	0.16	-0.03	-0.02	**-0.29**	-0.10	0.17	0.10	0.13	0.07	0.08	-0.04
18:1ω5c	**0.23**	0.01	0.05	**-0.28**	-0.20	0.09	0.05	0.05	0.02	0.19	-0.06
18:O	0.16	-0.01	-0.02	**-0.34**	-0.07	0.15	0.19	0.19	0.07	0.08	0.11
18:1ω7c7m/10Me 19:1ω7c	0.18	-0.04	0.02	-0.14	-0.14	0.13	0.14	0.15	0.14	0.05	-0.04
18:0ω8m/10Me 19:0	0.16	-0.03	0.01	**-0.25**	-0.08	0.11	0.18	**0.24**	0.15	0.05	0.06
cy19:0	0.10	0.00	0.03	**-0.39**	-0.01	0.03	0.27	**0.24**	0.15	0.14	0.09

Table 2.21 cont. Pearson's Correlation Coefficients between PLFA values and the biomass of each plant species. Values in bold are significant ($p<0.05$). Species names are given as the first three letters of the genus followed by the first three letters of the species name. Full names can be found in Table 2.1.

	ElyTra	*EriCae*	*FesBra*	*OxyCam*	*PenGor*	*PlanCan*	*PoaGla*	*PotPro*	*PulLud*	*ZygEle*
i15:0	-0.08	0.20	**0.26**	0.16	**-0.48**	-0.01	**0.27**	**0.37**	0.20	0.09
a15:0	-0.07	0.20	**0.25**	0.11	**-0.47**	-0.02	**0.30**	**0.39**	0.21	0.08
15:O	-0.08	0.13	0.17	0.07	**-0.46**	-0.19	**0.40**	-0.02	**0.25**	0.13
i16:1ω7c	-0.02	0.12	0.12	-0.07	**-0.33**	-0.18	**0.37**	0.05	**0.26**	0.13
i16:0	-0.04	0.17	0.20	0.09	**-0.47**	-0.01	**0.34**	**0.33**	0.22	0.09
16:1ω7c	0.07	0.04	**0.26**	0.18	-0.16	-0.22	**0.30**	0.05	**-0.24**	0.01
16:1ω5c	0.00	**0.24**	**0.22**	0.17	**-0.43**	-0.05	**0.25**	**0.26**	**0.25**	0.13
16:O	-0.03	**0.23**	0.18	0.10	**-0.48**	-0.05	**0.32**	**0.35**	**0.26**	0.07
i17:1ω8c	0.00	0.17	**0.24**	0.13	**-0.41**	-0.10	**0.32**	**0.24**	0.20	0.08
16:0ω6m/10Me 17:0	-0.01	0.18	**0.27**	0.18	**-0.50**	-0.04	**0.33**	**0.26**	**0.24**	0.08
i17:0	-0.04	0.19	0.21	0.16	**-0.52**	-0.04	**0.33**	**0.26**	**0.24**	0.09
a17:0	-0.04	0.17	0.19	0.11	**-0.49**	-0.04	**0.35**	**0.28**	**0.22**	0.08
cy17:0	-0.04	**0.23**	0.18	0.10	**-0.52**	-0.04	**0.34**	**0.28**	**0.31**	0.13
17:O	0.00	0.10	0.16	0.09	**-0.44**	-0.12	**0.41**	-0.05	**0.23**	0.06
17:0ω7m/10Me 18:0	-0.03	0.06	0.15	0.09	**-0.40**	-0.13	**0.37**	-0.06	**0.24**	0.05
18:2ω6,9	-0.05	**0.25**	0.04	0.07	**-0.37**	0.04	**0.26**	0.19	**0.29**	0.09
18:1ω9c	0.02	0.18	0.16	0.07	**-0.44**	-0.08	**0.34**	0.22	**0.27**	0.10
18:1ω7c	0.03	0.20	0.16	0.06	**-0.42**	-0.07	**0.31**	**0.24**	**0.29**	0.12
18:1ω5c	0.08	0.22	-0.05	-0.02	**-0.32**	-0.08	0.17	0.10	**0.39**	0.08
18:O	-0.02	0.20	0.14	0.09	**-0.51**	-0.08	**0.36**	**0.24**	**0.27**	0.09
18:1ω7c7m/10Me 19:1ω7c	-0.04	0.10	-0.01	-0.13	**-0.32**	-0.09	**0.34**	0.01	**0.30**	0.16
18:0ω8m/10Me 19:0	-0.05	0.00	0.12	0.00	**-0.35**	-0.12	**0.35**	-0.09	**0.26**	0.10
cy19:0	-0.04	0.18	0.08	0.05	**-0.46**	-0.06	**0.28**	0.22	**0.29**	0.21

Table 2.22. Pearson's Correlation Coefficients between PLFA values and the biomass of each plant functional group. Values in bold are significant ($p<0.05$).

	Graminoids	Forbs	Legumes
i15:0	**0.25**	-0.18	0.16
a15:0	**0.28**	-0.14	0.11
15:O	**0.38**	**-0.23**	0.09
i16:1ω7c	**0.32**	-0.08	-0.06
i16:0	**0.31**	-0.19	0.11
16:1ω7c	**0.25**	-0.10	0.06
16:1ω5c	0.21	-0.17	0.18
16:O	**0.28**	-0.16	0.11
i17:1ω8c	**0.28**	-0.18	0.14
16:0ω6m/10Me 17:0	**0.30**	**-0.24**	0.19
i17:0	**0.31**	**-0.24**	0.18
a17:0	**0.33**	-0.21	0.12
cy17:0	**0.30**	-0.17	0.11
17:O	**0.36**	**-0.25**	0.11
17:0ω7m/10Me 18:0	**0.35**	**-0.22**	0.11
18:2ω6,9	0.21	-0.09	0.08
18:1ω9c	**0.29**	-0.18	0.09
18:1ω7c	**0.27**	-0.14	0.08
18:1ω5c	0.14	-0.05	-0.01
18:O	**0.30**	-0.06	-0.12
18:1ω7c7m/10Me 19:1ω7c	**0.34**	-0.20	0.02
18:0ω8m/10Me 19:0	**0.32**	-0.21	0.11
cy19:0	**0.30**	-0.06	-0.12

2.4 Discussion

The objective of this study was to determine the roles of plant functional groups in controlling the soil microbial community under changing environmental conditions. The most significant and the most surprising result from this work was that through the methods used in this study, the soil microbial community appears quite insensitive to plant functional group removals. Overall, there were few clear responses by the microbial community to many of the applied treatments.

2.4.1 Aboveground Plant Responses

Fertilizer significantly increased total community biomass, caused mostly by the increase in graminoids biomass. This increase is likely due to nutrient limitation in this system. Fertilization decreased the biomass of legumes, caused mostly by a decrease in *Oxytropis campestris*. Because legumes make up the smallest portion of this plant community this decrease did not affect the response of overall biomass. The response of legumes is consistent with other studies that have reported decreased legume biomass under high nutrient levels (Piper 1995, Turkington *et al.* 1998, Piper *et al.* 2005, Barthram *et al.* 2006). Legumes are typically outcompeted in higher nutrient situations (Jensen 1996, Høgh-Jensen and Schjoerring 1997). The biomass of forbs was not significantly changed by fertilization. However, individual species did respond to fertilization, with two species increasing and one decreasing. Within the graminoids and legumes there were also varying responses to fertilization. This indicates that species within a functional group have different roles in this system and species identity may play a more important role than functional group. This is further supported by species-level responses to fungicide, discussed below, while there were no functional group responses to fungicide.

It seems unusual that such a nutrient-poor system would not have a larger response to fertilizer addition. However, the plants in this grassland are subjected to stressful conditions (Grime 1977) such as low temperatures, low nutrients and low moisture. Therefore many, or most, of the plants in this system are likely 'stress-tolerators' (*sensu* Grime 1977), are slow-growing, and respond slowly, or not at all, to environmental changes. Similar results have been reported from other studies conducted in the Kluane Lake region (Turkington *et al.* 1998, Graham and Turkington 2000, Hicks and Turkington 2000, Turkington *et al.* 2002) and in another low-productivity grassland in Germany (Storm and Suss 2008). It is temping to argue that something other than nutrients are limiting in our system, and an obvious candidate is water. However, water was applied to all plots in equal amounts and is therefore unlikely to be limiting. A water addition experiment in the Kluane area showed no response of plant biomass to irrigation (Carrier and Krebs 2002).

The application of the fungicide benomyl had no impact on the biomass of any of the three functional groups, but two grasses, *Calamagrostis purpurascens* and *Elymus calderi* decreased in biomass. These species may be more mycorrhizae-dependent than others at the site. Benomyl application in herb-dominated vegetation in Australia (O'Connor *et al.* 2002), and grasslands in Norway (Dhillion and Gardsjord 2004) demonstrated that individual species responded differently to benomyl and the greatest decreases were in those species that were more mycorrhizae-dependent. Dhillion and Gardsjord (2004) also reported an increase in total plant biomass with benomyl application, a result that is inconsistent with that found in our study. The majority of plants at our site are either not highly dependent on mycorrhizae to support growth or they are slow to respond to benomyl application, such as suspected for fertilization.

Legumes, graminoids and forbs increased in biomass when other functional groups were removed. This increase could be the result of increased nutrient availability.

Nutrient availability is more likely than an increase in the availability of space as there is often open space present in this system. Graminoids and forbs did not increase when legumes were removed and the removal of legumes also lowered total plant biomass, even though legumes made up the smallest portion of total biomass in the grassland. Legumes have been shown to have a disproportionately large impact on ecosystem functions such as vegetation cover, plant composition and nitrogen retention (Spehn *et al.* 2005, Stephan *et al.* 2000).This is likely due to the benefit gained by other plants from the nitrogen fixation by legumes. The negative impact of removing legumes further supports nutrient limitation in this system. Tilman (1997) has demonstrated that the inclusion of legumes in a grassland has been shown to increase total plant biomass by almost 60%.

2.4.2 Soil Property Responses

Soil moisture declined in fertilized plots. This decrease is likely caused by the increase in plant biomass after fertilization. The application of benomyl had the opposite effect and resulted in both an increase in soil moisture and soil nitrogen. Because mycorrhizae enhance the ability of plants to uptake water and nutrients from the soil (Leake *et al.* 2005), more water and nitrogen might remain in the soil due to decreased uptake by plants with decreased mycorrhizae. Fertilization also lowered soil pH, a result consistent with Sarathchandra *et al*. (2001), as fertilizer causes an increase in H^+ ions available in the soil, making it more acidic (Lorenz *et al.* 1994).

After five years of removing plant functional groups from this system, soil carbon, soil moisture, pH and the majority of the nutrients measured were unaffected by removal treatments. Similar results were reported from a New Zealand grassland where plant functional groups (similar to the groups used in the present study) were removed for

three years (Wardle *et al.* 1999). These authors found no change in soil carbon, nitrogen or pH. Our study detected a decrease in potassium with the removal of graminoids. A similar result was reported by both Wardle *et al*. (1999) and Bezemer *et al.* (2006). It has been shown previously that grasses are good competitors for potassium (Gray *et al.* 1953, Mengel and Steffens 1985) so it seems that graminoids may be using more soil potassium compared to other functional groups.

Soil nitrate has been shown to increase with the presence of legumes and decreases with the presence of grasses (Tilman *et al.* 2002). This was not the case in my study where soil nitrate levels were unaffected by the removal of any of the three functional groups. In our sites soil nitrate may be controlled by larger-scale site variables than in the Tilman *et al.* (2002) system. Data from the European BIODEPTH experiment show soil nitrogen is more affected by site variables (i.e. soil type, land-use history) than by plant diversity treatments (Spehn *et al.* 2005). Other site variables not measured in my study could be keeping the total nitrogen constant despite the removal of functional groups, such as the base material for the soil. Removal of legumes caused a decrease in soil ammonium. This makes up a very small fraction of the total nitrogen in the system (~2% of total nitrogen) as nitrogen in this system is mainly in the form of nitrate.

If the soil properties in this system are influenced mostly by the quality or identity of the litter rather than by the nature of root exudates, it might be expected that a change in the soil properties caused by plant composition would take longer to appear (Wardle *et al.*1999). It has been suggested that in low-nutrient habitats with short growing seasons plants will produce fewer root exudates (Lavorel *et al.* 2007, De Deyn *et al.* 2008) and will produce lower quality litter that will take longer to decompose (Chapin III 2003). In addition, soil carbon, which was unaffected by treatments in the present study and in other diversity and functional group experiments (Wardle *et al.* 1999, Zak *et al.* 2003), is relatively resistant to short-term changes and is an important soil property

(Wardle *et al.* 1999, Buckley and Schmidt 2001). Thus, it is quite likely that 5 years of treatment may not have been adequate to effect changes in the plant community and consequently to manifest as changes in soil properties.

2.2.3 Soil Microbial Community Responses

Fertilization in this system decreased the metabolic diversity of the soil microbial community. This was seen as a decreased ability of the soil microbial community to metabolize amino acids, organic acids and a phenolic acid. This is consistent with Sarathchandra *et al*. (2001) who reported decreased functional diversity of the microbial community after nitrogen addition; but others have reported an increase in SIR values with fertilization (Grayston *et al.* 2004, Lagomarsino *et al.* 2007). This decrease could be caused by an increased dominance of one group of bacteria that thrive under higher nutrient conditions. It could also be the result of the increased potassium, an important element in the regulation of root exudation. Under high levels of potassium, maize was found to exude less amino acids, organic acids (including citric acid; one of the organic acids tested in the current experiment), and sugars (Kraffczyk *et al.* 1984). Decreased exudation of amino acids and organic acids could explain the decreased ability of the soil microbial community to metabolize these compounds under higher nutrient levels.

In the present study PLFA 18:1ω7c/10Me19:1ω7c was used an as indicator of total microbial biomass. Fertilization resulted in an increase of this PLFA. Because this PLFA is not indicative of any particular bacterial group this result could be due to an increase in abundance of any one of several groups of bacteria, with no one group exhibiting a significant increase on its own.

This lack of consistency between the SIR and PLFA profiles is not uncommon (Buyer and Drinkwater 1997, Bailey *et al.* 2002, Grayston *et al.* 2004). These techniques

measure different aspects of the microbial community. SIR targets the culturable fraction of the community and measures potential physiological ability or function while PLFA targets the structure of the microbial community and measures membrane lipid composition, which is not necessarily related to physiological ability (Kirk *et al.* 2004, Leckie 2005). A decrease in SIR and an increase in one PLFA in response to fertilization can be explained as an increase in overall abundance of bacteria (i.e. the increased PLFA) probably leading to a decrease in evenness (i.e. decreased ability to metabolize different carbon sources).

Application of fungicide had no effect on any measurement of the soil microbial community. This is surprising considering the strong effects mycorrhizae have been shown to have on both the above- and belowground systems (Reynolds *et al.* 2003, Leake *et al.* 2005, Artursson *et al.* 2006). A study using long-term application (7 years) of the fungicide benomyl found increased SIR (Smith *et al.* 2000), but no such effect was detected in the present study. Application of benomyl caused an increase in soil moisture and total nitrogen and it decreased the biomass of three plant species, all of which may be expected to influence the soil microbial community. However, there may be numerous other factors that may influence the impact of mycorrhizae, such as the composition of the mycorrhizae community (Leake *et al.* 2005, Artursson *et al.* 2006) and the composition of the soil bacteria community (Artursson *et al.* 2006). Because some mycorrhizae show strong host specificity (Reynolds *et al.* 2003, Leake *et al.* 2005) the plant species identity can be important and the level of plant functional group may be too coarse and create too much variation in response to detect a change. There is evidence for this, as two graminoids and one forb were decreased by fungicide, but there were no effects on the graminoids or the forbs as a group.

Removal of plant functional groups exerted very little influence on the soil microbial community in this experiment. The only significant effect was an increase in

the PLFA stress ratio of cy19:0 to 18:1ω7c when legumes were removed and fertilizer was not added; the addition of fertilizer cancelled out the negative effect of removing legumes. This 'stress' makes sense because we know that legumes can have a positive effect on microbial biomass (Stephan *et al.* 2000, Wardle *et al.* 2003, but see Niklaus *et al.* 2006). The removal of legumes also significantly decreased ammonium in the soil. Because the addition of fertilizer cancelled out the negative response it is possible that the increased stress of the microbial community is at least partially caused by the decrease in ammonium. Our results agree with studies that have shown increases in PLFA stress ratios in bacteria subjected to starvation (Kieft *et al.* 1994, 1997).

Removing graminoids lowered potassium but exerted no effect on the microbial measurements. Potassium is an important nutrient for regulation of root exudation (Kraffczyk *et al.* 1984) but the changes in potassium levels elicited here by the removal of graminoids were evidently too low to influence exudation, or, the effects of exudation may only be playing a minor role in this community.

The manipulations imposed on this system were quite dramatic: for five years an entire plant functional group was absent from designated plots. It is therefore highly interesting that so few treatment effects were detected. There are many factors that can potentially influence the soil microbial community: plant community composition (Stephan *et al.* 2000) plant species identity (Grayston *et al.* 1998), soil chemical and physical properties (Hamman *et al.* 2007), land use history (Buckley and Schmidt 2001), and the soil food web community (Wardle 2005). In this stress-tolerant habitat the main controlling factor for the soil microbial community may be something other than what we considered, or, could take a longer period of time to result in noticeable changes. Other studies have also shown a lack of response in the soil microbial community to changes in the plant community (Wardle *et al.* 1999, Buckley and Schmidt 2001, Wardle *et al.* 2003, Potthoff *et al.* 2006). It is also likely that plant diversity manipulations have

stronger effects on aboveground ecosystem properties than on belowground ecosystem properties (Spehn *et al.* 2005). The remaining plant functional groups in this system may have compensated for the missing group (Suding *et al.* 2006), which would imply the roles of the different groups are not unique. While the plant functional groups in this study are known to have characteristics that significantly affect ecosystem function (Hooper and Vitousek 1997, Hooper and Vitousek 1998, Díaz and Cabido 2001), individual plant species responses to fertilization and fungicide indicate that in this system plant species identity may be important and functional group resolution is too rough.

Another reason for the lack of response in the soil microbial community could be the methods used to measure the microbes. SIR and PLFA analysis are relatively broad scale compared to molecular methods (DNA-based fingerprints or DNA sequencing, Kirk *et al*. 2004, Leckie 2005). SIR and PLFA group microbes using broad criteria such as microbes capable of metabolizing similar carbon sources and Gram positive or Gram negative bacteria. If microbial changes occurred within the broad groups detected by SIR and PLFA they would not be picked up by the methods used in this study.

Wardle *et al.* (2003) created artificially assembled plant communities consisting of the same functional groups used in our study. They found no effect of plant functional group richness (1 or all 3) or of plant species richness within a functional group (1 or 3 species) on SIR or PLFA profiles. This is consistent with the limited effect of removing plant functional groups in the present study on soil microbial profiles. Wardle *et al* (1999) also did a 3-year functional group removal experiment, and they only demonstrated significant effects on the soil community when all plants were removed.

Two of the main methods by which plants can influence the soil microbial community are through litter inputs and root exudation. Root exudates are more readily decomposed by the microbial community than plant litter. Therefore if the microbial

community is more strongly controlled by litter inputs than root exudates it would be expected to take a longer time for changes in the plant community to result in changes in soil microbial community structure. This is very likely because some literature points to higher rates of root exudation in more productive systems with faster-growing plants (Lavorel *et al.* 2007, De Deyn *et al.* 2008), although there appears to be little experimental evidence. Another consequence of plants living in a system with a short growing season and adapted to a low-nutrient environment, is lower quality litter (Chapin III 2003). This would further lengthen the time required for litter to decompose and exert an effect on the soil microbial community.

2.4.4 Conclusion

Although plant functional groups do not seem to be strong regulators of the soil microbial community based on the results of this study, close consideration of the data convinces us to think otherwise. The results indicate that plant root exudation does not play a major role in this community. Removal of mycorrhizae and a decrease in potassium, both of which influence root exudation, did not affect the microbial community. If litter is the main controlling factor for the soil microbial community it may take a greater number of growing seasons for the effects of changing the plant composition to alter the soil environment. The Kluane grassland system may require a longer timeline to fully understand what is controlling the soil microbial community and I feel this site should be maintained as a long-term biodiversity study site. Laboratory tests of the levels of root exudation occurring in this system will also help to understand the controls of the soil microbial community.

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3 CONCLUSIONS AND FUTURE DIRECTIONS

An on-going debate in the field of biodiversity-ecosystem functioning research (Naeem *et al*. 2002, Mooney 2002) concerns the design of experiments and the interpretation of results. Nevertheless, it remains a vitally important area of ecological research (Naeem *et al*. 2002) whose goal is to investigate the relationship between diversity and ecosystem functions (Srivastava and Vellend 2005). Species loss remains as important an issue as it was in the early 1990s, and the environment has become a front-burner political issue. Many components of ecosystems and the roles of species within ecosystems are still poorly understood, but if management practices are to be effective they must be based on a solid base of scientific knowledge.

3.1 Value of long-term and broad scale experiments

It is always intriguing in ecological field experiments when there are few, or idiosyncratic, responses to imposed treatments. Such results may simply indicate that we need further understanding. In the research described here we detected limited effects of plant functional group removal on the soil microbial community. This might suggest that plants have limited impacts on soil community composition. But in contrast, if litter quality has a large role to play in structuring the soil community, we may not detect the consequences of plant species lost for many growing seasons. In addition, the effect of species complementarity on the positive relationship between plant diversity and biomass has been shown to increase over time (Cardinale *et al.* 2007). For these reasons, long term studies are especially important to advance BDEF research. This area of research was initiated because of a concern about global and local biodiversity loss, and many of the consequences of biodiversity loss may not manifest themselves

for several years or decades. In order to develop effective management practices with the goal of maintaining current ecosystems in a productive state there must be knowledge of connections between the components of the ecosystem (i.e. plant-soil-microbe) and how alteration of these connections will influence the ecosystem, not just in the next year, but in the next decade, or several decades. An excellent example of this type of experiment is the long-term Cedar Creek plots in Minnesota (Tilman *et al*. 1997) which have shown a positive relationship between productivity and plant diversity (Tilman *et al*. 2002).

Much variation is seen in BDEF studies (Balvanera *et al*. 2006). In order to determine if there are general patterns studies need to be comparable across broad-scales and varying landscapes. For example, the BIODEPTH project was established to compare BDEF patterns across multiple sites throughout Europe (Hector *et al*. 1999). This allowed examination of large-scale controls over ecosystems. For example, soil nitrogen was mainly controlled by site variables as opposed to plant diversity treatments (Spehn *et al*. 2005). However, a positive relationship between plant diversity and productivity emerged when site-to-site variation was accounted for (Hector *et al*. 2002). This gives robust support to an overall positive effect of plant species diversity on ecosystem function.

3.2 Designs of experiments

Both the BIODEPTH and Cedar Creek projects are based on random assemblage experimental designs; different levels of diversity were created by randomly drawing species out of a pool and planting communities from seed (Tilman *et al*. 1997, Hector *et al*. 1999). There are inherent problems with this design (Díaz *et al*. 2003) because artificial assemblages do not account for compensation by remaining species after one or more are removed and natural proportions of species are often not

incorporated into the design. There are benefits of random assemblage designs for BDEF studies. Researchers have more control over influences on the system (Díaz *et al*. 2003) and this reduces variation and makes it easier to detect patterns (Balvanera *et al*. 2006).

Removal experiments provide a complementary method of doing BDEF research. A removal design facilitates monitoring of the responses of species to the removal of some of their neighbours. The design accounts for natural proportions of species and more closely mimics real-life species loss scenarios. Removal experiments such as we used in our research, and that by Wardle *et al*. (1999), are good candidates as long-term studies to increase our understanding of BDEF relationships.

3.3 Studying the microbial community

When experimental treatments have been imposed continuously over the long term, this often permits us to investigate other components of the ecosystem that may not be feasible in a short-term experiment. For example, research on the soil microbial community's responses to changing plant diversity contributes to an enormous gap in our knowledge (Balvanera *et al*. 2006). BDEF researchers, by making microbial analysis a part of their work, can fill this gap and realize other areas where knowledge is lacking. As our study demonstrates, responses of soil microbial communities, especially in low-productivity systems, may not be apparent for multiple growing seasons and are therefore not suited to short-term projects. Studying the microbial community is crucial because, along with the micro- and macro-invertebrates, they are a vital link in energy flow through the detrital food web and back into the producer community. Soil food web structure can influence the rates of nutrient cycling (Berg *et al*. 2001) and the addition of soil fauna has been shown to increase plant growth and plant nutrient content, likely caused partially by grazing of soil microbes (Wardle 2002). The detrital food web is also

a resource subsidy to the aboveground community. Consumption of soil organisms by aboveground consumers supplies energy to the aboveground food web from the detrital food web (Polis and Strong 1996). This allows aboveground consumers to be maintained at a level unexplained solely by herbivore consumption, and consumer abundance affects abundance of and flow of energy through many components of the aboveground system (Polis 1991, Polis and Strong 1996).

Although they play an important role in ecosystem functions and have unexpected influences on the aboveground energy flow, the effects of plant diversity on the soil fauna and how those changes could impact the system are still unclear. Long-term studies are needed to fully see the impacts of plant species loss (Wardle *et al*. 1999), especially since changes in the soil fauna may not be apparent until treatments impact the soil microbial community, the base of the soil food web.

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APPENDIX

Table A.1. Test of whether the canonical correlations between plant species biomass and soil properties are equal to zero. If the canonical correlation is not equal to zero (i.e. reject the null hypothesis), then it is considered significant. Values in bold are significant ($p<0.05$). Proportion is the amount of variation in the data explained by that canonical correlation.

Canonical Correlation	Proportion	Cumulative	df	F Value	Pr > F
1	0.233	0.233	294	**1.36**	**0.001**
2	0.196	0.429	260	**1.22**	**0.027**
3	0.123	0.552	228	1.09	0.227
4	0.111	0.663	198	1.00	0.487
5	0.082	0.745	170	0.90	0.784
6	0.077	0.822	144	0.82	0.919
7	0.043	0.866	120	0.71	0.987
8	0.038	0.904	98	0.66	0.992
9	0.036	0.940	78	0.61	0.995
10	0.026	0.966	60	0.50	0.999
11	0.017	0.982	44	0.40	1.000
12	0.008	0.990	30	0.31	1.000
13	0.007	0.997	18	0.29	0.998
14	0.003	1.000	8	0.19	0.992

Table A.2. Test of whether the canonical correlations between plant guild biomass and soil properties are equal to zero. If the canonical correlation is not equal to zero (i.e. reject the null hypothesis), then it is considered significant. Values in bold are significant ($p<0.05$). Proportion is the amount of variation in the data explained by that canonical correlation.

Canonical Correlation	Proportion	Cumulative	df	**F Value**	**Pr > F**
1	0.705	0.705	42	**1.80**	**0.004**
2	0.178	0.883	26	0.95	0.543
3	0.117	1.000	12	0.83	0.620