LINKING PLANT COMMUNITIES TO SOIL MICROBIAL COMMUNITIES AND PROCESSES IN OLD-FIELDS

By

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ABSTRACT

LINKING PLANT COMMUNITIES TO SOIL MICROBIAL COMMUNITIES AND PROCESSES IN OLD-FIELDS

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The resources that support soil microbial communities are primarily derived from plants, so the soil microbial community should respond to changes in plant diversity or productivity, particularly if changes in the plant community affects the quality or quantity of available resources. I investigated the role of soil and plants on the structure and function of the soil microbial community by conducting observational and experimental studies and two manipulative greenhouse experiments.

I examined the relationship between plant diversity and productivity and soil microbial community structure and function along a topographic gradient in a successional old-field in Michigan. Variation in plant productivity was confounded by changes in plant community diversity and edaphic characteristics, so I could not determine which of these variables caused the observed changes in the soil microbial community.

To further investigate the relationship between the soil microbial community and plant species diversity, I sampled soils from a set of experimental grassland plant communities established as part of the BIODEPTH experiment at Silwood Park, England. Plant species diversity, functional group diversity, and species composition varied across treatments. I found that plant diversity significantly affected soil

microbial community structure. However, N-mineralization rates and microbial respiration responded to variation in plant community composition, but not diversity.

In a greenhouse experiment I examined how variation in soil fertility influenced the soil microbial community. I found that soil origin had strong effects on the structure and function of the soil microbial community. Higher fertility soils had higher organic nitrogen pools and microbial activities and more eukaryotes in the microbial community. In addition, the presence of *Andropogon gerardi* also affected the structure and function of the soil microbial community. However, the magnitude of the plant effect on soil microbial respiration was inversely related to soil fertility.

In a second greenhouse experiment I further explored the plant species effect on the soil microbial community. I found strong effects of both plant species identity and soil origin on the structure and function of the soil microbial community. In particular, the presence of a legume (*Trifolium pratense*) increased soil nitrogen cycling processes. Plant species identity had a small effect on soil microbial community structure, but it was dwarfed by the soil origin effect.

Results from these studies indicate that several aspects of the plant community, including diversity, composition and individual plant species identities, can strongly influence the structure and function of the soil microbial community. However, other environmental factors that affect soil quality can have strong and persistant effects on the soil microbial community.

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I would like to thank my advisor, Kay Gross, for her unlimited support, patience, and guidance over the last six years. She has been particularly understanding this past year as I have faced health problems that interfered with my work. I will be forever grateful to Kay. I would also like to thank the other members of my committee, Steve Hamilton, Mike Klug, and Gary Mittelbach for their guidance and help in shaping the path of my graduate career.

This work could not have been completed without the help of the members of Kay Gross' lab. I would particularly like to thank Carol Baker, Karen Tindall, and Jaime Fiordalis for their technical assistance and help with field work; and Bryan Foster, Andrea Corbett, Melissa McCormack, Heather Reynolds, Wendy Goodfriend, Greg Houseman, Rich Smith, and Sarah Emery for their intellectual feedback and companionship.

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Evolution course at the W. K. Kellogg Biological Station who helped with the initial soil and plant sampling for the field survey at Lux Arbor.

Living and working at the Kellogg Biological Station can be a lonely endeavor. For their fellowship, dinner groups, game nights, and great parties, I am grateful to my fellow graduates students, particularly Eric Thobaben, Tara Darcy, and Natalie Dubois. I enjoyed singing with Eric in the Bach Festival Chorus and talking about the trivial but fun aspects of our lives. Stefanie Whitmire has been my constant companion for the past four years and I could not have made it through without her. I am eternally grateful to Stefanie.

For their encouragement, love, and support throughout my life, I would like to thank my parents, Sharon and Gary Broughton. I could never have made it this far without them. My brother, Keith Broughton, has also shown constant support for my chosen path.

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CHAPTER 1

INTRODUCTION

The role of species diversity and composition in ecosystems is increasingly under scrutiny due to concerns about the potential impacts of the current rapid decline in the Earth's biodiversity. Species diversity may have important effects on key ecosystem functions like nutrient cycling, water quality, and productivity (Rosenzweig 1995, Tilman 1996). Recent investigations into the relationship between diversity and function have focused mainly on how changes in primary producers and consumers affect ecosystem processes (Schläpfer and Schmid 1999, Rosenzweig 1995). The interaction between aboveground and belowground (soil) communities in mediating these processes has been less studied (Schläpfer and Schmid 1999, Ohtonen *et al.* 1997). To understand controls on diversity and the role of diversity in ecosystem function it is important to understand the relationships among organisms in the ecosystem. While considerable attention has been paid to factors that affect the composition and function of communities of macroorganisms, very little is known about the factors that affect the structure of soil microbial communities (Ohtonen *et al.* 1997, Tiedje 1995).

Resources available to soil microorganisms are primarily derived from plants. Most of the carbon and nitrogen entering the soil matrix results from litterfall, root exudates, or root death (Paul and Clark 1996). As a result, the composition and productivity of the plant community influences the soil microbial community. Similarly, the productivity

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or diversity of the plant community may be affected by processes mediated by soil microorganisms (*e.g.* N-mineralization rates). By performing key steps in the cycling of nutrients (carbon, nitrogen, and phosphorus, among others), the soil microbial community plays an essential role in the functioning of terrestrial ecosystems (Paul and Clark 1996). In most temperate grassland and forest systems, plant growth is limited by nitrogen (or a combination of nitrogen and phosphorus, Shaver and Chapin 1980, Schmidt *et al.* 1997, Jonasson *et al.* 1999). The soil microbial community controls the release of inorganic nitrogen to plants; however, the soil microbial community is most often limited by carbon (Zak *et al.* 1994). Therefore, the rate at which limiting nutrients are made available to plants is likely to be influenced by the amount and quality of carbon available to soil microorganisms. Consequently, changes in the plant community likely will change the soil microbial community and potentially affect ecosystem function.

Thesis Overview

I am interested in the influence of the plant community on the structure of soil microbial communities and the processes they mediate. The challenge is to distinguish between the direct effects of plants (through changes in soil carbon inputs) and indirect effects (due to soil characteristics) on the structure of the soil microbial community. In this dissertation, I explore the relationship between the plant and soil microbial communities through a combination of observational studies and manipulative experiments at two different scales: the plant community scale and the individual plant

species scale. I addressed the following questions through my research: (1) How does plant productivity affect the activity and structure of the soil microbial community? (2) How does plant community diversity affect the structure and function of the soil microbial community? (3) How does plant community composition affect the structure and function of the soil microbial community? (4) How do soil factors influence the structure and function of the soil microbial community and can plants mediate soil effects?

Chapter 2 examines the first two questions on the relationships between plant community productivity and diversity and the soil microbial community. I compared patterns of diversity in the plant and soil microbial communities along a productivity gradient in an old field at the Lux Arbor Reserve at the W. K. Kellogg Biological Station in southwestern Michigan. The sampled gradient had a high diversity – low productivity plant community on the ridge top that graded into a low diversity –high productivity plant community down-slope. There was a strong positive relationship between above ground plant biomass and soil microbial respiration at the site. However, this association was confounded by changes in edaphic characteristics (moisture and nitrogen) and with the composition of the plant community that also varied along the gradient. Distinguishing plant from soil effects on the soil microbial community is a necessary first step in determining factors that structure the composition and affect the function of soil microbial communities. In the following chapters I describe the results of field and greenhouse experiments designed to investigate the independent effects of plants and soils on the soil microbial community.

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To address questions of how plant community diversity and composition influence the structure and function of the soil microbial community, I sampled soil communities from a series of experimental grassland plots from the BIODEPTH experiment at Silwood Park, England. This research is described in Chapter 3. In the BIODEPTH experiment, the number of plant species and the number of plant functional groups were varied to create plant communities with different plant diversities on the same soil. I found that plant community biomass, composition, and diversity all affected the composition and several functional traits of the soil microbial community.

Many field studies have detected differences among soil microbial communities sampled from sites with contrasting plant communities (Zak *et al.* 1994, Grayston and Campbell 1996, Chapter 2), but in these studies plant and soil effects are confounded. Soil in different sites has been shaped by a variety of factors besides differences in plant community composition, such as parent material, disturbance and management regimes. Therefore, differences among soil microbial communities sampled from sites with different plant communities cannot be attributed solely to the differences in the plant communities because the soil characteristics and histories also differ. To investigate the direct effects of soil origin on the soil microbial community (question 4), I conducted a greenhouse experiment that compared soils from six different local plant communities. The six sites differed in fertility, soil organic matter, and plant productivity, and these factors had detectable, correlated effects on soil processes. To determine if plants could mediate these differences, a common plant species,

Andropogon gerardi, was grown in each soil. Interestingly, when soil microbial communities were grown in the presence of this species, the effect of soil origin on some functions (*e.g.* microbial respiration and N-mineralization rates) was diminished.

Because soil microorganisms are dependent on carbon, and most available carbon in soil comes from plants, the identity of the plant species supplying carbon to the microorganisms may influence the structure and function of the soil microbial community. In Chapter 5, I describe the results of a greenhouse experiment in which I compared the effects of three plant species, grown in two distinct soils, on the structure and function of the soil microbial community. Soils from two of the old fields (high and low fertility) used in the previous experiment were planted with all combinations of three plant species common to local old fields. The experiment allowed me to determine that (1) different plant species can have unique effects on the structure and function of soil microbial communities and (2) the effects of different plant species on soil microbial community structure and function are non-additive.

This dissertation suggests that both the origin of the soil and the presence of a plant influence the structure and functioning of the soil microbial community. In Chapter 6 I discuss the overall conclusions from this collection of field and greenhouse studies.

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Field Sites

The greenhouse experiments presented in Chapters 4 and 5 of this dissertation were conducted with soils from six successional old-fields at the W. K. Kellogg Biological Station of Michigan State University in southwestern Michigan (Kalamazoo County; 42° 24' N, 85° 24' W). The sites varied in fertility, species richness, and dominant plant type, but all were located on Kalamazoo sandy loam soil (Table 1.1, Figure 1.1, Burbank *et al.* 1992). The six sites also differed in past land use and time since abandonment ranging in age from 20 to 50 years. McKay (MK) field was abandoned from agriculture in 1973; a section was plowed once in 1981 and then re-abandoned (Burbank *et al.* 1992). Both the Upper (UL) and Lower (LL) Louden fields were abandoned from agriculture in 1951 (Burbank *et al.* 1992). The Bailey (Ba) field site was farmed until ten years prior to this sampling (K.L. Gross, personal communication). The Pond Lab Orchard (PL) and Field K (FK) sites had been abandoned for at least twenty years (Foster 1996).

Soils from all six sites were used in the first greenhouse experiment (Chapter 4; Table 1.1, Figure 1.1). Soils from two of the six sites (a high and a low fertility site) were used for the second greenhouse experiment (Chapter 5; sites FK & UL; Table 1.1, Figure 1.1).

Table 1.1. Plant productivity and diversity of abandoned old fields from which soil was collected for the greenhouse experiments. Values for peak aboveground plant biomass (an estimate of primary productivity), species richness, and mean percent organic matter are expressed as mean ± standard deviations (n = 6). Values that are significantly different for a given variable based on Fisher's LSD test are indicated by different letters.

Site	Dominant Plant Form	Peak Plant Biomass (standing + litter, g/m^2)	Species diversity (#/m^2)	Soil Organic Matter (%)
MK	Grass	188 ± 16 a	2.2 ± 0.4 a	2.40 ± 0.43 a
UL	Forb	320 ± 23 b	15.8 ± 1.0 e	3.17 ± 0.23 b
Ba	Forb	424 ± 52 c	11.3 ± 1.0 d	3.03 ± 0.17 b
LL	Grass	432 ± 27 c	8.5 ± 0.4 c	3.84 ± 0.32 c
PL	Grass	480 ± 48 c	5.7 ± 0.8 b	3.63 ± 0.28 c
FK	Grass	592 ± 22 d	1.3 ± 0.2 a	3.84 ± 0.20 c

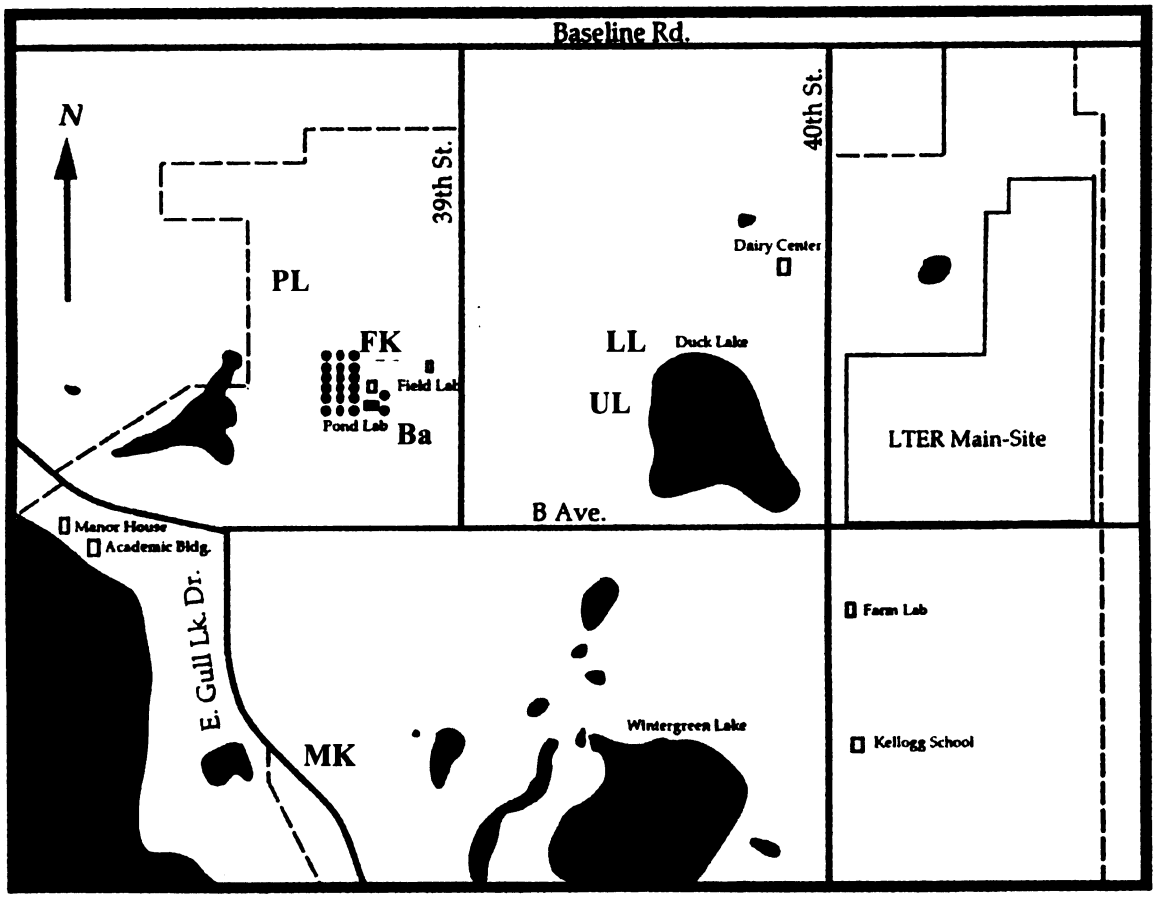

Figure 1.1. Map of the W. K. Kellogg Biological Station with codes illustrating the location of the six field sites (after Foster 1996). Table 1.1 describes these sites.

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CHAPTER 2

PATTERNS OF DIVERSITY IN PLANT AND SOIL MICROBIAL COMMUNITIES ALONG A PRODUCTIVITY GRADIENT IN A MICHIGAN OLD-FIELD

The following chapter was published as the article: Broughton, L.C. and K.L. Gross. 2000. Patterns of diversity in plant and soil microbial communities along a productivity gradient in a Michigan old-field. Oecologia 125: 420-427.

Introduction

A central question in ecology is why there are so many different organisms on the earth (Hutchinson 1959). Much of the work focusing on macroorganisms has emphasized the role of factors such as productivity, disturbance, energy, predation, resources, stochasticity, and colonization in determining the diversity of plant and animal communities (Rosenzweig 1995). Considerably less is known about what factors influence the abundance and diversity of microorganisms (Tiedje 1995). Microorganisms have rarely been incorporated into studies of mechanisms that may structure diversity-productivity relationships for plants and other macroorganisms (Ohtonen *et al.* 1997, Schläpfer and Schmid 1999). Although plant and soil communities are functionally linked, few studies have examined how patterns of diversity in plant and soil microbial communities co-vary.

Soil microbial communities are often limited by carbon (D. R. Zak *et al.* 1994) or nitrogen (Zak *et al.* 1990). Because the extant plant community is usually the main source for both of these resources, the composition and diversity of the soil microbial community may be closely associated with the plant community. It is difficult to assess composition and diversity of soil microbial communities. As a result, most investigations use techniques that assay different aspects of a subset of the microbial community. Two tools that are commonly used by ecologists to characterize the soil microbial community are the Biolog assay and fatty acid methyl ester (FAME) profiles. Biolog assays sole-carbon-source utilization by the microbial community and provides an index of functional diversity. Biolog profiles have been successfully used to differentiate soil microbial communities associated with different plant communities (*e.g.* J. C. Zak *et al.* 1994; Goodfriend 1998), especially when used in concert with other techniques, like fatty acid methyl ester (FAME) profiles (*e.g.* Buyer and Drinkwater 1997). FAME profiles reflect the phenotypic composition of the soil microbial community (Tunlid and White 1992) and can be used to distinguish among microbial communities with different compositions (Haack *et al.* 1995; Cavigelli *et al.* 1995).

I investigated the relationship between the structure and activity of the soil microbial community and its relationship to the plant community within an ecologically variable site. I hypothesized that the structure of the soil microbial community would vary at this site in relation to: (1) soil characteristics, (2) plant productivity, and (3) plant diversity. I investigated the relationship between the soil microbial community and

these three variates along a topographic productivity gradient in a mid-successional old-field in southwestern Michigan.

Methods

Site Description

The study site was in a mid-successional abandoned field at the Lux Arbor Reserve of the W. K. Kellogg Biological Station (KBS) in southwestern Michigan. This site had been abandoned approximately 25 years from agricultural production and during that period had not been grazed, burned, or otherwise managed. Successional fields in this area typically attain a stable species composition of herbaceous perennial 5 to 25 years after abandonment (Huberty *et al.* 1998). There has been no apparent change in the plant community at this site over the past ten years (K.L. Gross, personal communication). The study site was located along a gentle slope, approximately 15° from the top to the bottom of the hill, along which there were apparent changes in plant species composition and productivity. The soil at the site is Kalamazoo sandy loam soil and does not vary across the study area.

Sampling Design and Characterization of the Gradient

I established five parallel transects, 7.5 m apart, perpendicular to the slope of the hill and sampled soil and vegetation in seven 0.25 m^2 plots placed at 10 m intervals along

each transect (n = 7 per transect). To characterize the gradient, I measured light at ground level, soil moisture, soil inorganic nitrogen, and aboveground plant biomass.

Aboveground plant biomass and species composition were sampled in June 1996 by clipping the plants at ground level (0 cm above the soil surface), sorting by species, drying at 60°C for 48 hours, and weighing. To better estimate peak plant biomass, particularly at the more mesic end of the gradient, which was dominated by warm-season grasses, the same plots were re-clipped in July 1996. Samples were processed and treated as before. Peak plant biomass was calculated as June biomass + July biomass (both living and standing dead).

Light availability at ground level was determined prior to clipping in June and July. Measurements were made at midday (1100-1400 hours EDT) using a Sunfleck PAR Ceptometer (Decagon Devices, Inc.). I measured photosynthetically active radiation (PAR) in full sun 1 m above the plots and took four measurements of PAR at ground level within each plot (cardinal directions). I averaged these four data points to obtain an estimate of the percentage of full sunlight penetrating to ground level.

Soils were also sampled in June and July. For the soil analyses, I aggregated five 2.5 cm diameter by 10 cm deep soil cores taken from each 0.25 m^2 plot in an X-shaped pattern. Samples were placed in sealed plastic bags and kept on ice for up to 6 hours until they could be returned to the laboratory. There, they were passed through a 2-mm sieve and sub-sampled for gravimetric soil moisture and nitrogen content within 24

hours of sampling. Samples were kept at 4°C until processed. Gravimetric soil moisture was determined by weight loss after drying 10 to 15 g soil at 105°C for 24 hours. For the nitrogen assays, I extracted 20 g fresh soil in 100 ml 1 M KCl. These samples were shaken for 1 minute, settled for 24 hours at room temperature, and filtered through a 1 μm Gelman glass filter. The NO_3^- and NH_4^+ concentrations of the extracts were determined using an Alpkem Auto-Analyzer. The remaining soil was used to characterize the soil microbial community. Soil for FAME analyses was kept at -20°C until the fatty acids were extracted.

Characterization of the Soil Microbial Community

I characterized the soil microbial community from samples taken in July using a modified substrate-induced respiration (SIR) method, carbon-source utilization (Biolog) and FAME profiles. SIR assesses the microbial biomass of the soil microbial community and is a good indicator of microbial respiration (Hassink 1993). For SIR microbial biomass, soil slurries were shaken with and without glucose in Erlenmeyer flasks sealed with parafilm, and the headspace CO_2 was measured. For the control, I combined 25 g soil and 25 ml water in 125 ml Erlenmeyer flasks, and for the glucose-addition I substituted 25 ml 30 mg ml^{-1} glucose for the water. Both sets were shaken for 2 hours at 22°C. After 2 hours, I transferred 5 ml of the headspace gas to a serum vial and measured the initial CO_2 on an ADC series EGA infrared CO_2 gas analyzer

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(The Analytical Development Co. Ltd., Hoddesdon, Herts., UK). The flasks were shaken for another 38 hours and headspace CO_2 again measured.

For the Biolog assay, 1 g of fresh, sieved soil was shaken with 99 ml of 1% phosphate buffer solution for 20 min and 150 µl of the solution was transferred into each well of a GN Biolog microtiter plate (95 Carbon sources; Biolog, Inc., Hayward, CA 94545). Three replicate plates were inoculated for each plot. The plates were incubated at 25°C in the dark and optical densities were measured after 24 and 48 hours using an Emax precision microplate reader (Molecular Devices Corp., Menlo Park, CA). It is well known that inoculation densities from a standard amount of soil can vary for samples taken from different environments (Konopka *et al.* 1998). Optical density measures are often standardized to account for differences in inoculation densities; however, the standardizations have been criticized for not accurately reflecting growth across samples with different compositions (Konopka *et al.* 1998). Therefore, instead of standardizing optical densities, I chose to take advantage of differences and used average well color development (AWCD) from Biolog (corrected within plate for water reading) as an index of microbial respiration. Because profiles at 24 h and 48 h were similar only the results from the 48 h time point are presented here.

To obtain fatty acids for FAME analysis, I first extracted the lipids from whole soil samples for 2 h using a mixture of dichloromethane (DCM):methanol:phosphate buffer (1:2:0.8 v/v/v), following a modified Bligh-Dyer procedure (Bligh and Dyer 1959). I then saponified the samples using 1 ml NaOH (15% w/v) in methanol (50% v/v) at

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100°C for 30 min and methylated the sample with 2 ml 6N HCl in methanol at 80°C for 10 min. I extracted the fatty acid methyl esters into 1.25 ml (1:1 v/v) methyl-*tert*-butyl ether-hexane for 10 min and washed the extract with 3 ml 1.2% NaOH. FAME analyses were carried out using a HP 5890 series II gas chromatograph (Hewlett Packard Co., Palo Alto, CA) equipped with a 7673 autosampler and flame ionization detector (Microbial ID Inc., Newark, DL). Peaks were identified by comparison with an external standard. I performed all analyses on the fatty acid proportions of the total peak area to correct for differences in overall peak area.

I describe fatty acids using standard nomenclature where the total number of carbon atoms appears before the colon and the total number of C-C double bonds appears after it. Cyclo-propane analogs are indicated by "cyclo," and the location of the epoxy bond is indicated by a "c" followed by two numbers. If the cis or trans configuration is unknown, the word "at" is used. The number following "cis", "trans" or "at" indicates the location of the double bond in relation to the carboxyl end of the molecule. A number before "OH" indicates the location of the hydroxyl group in relation to the carboxyl end of the molecule. Those fatty acids with the same retention time are grouped as "sum in feature" and given a unique number designation.

Statistical Analyses

To obtain an index of productivity along the gradient I performed a principal components analysis (PCA) on those variates expected to be closely related to

productivity: light at ground level, gravimetric soil moisture, soil inorganic N, and peak plant biomass. To examine the productivity-diversity patterns, I regressed plant species diversity, number of carbon sources metabolized (Biolog), AWCD (from Biolog), and SIR microbial biomass against this index of productivity. Changes in plant community composition along the gradient were evaluated with indirect gradient correspondence analysis on species-specific aboveground plant biomass. To visually compare the plant and soil community patterns, I performed *K*-means cluster analysis on plant species-specific biomass data, carbon source utilization profiles (Biolog), and fatty acid methyl ester profiles (FAME). Multidimensional scaling (MDS) was used to predict the number of clusters expected for the Biolog and FAME profile data. As there were more parameters than samples for the Biolog data, I randomly split the parameters into two subgroups that were run through all analyses independently. The results of these two independent analyses were consistent, so the data from only one is presented.

Results

Characterization of the Gradient

Light at ground level, gravimetric soil moisture, soil inorganic N, and peak plant biomass all co-varied along the topographic gradient. Light availability at ground level (%PAR) decreased from 85% at the crest of the hill to 3% at the base of the hill. Gravimetric soil moisture increased from 15% to 36%, soil inorganic N increased from

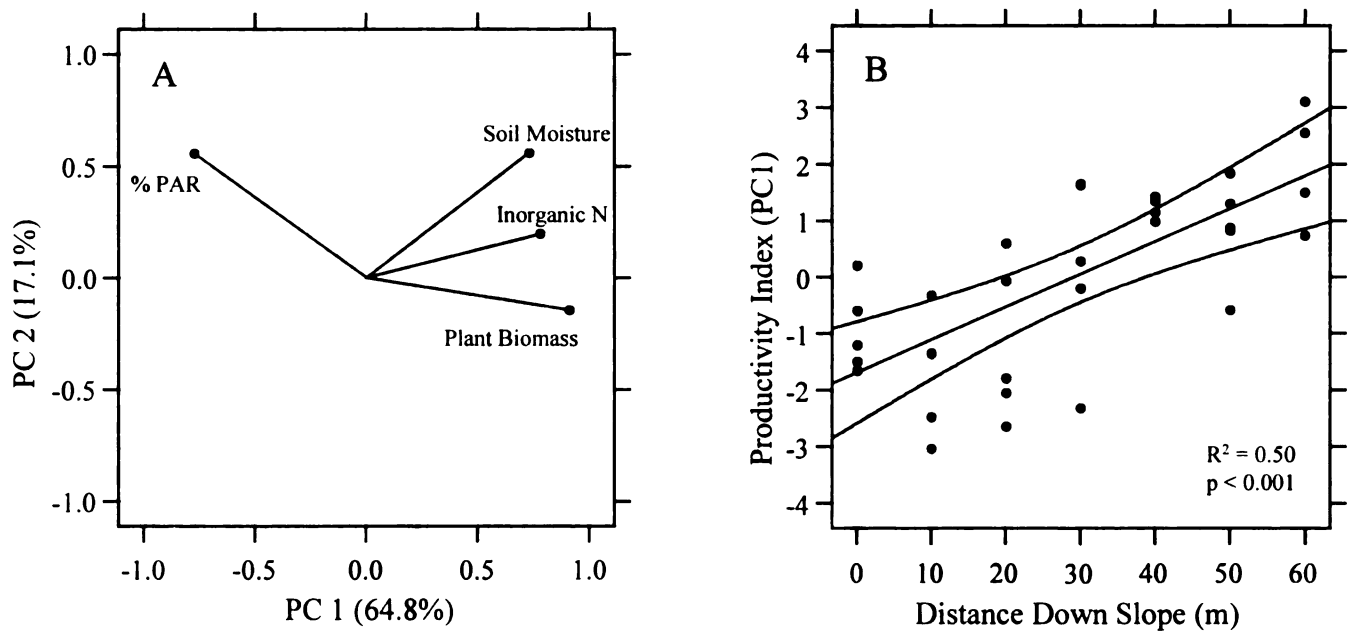

Figure 2.1. (A) Factor loadings plot for PC 1 and 2 from the PCA of the June 1996 sampling for light at ground level, soil moisture, soil inorganic N, and peak plant biomass; PC 1 and 2 account for 64.8% and 17.1% of the variation, respectively. Correlations between factor 1 and light ($r = -0.77$), moisture ($r = 0.73$), N ($r = 0.80$), and biomass ($r = 0.91$) were significant at $p < 0.001$; correlations between factor 2 and light ($r = 0.56$) and moisture ($r = 0.56$) were significant at $p < 0.005$. (B) The relationship between PC 1 and distance down slope for 34 of the 35 sampling points, $y = -1.69 + 0.058x$; Lines depict regression and 95% confidence intervals.

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3.29 to 18.58 μg N/ g dry soil, and above-ground plant biomass increased from 89.2 to 309.6 g m^{-2} along the slope.

I performed a PCA to obtain an index of productivity that incorporated these measures of resource availability plus above-ground plant biomass. One sample point at the bottom of the hill was excluded from the analysis because of an abnormally high inorganic soil N value (10-fold higher than the median). The first principal component based on resource levels measured in June and peak plant biomass (June + July) accounted for 64.8% of the variation in the data set (λ = 2.594). Soil moisture, nitrogen, and peak plant biomass were positively correlated with PC1, whereas light at ground level was negatively correlated with PC1 (Figure 2.1A). PC2 accounted for an additional 17.1% of the variation, but showed no pattern in relation to the gradient. Therefore, I used PC1 as an index of productivity in the remaining analyses (r^2= 0.50, Figure 2.1B). A PCA performed on the same variates from the July sampling was indistinguishable from the PCA on the June data, so I will present and use only the June results here.

Plant and Soil Microbial Community Relationships with Productivity

Plant species richness declined with increasing productivity (PC1) at this site, but productivity accounted for little of the variation in diversity (r^2 = 0.17, Figure 2.2A). This relationship was clearly driven by two low diversity points at the high end of the

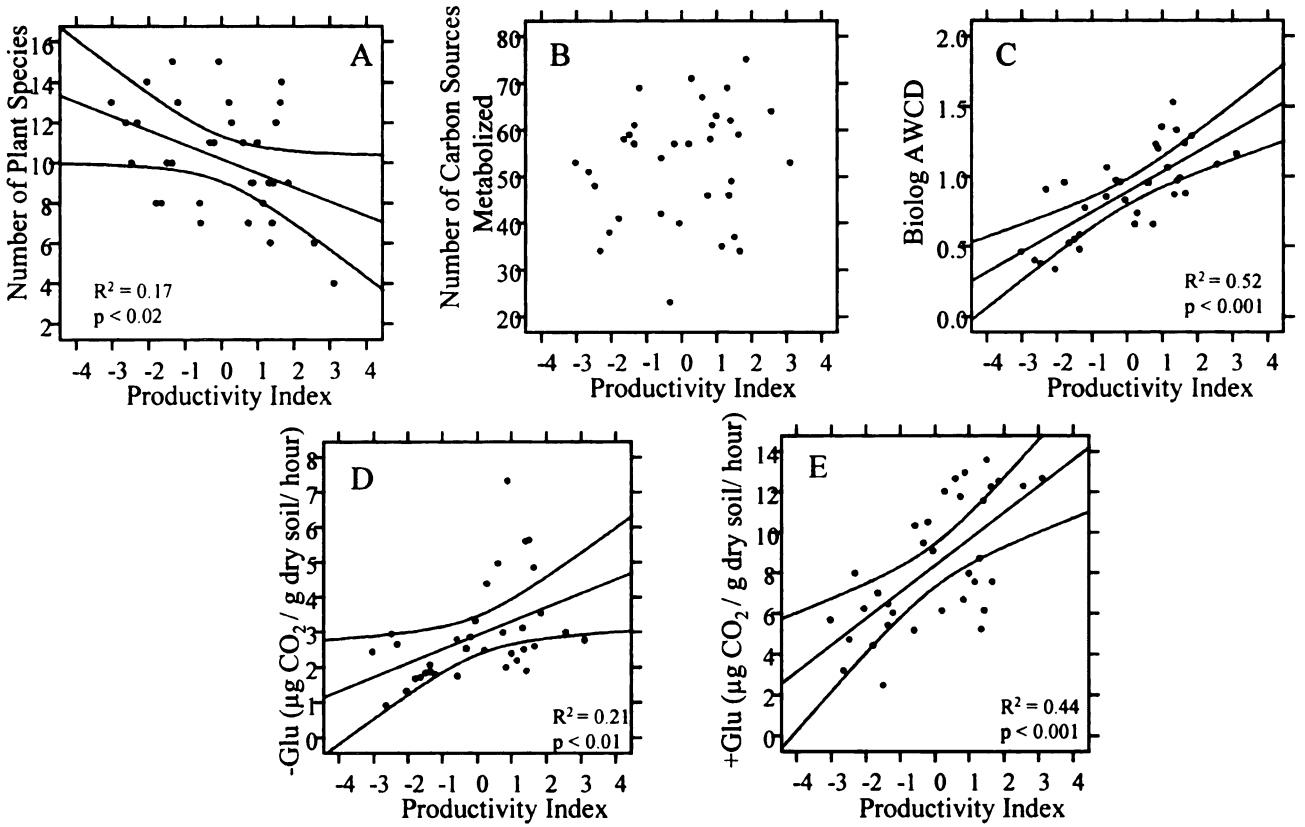

Figure 2.2. The relationship between productivity index and several variates describing the plant and microbial communities at this site. (A) Plant species richness, y = 10.18 - 0.71x; (B) The number of carbon sources metabolized by the soil microbes after 48 hours; (C) Biolog average well color development after 48 hours, y = 0.89 + 0.14x; (D)µg CO_2-C/ g dry soil/ hour evolved from SIR control after 40 hours, y = 0.54x – 1.55; (E) µg CO_2-C/ g dry soil/ hour evolved from the SIR glucose addition treatment after 40 hours, y = 0.33x – 2.80. Lines depict regression and 95% confidence intervals, n = 34.

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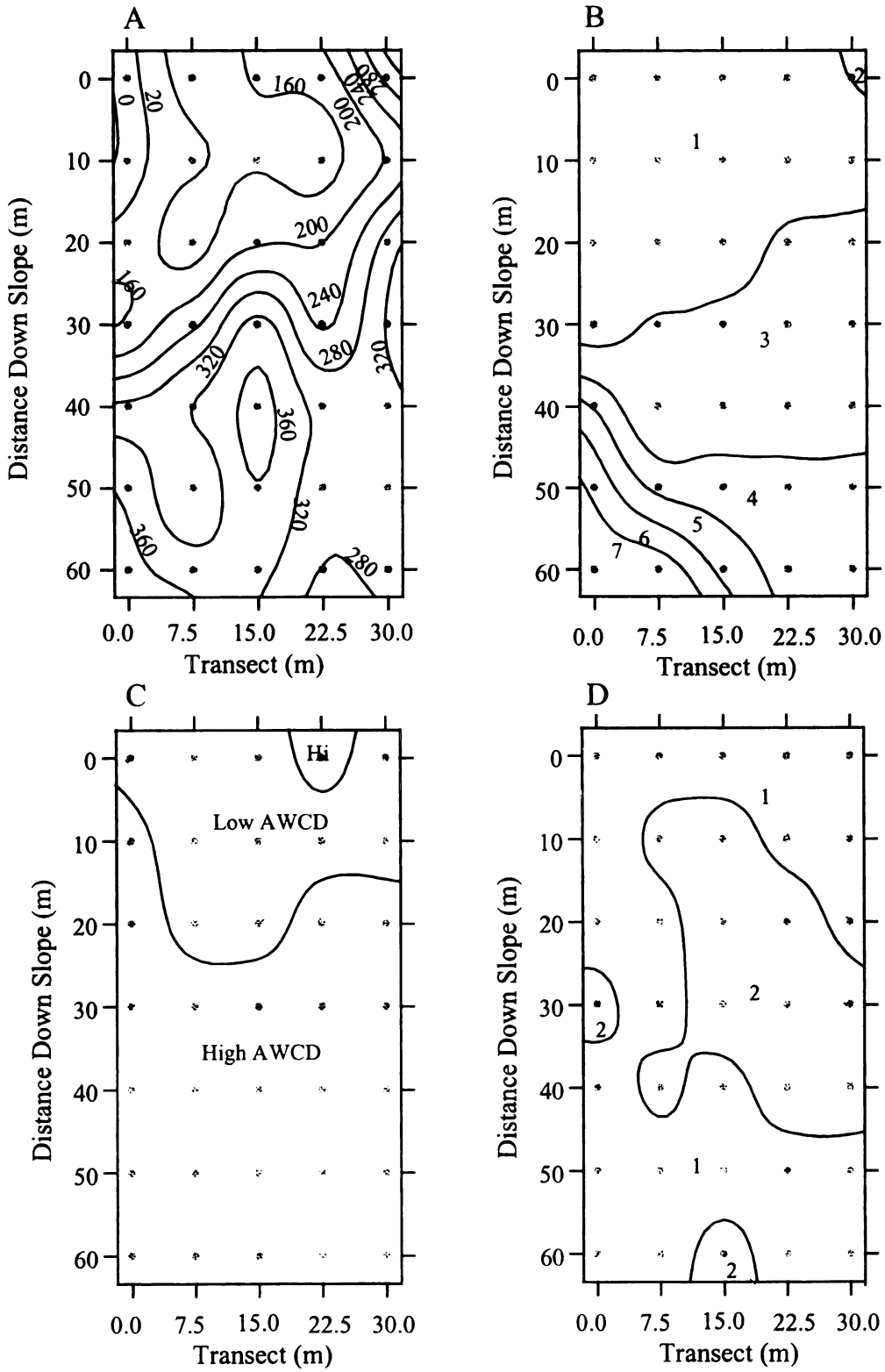
productivity gradient (Figure 2.2A). However, averaging over transects there was a clear decline in species richness across the gradient: an average of 13 species per plot (0.25 m^2) were found at the top of the hill, where productivity was lowest, while as few as 4 species per plot were found at the bottom of the hill, where productivity was highest (Figure 2.2A). Although the number of carbon sources metabolized by the microbial community varied from 23 to 75 across this gradient, there was no relationship between the number of carbon sources metabolized and productivity (Figure 2.2B). However, AWCD at 48 hours, which could be indicative of either microbial respiration or biomass, increased from 0.4 to 1.4 as productivity increased (r^2 = 0.52, Figure 2.2C). Similarly, SIR basal activity rate after 40 hours also increased along the gradient (r^2 = 0.21, Figure 2.2D), as did the rate at which glucose was consumed between 2 and 40 hours (r^2 = 0.44, Figure 2.2E). I used the SIR control and experimental treatments separately in this analysis as measures of microbial respiration because the SIR time course was insufficient to determine microbial biomass.

Compositional Shifts in the Plant and Soil Microbial Communities

To visually compare patterns in the plant and microbial communities, I performed separate *K*-means cluster analyses on the plant species biomass data and the Biolog and FAME profiles of the soil microbial communities. A plot of above-ground plant biomass across the study site clearly shows the topographic-productivity gradient (Figure 2.3A) and allows visual comparisons to plant diversity and microbial community measures (Figure 2.3B-D). There were compositional changes in the plant

Figure 2.3. Results from K-means cluster analysis evaluating the changes in production and composition of the plant and soil communities in relation to transect position at this site. (A) Contour plot of above-ground plant biomass, values are g/ m^2; (B) Plant species composition: cluster 1 has no dominant species, cluster 2 is dominated by *Rubus sp.*, cluster 3 by *Solidago canadensis*, cluster 4 by *S. canadensis* and *Poa pratense,* cluster 5 by *Agropyron repens*, cluster 6 by *Poa pratense*, and cluster 7 by *Polygonum amphibium* var. *emersum* (see Table 2.1 for species lists). (C) Biolog™ carbon source utilization profiles separated into two clusters based on AWCD; and (D) FAME profiles: cluster 2 had smaller proportions of 18:1 cis 9, 16:0, and summed in feature 9 (18:2 cis 9, 12 and 18:0 anteiso, Table 2.2) than cluster 1.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community along the productivity gradient. MDS on the species biomass data indicated three to seven valid clusters: I chose to illustrate seven in order to more completely portray the variation in plant composition (Figure 2.3B). The cluster analysis revealed an inverse relationship between plant community diversity and productivity. More specifically, the cluster analysis showed a shift in the plant community from the top of the slope, where there was a mixed community of forbs and no clearly dominant species (Table 2.1, cluster 1), to a mid-slope region dominated by perennial herbs (Table 2.1, clusters 3-4) to a low diversity community dominated by *Agropyron repens* near the bottom of the slope (Table 2.1, clusters 5-7, Figure 2.3B). Plots at the bottom of the hill with highest soil moisture and productivity were dominated by *Polygonum amphibium* var. *emersum* (water smartweed) (Figure 2.3B).

MDS of the Biolog profiles indicated two strong clusters; however, cluster formation relied solely on AWCD and not number or type of carbon sources. This is consistent with the soil microbial community - productivity relationship (Figure 2.2B,C). The number of carbon sources was not related to the productivity index (Figure 2.2B), while AWCD was significantly related to the productivity index (Figure 2.2C). Similarly, in the *K*-means cluster analysis, sites located at the top of the slope were characterized by lower AWCD, while the sites at the base of the hill had higher AWCD (Figure 2.3C). There was no difference in the number or types of carbon sources metabolized across the gradient (data not shown).

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Table 2.1. Plant species composition in the clusters from the *K*-means cluster analysis shown in Figure 2.3B. Plant species are listed from most common (by biomass) to least common. Only species that have a total biomass > 1 g for the cluster are listed. Nomenclature follows Gleason and Cronquist (1991).

Cluster (# plots)	Plant species
1 (13)	*Centaurea maculosa, Rubus occidentalis, Hieracium* sp., *Achillea millifolium, Rubus allegheniensis, Rumex acetosella, Poa compressa, Panicum* sp., *Agropyron repens, Solidago canadensis, Potentilla recta, Solidago graminifolia, Poa pratense, Aster pilosus, Plantago lanceolata, Phleum pratense, Cerastium vulgatum, Daucus carota, Trifolium pratense, Lespedeza capitata, Dactylis glomerata*
2 (2)	*Rubus occidentalis, Poa pratense, Solidago canadensis, Phleum pratense, Hieracium* sp., *Rumex acetosella, Agropyron repens, Polygonum amphibium* var. *emersum, Panicum* sp.
3 (9)	*Solidago canadensis, Rubus occidentalis, Poa pratense, Achillea millifolium, Monarda fistulosa, Phleum pratense, Poa compressa, Rumex acetosella, Agropyron repens, Daucus carota, Potentilla recta, Solidago graminifolia, Cornus racemosa, Apocynum cannabinum, Hieracium* sp., *Lespedeza capitata, Rubus allegheniensis, Centaurea maculosa, Trifolium pratense, Cerastium vulgatum, Taraxacum officinale, Rumex crispus, Hypericum perforatum*
4 (6)	*Poa pratense, Solidago canadensis, Agropyron repens, Achillea millifolium, Monarda fistulosa, Phleum pratense, Aster strigosa, Potentilla recta, Taraxacum officinale, Galium aparine, Daucus carota, Rumex crispus, Solidago graminifolia, Rumex acetosella*
5 (2)	*Agropyron repens, Solidago canadensis, Monarda fistulosa, Polygonum amphibium* var. *emersum, Rubus occidentalis, Solidago graminofolia, Galium aparine, Poa pratense, Achillea millifolium*
6 (1)	*Poa pratense, Agropyron repens, Solidago canadensis, Achillea millifolium, Polygonum amphibium* var. *emersum*
7 (2)	*Polygonum amphibium* var. *emersum, Agropyron repens, Polygonum persicaria, Solidago canadensis, Rumex acetosella, Poa pratense, Poa compressa*

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Although there was variation in the FAME profiles from these samples, MDS showed no distinct clusters in this data set along the productivity-diversity gradient. Fatty acids used in MDS and cluster analyses are listed in Table 2.2. When I forced the cluster analysis to create two clusters, soils from cluster 1 had larger proportions of fatty acids 18:1 cis 9, 16:0, and summed in feature 9 (18:2 cis 9, 12 and 18:0 anteiso, Table 2.2) than soils from the cluster 2 (Figure 2.3D). However, the cluster-based FAME profiles did not show any pattern concordant with peak plant biomass (Figure 2.3A), plant diversity (Figure 2.3B), or Biolog AWCD patterns (Figure 2.3C).

Discussion

I had hypothesized that the structure of the soil microbial community at this site would be related to plant community diversity, plant productivity, or soil characteristics. Because these three factors covaried at this site (Figure 2.1), I combined them into an index of productivity, but still could not detect any relation to the soil microbial community structure. Neither Biolog nor FAME assays of the soil microbial community were strongly related to variation in productivity. There were changes in the diversity and composition of the plant community associated with soil fertility and plant biomass; however, these differences in plant community composition had no detectable effect on the composition of the soil microbial community.

I did find evidence, however, that suggested the respiration (or biomass) of the soil microbial community varied in relation to plant productivity, paralleling the edaphic

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Table 2.2. Fatty acids used in *K*-means cluster analysis of FAME profiles.

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11:0 iso 3OH	17:1 cis 10
C9 dicarboxylic acid	17:0 cyclo
14:0	18:3 cis 6, 12, 14
15:0 iso	18:1 cis 9
15:0 anteiso	18:0
15:1 cis 7	19:0 cyclo C11-12
15:0	18:0 2OH
16:0 iso	20:4 cis
16:1 cis 9	20:0
16:1 cis 11	22:0
16:0	23:0
iso 17:1 G	22:0 2OH
anteiso 17:1 at 9	24:0
17:0 iso	23:0 2OH
summed feature 9: 18:2 cis 9, 12; 18:0 anteiso	summed feature 10: 18:1 cis 11; 18:1 trans 9; 18:1 trans 6

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gradient. I have two lines of evidence that support the idea that soil microbial community respiration (or biomass) increases with plant productivity at this site. First, I detected an increase in AWCD of the Biolog plates in relation to productivity (Figure 2.2C). Although Biolog AWCD is not a direct measure of respiration, it is strongly related to inoculum density (Garland and Mills 1991, Haack *et al.* 1995) and, as such, can be interpreted as an indicator of total number of bacteria (biomass). Conversely, two wells with the same inoculation density may differ in AWCD because of differences in microbial respiration (Konopka *et al.* 1998). In either case, the higher AWCD in the sites at the base of the hill indicates a more productive microbial community and this corresponds to areas along the gradient where the plant community is also the most productive. This is consistent with the higher amounts of N, moisture, and plant biomass at the base of the hill, which should make more C available to the microorganisms. Secondly, the modified SIR analysis indicates higher rates of respiration at the base of the hill where productivity was highest (Figure 2.2D,E).

Most studies that have reported changes in soil microbial community composition across community types have sampled sites that differed in plant species composition, productivity, and soil type. From these studies, it is unclear whether the plant community or the underlying edaphic factors are influencing the soil microbial community structure. For example, J. C. Zak *et al.* (1994) used Biolog to investigate changes in functional diversity of the soil microbial community from grasslands located along an elevational and moisture gradient in New Mexico. They found differences in the Biolog profiles of the soil microbial community from six distinct plant communities

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along this gradient. However, because soil characteristics also varied among these sites, it is not clear whether the differences in Biolog profiles were due to changes in plant community composition, edaphic factors, or some other variable. Similarly, Goodfriend (1998) found that Biolog distinguished among the soil microbial communities of eight sites representing a variety of wetlands in the southwestern United States. However, it was not clear whether plant community composition or edaphic characteristics were more important in influencing the grouping of those Biolog profiles into habitat types.

Several authors have argued that phospholipid fatty acids (PLFA's), a subset of fatty acids present in the phospholipid membrane, may provide a more sensitive indicator to distinguish among microbial communities. Phospholipid fatty acids break down easily in the soil and are thus thought to represent the active soil microbial community (Bossio and Scow 1998). Zelles *et al.* (1992) used PLFA profiles to compare soil microbial community patterns in grassland and agricultural fields under different management regimes and found that profiles differed among the different fields, but they did not distinguish between plant community and edaphic effects. Bossio *et al.* (1998) concluded that soil type has stronger effects on the soil microbial community structure than plant community type. They found that the addition of a cover crop (an increase in plant community diversity over time) was less influential in changing PLFA profiles than soil type. The differences in edaphic characteristics at this site, although substantial, were not as striking as differences between soil types would be.

There are several possible reasons why I did not detect changes in the soil microbial community composition along this gradient: (1) there is no connection between the structure of the soil microbial community and the soil characteristics, plant diversity, or plant productivity; (2) the soil microbial community structure is very stable and affected mainly by factors like long-term plant community composition or historical C inputs to the soil; (3) the spatial scale or time of year I sampled was inappropriate for detecting differences in the soil microbial community; or (4) the techniques I used to assay the soil microbial community were not specific enough to detect what differences were there.

The first two reasons seem unlikely because there should be a linkage between the microbial (consumer) community and the resources (plant carbon) that they utilize (Paul and Clark 1996). Much of the carbon available to soil microorganisms is being provided to the soil microorganisms each year by the extant plant community, and although this is a successional community, the plant community composition at this site has remained stable for the past decade (K. L. Gross, personal communication). Even if soil microbial community structure is not affected by plant community composition, increasing plant diversity or productivity should provide additional resources to the extant soil microbial community and thus influence soil microbial community composition. Additionally, past agricultural use at this site likely would have depleted soil C (Drinkwater *et al.* 1998, Robertson *et al.* 1993), and therefore made the current community inputs of C important in determining the structure and activity of the soil microbial community.

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It is difficult to know if sampling at a different time of year or spatial scale would have revealed associations between the plant and microbial communities at this site. Both temporal and spatial scales are important in the observation of ecological phenomena. I chose to sample in mid-summer on the assumption that at this time of year both the plant and soil microbial communities would be most active. Other researchers have revealed associations between plant and microbial communities using soil sampled in mid-summer. Bossio *et al.* (1998) detected differences in PLFA patterns of soil sampled in July from different agricultural treatments in California. Similarly, using carbon source utilization patterns, Westover *et al.* (1997) differentiated among rhizosphere soils sampled in August from several grass species in Washington.

It is possible that if this sampling had been done at a smaller, more fine-grained scale I might have detected associations between microorganisms and specific plant species. Westover *et al.* (1997) detected differences among soil microbial communities of rhizosphere soils of several grass species in both the field and greenhouse. Grayston and Campbell (1996) used Biolog to differentiate between the microbial communities of rhizosphere soils from two tree species, *Larix eurolepis* and *Picea sitchensis.* However, others have found associations between plant and microbial communities at spatial scales similar to the scale used in this study. Plant community composition is more likely to affect soil microbial community composition than plant diversity or productivity. A recent experimental study by Wardle *et al.* (1999) did detect differences in PLFA composition of the soil microbial community that were

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significantly related to the plant removal treatments. This suggests that 3-4 years of abandonment is sufficient to detect changes in the soil microbial community. Broughton *et al.* (2001) saw a similar relationship between plant community composition and PLFA patterns of the soil microbial community at the Silwood Park BIODEPTH site after three years (results presented in Chapter 3).

The inadequacy of tools to assess microbial diversity has been a long-standing limitation to this understanding of soil microbial communities (Tiedje 1995). While there are clearly limitations to the ability of functional tools such as Biolog and PLFA to distinguish among microbial communities, as noted above, a number of studies have used these tools to successfully differentiate among communities (Zelles *et al.* 1992, 1995; J. C. Zak *et al.* 1994; Goodfriend 1998). The development of molecular techniques more sensitive to shifts in composition may reveal natural shifts from one closely related microorganism to another along a gradient, just as there are shifts among closely related plant species along gradients. Additionally, molecular techniques may allow us to better address the roles of dominance and plasticity in structuring soil microbial communities.

The correlative nature of this study does not allow us to determine what factors may underlie the observed variation in the soil microbial community at this site. Despite our expectation that there should be a close association between the plant community and the soil microbial community, at this spatial scale (within a site), using these tools, I were not able to detect any association between plant community composition and soil

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microbial community composition. The similarity between patterns of plant biomass and soil microbial respiration is intriguing, however, and suggests that the resources that limit each of these communities co-vary. In contrast, the differences between patterns of plant diversity and soil microbial community structure suggest that different mechanisms are responsible for structuring diversity in these associated communities.

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CHAPTER 3

LINKING PLANT COMMUNITY DIVERSITY TO SOIL MICROBIAL COMMUNITIES: AN EXPERIMENTAL EVALUATION FROM THE BIODEPTH EXPERIMENT

These results have been submitted to *Journal of Ecology* in an article: Broughton, L.C., K.L. Gross, and A. Hector. 2001. Linking plant community diversity to soil microbial communities: an experimental evaluation from the BIODEPTH experiment. Journal of Ecology (submitted).

Introduction

Most studies to date investigating the relationship between species diversity and ecosystem function have focused on how changes in primary producers and consumers affect ecosystem processes (Schläpfer and Schmid 1999, Rosenzweig 1995). The interaction between above-ground and below-ground (soil) communities in mediating these processes has been less studied (Schläpfer and Schmid 1999, Ohtonen *et al.* 1997). While considerable attention has been paid to factors that affect the composition and function of communities of macroorganisms, very little is known about the factors that affect the structure of soil microbial communities (Ohtonen *et al.* 1997, Tiedje 1995).

Soil microorganisms play an essential role in the functioning of terrestrial ecosystems because the soil microbial community provides key steps in the cycling of nutrients

(carbon, nitrogen, and phosphorus, among others) through the ecosystem (Paul and Clark 1996). In most temperate grassland and forest systems, plant growth is limited by nitrogen (or a combination of nitrogen and phosphorus, Shaver and Chapin 1980, Schmidt *et al.* 1997, Jonasson *et al.* 1999). The soil microbial community controls the release of inorganic nitrogen to plants; however, the soil microbial community is most often limited by carbon. Therefore, the rate at which limiting nutrients are made available to plants is likely to be influenced by both the amount and quality of carbon provided by plants and available to soil microorganisms.

Because plant species differ in carbon content and quality, plant species identity has the potential to affect nutrient process rates through litter quality effects, which consequently affect the soil microbial community (Paul and Clark 1996, Wardle and Giller 1996). As a result, the composition and productivity of the plant community may influence the soil microbial community. Similarly, differences in the productivity or diversity of the plant community may be affected by processes mediated by soil microorganisms (*e.g.* N-mineralization rates). Consequently, changes in the plant community and the resulting change in the soil microbial community potentially affect ecosystem function.

To determine the effect of plant community diversity on soil microbial community diversity and processes, I studied the soil microbial community in experimental plant communities at the BIODEPTH site in Silwood Park, UK, where plant community structure and diversity were experimentally manipulated. I asked the following

questions: (1) How does plant community diversity affect the structure of the soil microbial community? (2) How does plant community composition affect the structure of the soil microbial community? (3) How does plant productivity affect the relationship between plant community diversity and the soil microbial community? and (4) Do specific functional groups or plant species have detectable effects on the soil microbial community?

Materials and Methods

Site

The study was conducted at the Imperial College site at Silwood Park, Ascot, UK (National Grid Reference 51°22'N, 00°37'W) and was part of the BIODEPTH experimental network of sites (BIODiversity and Ecosystem Processes in Terrestrial Herbaceous systems, Hector *et al.* 1999). The site was previously used for horse-grazing and has sandy-loam soil with an average pH of 5.26. In Fall 1995, the field was fenced, herbicided (Round Up, Dow Elanco), and tilled (Hector *et al.* 2000). The soil was fumigated in April 1996 with methyl bromide (Check Fumigation Ltd., Reading, UK) to remove the soil seed bank. Fumigation should also have killed much of the soil microbial community. In May 1996, two replicate blocks each with 33 plant assemblages, plus no-plant controls, were established in 2 x 2 m plots. The assemblages consisted of different combinations of plant species that varied in species richness and functional group richness (Hector *et al.* 1999). The species sown were all

herbaceous perennials representative of grassland species common to this part of England. Maximum species richness was 11 species per 2 m x 2 m quadrat, the average number of species in the area. Plant species were classified into one of three functional groups: legumes, non-leguminous forbs, and grasses. To address concerns about individual species effects on species richness curves (see Huston 1997), species thought to have strong effects on productivity were included in all mixtures. To minimize plant biomass effects, all mixtures included at least one grass species. This limits our ability to detect the effects of individual plant species and to evaluate the effects of grasses. Plant assemblages were maintained by hand-weeding for all 4 years of the experiment. Several undisturbed reference plots were also established adjacent to the manipulated plots. Peak plant biomass was clipped 5 cm above-ground level both years to provide an estimate of annual net primary productivity (Hector *et al.* 1999).

Soil Sampling and Analysis

To determine the relationship between plant diversity and the soil microbial community, I selected a subset of plots, encompassing the full range of species and functional group diversity, to sample for soil and microbial characteristics. In October of year 3 of the experiment (1998) I sampled soils from 28 plots (2 replicates of 14 different plant compositions, Table 3.1). The following year (September 1999, year 4), I sampled soils from 36 plots (2 replicates of 18 different plant compositions, Table 3.1). These included 12 of the 14 plots sampled in year 3, plus an additional 6 plots to expand the coverage of the species richness gradient. I were unable to re-sample the

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Table 3.1. (A) Plant species pool used in establishing the experimental communities and (B) the plant communities sampled for the soil microbial community. Plots sampled only in year 3 (1998) of the experiment are in italics. The control and *Rumex* plots were not maintained through year 4 and could not be re-sampled. Plots sampled in both years are indicated in bold. Other plots were sampled only in year 4 (1999) of the experiment. Communities are grouped by number of plant functional groups (FG) present.

(a)

Grasses	Abbrev.	Legumes	Abbrev.	Forbs	Abbrev.
Agrostis capillaris	AgC	*Lotus corniculatus*	LC	*Achillea millefolium*	AM
Alopecurus pratensis	AP	*Medicago lupulina*	ML	*Cerastium fontanum*	CF
Anthoxanthum odoratum	AO	*Trifolium repens*	TR	*Hypochaeris radicata*	HR
Arrhenatherum elatius	AE	*Trifolium pratense*	TP	*Plantago lanceolata*	PL
Cynosurus cristatus	CC	*Vicia hirsuta*	VH	*Potentilla erecta*	PE
Dactylis glomerata	DG	*Vicia sativa*	VS	*Rumex acetosa*	RA
Festuca rubra	FR	*Vicia tetrasperma*	VT	*Stellaria graminea*	SG
Holcus lanatus	HL			*Taraxacum officinale*	TO
Luzulla campestris	LC			*Veronica chamaedrys*	VC
Phleum pratense	PhP				
Trisetum flavescens	TF				

Table 3.1 (cont'd).

(b) Trt Code	0 FG	Trt Code	1 FG	Trt Code	2 FG	Trt Code	3 FG
Co	*co*	**1**	**AE**	15	AE, LC	**Ref**	**reference**
	n-	**2**	**FR**				**plots**
	tro	**3**	**HR**	16	AE, FR, LC,		
	ls	*4*	*RA*		TR	**11**	**AE, TR,**
		5	**LC**				**RA, HR**
		6	**TR**	18	AgC, AE, FR,		
					HL, AM, HR,	**12**	**FR, AE,**
		13	AgC, FR,		RA, PL		**LC, TR,**
			HL, AE				**HL, RA,**
				17	AgC, AE, FR,		**PL, AM**
		14	AgC, AE,		HL, TR, LC,		
			FR, HL,		TP, VS	19	HL, AgC,
			AP, AO,				LC, TR, FR,
			CC, TF	**8**	**AgC, AE,**		PL, RA, HR
					FR, HL, RA,		
		7	**AgC, AE,**		**PL, AM, SG,**	**10**	**AgC, AE,**
			FR, HL,		**VC, PE, TO**		**FR, HL,**
			AP, AO,				**LC, TR,**
			CC, TF,	**9**	**AgC, AE,**		**AM, CF,**
			DG, PhP,		**FR, HL, TR,**		**HR, PL,**
			LZ		**LC, TP, VS,**		**RA**
					ML, VH, VT		

control (no plant) and *Rumex* monoculture plots because the plots were not maintained in the fourth year. In year 4, I focused on a subset of the soil and microbial variables. In both years the sampled plots included undisturbed reference plots and combinations of 4, 8, and 11 species varying from 1 to 3 functional groups.

I sampled soil to a depth of 10 cm, then sieved the sample through a 3.35 mm sieve, and stored it in sealed plastic bags at 4°C until analyzed. All analyses were done at the W.K. Kellogg Biological Station of Michigan State University within 3 days of sampling.

I determined gravimetric soil moisture for each sample by drying 10 g soil at 105°C for 48 hours (Nelson and Sommers 1982). A subsample of the dried soil was ashed at 500°C for 4 hours to determine organic matter content (Nelson and Sommers 1982). Soil pH was determined using a Corning pH meter 420 after mixing 5 g of air-dried soil in 50 ml millipure H_2O. For nitrogen analyses, I extracted 20 g of fresh soil in 100 ml 1M KCl. The samples were shaken for 1 min and allowed to settle for 24 h at room temperature. The supernatant mixture was filtered through a 1-μm Gelman glass-fiber filter and NO_3^- and NH_4^+ concentrations were measured using Alpkem auto-analyzer. To determine potential N-mineralization and nitrification rates, a companion 20 g sample was incubated for 21 days at 25°C and 15% humidity and then extracted using the same methods as above.

I determined microbial biomass using the chloroform fumigation incubation method (Paul *et al.* 1999b). Two 25 g soil samples were pre-incubated for 5 days then one sample was fumigated with chloroform for 24 hours to kill the microorganisms. After a vacuum was created and the chloroform evaporated, 0.5 g of original soil was added to both samples. I measured initial headspace CO_2 and accumulated CO_2 after 10 days on an ADC series EGA infrared CO_2 gas analyzer (The Analytical Development Co. Ltd., Hoddesdon, Herts., UK). I calculated microbial biomass as [1.73 * (10 day accumulated CO_2-C – initial CO_2-C for the fumigated samples) – 0.56 * (10 day accumulated CO_2-C – initial CO_2-C for the control samples)] (Paul *et al.* 1999b). To determine microbial respiration I used a separate set of 10 g soil samples that were pre-incubated 5 days in a 160 ml glass qorpak bottle. I measured initial headspace CO_2 and accumulated CO_2 after 1 and 5 days and calculated the rate of CO_2-C respired per day.

Community-level physiological profiles (CLPP) were determined using both Biolog and Ecolog plates (Biolog, Inc., Hayward, Calif., USA). For both assays, 1 g of fresh, sieved soil was shaken with 99 ml 1% phosphate buffer solution for 20 min. 150 µl of the mixture was transferred to each well of the microtiter plate (GN Biolog, 95 Carbon sources + 1 non-Carbon control; or Ecolog, 3 replicates of 31 Carbon sources + 1 non-Carbon control). The plates were incubated in the dark at 25 °C and optical densities were measured at 14 h intervals from 0 h to 64 h using an Emax precision microplate reader (Molecular Devices Corp., Menlo Park, Calif., USA). Because the 5 incubation

times gave consistent results and the Biolog and Ecolog plates were similar in their results, I present here data only from the 64 h Ecolog measurements.

For the PLFA analysis, I extracted lipids from 6 g whole soil samples for 2 h using a mixture of dichloromethane (DCM): methanol: phosphate buffer (1:2:0.8 v/v/v), following a modified Bligh-Dyer procedure (Bligh and Dyer 1959). Phase separation was achieved by adding DCM and saturated sodium bromide solution (1:4 v/v). I isolated the phospholipid fatty acids from the dried lipid extracts by solid phase extraction. The lipid material was added to a polar column consisting of 100 mg silica (Varian Bond Elut LRC Columns, Product # 1211-3010). Lipids of low or intermediate polarity were eluted with chloroform and acetone and discarded. Subsequently, phospholipid fatty acids were eluted with 1.5 ml methanol for preparation of fatty acid methyl esters. I saponified the samples using 1 ml NaOH (15% w/v) in methanol (50% v/v) at 100°C for 30 min and methylated the samples with 2 ml 6M HCl in methanol at 80°C for 10 min. I extracted the fatty acid methyl esters into 1.25 ml (1:1 v/v) methyl-tert-butyl etherhexane for 10 min and washed the extract with 3 ml 1.2% NaOH. Phospholipid amounts were measured using a HP 5890 series II gas chromatograph (Hewlett Packard Co., Palo Alto, Calif., USA) equipped with a 7673 autosampler and flame ionization detector (Microbial ID Inc., Newark, Del., USA). Peaks were identified by comparison with an external standard. I performed all analyses on the phospholipid fatty acid proportions of the total peak area to correct for differences in overall peak area.

I describe fatty acids using standard nomenclature where the total number of carbon atoms appears before the colon and the total number of C-C bonds appears after it. Cyclo-propane analogs are indicated by "cyclo," and the location of the epoxy bond is indicated by a "c" followed by two numbers. If the cis or trans configuration is unknown, the word "at" is used. The number following "cis," "trans," or "at" indicates the location of the double bond in relation to the carboxyl end of the molecule. Fatty acids with the same retention time are grouped as "sum in feature" and given a unique number designation. For analysis, I included only those phospholipid fatty acids that were present in all samples and reported their abundance as the proportion of the total phospholipid fatty acid amount in each sample. Of the 25 lipids detected, 16 phospholipid fatty acids met this criterion in both years.

Statistical Analysis

Using an analysis of variance (ANOVA) model and sequential (Type 1) sums of squares, I tested the effect of number of species (richness), number of functional groups, block, and plant community composition (MIXTURE) on the following response variables: pH, soil moisture, soil organic matter, total N, N-mineralization rate, nitrification rate, number of culturable bacteria, microbial respiration, microbial biomass, CLPP, and PLFA profiles (Table 2). The effect of number of species was tested separately from the effect of number of functional groups; therefore, both tests were non-conservative. Hector *et al.* (1999) found that aboveground plant biomass increased with increased numbers of plant species at this site (and most of the other

Table 3.2. Analysis of variance model with sequential sums of squares used to evaluate the effects of the plant community manipulations on soil and microbial community parameters.

Source of variation	Mean square	Variance ratio
Main GLM model (ANOVA):		
Block	MS_B	MS_B / MS_{B*M}
Diversity	MS_D	MS_D / MS_M
Mixture	MS_M	MS_M / MS_{B*M}
Block*Mixture	MS_{B*M}	--
[Where Diversity is 1) species diversity, 2) functional group diversity, 3) presence/absence of legumes, or 4) presence/absence of forbs]		
Plant Biomass as a covariate (ANCOVA):		
Plant Biomass (covar)	MS_C	MS_C / MS_M
Block	MS_B	MS_B / MS_{B*M}
Diversity	MS_D	MS_D / MS_M
Mixture	MS_M	MS_M / MS_{B*M}
Block*Mixture	MS_{B*M}	--

BIODEPTH sites, species number, $p<0.001$; functional group number, $p<0.01$). Consequently, I used aboveground plant biomass as a covariate in these analyses to investigate the influence of plant diversity independent of plant biomass effects (Table 3.2).

I used Principal Components Analysis (PCA) to determine if there was an underlying structure to the soil microbial community as detected by CLPP and PLFA. I used these principal component axes as response variables in the ANOVA and ANCOVA to test for main treatment effects of plant species richness, functional group richness, and community composition on soil microbial community metabolic activity and structure.

Results

Legacies of Site Preparation

The effect on the soil microbial community of soil fumigation with methyl bromide during site preparation is clearly illustrated by the soil microbial biomass data. In both years, the undisturbed (non-fumigated) reference plots had the highest microbial biomass, as much as four times higher than the manipulated plots (Figure 3.1). Even after 4 years (1999 sampling), the biomass of the soil microbial communities had not recovered from the disturbance effect of fumigation (Figure 3.1B).

Effects of Species Richness and Functional Group Richness Treatments on the Soil Microbial Community

The initial analyses of these data excluded aboveground plant biomass as a covariate and I found little effect of species richness or functional group richness on soil or microbial community parameters (Table 3.3A). There were a few exceptions. In year 3, the numbers of colony forming units (CFU's) after 48 hours increased at higher species richness (Figure 3.2A, r^2=0.29, p< 0.01). Similarly, number of CFU's after 48 hours increased at higher functional group richness (Figure 3.2B, r^2=0.29, p< 0.01). However, neither of these relationships was significant when aboveground plant biomass was used as a covariate in the year 3 analysis (Table 3.3A). The number of culturable bacteria was significantly correlated with aboveground plant biomass in year

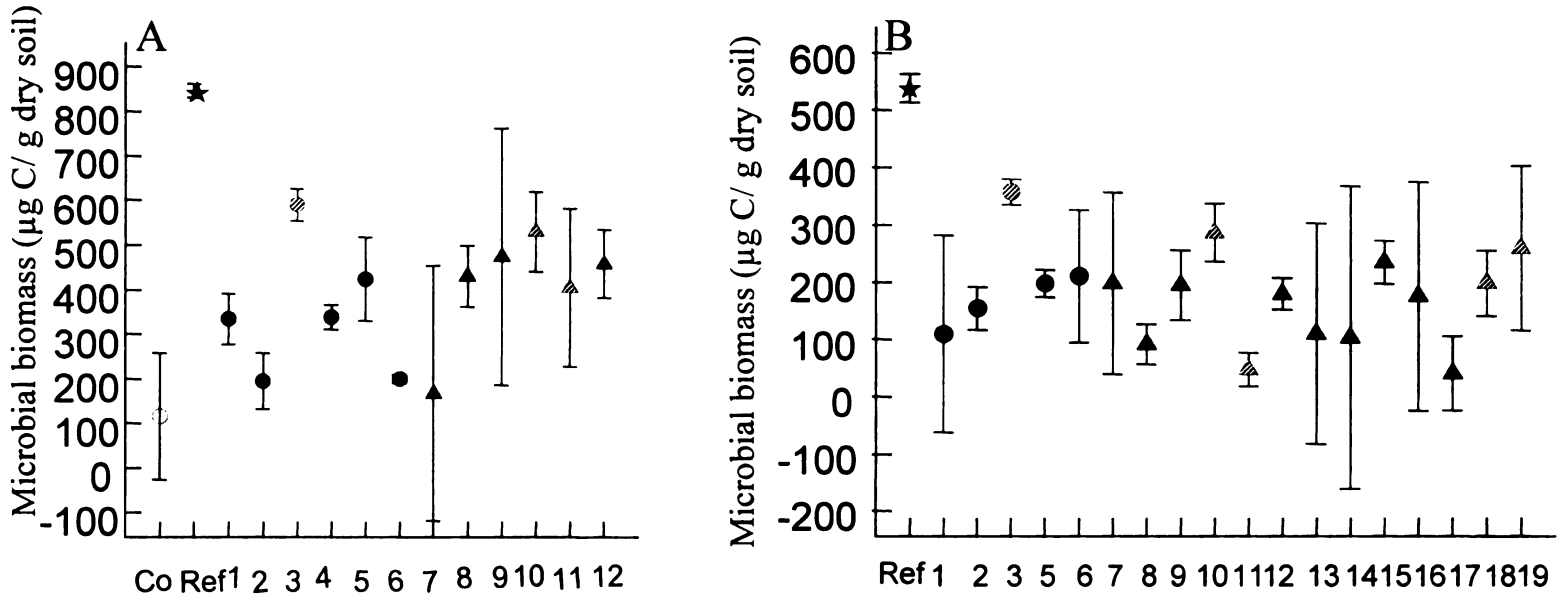

Figure 3.1. Plant community composition effects on soil microbial biomass in (A) year 3 and (B) year 4 of the Silwood Park BIODEPTH study. Values are Mean ± 1 SE. Plant community identification codes are from Table 3.1B. No-plant control plots are represented by a blank circle, while non-manipulated reference communities are represented by stars. Monocultures are indicated by circles and mixtures by triangles. Plots that have *Hypochaeris radicata* have hatch-marks.

Table 3.3. Significance and direction (for diversity) of treatment effects on soil and microbial parameters for both years as detected by Analysis of Variance using Type I sums of squares (model in Table 3.2) for (a) species diversity, functional group diversity, and composition and (b) presence/absence of legumes or forbs. ANCOVA results using above-ground plant biomass as the covariate are shown in parentheses if the effect changed in significance. NS=not significant

(a) Variable:	Plant Biomass	Species Diversity	FG Diversity	Composition
Year 3				
pH	<0.05	NS	NS	< 0.05 (< 0.05)
Soil moisture	<0.10	+ < 0.01 (< 0.05)	NS	NS
Soil organic matter	NS	NS	NS	NS
Total N	NS	NS	NS	NS
N-mineralization rate	NS	NS	NS	NS
Nitrification rate	NS	NS	NS	< 0.05 (NS)
Culturable bacteria	+ <0.05	+ < 0.05 (NS)	+ < 0.05 (NS)	NS
Microbial respiration	NS	NS	NS	< 0.01 (< 0.01)
Microbial biomass	NS	NS	NS	NS
CLPP PC1	+ <0.01	+ < 0.05 (NS)	+ < 0.05 (< 0.10)	NS
CLPP PC3	NS	NS	+ < 0.10 (<0.05)	NS
PLFA PC1	- <0.05	NS	NS	NS
PLFA PC2	NS	- <0.05 (<0.10)	NS	NS
PLFA PC4	NS	NS	NS	<0.05
Year 4				
Soil moisture	NS	NS	NS	NS
Total N	+ <0.10	- <0.10 (NS)	NS	NS
N-mineralization rate	NS	NS	NS	NS
Nitrification rate	NS	NS	NS	NS
Microbial respiration	NS	NS	NS	NS
Microbial biomass	NS	NS	NS	NS
PLFA	NS	NS	NS	NS

Table 3.3 (cont'd).

(b) Variable:	Plant Biomass	+/- Legumes	+/- Forbs
Year 3			
pH	NS	NS	NS
Soil moisture	+ <0.05	NS	+ <0.10 (<0.10)
Soil organic matter	NS	NS	NS
Total N	NS	NS	NS
N-mineralization rate	NS	NS	NS
Nitrification rate	NS	NS	NS
Culturable bacteria	+ <0.01	NS	+ < 0.05 (NS)
Microbial respiration	NS	NS	+ < 0.05 (<0.05)
Microbial biomass	NS	NS	+ < 0.05 (<0.05)
CLPP PC4	NS	+ < 0.05 (0.10)	+ < 0.10 (< 0.10)
CLPP PC5	NS	NS	- < 0.01 (<0.01)
PLFA	NS	NS	NS
Year 4			
Soil moisture	NS	NS	NS
Total N	NS	+ <0.05 (<0.05)	NS
N-mineralization rate	NS	+ <0.05 (<0.10)	NS
Nitrification rate	NS	NS	NS
Microbial respiration	NS	NS	+ <0.10 (<0.10)
Microbial biomass	NS	NS	NS
PLFA	NS	NS	NS

Table 3.4. Fatty acids used in principal components analysis of PLFA profiles.

Phospholipid Fatty Acids
C9 Dicarboxylic acid
14:0
15:0 iso
15:0 anteiso
16:0 iso
16:1 cis 9
16:1 cis 11
16:0
17:0 iso
17:0 anteiso
17:0 cyclo
18:2 cis 12
18:1 cis 9
18:0
19:0 cyclo c11-12
summed feature 8: 18:1 trans 9

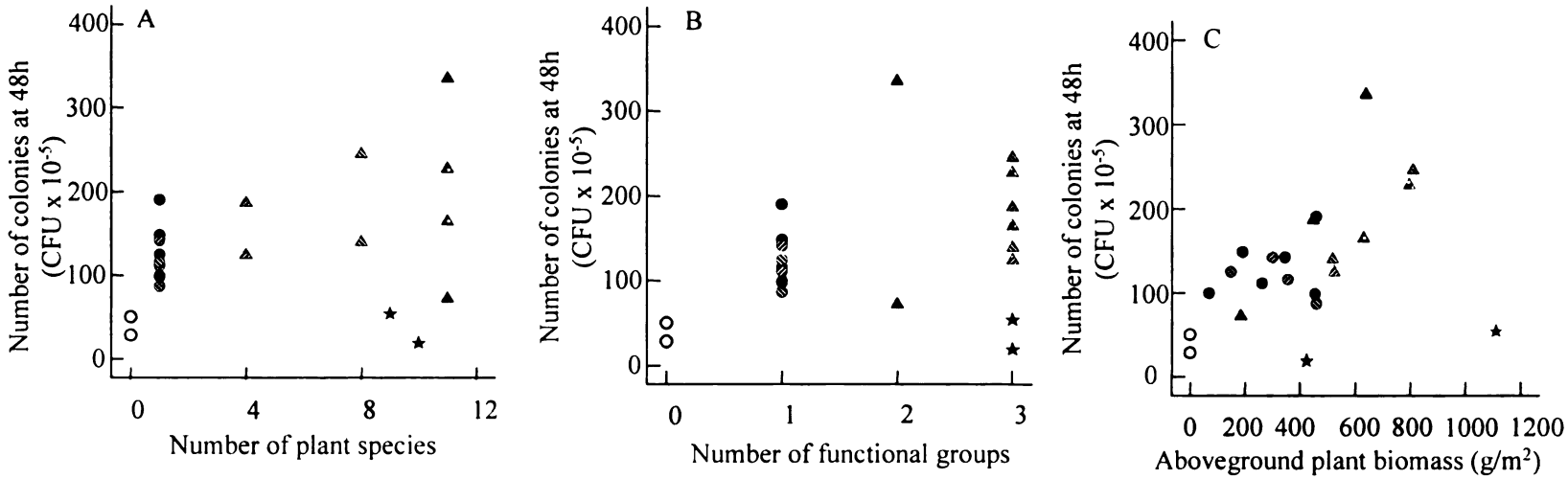

Figure 3.2. Relationship between number of culturable soil bacteria and (A) plant species richness (R^2=0.294, $p<0.01$), (B) number of plant functional groups (R^2=0.288, $p<0.01$), and (C) aboveground plant biomass in year 3 (R^2=0.573, $p<0.01$). No-plant control plots are represented by blank circles, while non-manipulated reference communities are represented by stars. Monocultures are indicated by circles and mixtures by triangles. Plots that have *Lotus corniculatus* have hatch-marks slanting up to the right; plots that have *Hypochaeris radicata* have hatch-marks slanting down to the right. The two plots with both species are mottled.

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3 (Figure 3.2C, r^2= 0.57, p< 0.01). PLFA PC2 was also significantly related to species number (Table 3.3A). Soil moisture and CLPP PC1 were both positively correlated with plant species number, while CLPP PC1 and PC3 were positively correlated with number of functional groups (Table 3.3A). These relationships were still significant when plant biomass was included as a co-variate; however, when the no-plant control plots were excluded from the analyses, the relationships were not significant.

Effects of Functional Groups or Individual Species on the Soil Microbial Community

Because grass species were present in all mixtures, I were only able to test for the effect of presence/absence of forbs and legumes on the soil and microbial community parameters (Table 3.2). The presence of forbs had a significant positive effect on soil moisture, number of culturable bacteria, microbial respiration, microbial biomass in year 3, and microbial respiration in year 4 (Table 3.3B). CLPP PC4 and PC5 also distinguished among plots with and without forbs in year 3 (Table 3.3B). The presence of legumes influenced CLPP PC4 in year 3 and corresponded with increased Total N and N-mineralization rates in year 4.

The small number of plots sampled only allowed us to evaluate the direct effects of a few species on the soil microbial community; most species were either in all or only a very few mixtures. I were able to detect the effects of two species, *Lotus corniculatus* (legume) and *Hypochaeris radicata* (forb), on soil and microbial parameters. Soil from *L. corniculatus* monocultures had a significantly lower pH than all other plots. The *L.*

corniculatus monocultures also were distinguished from the other plots by having higher values for CLPP on the third PC (Figure 3.3), indicating the soil microorganisms in these plots were better able to metabolize α-D-lactose. *Hypochaeris radicata* monocultures had the highest soil microbial biomass of manipulated plots in both years (Figure 3.1A,B), suggesting a strong plant species effect. However, I saw no evidence that the presence of this species in mixtures increased the overall soil microbial biomass for the mixture.

Effects of Plant Community Composition (Mixture) on the Soil Microbial Community

I were able to detect plant community composition effects on the soil microbial community even when aboveground plant biomass was a covariate. Year 3 PLFA PC4 was significantly related to plant species composition. Soil microbial respiration was significantly related to plant community composition in year 3 (Figure 3.1A, Table 3.3A, $p< 0.05$), but not in year 4 (Figure 3.1B). Soil microbial biomass in these plots was consistent across years ($r^2=0.39$, $p <0.01$), although not related to diversity or composition of the plant community.

Changes in Soil Microbial Community Composition

There was considerable variation in CLPP among plots, and much of this could be accounted for by the plant diversity or composition treatments. PC1 explained 46.1% of the variation and was correlated with increased species diversity and increased

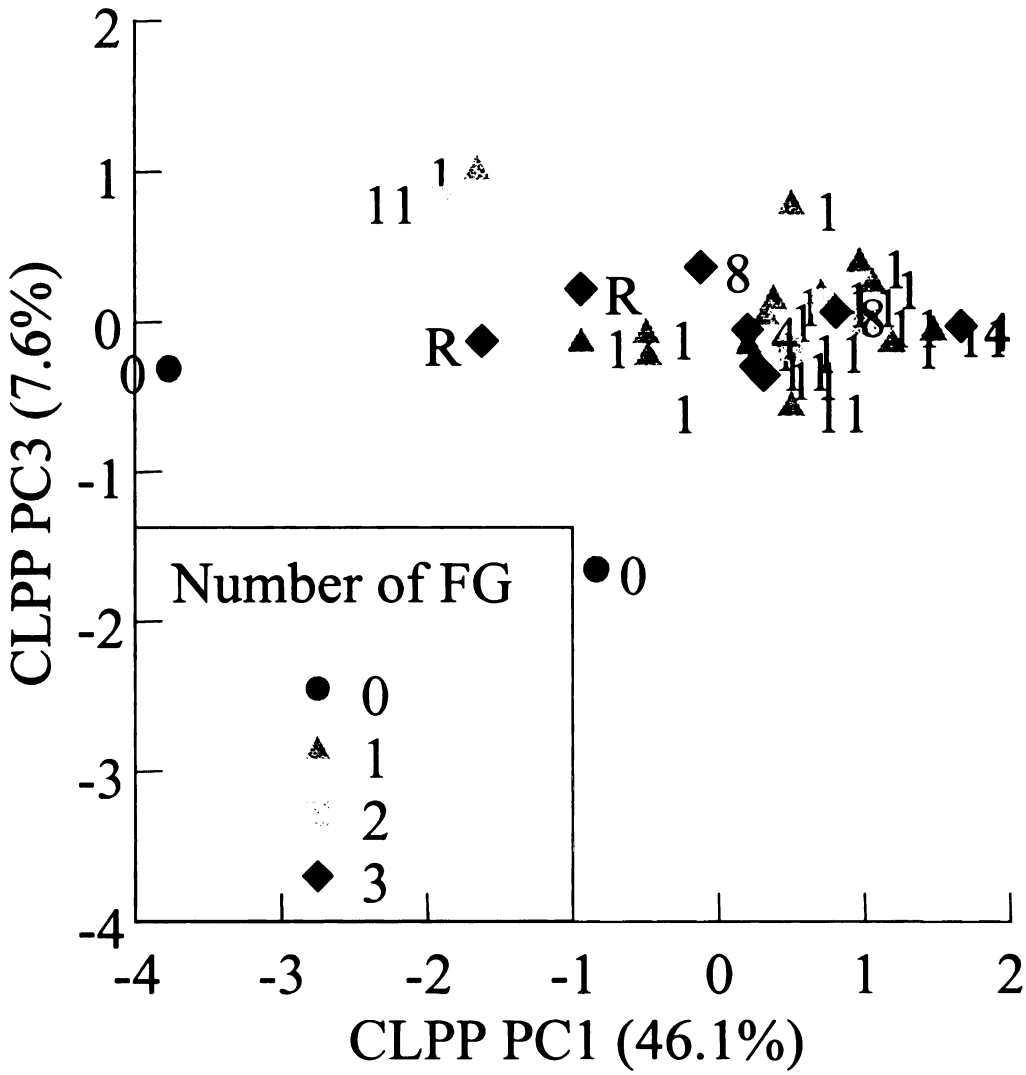

Figure 3.3. Plant diversity effects on the metabolic activity of the soil microbial community as measured by Community Level Physiological Profiles (CLPP) in year 3 (n = 28). Principal component axis 1 accounted for 46.1% of the total variation. Principal component axis 3 accounted for 7.6% of the overall variation and was driven by the ability to metabolize alpha-D-lactose. Significance values for diversity effects are listed in Table 3.3A. Number of functional groups is represented by symbol shape. No-plant control plots are represented by circles, and non-manipulated control communities labeled with "R". Number of plant species (0, 1, 4, 8, or 11) are labeled.

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numbers of functional groups (Table 3.3A), while PC2 accounted for an additional 15.0% of the variation, but was not significantly correlated with any explanatory variables. However, PC3 (which accounted for 7.6% of the variation in the CLPP data set) was significantly related to number of functional groups (Figure 3.3, Table 3.3, $p<0.05$). PC3 was driven by the ability to metabolize α-D-lactose. This relationship was still significant when aboveground plant biomass was included as a covariate in the analysis, indicating that the functional group diversity effect was independent of any plant biomass effect. CLPP PC4 accounted for 6.8% of the variation and was positively correlated with both the presence of legumes and the presence of forbs (Table 3B, $p<0.05$, $p<0.10$), while PC5 accounted for 5.4% of the variation and was negatively correlated with the presence of forbs (Table 3B, $p<0.01$).

The variation in PLFA profiles of the soil microbial community showed structure at two levels. PC1 (45.3% of the variation) was significantly related to Block ($p<0.01$), indicating location in the field was important in structuring the soil microbial community at this site. Soils from the first block contained more C9 dicarboxylic acid, 14:0, 15:0 iso, 16:0 iso, 17:0 iso, and 17:0 anteiso; while the second block contained more 16:1 cis 11, 18:2 cis 12, 18:1 cis 9, and summed in feature 8: 18:1 trans 9, indicating a higher proportion of eukaryotes, most likely fungi (Cavigelli *et al.* 1995). PC2 accounted for 21.5% of the variation and was significantly correlated with species diversity (Table 3.3A, $p<0.05$). PC3 accounted for an additional 16.8% of the total variation, but was not significantly correlated with plant diversity, plant productivity, or aboveground biomass. A much smaller amount of the variation in PLFA profiles (PC4,

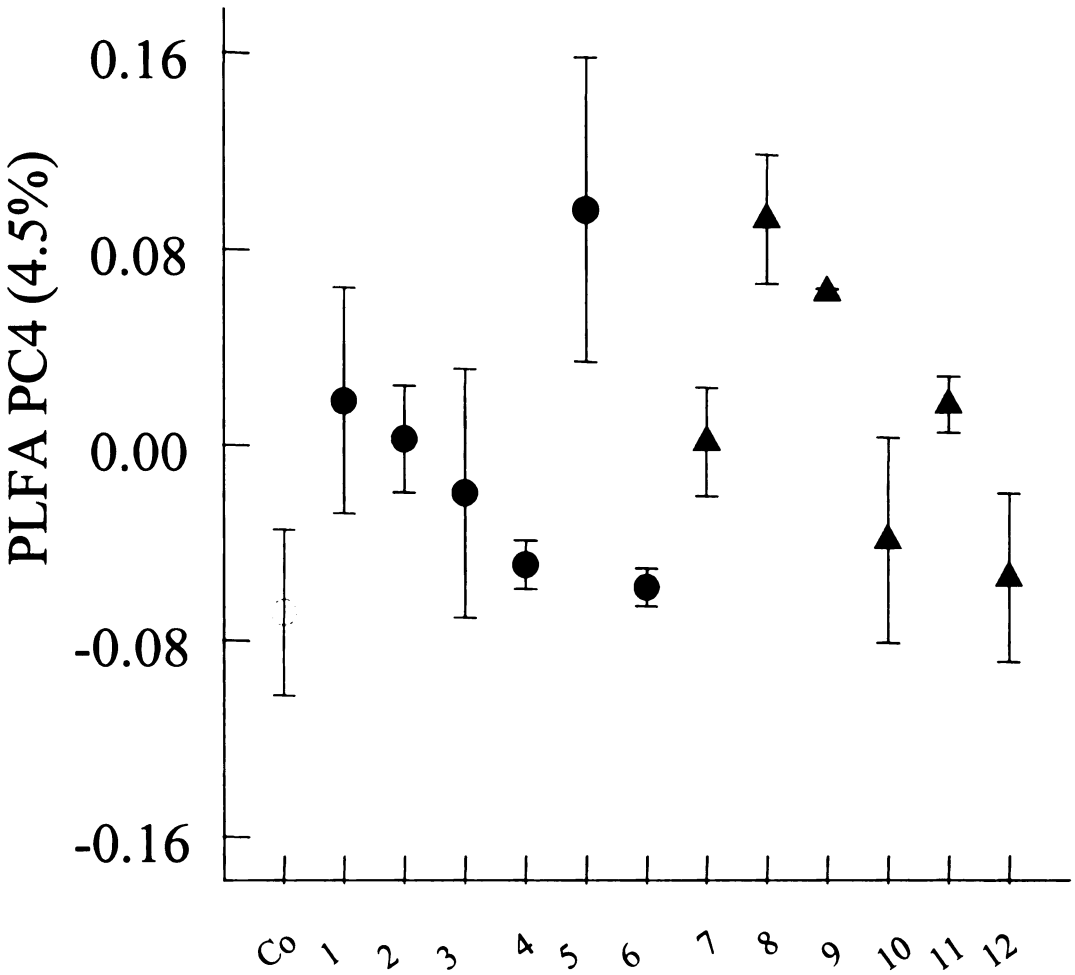

Figure 3.4. Plant community composition effects on the structure of the soil microbial community, measured with Phospholipid Fatty Acid profiles of 1998 soil samples. Principal component axis 4 accounted for 4.5% of the overall variation and reflected the amount of 15:0 anteiso in the PLFA profiles. Plant community identification codes are from Table 3.1B. No-plant control plots are represented by a blank circle. The reference plots were not included in this analysis. Monocultures are indicated by circles and mixtures by triangles.

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3.9%) was significantly related to plant community composition (Figure 3.4, Table 3.2, $p < 0.05$). PC4 reflected the amount of 15:0 anteiso in the PLFA profiles. As with microbial biomass and CLPP, this relationship was independent of plant biomass.

Discussion

I expected to find that the composition and diversity of the plant community would significantly affect soil microbial diversity and productivity. I found that plant community diversity and composition affected soil microbial community structure rather than processes (Table 3.3A,B). Although I expanded the number of plots and range of treatments sampled in year 4, I detected fewer diversity and composition effects than in year 3 and the significant variables were inconsistent across years (Table 3.3A,B). In most cases, the initially detected effects of plant species number or number of functional groups on the soil microbial community were reduced or became non-significant when above-ground plant biomass was included as a covariate in these analyses. Plant diversity effects above and beyond those effects on the microbial community that could be accounted for by productivity were detected for CLPP and PLFA profiles in year 3. However, CLPP profile differences seem to be driven primarily by the no plant control plots. Only year 3 did I detect a shift in the soil microbial community (PLFA PC2) in response to plant species diversity (Table 3.3A).

Plant Diversity Effects on the Soil Microbial Community

A number of studies have suggested that there should be a relationship between plant diversity and soil microbial diversity (Ohtonen *et al.* 1997, Wardle and Giller 1996, Schläpfer and Schmid 1999). In most field studies of plant community effects on soil microbial communities several explanatory variables (*e.g.* plant diversity, plant productivity, and plant community composition) are confounded. For example, J.C. Zak *et al.* (1994) detected differences in the structure of the soil microbial community (using Biolog) along an elevational and moisture gradient in the Chihuahuan Desert at the Jornada Long-Term Ecological Research site. Similarly, Goodfriend (1998) used CLPP patterns to distinguish among the communities at eight sites representing a variety of wetland communities. Broughton and Gross (2000) examined characteristics of the soil microbial community composition along a natural topographic, productivity and diversity gradient at a site in southwestern Michigan and found a correlation between the productivity of the plant and soil microbial communities, but no relationship between plant diversity and soil microbial community composition (results in Chapter 2). However, in all of these studies, the plant communities sampled were from different sites in which there were likely concomitant changes in soil characteristics, so the influence of plant community composition differences could not be assessed independently of differences in edaphic characteristics.

In contrast, Wardle *et al.* (1999) showed plant effects on the soil microbial community, which were not confounded by either soil or management effects. They removed

subsets of the plant community (that varied in number and functional group composition) from a New Zealand perennial grassland. PLFA patterns distinguished among soils from the plant removal treatments, suggesting plant community composition effects on soil microbial community structure (Wardle *et al.* 1999).

The BIODEPTH experiment provides a unique opportunity to examine both species diversity (through number and functional group) and composition effects on the soil microbial community. Because the Silwood Park site preparation included fumigation with methyl bromide after tillage to destroy the seed bank, the soil microbial community was "standardized" before the initiation of treatments. This allowed us to control for the effects of soil factors and focus solely on the manipulated plant diversity and composition treatments as explanatory factors for the soil microbial community.

A recent paper by Stephan *et al.* (2000) from the Swiss BIODEPTH site reported a relationship between plant species richness and functional diversity of the culturable soil microbial community as measured by CLPP. They found that increased plant species richness and plant functional diversity increased the overall catabolic activity diversity in CLPP. However, Stephan *et al.* (2000) did not include a measure of plant biomass as a covariate in these analyses, so it is not clear the extent to which the plant diversity effect on the culturable soil bacteria is due to a correlated plant productivity effect on the microorganisms. Hector *et al.* (1999) reported a strong relationship between plant diversity and plant biomass at the Swiss site. At higher plant diversities, aboveground plant biomass is greater, likely making labile carbon available to the soil

microorganisms. Unlike the Silwood Park site, soils at the Swiss site were not fumigated prior to the establishment of the diversity treatments (Hector *et al.* 1999, Spehn *et al.* 2000a). These two sites also differed in the range of species diversity used in the experiment: the Silwood site had a maximum species richness of 11, whereas the Swiss site had a maximum of 32 (Hector *et al.* 1999, Spehn *et al.* 2000b). These differences in range of species diversity examined at the two sites is reflective of the natural diversity at these sites. This may also affect the ability to detect plant species diversity effects on the soil microbial community at these two sites.

CLPP catabolic activity has been found to be strongly related to innoculum density (Garland and Mills 1991, Haack *et al.* 1994) and, thus, can be an approximate indicator of overall bacterial number. I did not detect a significant relationship between CLPP overall catabolic activity and plant diversity at the Silwood site. However, the increase in culturable bacteria with both increased plant species diversity (Figure 3.2A) and increased plant functional group diversity (Figure 3.2B) reflects the underlying relationship between plant diversity and plant biomass. The number of culturable bacteria in the soil is clearly correlated with the overall aboveground plant biomass of the plot (Figure 3.2C).

Plant Community Composition Effects on the Soil Microbial Community

In year 3 (but not year 4), I found plant community composition effects on some soil and microbial parameters (Table 3.3A). Some previous studies have shown plant

community composition to be more important than species number or number of functional groups in influencing ecosystem processes. Hooper and Vitousek (1998) concluded that plant community composition better explained variation in nutrient cycling processes in a Californian serpentine grassland than number of functional groups. In a comprehensive study using plant removals, Wardle *et al.* (1999) found significant effects of plant community composition on several different trophic levels, including the soil microbial community, and ecosystem properties. These results suggest that individual plant species may influence communities and processes independent of any diversity or productivity effects.

I also found that plant community composition significantly affected some soil and microbial parameters at the Silwood site, but these effects varied across years. The presence of legumes was positively correlated with CLPP PC4 (year 3), Total N (year 4), and N-mineralization rates (year 4; Table 3.3B). As legumes are symbiotic with nitrogen-fixing bacteria in their roots, it is not surprising to find an effect of legumes on soil microbial processes. However, I were not able to determine whether the legume effects were due to a particular legume species, because both *L. corniculatus* and *Trifolium repens* were present in all mixtures containing legumes. However, *L. corniculatus* monocultures differed from the other monocultures and mixtures in both pH and CLPP profiles. At the Swiss BIODEPTH site, Stephan *et al.* (2000) found that legumes had positive effect on overall CLPP catabolic activity, and Spehn *et al.* (2000a) reported a positive effect of legumes on microbial biomass. In contrast to this study, Stephan *et al.* (2000) were able to detect the effect of the presence of a specific

legume, *T. repens*, on the soil microbial community. The presence of *T. repens* was positively correlated with CLPP catabolic activity and number of carbon sources metabolized at the Swiss site (Stephan *et al.* 2000). Although both the Silwood and Swiss sites had *L. corniculatus* and *T. repens* grown in monocultures and mixtures; differences between these sites in the specific effects of these species suggest a species by environment interaction as seen for aboveground biomass (Table 3.3, Hector *et al.* 1999).

Legacy Effects on the Soil Microbial Community

N-mineralization rates did not differ significantly among mixtures; however, the reference plots consistently had lower N-mineralization rates than the treatment plots. N-mineralization rates typically are higher in earlier successional sites and decline over time (Schlesinger 1997). These differences in N-mineralization rates may reflect the successional status of the treatment and reference plots. This temporal change may reflect the immobilization of nitrogen by the soil microbial community in later successional plots and a more mature soil microbial community (Schlesinger 1997).

Microbial biomass measurements from the reference communities at Silwood indicate that the treatment plots still had not recovered from fumigation after 4 years. Because microbial respiration and microbial biomass may be correlated with aboveground plant biomass (Broughton and Gross 2000), and plant diversity is correlated with aboveground plant biomass, I would expect to see higher microbial activities and

biomasses at higher plant diversity levels. Spehn *et al.* (2000a) observed a positive relationship between soil microbial biomass and plant species diversity at the Swiss site where the soil was not fumigated. The fact that I did not see a relationship between plant diversity and soil microbial biomass at the Silwood site suggests that the soil microbial community is still recovering from the severe disturbance of methyl bromide application. Alternatively, it may be that different relationships between plant diversity and soil microbial biomass emerge under different local conditions. Studies at other BIODEPTH sites may help to resolve this issue. Disturbance effects on the soil microbial community may persist for decades and make it difficult to detect current plant species or diversity effects on the soil microbial community. Buckley and Schmidt (2001) found that there was little difference between the soil microbial communities of a continuously tilled agricultural site and a companion successional site (abandoned for 12 years) in southwestern Michigan. A nearby reference field (never-tilled) had a distinct soil microbial community (detected using rRNA) from either the tilled or successional fields (Buckley and Schmidt 2001).

The results from this study provide some evidence that there is an overall plant diversity effect on the soil microbial community. However, both the productivity and the composition of the plant community can have effects on the biomass and structure of the soil microbial community. In addition, disturbance effects on the soil microbial community may persist for quite some time. Longer-term studies that can separate soil microbial community function from structure are necessary to better investigate the role

that plants play in how the soil microbial community mediates the flow of carbon and nitrogen through the ecosystem.

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CHAPTER 4

PLANT-MEDIATED EFFECTS OF SOIL ORIGIN ON THE COMPOSITION AND FUNCTION OF SOIL MICROBIAL COMMUNITIES

Introduction

While considerable attention has been paid to factors that affect the composition and function of communities of macroorganisms, very little is known about the factors that affect the structure and function of soil microbial communities (Ohtonen *et al.* 1997, Tiedje 1995). To understand how changes in the structure of the soil microbial community affect ecosystem functions, I must first investigate what factors influence soil microbial community structure and function. Plant community composition can be stable for long time periods, but can also vary depending on factors such as disturbance history and successional status. In contrast, soil characteristics change much more slowly on average than the plant community (*e.g.* soil quality). Consequently, soil characteristics may have a more consistent effect on the soil microbial community than plants. While the soil has a large reserve of relatively recalcitrant carbon that is less available to microorganisms, much of the labile carbon available to the soil microbial community is derived from recent plant production (Paul and Clark 1996). Because the plant community is dynamic and the main source of carbon for the soil microbial community, the current plant community should have a big effect on structure and function. To date, many studies have investigated the role of edaphic factors in structuring the soil microbial community, while fewer have addressed the effects of plants (Metting 1993, Schläpper and Schmid 1999, Hooper *et al.* 2000). Additionally,

very few have sought to distinguish between the effects of soil and the effects of plants on the soil microbial community.

Several studies that have reported plant community effects on soil microbial communities confound direct plant-mediated effects with soil effects. For example, J.C. Zak *et al.* (1994) detected differences in the structure of the soil microbial community (using Biolog) from plant communities that occur along an elevational and moisture gradient in the Chihuahuan Desert at the Jornada Long-Term Ecological Research site. Similarly, Goodfriend (1998) used Biolog to distinguish among the soil bacterial communities at 8 sites representing a variety of wetlands across a salinity gradient. However, in both these studies, the plant communities sampled were from different sites in which there were likely concomitant changes in soil characteristics, so the influence of plant community composition differences could not be assessed independently of differences in edaphic characteristics. Other studies in single sites have found little change in microbial communities in soils sampled from different plant communities (*e.g.* Buckley and Schmidt 2001). After 10 years of plant community divergence resulting from vastly different agricultural management, Buckley and Schmidt (2001) could detect no differences among rRNA patterns of the soil microbial communities among treatments at the Kellogg Biological Station's Long Term Ecological Research site, suggesting that the soil microbial community structure was still dominated by the influence of the past land use and soil quality or, perhaps, that the various plant forms contributed similarly to the sustenance of the microbial assemblage despite our perception that they might differ in this respect. The plant communities

sampled ranged from successional fields to poplar plantations to conventional corn. All had been under similar management (conventional corn) prior to treatment implementation (Robertson *et al.* 1993).

In this study, I am interested in distinguishing the relative importance of variability in soil characteristics and the current plant community in controlling soil microbial community structure and composition (see Figure 1.1). Specifically, I used a manipulative greenhouse experiment to investigate whether (1) soils from different plant communities that differ in fertility vary in the composition and function of the soil microbial community and (2) plants can mediate these effects.

Methods

Site Descriptions

I selected six successional old-fields at the W. K. Kellogg Biological Station of Michigan State University in southwestern Michigan to compare the soil microbial communities of sites with different plant communities. The sites varied in fertility, species richness, and dominant plant type, but all were located on Kalamazoo sandy loam soil. I determined plant species composition at each site in six 0.5 m x 2 m plots in August 1998. To estimate above-ground net primary productivity (ANPP), I clipped aboveground biomass at ground level from a 0.5 m x 0.5 m plot located within the plots used to assess species diversity and composition.

The six sites also differed in past land use and ranged in time since abandonment from 20 to 50 years. McKay field was abandoned from agriculture in 1973; a section was plowed once in 1981 and then re-abandoned (Burbank *et al.* 1992). Both the Upper and Lower Louden fields were abandoned from agriculture in 1951 (Burbank *et al.* 1992). The Bailey field site was farmed until ten years prior to this sampling (K.L. Gross, personal communication). The Pond Lab Orchard and Field K sites had been abandoned for at least twenty years (Foster 1996).

Experimental Design

To determine if site differences in ANPP and species composition had detectable effects on the soil microbial community I incubated soils from each site in the greenhouse and evaluated the soil microbial communities 12 to 16 weeks later. To determine if plants could mediate these differences, I sowed half the pots with *Andropogon gerardi*, a C_4 grass native to Michigan prairies. I collected approximately 10 kg of soil in June 1998 from the top 15 cm of each field in the same area from which species diversity and plant biomass were sampled. The soil was sieved to 4 mm and thoroughly mixed. Soil was stored at room temperature until the experiment was established in the greenhouse (less than 2 weeks).

I used a randomized complete block design for the greenhouse experiment to test for the effects of soil origin and plant effects on the soil microbial community: 6 soils x 2

treatments, with 8 replicates of each. The two treatments were control (no-plant) and plant (*Andropogon gerardi*). I chose *A. gerardi* because it is a native C_4 grass that can grow in all of these fields, although it was not present in our soil collection sites and is rare in these communities because it is out-competed by naturalized C_3 grasses (Foster 1996). This allowed us to measure the effects of a relatively novel plant on the soil microbial communities present in each site. Seeds of *A. gerardi* were collected from local fields in autumn 1997, and stored at room temperature in the laboratory until used for these experiments the following summer.

Soils were placed in 5 cm diameter x 20 cm deep pots and kept well-watered with de-ionized water to avoid adding nutrients or contaminants. Temperature in the greenhouse ranged from 25 to 40 °C; light availability was controlled through a 12 h light/ 12 h dark cycle.

Treatments were randomly assigned within replicates. *Andropogon gerardi* was added as 2 week old seedlings; all seedlings were germinated in a sterile sand medium in a growth chamber and were less than 2 cm in height when transplanted. I estimated initial biomass by drying a representative subset of the seedlings at the time of transplantation. The experiment ran for a total of 16 weeks. I harvested the experiment in two segments because of the number of samples and the time required to process each sample: 4 replicates were harvested at week 12, and the remaining 4 replicates were harvested at week 16. Thus, time was an additional factor in the ANOVA.

Data Collection

Differences among sites and treatments in soil fertility were assessed by (1) growth of *Andropogon gerardi*, (2) inorganic nitrogen pools and N-mineralization rate, and (3) soil organic matter. Shoot and root biomass of *Andropogon gerardi* were harvested separately and dried at 60°C for 48 h. I separated root biomass from the soil during sieving; roots were rinsed thoroughly in de-ionized water before drying. I used the change in total plant biomass to estimate the relative growth rate (RGR) as [ln (total plant biomass) – ln (initial plant biomass)] / number of days between harvest and planting.

I sieved the soil through a 2 mm sieve, and stored it in sealed plastic bags at 4°C until analysis. All analyses were done within 3 days of sampling, except PLFA. Soil for PLFA analyses was kept at –80°C until the fatty acids were extracted.

I determined gravimetric soil moisture for each sample by drying 10 g soil at 105°C for 48 hours (Nelson and Sommers 1982). A subsample of the dried soil was ashed at 500°C for 4 hours to determine organic matter content (Nelson and Sommers 1982). For nitrogen analyses, I extracted 20 g of fresh soil in 100 ml 1M KCl. The samples were shaken for 1 min and allowed to settle for 24 h at room temperature. The supernatant mixture was filtered through a 1-μm Gelman glass filter and $NO3^-$ and $NH4^+$ concentrations were measured using Alpkem auto-analyzer. To determine potential N-mineralization and nitrification rates, a companion 20 g sample was

incubated for 21 days at 25°C and 15% humidity and then extracted using the same methods as above. The remaining soil was used to characterize the soil microbial community.

I assessed differences in soil microbial community production among the sites and treatments by (1) microbial biomass C, (2) microbial respiration, and (3) plate counts (number of colony-forming units). I determined microbial biomass using the chloroform fumigation incubation method (Paul *et al.* 1999). Two 25 g soil samples were pre-incubated for 5 days then one sample was fumigated with chloroform for 24 hours to kill the microorganisms. After a vacuum was created and the chloroform evaporated, 0.5 g of original soil was added to both samples. I measured initial headspace CO_2 and accumulated CO_2 after 10 days on an ADC series EGA infrared CO_2 gas analyzer (The Analytical Development Co. Ltd., Hoddesdon, Herts., UK). I calculated microbial biomass as [1.73 * (10 day accumulated CO_2-C – initial CO_2-C for the fumigated samples) – 0.56 * (10 day accumulated CO_2-C – initial CO_2-C for the control samples)] (Paul *et al.* 1999). To determine microbial respiration I used a separate set of 10g soil samples that were pre-incubated 5 days in a 160 ml glass qorpak bottle. I measured initial headspace CO_2 and accumulated CO_2 after 1 and 5 days.

I determined the number of colony-forming units by mixing 5 g of fresh, sieved soil into 1% phosphate buffer to reach a final dilution of 10^{-6} g soil/ ml. I plated this solution on minimal media (R2A agar plates) and incubated the plates at 25 °C and then counted the number of colony-forming units after 24 h and 48 h.

I assessed soil microbial community structure differences among sites and treatments by (1) Community-level physiological profiles (CLPP) and (2) phospholipid fatty acid (PLFA) profiles. Community-level physiological profiles (CLPP) were determined using Biolog GN plates (Biolog, Inc., Hayward, Calif., USA) and reflect the range and amount of carbon sources or resources that can be metabolized by the community (Konopka *et al.* 1998). For the assay, 1 g of fresh, sieved soil was shaken with 99 ml 1% phosphate buffer solution for 20 min. 150 µl of the mixture was transferred to each well of the microtiter plate (GN Biolog, 95 Carbon sources + 1 non-Carbon control). The plates were incubated in the dark at 25 °C and optical densities were measured at 24 h intervals from 0 h to 96 h using an Emax precision microplate reader (Molecular Devices Corp., Menlo Park, Calif., USA). Because the 5 incubation times gave consistent results, I present here data only from the 96 h Biolog measurements. Optical densities (intensity of resource use) were used in the RDA analysis.

For the PLFA analysis, I extracted lipids from 6 g whole soil samples for 2 h using a mixture of dichloromethane (DCM): methanol: phosphate buffer (1:2:0.8 v/v/v), following a modified Bligh-Dyer procedure (Bligh and Dyer 1959). Phase separation was achieved by adding DCM and saturated sodium bromide solution (1:4 v/v). I isolated the phospholipid fatty acids from the dried lipid extracts by solid phase extraction. The lipid material was added to a polar column consisting of 100 mg silica (Varian Bond Elut LRC Columns, Product # 1211-3010). Lipids of low or intermediate polarity were eluted with chloroform and acetone and discarded. Subsequently,

phospholipid fatty acids were eluted with 1.5 ml methanol for preparation of fatty acid methyl esters. I saponified the samples using 1 ml NaOH (15% w/v) in methanol (50% v/v) at 100 °C for 30 min and methylated the sample with 2 ml 6M HCl in methanol at 80 °C for 10 min. I extracted the fatty acid methyl esters into 1.25 ml (1:1 v/v) methyl-tert-butyl etherhexane for 10 min and washed the extract with 3 ml 1.2% NaOH. Phospholipid amounts were measured using a HP 5890 series II gas chromatograph (Hewlett Packard Co., Palo Alto, Calif., USA) equipped with a 7673 autosampler and flame ionization detector (Microbial ID Inc., Newark, Del., USA). Peaks were identified by comparison with an external standard. For analysis, I included only those phospholipid fatty acids that were present in greater than 50% of samples and reported their abundance as the square root of the proportion of the total phospholipid fatty acid amount in each sample (Hellinger transformation). Of the 70 lipids detected, 30 phospholipid fatty acids met this criterion (Table 4.3).

Statistical Analyses

I used a randomized complete block design (ANOVA) model to test the effects of soil origin, presence/absence of plant, and time on the following response variables: soil moisture, soil organic matter, plant biomass, relative growth rate, total N, N-mineralization rate, nitrification rate, number of culturable bacteria, microbial respiration, and microbial biomass.

I used a modified redundancy analysis (RDA, Legendre & Anderson 1999) to determine the relationship between the environmental factors (soil origin, presence/absence of plant, time of harvest) and the two measures of soil microbial community structure, CLPP and PLFA. This is a relatively new, powerful technique for multivariate analysis. RDA is a multiple regression technique that reduces the number of variables necessary to explain the variation in a data set by creating composite variables. In addition, RDA compares a second matrix that describes the environment in which the original variables were measured. This new technique also uses permutations to allow for statistical tests of how these composite variables vary with the explanatory variables to determine the strengths of the significance of any environmental correlations with measures of the soil microbial community.

Because the CLPP and PLFA data matrices have many zeros, I transformed the CLPP and PLFA data using a Hellinger transformation (a square root transformation of relative abundance, Legendre & Gallagher, in press). The RDA procedure involved: (1) the creation of a matrix of dummy variables corresponding to the randomized complete block design (modeled from the experimental design: soil origin, presence/absence of *Andropogon gerardi*, time at harvest), (2) redundancy analysis of the relationship between the principal coordinates (matrix of optical density or phospholipid fatty acid data) and the environmental variables (matrix of dummy variables in (1)), and (3) implementation of a Monte Carlo permutation test to estimate the statistical relationship between the two matrices (Legendre & Anderson 1999). This analysis allows us to test which factors from the experimental design (soil origin,

presence/absence of *Andropogon gerardi*, time at harvest) are significantly related to the variation in the CLPP and PLFA patterns. The modified RDA is a better statistical technique than regular ordination techniques because it allows for significance testing.

Results

Plant Communities

The six field sites varied in ANPP, species diversity, and soil organic matter (Table 4.1). McKay Field (MK) had the lowest ANPP and a low species diversity and was dominated by *Agropyron repens*, a C_4 perennial grass. Both Bailey (Ba) and Upper Louden (UL) had moderate ANPP and high species diversities and were dominated by diverse forb communities. In contrast, Lower Louden (LL) and the Pond Lab Orchard (PL) field had moderate ANPP and species diversities and were dominated by graminoids. Lower Louden was dominated by *Bromus inermis*, a C_3 perennial grass, although perennial forbs such as *Solidago canadensis*, *Daucus carota*, *Taraxacum officionale*, and *Hieracium* sp. contributed significant biomass to total ANPP. The Pond Lab Orchard site was dominated by several C_3 species: *Bromus inermis*, *Agropyron repens*, and *Poa pratensis*. Field K (FK) had the highest ANPP and lowest species diversity and was dominated by *Bromus inermis* (Table 4.1)

Table 4.1. Plant productivity and diversity of abandoned fields from which soil was collected for the greenhouse experiment. Values for Annual Net Primary Productivity, species richness, and mean percent organic matter are expressed as mean ± standard deviations. Values that are not significantly different for a given variable based on Fisher's LSD test have the same letter.

Site	Dominant Plant Form	Peak Plant Biomass (standing + litter, g/m^2)	Species diversity ($\#/m^2$)	Soil Organic Matter (%)
MK	Grass	188 ± 16 a	2.2 ± 0.4 a	2.40 ± 0.43 a
UL	Forb	320 ± 23 b	15.8 ± 1.0 e	3.17 ± 0.23 b
Ba	Forb	424 ± 52 c	11.3 ± 1.0 d	3.03 ± 0.17 b
LL	Grass	432 ± 27 c	8.5 ± 0.4 c	3.84 ± 0.32 c
PL	Grass	480 ± 48 c	5.7 ± 0.8 b	3.63 ± 0.28 c
FK	Grass	592 ± 22 d	1.3 ± 0.2 a	3.84 ± 0.20 c

Effects of Soil Origin on Soil and Microbial Processes

The differences among sites in plant community productivity were reflected in the growth of *Andropogon gerardi* in the greenhouse ($p < 0.001$, $F = 40.3$, Table 4.2A, Figure 4.1A). *A. gerardi* grown in soils from more productive sites had greater total biomass (Table 4.2A, Figure 4.1). The positive relationship between *A. gerardi* production and 1998 field above-ground plant biomass (Figure 4.1) suggests that the ranking of sites based on plant productivity also reflected differences in fertility. Interestingly, time had no effect on any of the measured plant growth or soil or

Table 4.2. Effect of soil origin and presence of *A. gerardi* on (A) plant and (B) soil and microbial variables as detected by Analysis of Variance. NS = not significant, $p > 0.05$. The time factor investigates the results of harvesting half the experiment at 12 weeks, the other half at 16 weeks.

(A)

Plant Variable	BLOCK	SOIL	TIME	SOIL*TIME
Total Biomass (g)	NS	< 0.001	NS	NS
Root Biomass (g)	0.045	< 0.001	NS	NS
Shoot Biomass (g)	NS	< 0.001	NS	NS
RGR (g/day)	NS	< 0.001	< 0.001	NS
Plant Height (cm)	NS	< 0.001	NS	NS

(B)

Soil or Microbial Variable	BLOCK	SOIL	PLANT	TIME	SOIL*PLANT
Percent Organic Matter	< 0.01	< 0.001	NS	--	NS
Soil Moisture	NS	< 0.01	< 0.05	NS	< 0.05
Total Inorganic Nitrogen	NS	< 0.001	< 0.001	NS	< 0.001
N-mineralization Rate	NS	NS	< 0.001	NS	< 0.05
Nitrification Rate	NS	NS	< 0.001	NS	< 0.01
Microbial respiration	NS	< 0.001	< 0.001	--	< 0.05
Culturable Bacteria (CFU's)	NS	< 0.001	NS	--	< 0.05
Microbial Biomass	< 0.01	NS	NS	NS	NS

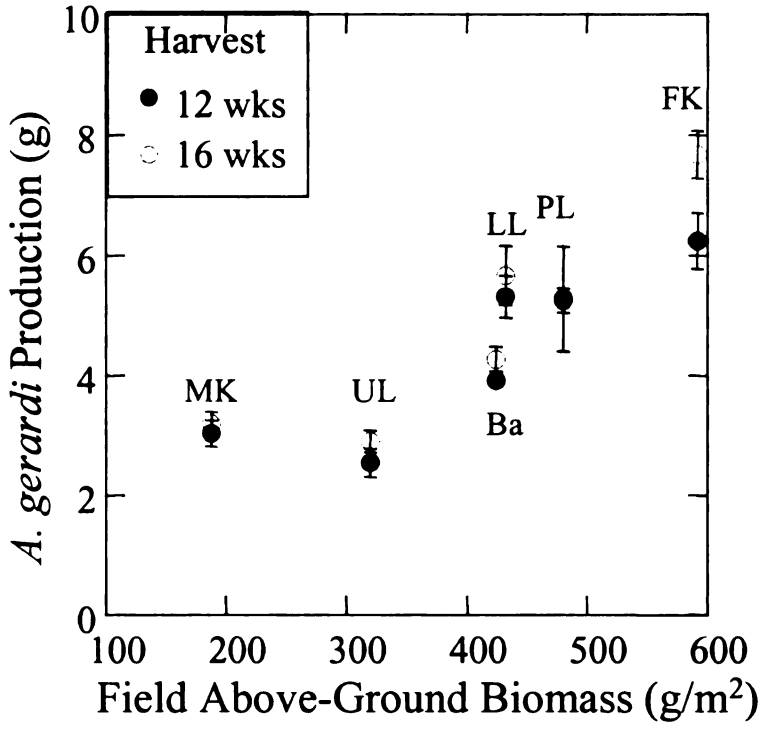

Figure 4.1. Total plant biomass of *Andropogon gerardi* produced at 12 and 16 weeks in relation to variation among sites in 1998 field above-ground plant biomass. Soils are coded as in Table 4.1. Values are mean ± standard error, n = 8. Significance values from the ANOVA are listed in Table 4.2A.

microbial variables measured, indicating that the 4 weeks difference in harvesting replicates had no discernable effect on the results (Table 4.2A&B). Consequently I combined data from the two sampling intervals for the subsequent analyses.

Soil origin significantly affected total inorganic nitrogen ($p < 0.001$, $F = 14.9$, Table 4.2B, Figure 4.2A), microbial respiration ($p < 0.001$, $F = 17.8$, Table 4.2B, Figure 4.2D), and the number of colony-forming units ($p < 0.001$, $F = 12.2$, Table 4.2B, Figure 4.2E), but did not influence N-mineralization rate (Table 4.2B, Figure 4.2B), nitrification rate (Table 4.2B, Figure 4.2C), or microbial biomass (Table 4.2B, Figure 4.2F). In general, sites with higher fertility soils had higher soil microbial respiration, and higher nitrogen pools in the absence of plants.

Effects of **Andropogon gerardi** *on Soil and Microbial Processes*

The presence of *Andropogon gerardi* significantly affected several soil and microbial characteristics and processes (Table 4.2B). The presence of *Andropogon gerardi* decreased soil moisture ($p < 0.05$, $F = 4.5$, Table 4.2B), total inorganic nitrogen ($p < 0.001$, $F = 643.6$, Table 4.2B, Figure 4.2A), N-mineralization ($p < 0.001$, $F = 14.9$, Table 4.2B, Figure 4.2B) and nitrification rates ($p < 0.001$, $F = 18.6$, Table 4.2B, Figure 4.2C), and increased soil microbial respiration ($p < 0.001$, $F = 322.7$, Table 4.2B, Figure 4.2D). The most dramatic effect was on total inorganic nitrogen; the presence of *Andropogon gerardi* reduced nitrogen to similar low levels in all soils (Figure 4.2A).

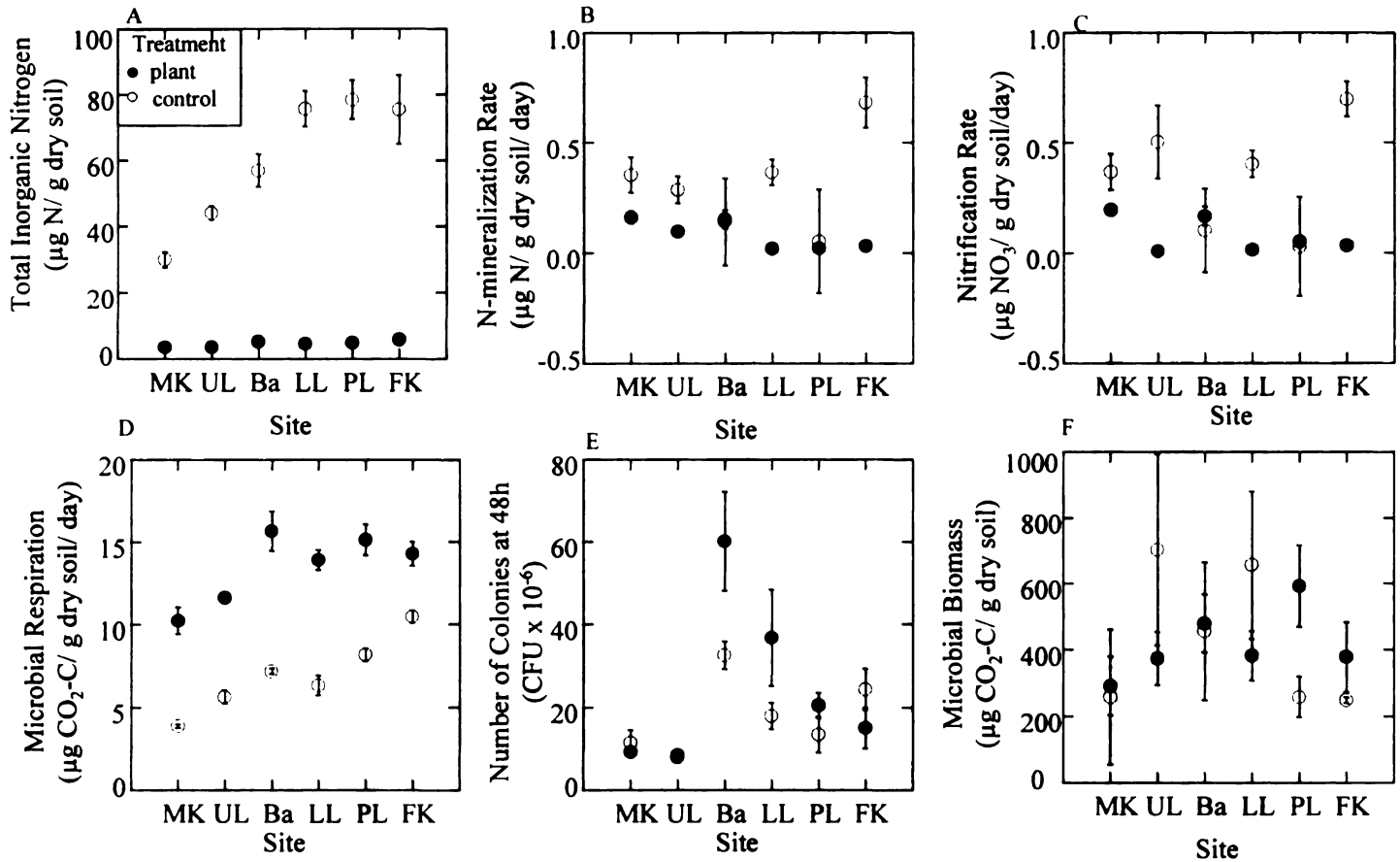

Figure 4.2. Variation among sites in (A) total inorganic nitrogen, (B) N-mineralization rate, (C) nitrification rate, (D) microbial respiration, (E) number of culturable bacteria, and (F) microbial biomass. Sites are arranged in order of increasing productivity, as listed in Table 4.1. For each variable, samples are distinguished between treatments with *Andropogon gerardi* (solid) and no-plant controls (open symbol). Values are mean ± standard error, $n = 8$ for all except number of culturable bacteria and microbial respiration which have $n = 4$. Significance values from the ANOVA are listed in Table 4.2B.

Similarly, the presence of *A. gerardi* decreased N-mineralization and nitrification rates to similar low levels in all soils (Figure 4.2B&C). In contrast, soil microbial respiration increased in the presence of *A. gerardi*, but the magnitude of this effect decreased with fertility (Figure 4.2D). Although the number of culturable bacteria varied across sites (Table 4.2B), and there was a significant plant x site interaction, there was no consistent effect of *A. gerardi* on this variable across sites. The presence of *A. gerardi* also did not have a consistent effect on either the number of culturable bacterial colonies (Table 2B, Figure 2E) or microbial biomass (Table 2B, Figure 2F).

Plant and Soil Effects on Soil and Microbial Processes

For some variates, the soil x plant treatment interaction (*i.e.* the magnitude of the *A. gerardi* effect) varied across the sites and appeared to be related to soil fertility. To evaluate this relationship, I estimated the magnitude of the relative "plant effect" on these variables by calculating the relative difference in the variable in the plant versus no-plant treatments ((plant – control)/ control).

The magnitude of the effects of *A. gerardi* on soil and microbial variables is illustrated in Figure 4.3. For total nitrogen, the magnitude of the effect of *A. gerardi* varied with site fertility and was inversely related to plant biomass (Figure 4.3A, sites are ranked by fertility as per Figure 4.1, $R^2 = 0.18$, $p < 0.01$). The effect of *A. gerardi* on soil microbial respiration also varied with site fertility and was inversely related to plant

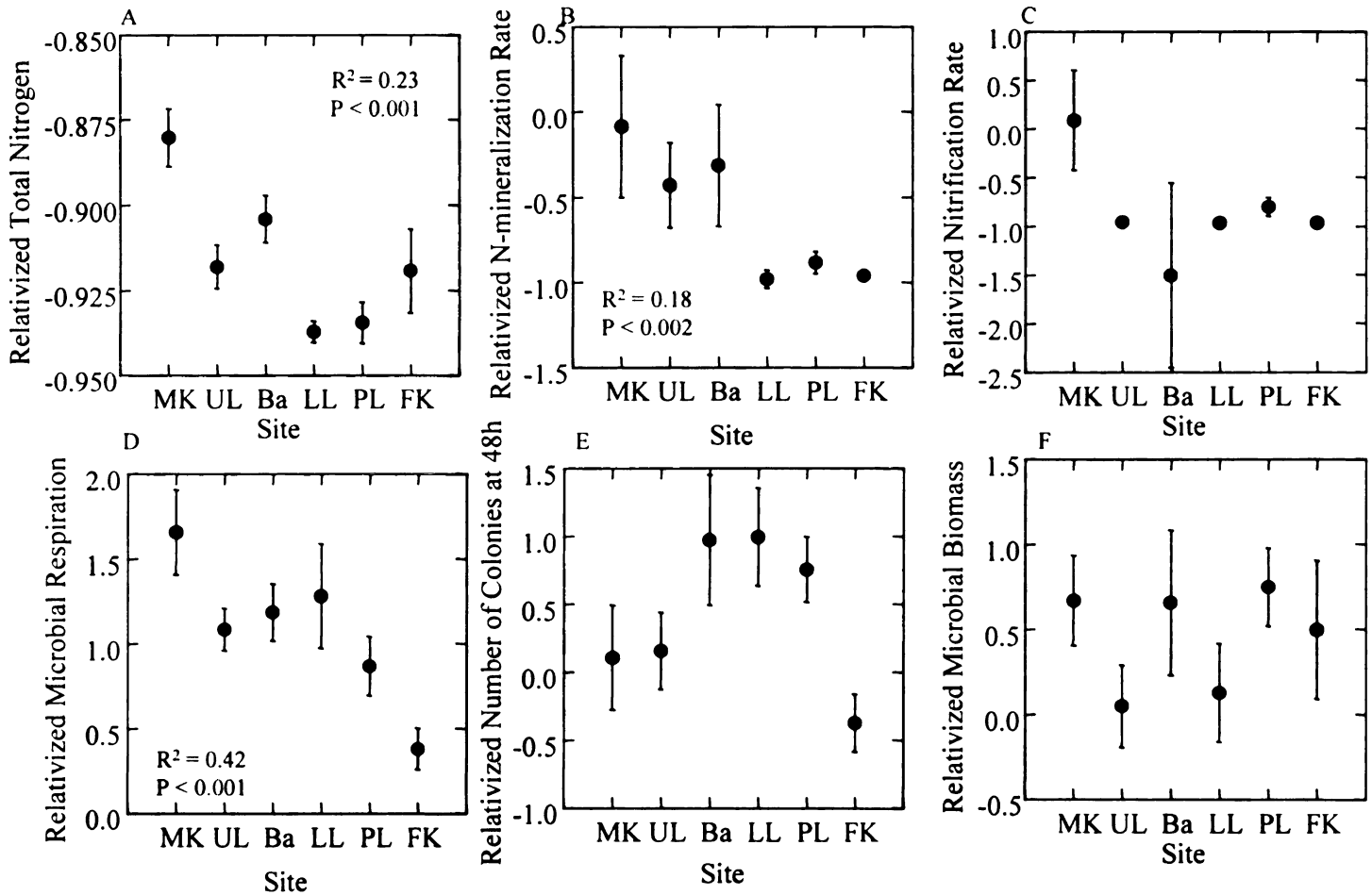

Figure 4.3. The magnitude of the plant effect on soil and microbial processes across sites in relation to the plant biomass gradient for (A) total inorganic nitrogen, (B) N-mineralization rate, (C) Nitrification rate, (D) microbial respiration, (E) number of culturable bacteria, and (F) microbial biomass. Values are mean ± standard error, n = 8 except number of culturable bacteria and microbial respiration where have n = 4.

biomass (Figure 4.3D, $R^2 = 0.26$, $p < 0.01$). However, the significant site x plant interactions observed for N-mineralization rate (Table 4.2B, $p < 0.05$, $F = 2.7$), nitrification rate (Table 4.2B, $p < 0.01$, $F = 3.6$), and number of culturable bacteria (Table 4.2B, $p < 0.05$, $F = 3.0$) were not related to site fertility or *Andropogon gerardi* production (Figure 4.3B, C, & E). Similarly, variation in microbial biomass was not related to the magnitude of the plant response (Table 4.2B, Figure 4.3F).

Plant and Soil Effects on Soil Microbial Community Structure

CLPP of the soil microbial community varied among the soils from the six sites and also responded to the presence of *A. gerardi* (Table 4.3, Figure 4.4). The RDA shows that the presence of *A. gerardi* (Trt) was significantly related to the ordination of the CLPP profiles, as was soil origin (Table 4.3A). Figure 4.4 shows the separation of samples coded by treatment. Axis 1 of the RDA accounted for 20.4% of the variance in optical density data, 35.9% of the variance in the optical density-environment relationship and had an optical density -environment correlation of 0.926 (Table 4.3B). Axis 2 accounted for 12.0% of the variance in optical density data, 22.9% of the variance in the optical density -environment relationship and had an optical density -environment correlation of 0.946. Axis 3 accounted for 7.1% of the variance in optical density data, 12.5% of the variance in the optical density -environment relationship and had an optical density -environment correlation of 0.921. Axis 4 accounted for 5.7% of the variance in optical density data, 10.0% of the variance in the optical density -environment relationship and had an optical density -environment correlation of 0.897.

Table 4.3. Effect of soil origin and presence of *A. gerardi* on the soil microbial community as detected by Distance-Based Redundancy Analysis (db-RDA) of CLPP profiles. (A) Significance values for the permutation tests on the environmental factors of the RDA. (B) Variance explained by species data and species-environment correlations for the RDA.

(A)

Factor	Lambda	F-stat	p-value	% variance
Plant	0.08	5.593	0.0010	7.6
Soil	0.36	5.340	0.0010	36.4
Plant*Soil	0.57	4.290	0.0010	56.7

(B)

Axis	Eigenvalue	Species-Environment Correlation	Cumulative % Variance of Species Data	Cumulative % Variance of Species-Environment
1	0.204	0.926	20.4	35.9
2	0.120	0.946	32.3	57.0
3	0.071	0.921	39.4	69.5
4	0.057	0.897	45.1	79.5

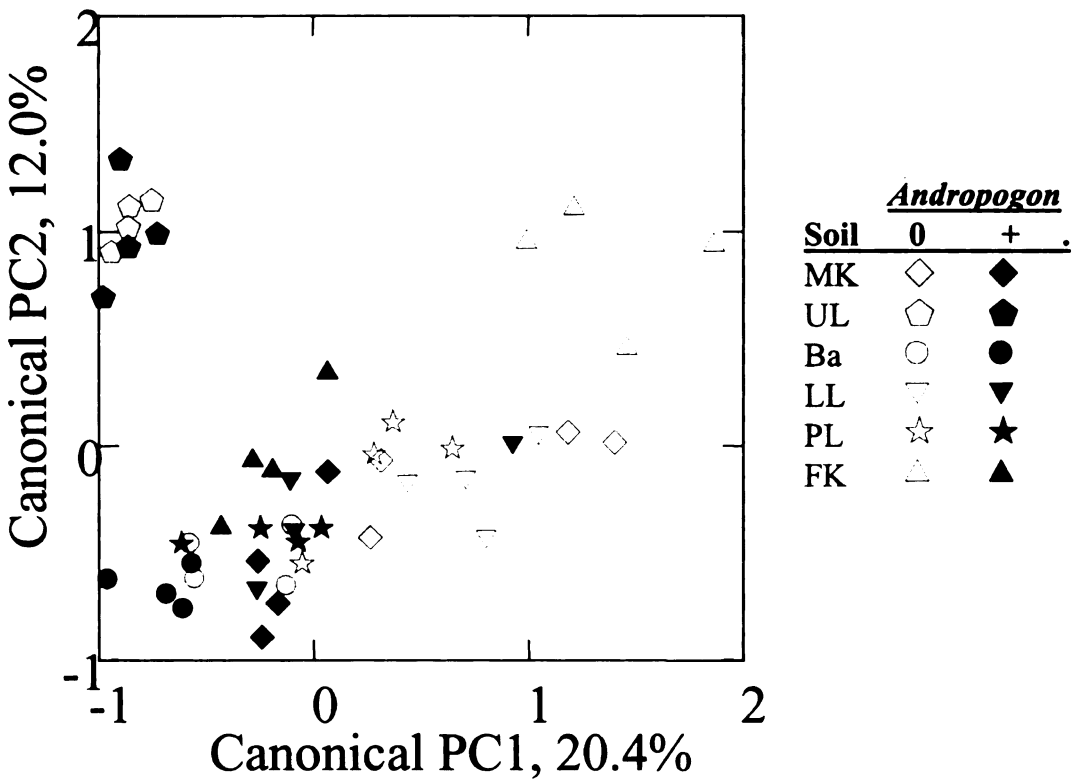

Figure 4.4. Soil origin and plant effects on the structure of the soil microbial community as measured by CLPP. CLPP patterns are distinguished between soil microbial communities from the *A. gerardi* (solid symbols) and no plant treatments (open symbols). Soil microbial communities in soils from different sites are indicated by symbols: circles, BA; triangles, FK; upside-down triangles, LL; diamonds, MK; stars, PL; pentagons, UL. Significance values from the RDA are listed in Table 4.4.

There were also some significant interactions between the presence of *A. gerardi* and soil origin (Table 4.3A), indicating that the presence of a plant did not have uniform effects on the CLPP profiles across all soils. In general, CLPP patterns in the presence of *A. gerardi* loaded lower on canonical PC1 and canonical PC2; however, CLPP patterns from UL soil were markedly different from all other soils regardless of the presence of *A. gerardi* (Figure 4.4).

The RDA for the PLFA profiles of the soil microbial community also revealed variation among the six sites and detected an effect of the presence of *A. gerardi* (Table 4.5, Figure 4.5). Axis 1 accounted for 20.8% of the variance in phospholipid fatty acid data, 47.2% of the variance in the phospholipid fatty acid-environment relationship and had a phospholipid fatty acid-environment correlation of 0.879 (Table 4.5B). Axis 2 accounted for 9.2% of the variance in phospholipid fatty acid data, 21.2% of the variance in the phospholipid fatty acid-environment relationship and had a phospholipid fatty acid-environment correlation of 0.890 (Table 4.5B). Axis 3 accounted for 5.1% of the variance in phospholipid fatty acid data, 11.6% of the variance in the phospholipid fatty acid-environment relationship and had a phospholipid fatty acid -environment correlation of 0.790 (Table 4.5B). Axis 4 accounted for 3.0% of the variance in phospholipid fatty acid data, 6.7% of the variance in the phospholipid fatty acid-environment relationship and had a phospholipid fatty acid-environment correlation of 0.630 (Table 4.5B). The presence of *A. gerardi* (Trt) was significantly related to the ordination of the PLFA profiles, as were all levels of soil origin (MK, BA, LL, PL, and FK), and the time at harvest (Table 4.5A).

Table 4.4. Fatty acids used in principal components analysis of PLFA profiles. I describe fatty acids using standard nomenclature where the total number of carbon atoms appears before the colon and the total number of C-C bonds appears after it. Cyclo-propane analogs are indicated by "cyclo," and the location of the epoxy bond is indicated by a "c" followed by two numbers. If the cis or trans configuration is unknown, the word "at" is used. The number following "cis," "trans," or "at" indicates the location of the double bond in relation to the carboxyl end of the molecule. Fatty acids with the same retention time are grouped as "sum in feature" and given a unique number designation.

Phospholipid Fatty Acids
C9 Dicarboxylic acid
14:0 iso
14:0
15:0 iso
15:0 anteiso
15:0
16:0 iso
16:1 cis 7
16:1 cis 9
16:1 cis 11
16:0
iso 17:1 G
17:0 iso
17:0 anteiso
17:0 cyclo
16:1 2OH
18:1 9 trans alcohol
18:2 cis 12
18:1 cis 9
18:1 cis 13
18:0
19:1 at 11 alcohol
19:0 cyclo c11-12
19:0 cyclo 11-12 2OH
22:0
22:0 2OH
24:0
Coprostane
Unknown 25.339
Summed feature 8: 18:1 trans 9

Table 4.5. Effect of soil origin, time of harvest, and presence of *A. gerardi* on the soil microbial community as detected by Distance-Based Redundancy Analysis of PLFA profiles. (A) Significance values for the permutation tests on the environmental factors of the RDA. (B) Variance explained by species data and species-environment correlations for the RDA.

(A)

Factor	Lambda	F-stat	p-value	% variance
Plant	0.06	9.189	0.0010	6.4
Time	0.01	1.859	0.0540	1.3
Soil	0.31	9.037	0.0010	31.3
Plant*Time	0.09	4.351	0.0010	9.0
Soil*Plant	0.42	5.607	0.0010	42.1
Soil*Time	0.36	4.799	0.0010	36.4
All	0.51	3.285	0.0010	51.2

(B)

Axis	Eigenvalue	Species-Environment Correlation	Cum % Variance of Species Data	Cum % Variance of Species-Environment
1	0.208	0.879	20.8	47.2
2	0.092	0.890	30.0	68.0
3	0.051	0.790	35.1	79.6
4	0.030	0.630	38.1	86.3

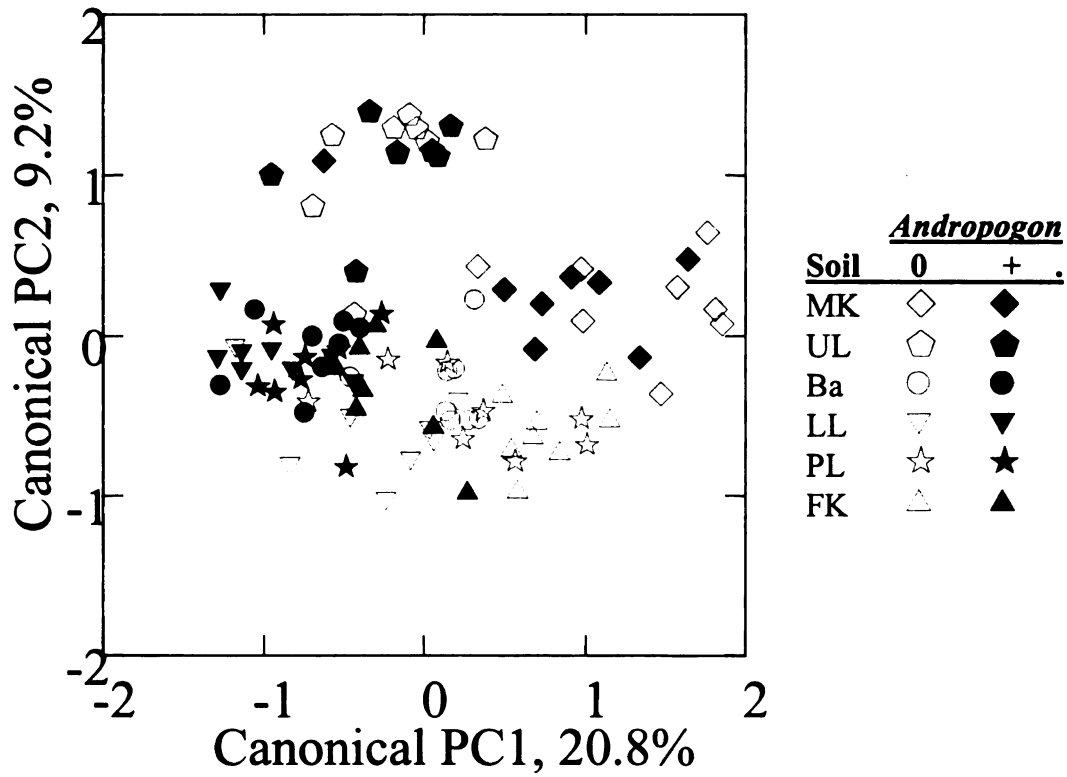

Figure 4.5. Distance-based Redundancy Analysis of PLFA profiles, investigating the effects of soil origin, plant, and time effects on the structure of the soil microbial community. The PLFA profiles are distinguished between soil microbial communities from the *A. gerardi* (solid symbols) and no plant treatments (open symbols). Soil microbial communities in soils from different sites are indicated by symbols: circles, BA; triangles, FK; upside-down triangles, LL; diamonds, MK; stars, PL; pentagons, UL. Labels for phospholipid fatty acids are listed in Table 4.3. Significance values from the RDA are listed in Table 4.5.

Figure 4.5 shows the separation of samples by environmental factors and fatty acids. There was a significant interaction between the presence of *A. gerardi* and time at harvest (Table 4.5A, Plant*Time). Soils with plants tended to cluster higher on canonical PC1 (Figure 4.5). This was related to higher amounts of high carbon chain phospholipid fatty acids (22:0, 24:0, 22:0 2OH, and unknown 25.339), indicating more eukaryotes were present in soils with plants.

Additionally, there was also a significant interaction between the presence of *A. gerardi* and soil origin (Table 4.5A: Soil*Plant), indicating that the presence of a plant did not have uniform effects on the PLFA profiles across all soils. The higher fertility soils (Ba, LL, PL, and FK) had similar PLFA patterns and responded the same way to the presence of *A. gerardi* (an increase in canonical PC1, Figure 4.5); however, the PLFA patterns of the soils from the two low productivity sites did not change in response to the presence of *A. gerardi* and were different from the patterns of the high fertility sites (Figure 4.5). The UL site was different from all other sites in the amounts of some monounsaturated fatty acids on canonical PC2 (UL site had higher amounts of 18:1 cis 13 and lower amounts of summed in feature 8). Soils from the MK site showed a smaller elevation along canonical PC2 and increased numbers of eukaryotes (higher canonical PC1).

Discussion

Growing *A. gerardi* in these soils provided an independent assay of the potential productivity of each of these sites (*A. gerardi* production) and a direct test of the "plant" effect on soil microbial community structure and processes. This study suggests the origin of the soil and the presence of a plant both influence the structure and functioning of the soil microbial community. Most previous studies have been unable to distinguish between the effects of plants and the effects of soil origin on the structure of the soil microbial community. For example, Zelles *et al.* (1992) distinguished among the soil communities of grassland and agricultural fields using PLFA; however, both the soils and the plant communities differed among sites. Zelles *et al.* (1992) were able to distinguish among management regimes, but it was not possible to determine the relative effects of the soil versus the plants on these differences. In addition, those studies which have attempted to distinguish between soil and plant effects on the soil microbial community often did not measure the soil effect independent of any plant influence. Grayston and Campbell (1996) used CLPP patterns to differentiate between the soil microbial communities from the rhizospheres of hybrid larch (*Larix eurolepis*) and Sitka spruce (*Picea sitchensis*) trees in woodland and forest sites. However, the study does not estimate the magnitude of the plant effect on the CLPP patterns because there was no independent measure of the CLPP patterns of the soil microbial community in the absence of plants in these sites (Grayston and Campbell 1996).

Site Fertility Effects on Soil Microbial Processes

In this study, soil origin had a significant influence on soil properties controlled by the soil microbial community and on the structure of the soil microbial community itself. This suggests that historical factors of the soil can have persistent effects on the soil microbial community, while the extant plant community is a major source of labile carbon and can influence the structure of the soil microbial community and, consequently, ecosystem functioning through the soil microbial community. I have presented site fertility as the driving factor explaining the relationships between soil origin and the soil and microbial properties that I measured in this study. However, other factors besides site fertility differed among these sites (Table 4.1). There were some differences in percent soil organic matter across sites, and species diversity varied dramatically among sites. Bossio *et al.* (1998) have shown that enrichment of organic matter through agricultural management produces recognizable differences in the PLFA patterns from the soil microbial communities from various management regimes (organic, low-input, and conventional farming). The results from this study do not change if I rank the sites by soil organic matter rather than field above-ground biomass (data not shown). Both soil organic matter and above-ground biomass are surrogates for site fertility. Bossio *et al.* (1998) suggested that higher soil organic matter should lead to greater soil microbial biomass, but this study does not support this assertion. Increased organic matter inputs generally occur in agricultural systems that are being managed organically; perhaps natural gradients in soil organic matter should not be

expected to reflect the same pattern because the systems have already had time to reach an equilibrium in soil organic matter turnover.

Historical Plant Diversity Effects on Soil Microbial Processes

Another major difference among these field sites was the current plant species composition and diversity. There appears to be a unimodal relationship between above-ground plant biomass and species diversity across these six sites with low diversity, grass-dominated communities at both the lowest (MK) and highest (FK) fertility sites (Table 4.1). However, the soil and microbial properties of field MK were consistently more similar to the other low fertility site (UL) rather than FK, the other low species diversity site (Figure 4.2). This suggest that it is fertility more than diversity or composition that influences the soil microbial community.

To better understand the impact of the global decline in species diversity due to human activities, many researchers have been investigating the relationship between species diversity and ecosystem function. The 'rivet hypothesis' proposes that each species contributes something unique to ecosystem function, and so ecosystem function declines as biodiversity declines (Ehrlich and Ehrlich 1981, Lawton 1984). A contrasting hypothesis suggests that species are redundant and that ecosystem function only declines when functional groups are missing from an ecosystem (Walker 1992, Lawton 1994). Finally, Lawton (1994) proposed that ecosystem function changes when a species is lost, but the direction and amount of that change are not predictable.

Observational studies and manipulative experiments investigating these theories have provided mixed results. My work in Chapter 2 (Broughton and Gross 2000) showed a significant effect of plant species diversity on the respiration or biomass of the soil microbial community, but these results were confounded with plant productivity and edaphic changes. My work at the Silwood, England BIODEPTH site (Chapter 3) found a positive relationship between plant diversity and two measures of microbial community structure (CLPP and PLFA). The Swiss BIODEPTH experiment has shown positive relationships between plant diversity and plant biomass (Spehn *et al.* 2000a, Spehn *et al.* 2000b), soil microbial respiration and functional diversity (Stephan *et al.* 2000), microbial biomass (Spehn *et al.* 2000a), and earthworm population density (Spehn *et al.* 2000a). In contrast, Wardle has consistently shown no relationship between diversity and ecosystem function in a series of plant removal experiments in New Zealand perennial grasslands (Wardle *et al.* 1999, Wardle *et al.* 2000, Wardle and Nicholson 1996). In a plant removal study in a North American grassland, Symstad *et al.* (1998) showed a positive relationship between plant species diversity and productivity, but no relationship between plant diversity and other ecosystem functions. Mikola and Setälä (1998) found unpredictable ecosystem functioning responses to changes in species diversity when studying the phenomenon in a simple (three trophic level) decomposer food web from the soil of a pine forest in Finland.

In this experiment, I found no relationship between species diversity and soil microbial processes, regardless of the presence of *Andropogon gerardi*. Soils from communities

with high plant species diversity did not have higher or more efficient ecosystem processes or higher soil microbial activities or biomass, as suggested by some recent theory (Loreau 2000). These results are consistent with Symstad *et al.*'s (1998) plant species removal study in which they detected the effect of declining species richness on productivity, but not nitrogen retention, suggesting that the relationship between species diversity and ecosystem processes is not necessarily consistent or predictable. In this study, the one exception was microbial biomass. The pots without *A. gerardi* showed a positive relationship between the microbial biomass and the plant species diversity of the communities from the soils were taken. This relationship disappeared when *A. gerardi* was grown in the soil, however, suggesting that the present plant community can have a large effect on the present soil microbial community.

Several studies have shown plant composition (rather than plant diversity) effects on ecosystem functioning. The researchers reason that the quality of the carbon available to the microbial community is important and so the identity of the plant species providing that carbon should influence how ecosystem functions change (Paul and Clark 1996). Wardle *et al.* (1999) saw plant composition effects on PLFA patterns in the soils from a plant removal experiment in New Zealand grasslands, while Symstad *et al.* (1998) found plant composition effects on productivity and nitrogen retention. Hector *et al.* (2000) detected a relationship between the species composition of litter and the decomposition rate at the Silwood Park, England BIODEPTH site. Hooper and Vitousek (1998) investigated the relationship between plant composition and nutrient cycling in experimental plots on serpentine soil in California. They determined that

plant community composition accounted for much more of the variation in nutrient cycling processes than did plant functional group diversity alone (Hooper and Vitousek 1998). Knowing the identity of the plant species involved allowed for a much better explanation of changes in inorganic N pools, soil moisture, microbial biomass, and microbial immobilization as a result of the experimental manipulations (Hooper and Vitousek 1998). I found some evidence to support this view in the relationships between plant composition and microbial respiration and soil PLFA patterns at the Silwood Park, England BIODEPTH site (Chapter 3). Historical plant composition effects may be contributing some of the variation in this study in the effects of soil origin on soil and microbial processes, but I are unable to test this assertion due to the design of the study.

The 'Plant' Effect on Soil Microbial Processes

Not only was soil origin important in structuring the soil microbial community in this experiment, the presence of a plant also had important effects on the structure and function of the soil microbial community. The presence of *A. gerardi* drove down nitrogen pools, but increased microbial respiration without affecting microbial biomass, suggesting an increase in the turnover rate of the soil microbial community and faster nutrient cycling.

Other studies have shown plant effects on soil and microbial processes. Bachmann and Kinzel (1992) detected differences in the amounts of amino acids and sugars and the

rates of CO_2 evolution and some enzyme activities in the rhizosphere soils of six different plant species grown in four different soils. As in our study, Bachmann and Kinzel (1992) were able to detect strong plant effects regardless of soil origin and strong plant-soil interactions, although the magnitude and direction of change for enzyme activities and resource amounts were not consistent for different plant species. However, Bachmann and Kinzel (1992) did not measure nitrogen transformation rates, nitrogen pools, or microbial respiration or biomass. Groffman *et al.* (1996) investigated the relative roles of plant versus soil effects on the soil microbial community by measuring microbial biomass and activity and nitrogen transformation rates of soil taken from a range of old-field sites. The two experiments used various combinations of 10 plant species in monoculture and 4 soils that had been established for 4 years. Groffman *et al.* (1996) concluded that the main driver for microbial biomass and activity was soil type rather than plant species, although they suggested that plant effects might become more important after a longer period of time.

In this study, the magnitude of the plant effect on microbial respiration and total inorganic Nitrogen pools was larger in higher fertility soils than low fertility soils. One possible explanation for this result is that in lower fertility soils, there is a greater possibility that the resources supporting the soil microbial community are coming from the extant plant community. Consequently, the presence of a plant constantly providing resources to the soil microbial community could allow for a more active microbial community. This is in contrast to the possibility of increasing the soil microbial

biomass using the influx of new resources, which Bossio *et al.* (1998) suggest is often the result of increasing organic matter inputs.

Plant and Soil Effects on Soil Microbial Community Structure

Both soil origin and the presence of *Andropogon gerardi* had significant effects on potential metabolic diversity (measured with CLPP patterns) and phenotypic diversity (measured with PLFA profiles) of the soil microbial community in this study. Communities from field UL soils had strikingly different PLFA and CLPP patterns from communities grown in other soils; additionally, the presence of *A. gerardi* had no effects on CLPP and PLFA patterns for UL soils. The UL field is a highly diverse, low productivity site and the composition of the soil microbial community does not seem to respond quickly to changes in the plant community.

The soil microbial communities detected in MK soil (another low fertility site) had PLFA patterns similar to field UL, but had similar CLPP patterns similar to the higher productivity sites. This indicates that structurally different microbial communities (from fields MK and UL) are capable of consuming the same resources. Other studies have found that community function may not change when community structure does. Buyer and Drinkwater (1997) detected differences in PLFA patterns between replicates of manipulations of different management treatments involving different crop residues but saw no differences in CLPP patterns. Similarly, Ibekl and Kennedy (1998)

determined that PLFA profiles were more sensitive than CLPP patterns to differences in the soil microbial community grown in two different soils under six plant treatments.

Like field UL, field MK is a low productivity field; however, MK also has low organic matter is dominated by a grass, *Agropyron repens*. If the long chain phospholipid fatty acids indicate mycorrhizae, the historical presence of *A. repens* plants in MK may explain why microbial communities incubated in MK soils have a higher proportion of eukaryotes than microbial communities grown in other soils, regardless of the presence of *Andropogon gerardi*. Although, the long chain phospholipid fatty acids may only be indicative of more plant material present in the soil.

A central goal in ecology is to determine the factors controlling the abundance and distribution of species. Microorganisms in their natural habitats are only recently being studied in ecology. This study suggests that both the history of the soil and the plants presently growing in a community affect the structure and function of the soil microbial community. Further studies should eludicate the relative importance of these two factors in influencing the soil microbial community.

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CHAPTER 5

AN EXPERIMENTAL EVALUATION OF THE EFFECTS OF DIFFERENT PLANT SPECIES ON THE STRUCTURE AND FUNCTION OF SOIL MICROBIAL COMMUNITIES

Introduction

Most of the carbon and nitrogen entering the soil results from litterfall, root exudates, or root death of plants (Paul and Clark 1996). Because organic inputs from plants species can differ in quantity, timing, and biochemistry, plant species identity has the potential to affect microbial process rates through litter quality and root exudation effects. As a result, the composition and productivity of the plant community may influence the soil microbial community. Conversely, the productivity or diversity of the plant community may be affected by processes mediated by soil microorganisms (*e.g.* N-mineralization rates). Consequently, changes in the plant community and the accompanying change in the soil microbial community potentially affect ecosystem function.

To date, many studies have investigated the role of edaphic factors in structuring the soil microbial community, while fewer have addressed the effect of the plant community. Additionally, very few have sought to distinguish between the effects of soil and the effects of plants on the soil microbial community. Most studies of plant community effects on soil microbial communities confound direct plant effects with soil effects. For example, J.C. Zak *et al.* (1994) detected differences in the structure of the soil microbial community (using Biolog) along an elevational and moisture gradient

in the Chihuahuan Desert at the Jornada Long-Term Ecological Research site. Similarly, Goodfriend (1998) used Biolog to distinguish among the microbial communities at eight sites representing a variety of wetlands. However, in both of these studies, the plant communities sampled were from different sites in which there were likely concomitant changes in soil characteristics Consequently, the influence of plant community composition differences could not be assessed independently of differences in edaphic characteristics.

It remains unclear how the plant community affects the soil microbial community relative to effects of soil environment on the microbial community. In an earlier greenhouse experiment I used field soils from six sites to distinguish the relative importance of direct soil effects versus plant-mediated soil effects on the soil microbial community in a controlled environment. Using a single species, *Andropogon gerardi*, I found that microbial communities grown in the six soils differed in both structure and function and that *A. gerardi* mediated those differences. To follow up on those results and determine if plant species differed in their effects on the structure and function of the soil microbial community, I conducted a greenhouse experiment in which soils from two old fields that differed in fertility were planted with all combinations of three plant species common to local old fields. I hypothesized that (1) plant species have unique effects on the structure and function of soil microbial communities, (2) the effects of different plant species on soil microbial community structure and function are non-additive, and (3) soil microbial communities close to the roots (rhizosphere) are different from the communities in the bulk soil. To test whether soil microbial

communities in rhizosphere soil differed from those in bulk soil, I excluded roots from a cylinder of soil in each pot.

Methods

Site and Species Descriptions

Soil was collected in October 1999 and October 2000 from two of the six successional old-fields at the W. K. Kellogg Biological Station in southwestern Michigan sampled for the experiment in Chapter 4. The sites differed in fertility, species diversity, dominant plant type, and years since abandonment, but both were located on Kalamazoo sandy loam soil (Table 5.1). To estimate plant species diversity, I determined species composition in six 0.5 m x 2 m plots at each site in August 1998. To estimate annual net primary productivity (ANPP), I clipped aboveground biomass at ground level from a 0.5 m x 0.5 m plot located within the plots used to assess species diversity.

The Upper Louden (UL) field had moderate ANPP, high species diversity and was dominated by diverse forb communities. In contrast, Field K (FK) had high ANPP, low species diversity, and was dominated by *Bromus inermis* (Table 5.1). The UL field had been abandoned from agriculture for over fifty years. Field K was once used as pasture, but had been unmanaged for over twenty-five years.

Table 5.1 Plant species by functional group and aboveground biomass at the two field sites from which soil was collected. Functional groups are coded by Grass (G), Forb (F), Legume (L), and Woody (W). Plant species used in this greenhouse experiment are in bold.

Field	Species	Functional group	Biomass (g/m^2)	% of Total Biomass
FK	***Bromus inermis***	**G**	**589.6**	**98.7**
	Agropyron repens	G	8.0	1.3
	Total		597.6	
UL	*Poa compressa*	G	48.8	15.6
	Trifolium pratense	**L**	**34.4**	**11.0**
	Andropogon virginicus	G	30.6	9.8
	Hieracium sp.	F	30.5	9.8
	Danthonia spicata	G	26.3	8.4
	Rudbeckia hirta	F	20.6	6.6
	Solidago nemoralis	F	20.0	6.4
	Solidago canadensis	**F**	**14.4**	**4.6**
	Aster sp.	F	14.4	4.6
	Antennaria plantaginifolia	F	9.4	3.0
	Rubus sp.	W	9.3	3.0
	Panicum sp.	G	8.1	2.6
	Achillea millifolium	F	8.0	2.6
	Centaurea maculosa	F	7.0	2.3
	Chrysanthemum leucanthemum	F	5.5	1.8
	Aster sp.	F	4.4	1.4
	Rumex acetosella	F	3.8	1.2
	Panicum sp.	G	3.8	1.2
	Solidago speciosa or *juncea*	F	3.7	1.2
	Other (5 species)	3F,2G	9.2	2.9
	Total		312.3	

To maximize the possible differences among the plant species, I selected three perennial species that are representative of different functional groups in old-field communities: *Solidago canadensis* (forb), *Trifolium pratense* (legume), and *Bromus inermis* (grass). *Solidago canadensis* is a native herbaceous perennial dicot that is

commonly dominant in higher productivity fields of southwestern Michigan (Foster 1996, Werner *et al.* 1980). *Trifolium pratense* is an herbaceous perennial legume naturalized from Eurasia that occurs in fields across a broad range of productivities (Scoggin 1978b). *Bromus inermis* is a C_3 perennial grass dominant in high productivity fields in southwestern Michigan and is naturalized from Eurasia (Scoggin 1978a).

Experimental Design

To distinguish direct plant effects versus plant-mediated soil effects on the soil microbial community, I grew each species alone and in all combinations in soil from the two sites described above in a greenhouse experiment. I also included no-plant controls. There were root exclosures in each pot to separate bulk from rhizosphere soil.

Soil for these experiments was collected from both field sites from the top 15 cm in the same area from which species diversity and plant biomass were sampled. Because of sample processing constraints, I conducted the experiment in three time blocks. Soil collected in December 1999 was used for the first two time blocks, while soil collected in October 2000 was used for the third time block. The soil was sieved to 4 mm and mixed to reduce variability. Soil was stored at room temperature until the experiment was established in the greenhouse (less than 2 months).

I used a randomized complete block factorial design for the greenhouse experiment in which I varied soil (2 sources - Soil) and plant species (3 species in monoculture and all

combinations, including no plants), with 3 replicates of each treatment (Replicate) at 3 different times (Time). The plant treatments used are described in Table 5.2. Soils were placed in 5 cm diameter x 20 cm deep pots and kept well-watered with de-ionized water to avoid adding nutrients or contaminants. Treatments were randomly assigned to pots within replicate. To compare soil microbial communities between bulk and rhizosphere soil, I included one root exclosure tube in each pot. Root exclosures were sewn into 15 cm x 2 cm diameter cylinders from 20-micron mesh, and the seams were sealed with silicone sealant. The exclosure tube was filled with soil and placed in the center of the pot.

All plant species were added as 1 month old seedlings; all seedlings were germinated in a sterile sand medium in a growth chamber and were less than 2 cm in height at transplantation. One plant per species was added to each pot; for mixtures, seedlings were planted at equal distances from each other with the root exclosure in the center. If the transplant was unsuccessful, the seedling was replaced for up to four weeks into the experiment.

Each segment of the experiment ran for a total of 18 weeks to ensure that the roots had filled the pot. I ran the experiment in three time blocks because of the number of samples and the time required to process each sample. Thus, time was an additional

Table 5.2. Treatments used in the Randomized Complete Block Design to test for the effects of plant species on the soil microbial community. Each treatment was replicated three times within three Time blocks on two different soils. Species planted were G = *Bromus inermis*, F = *Solidago canadensis*, L = *Trifolium pratense.* 0 = absent, + = present, C = control (no plants).

Plant Treatment	*Bromus inermis*	*Solidago canadensis*	*Trifolium pratense*
C	0	0	0
G	+	0	0
F	0	+	0
L	0	0	+
FL	0	+	+
GF	+	+	0
GL	+	0	+
GFL	+	+	+

factor in the ANOVA. The first three replicates grew from December 1999 until May 2000, the second three replicates grew from February to June 2000, and the third set of three replicates grew from November 2000 until March 2001. Temperature in the greenhouse from 25 to 40 °C; light availability was controlled through a 12 h light/ 12 h dark cycle.

Data Collection

Differences among treatments were assessed by (1) plant growth, (2) the inorganic nitrogen pool, and (3) N-mineralization rate. At harvest, I determined the dry mass of above and belowground plant biomass. I separated root biomass from the soil (and other species' roots) during sieving. To avoid confusing roots from different plant species, root systems were kept intact. Roots were rinsed thoroughly in de-ionized water before drying. The root and shoot biomass for each species were measured separately. All plant material was dried at 60°C for 48 h. Shoot biomass was dried separately from root biomass.

After the roots were removed, soil from the entire pot (rhizosphere influenced) was passed through a 2 mm sieve and stored in sealed plastic bags at 4°C until analyzed. Soil from the exclosures was sieved and stored separately. All analyses were done within 3 days of sampling, except PLFA. Soil for PLFA analyses was kept at –80°C until the fatty acids were extracted.

I determined gravimetric soil moisture, total inorganic nitrogen, and N-mineralization and nitrification rates for each sample as described in Chapter 4. I assessed differences in soil microbial community production among treatments by (1) microbial biomass C and (2) microbial respiration. The method used to determine microbial respiration and the chloroform fumigation incubation method used to determine microbial biomass are described in Chapter 4 (see also Paul *et al.* 1999). I assessed soil microbial community structure differences among treatments by PLFA profiles as described in Chapter 4. For analysis, I included only those phospholipid fatty acids that were present in greater than 50% of samples and reported their abundance as the square root of the proportion of the total phospholipid fatty acid amount in each sample (Hellinger transformation). Of the 70 lipids detected, 31 phospholipid fatty acids met this criterion for the entire experiment (n = 144) and 34 phospholipid fatty acids met this criterion for the subset (n = 58) analyzed with root exclosures (Table 5.4).

Statistical Analyses

I used a randomized complete block design (ANOVA) model to test the effects of soil origin, presence/absence of each plant species, and time on the following response variables: soil moisture, plant biomass, relative growth rate, total N, N-mineralization rate, nitrification rate, microbial respiration, and microbial biomass (Table 5.3). The plant treatments were analyzed in three ways. (1) I included all 8 plant treatments as independent factors in the ANOVA (Table 5.3A) to assess the effect of each plant treatment on the soil and microbial characteristics. (2) Because in Chapter 4 I found

large effects of the presence of a plant on many soil and microbial variables, to determine the effects of the plants on soil and microbial characteristics, I ran the ANOVA with only the 7 plant treatments, excluding the no-plant control (Table 5.3B). (3) To determine the compositional effects of *Bromus inermis, Solidago canadensis*, and *Trifolium pratense* on soil and microbial characteristics, I used a 2 x 2 x 2 factorial design for the plant treatments in the ANOVA (Table 5.3C). As carbon should be limiting to the microorganisms, the size of the microbial community should depend on the input of carbon to the system. Because the plant material is the primary source of new carbon to microorganisms, then the microbial biomass should be proportional to the belowground plant biomass in any one spot. To determine if significant treatment effects on soil community structure or function were the indirect results of changes in plant biomass, I also used root, shoot, and total plant biomass as co-variates in all three analyses.

I used a modified redundancy analysis (RDA, Legendre & Anderson 1999) to determine the relationship between the environmental factors (soil origin, presence/absence of each plant species, time of harvest) and the PLFA measure of soil microbial community structure. I transformed the PLFA data using a Hellinger transformation because this transformation does a good job of handling data matrices with many zero values (Legendre & Gallagher, in press). The RDA procedure involved: (1) the creation of a matrix of dummy variables corresponding to the randomized complete block design (modeled from the experimental design: soil origin, presence/absence of each plant, time at harvest), (2) redundancy analysis of the

relationship between the principal coordinates (matrix of optical density or phospholipid fatty acid data) and the environmental variables (matrix of dummy variables in (1)), and (3) implementation of a Monte Carlo permutation test to estimate the statistical relationship between the two matrices (Legendre & Anderson 1999). This analysis allows me to test which factors from the experimental design (soil origin, presence/absence of each plant, time at harvest) are significantly related to the variation in the PLFA patterns. The modified RDA is a better statistical technique than regular ordination techniques because it allows for significance testing.

Results

Plant Communities

As presented in Chapter 4, the two field sites differed significantly in peak aboveground plant biomass (live and litter) and species composition (Table 5.1). The Upper Louden (UL) field had 320 ± 23 g/m^2 aboveground plant biomass and 15.8 ± 1.0 species per square meter, while Field K (FK) had 592 ± 22 g/m^2 aboveground plant biomass and 1.3 ± 0.2 species per square meter. Field K was dominated by *Bromus* inermis with very little *Agropyron repens* (Table 5.1). Upper Louden field supported a complex forb-dominated community, which included *Poa compressa, Trifolium pratense, Andropogon virginicus,* several *Hieracium* species, *Danthonia spicata, Rudbeckia hirta,* several *Solidago* species, *Antennaria plantaginifolia, Achillea*

millefolium, Centauria maculosa, Chrysanthemum leucanthemum, several *Panicum* species, and a species of *Rubus* (Table 5.1).

Time Effects on Soil and Microbial Processes

Time significantly affected most soil and microbial variables (Table 5.3A). The first two time groups used soil collected in 1999, while the last time group used soil collected in 2000. The last experimental group (time 3) had significantly higher total inorganic nitrogen pools ($p < 0.001$, $F = 83.3$), N-mineralization rates ($p < 0.001$, $F = 43.5$), and nitrification rates ($p < 0.001$, $F = 39.4$). Microbial respiration ($p< 0.001$, $F = 8.0$) and microbial biomass ($p < 0.001$, $F = 17.8$) also differed significantly among time blocks (Table 5.3A). The time effect seems to be the result of the two different soil collection times. Because patterns were consistent among time blocks and there were no significant interactions between Time and other factors, I did not investigate the Time effects in any further detail.

Plant Species Responses to Soil Types

Plants grown in soil from field FK, the higher fertility site, attained higher biomass than those grown in soil from field UL (Figure 5.1A-C, Table 5.3B). There were also significant differences among the seven plant treatments in total plant biomass (Figure 5.1A, $p < 0.001$, $F=5.2$), root biomass (Figure 5.2A, $p < 0.001$, $F = 11.2$), and shoot biomass ($p< 0.05$, $F = 2.6$). In FK soil, *Bromus inermis* had the highest biomass and

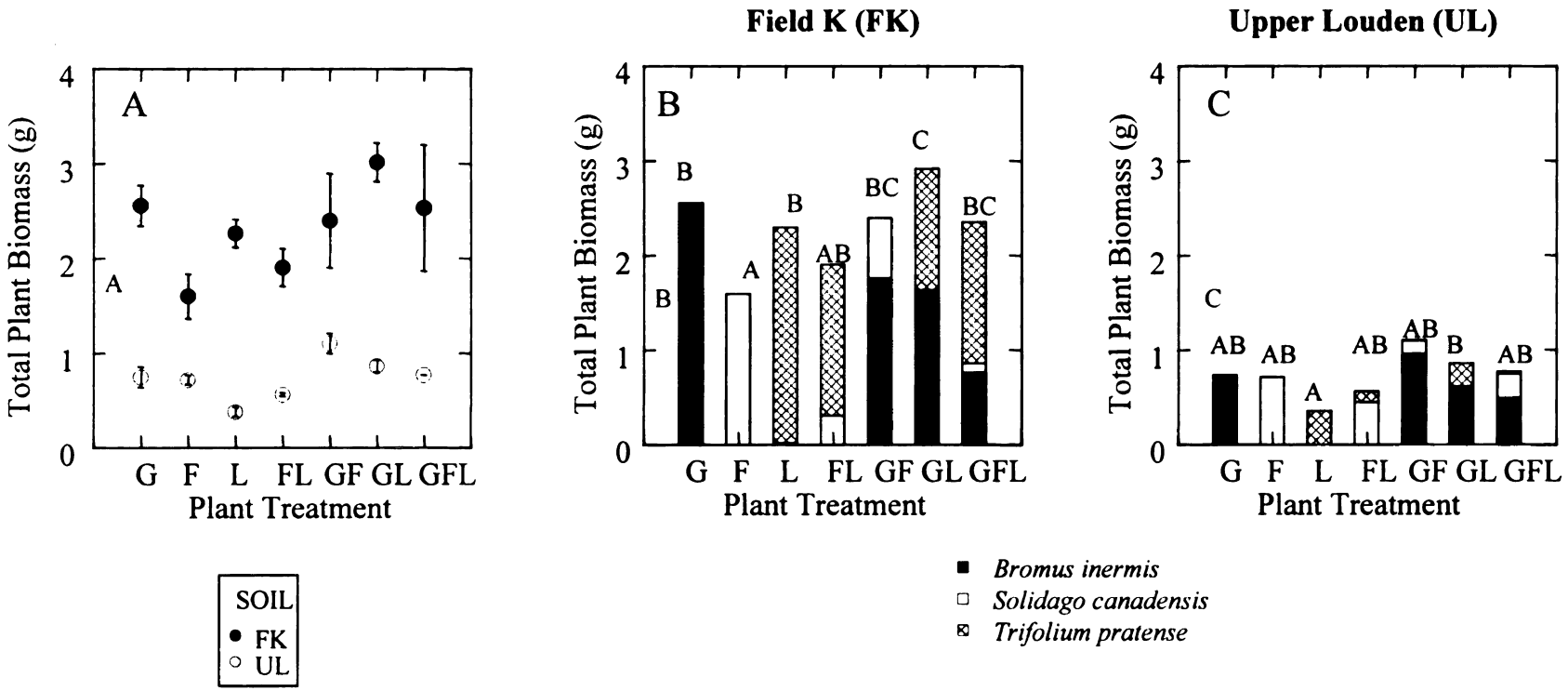

Figure 5.1. Total plant biomass of *Bromus inermis, Solidago canadensis,* and *Trifolium pratense*, coded by A) soil origin and plant treatment, treatment codes are in Table 5.2; and plant species by plant treatment for soil from B) Field K (FK) and C) Upper Louden (UL). For (A), values are mean ± standard error; for (B) and (C), values are means. Significance values from the ANOVA are listed in Table 5.3A.

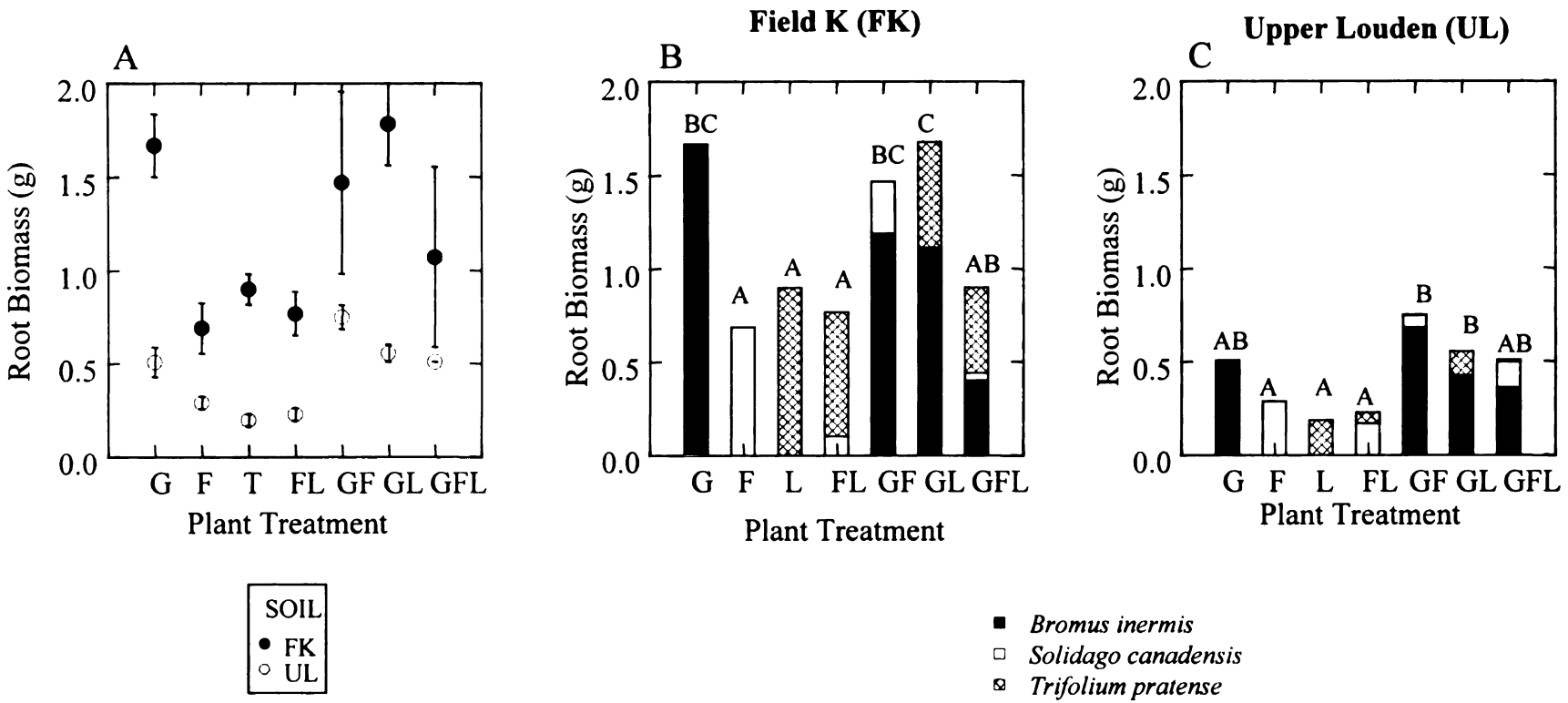

Figure 5.2. Root biomass of *Bromus inermis, Solidago canadensis,* and *Trifolium pratense*, coded by (A) soil origin and plant treatment, treatment codes are in Table 5.2; and plant species by plant treatment for soil from (B) Field K (FK) and (C) Upper Louden (UL). For (A), values are mean ± standard error; for (B) and (C), values are means. Significance values from the ANOVA are listed in Table 5.3A.

Table 5.3. Summary of ANOVA results analyzing effect of soil origin and (A) individual plant treatments, (B) individual plant treatments without no-plant controls, or (C) factorial plant treatments on plant and soil and microbial variables. Species codes in 3C are: BROIN, *Bromus inermis*; SOOCA, *Solidago canadensis*; TRFPR, *Trifolium pratense*. NS = not significant, $p > 0.05$. ANCOVA results, using total plant biomass as the covariate, are shown in parentheses if the effect changed in significance.

(A)

Variable	SOIL	TRT	TIME	SOIL*TRT
Total Biomass (g)	< 0.001	< 0.001	< 0.05	< 0.001
Root Biomass (g)	< 0.001	< 0.001	< 0.001	< 0.001
Shoot Biomass (g)	< 0.001	< 0.001	NS	< 0.001
Root to Shoot Ratio	<0.01	< 0.001	NS	< 0.05
Total Inorganic Nitrogen	< 0.05 (NS)	< 0.001	< 0.001	NS
N-mineralization Rate	< 0.01 (< 0.05)	NS	< 0.001	NS
Nitrification Rate	< 0.01 (< 0.05)	NS	< 0.001	NS
Microbial respiration	< 0.001 (< 0.01)	< 0.001	< 0.001	< 0.01 (< 0.05)
Microbial Biomass	NS	NS	< 0.001	NS

(B)

Variable	SOIL	TRT	TIME	SOIL*TRT
Total Biomass (g)	< 0.001	< 0.001	< 0.05	NS
Root Biomass (g)	< 0.001	< 0.001	< 0.001	< 0.05
Shoot Biomass (g)	< 0.001	< 0.05	NS	< 0.001
Root to Shoot Ratio	<0.01	< 0.001	< 0.05 (NS)	NS
Total Inorganic Nitrogen	< 0.05	NS	< 0.001	NS
N-mineralization Rate	< 0.01 (< 0.05)	NS	< 0.001	NS
Nitrification Rate	< 0.01 (< 0.05)	NS	< 0.001	NS
Microbial respiration	< 0.001 (< 0.01)	NS	< 0.01	< 0.05
Microbial Biomass	NS	NS	< 0.001	NS

(C)

Variable	SOIL	BROIN	SOOCA	TRIPR	TIME
Total Biomass (g)	< 0.001	< 0.001	< 0.05	< 0.001	< 0.05
Root Biomass (g)	< 0.001	< 0.001	NS	< 0.01	< 0.001
Shoot Biomass (g)	< 0.001	< 0.01	< 0.001	< 0.001	NS
Root to Shoot Ratio	< 0.001	< 0.001	< 0.05	< 0.05	< 0.05
Total Inorganic	NS	< 0.001	< 0.001	< 0.01	< 0.001
Nitrogen	(< 0.05)	(< 0.01)		(< 0.05)	
N-mineralization	< 0.001	NS	NS	< 0.05	< 0.001
Rate	(NS)			(NS)	
Nitrification Rate	< 0.001	NS	NS	< 0.05	< 0.001
	(NS)			(NS)	
Microbial respiration	< 0.001	< 0.01	< 0.001	< 0.01	< 0.001
	(< 0.05)	(NS)	(< 0.01)	(NS)	(< 0.05)
Microbial Biomass	NS	NS	NS	NS	< 0.001

Trifolium pratense had the next highest biomass (Figure 5.1A-C). In UL soil, *Bromus inermis* and *Solidago canadensis* had higher biomass than *Trifolium pratense* (Figure 5.1A-C). However, the diversity effect on productivity was non-additive: mixtures did not have higher biomass than monocultures (Figure 5.1A). *Bromus inermis* and *Trifolium pratense* dominated mixtures in FK soil (Figure 5.1B), while *Bromus inermis* tended to dominate the mixtures in UL soil (Figure 5.1C).

Plants grown in the higher fertility soil (FK) had higher root biomass than those grown in soil from field UL (Figure 5.2A-C, $p < 0.001$, $F = 21.9$). *Bromus inermis* had the highest root biomass in both FK and UL soils (Figure 5.2B,C, Table 5.3C).

Bromus inermis and *Trifolium pratense* grown in the lower fertility UL soil had higher root to shoot ratios than plants grown in FK soil (Table 5.4). *Bromus inermis*, the grass, had two times higher root to shoot ratios than both *Solidago canadensis* and *Trifolium pratense*, regardless of soil origin ($p < 0.001$, $F = 42.5$). Plant species did not appear to change their root to shoot ratios in response to the presence of competitors (Table 5.4).

Effects of Soil Origin on Soil and Microbial Processes

Soils from the FK site had significantly higher total inorganic nitrogen pools ($p < 0.05$, $F = 5.7$, Figure 5.3A), N-mineralization rates ($p < 0.01$, $F = 9.0$, Figure 5.3B), nitrification rates, ($p < 0.01$, $F = 8.5$, Figure 5.3C), and microbial respiration ($p < 0.001$,

Table 5.4. Root to shoot ratios for each plant species in each soil and treatment. Values are mean ± standard error. Significance values from the ANOVA are listed in Table 5.3C.

		2 species Mixtures			3 species mixtures
Field K (FK)	**Monoculture**	**+ G**	**+ F**	**+ L**	**GFL**
Bromus inermis (G)	1.95 ± 0.17	---	1.86 ± 0.50	2.14 ± 0.28	1.06 ± 0.06
Solidago canadensis (F)	0.70 ± 0.09	0.79 ± 0.18	---	0.49 ± 0.02	0.47 ± 0.47
Trifolium pratense (L)	0.67 ± 0.06	0.83 ± 0.20	0.70 ± 0.08	---	0.44 ± 0.02
Upper Louden (UL)	**Monoculture**	**+ G**	**+ F**	**+ L**	**GFL**
Bromus inermis (G)	2.79 ± 0.61	---	2.72 ± 0.27	2.25 ± 0.15	2.77
Solidago canadensis (F)	0.69 ± 0.07	0.87 ± 0.17	---	0.69 ± 0.17	1.17
Trifolium pratense (L)	1.22 ± 0.15	1.24 ± 0.19	1.04 ± 0.23	---	1.00

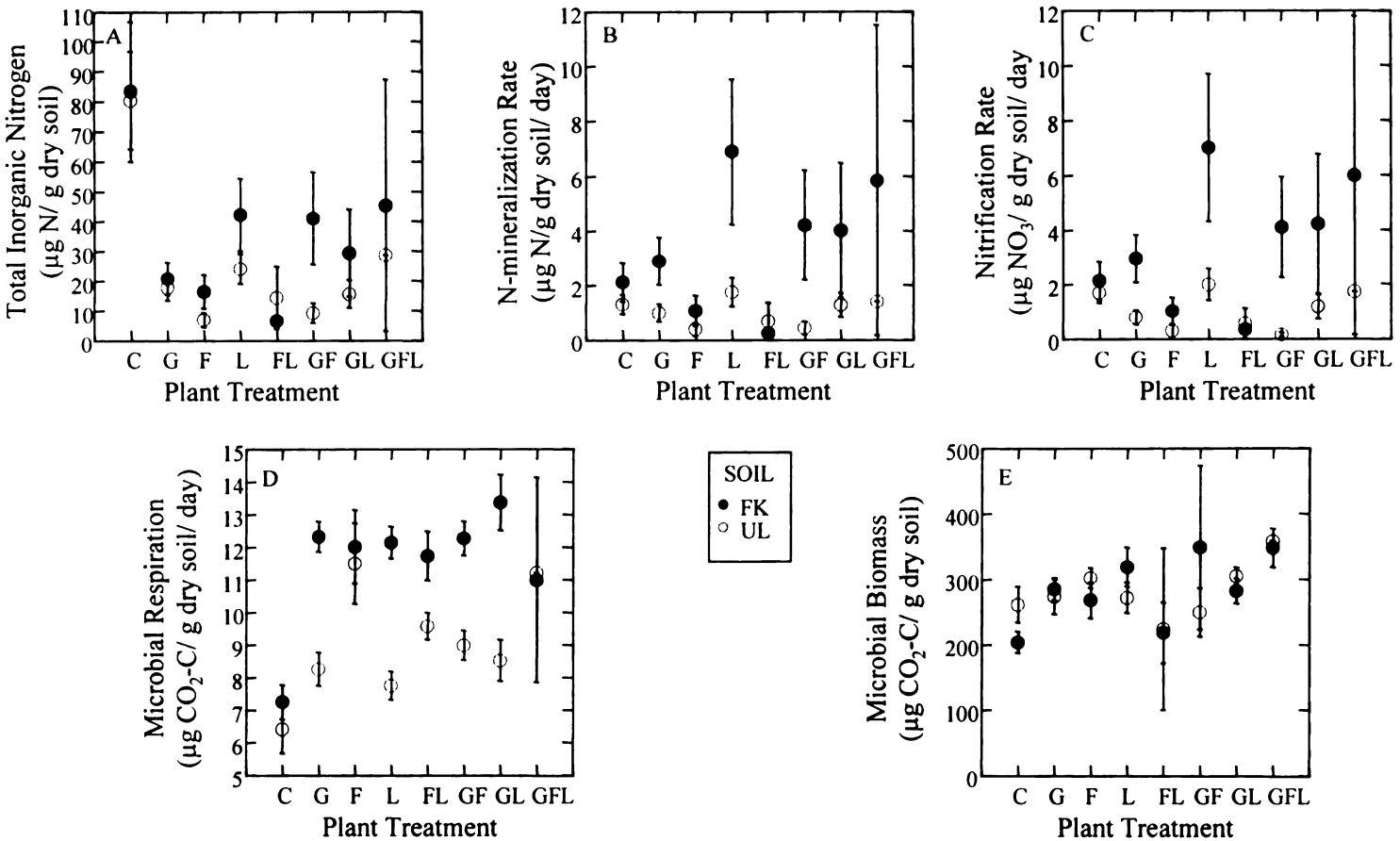

Figure 5.3. Variation among plant treatments in (A) total inorganic nitrogen, (B) N-mineralization rates, (C) nitrification rate, (D) microbial respiration, and (E) microbial biomass. For each variable, samples are distinguished between the two sites: FK (filled circles) and UL (hollow circles). Values are mean ± standard error. Significance values from the ANOVA are listed in Table 5.3A.

F = 22.6, Figure 5.3D) than those from the UL sites. However, soil origin did not affect soil microbial biomass (Table 5.3A, Figure 5.3E).

The differences in soil and microbial variables between the two sites were partly due to differences in plant biomass produced on the two soils (Figure 5.1A-C, Table 5.3A,C). When the analyses were re-run with total plant biomass as a covariate, total inorganic Nitrogen was no longer affected by soil origin (Table 5.3A) and N-mineralization and nitrification rates were not significantly influenced by soil origin (Table 5.3C). In contrast, when root biomass was the covariate, total inorganic Nitrogen ($p < 0.05$, $F = 4.5$) and N-mineralizaton ($p < 0.01$, $F = 8.6$) and nitrification rates ($p < 0.01$, $F = 8.3$) were still significantly affected by soil origin.

Plant Effects on Soil and Microbial Processes

The presence of any plant significantly reduced total inorganic Nitrogen (Figure 5.3A, $p < 0.001$, $F = 13.0$) and significantly increased microbial respiration (Figure 5.3D, $p < 0.001$, $F = 8.7$) on both soils. However, the composition of the plant community had no effect on either of these variables and there were no significant plant treatment effects when the no-plant controls were excluded from the analysis (Table 5.3B).

This experimental design allowed me to test for the effects of the presence of specific plant species on these processes (Table 5.3C). The presence of *Trifolium pratense* was significantly correlated with higher N-mineralization rates (Figure 5.3B, Figure 5.4B,E,

$p < 0.05$, $F = 5.1$) and nitrification rates (Figure 5.3C, $p < 0.05$, $F = 5.1$) and this effect was still detectable when root biomass was used as the covariate ($p < 0.05$, $F = 4.5$; $p < 0.05$, $F = 4.5$, respectively). However, increasing the number of plant species (or functional groups, the same in this design) did not have additive effects on any of the soil or microbial processes. Mixtures did not have detectably higher nitrogen pools or process rates than monocultures (Figures 5.3A-E).

Interactive Effects of Plant Species and Soil Origin on Soil and Microbial Processes

In this study there were few significant interactions between soil origin and plant treatments that affected soil and microbial processes (Table 5.3A-B). Plant biomass was significantly affected by the interaction between soil origin and plant treatments (Figure 5.1A, $p < 0.001$, $F = 28.3$). For example, *Solidago canadensis* had the lowest total biomass in monoculture in FK soil, while *Trifolium pratense* had the lowest total biomass in monoculture in UL soil (Figure 5.1A). Additionally, microbial respiration was significantly affected by the interaction between soil origin and plant treatment (Table 5.3A, $p < 0.01$, $F = 3.1$), primarily because the control, *Solidago canadensis* monoculture, and three species mixture (GFL) did not differ significantly in microbial respiration between the two soils, FK and UL (Figure 5.3D). Although there was no significant interaction between soil origin and plant treatment for N-mineralization and nitrification rates (Table 5.3A,B), in mixtures in the FK soil both tended to decrease in the presence of *Solidago canadensis* and increase in the presence of *Trifolium pratense*,

but were less responsive in the UL soil (Figure 5.3B-C). Total plant biomass and root biomass had no effect on this relationship.

Plant and Soil Effects on Soil Microbial Community Structure

PLFA profiles of the soil microbial community varied between the soils from the two sites and also responded to the presence of the three plant species (Table 5.6A). Soil origin accounted for 12% of the variation in PLFA profiles ($p < 0.001$, $F = 24.4$), while Time accounted for 10% ($p < 0.001$, $F = 20.4$) and Replicate an additional 5% of the variation ($p < 0.001$, $F = 10.6$). The three plant species had small but significant effects PLFA profiles, each accounting for only 1% of the variation (Table 5.6A). Axis 1 from the Redundancy Analysis accounted for 20.7% of the variance in phospholipid fatty acid data, 41.1% of the variance in the phospholipid fatty acid -environment relationship and had a phospholipid fatty acid -environment correlation of 0.853 (Table 5.5B). Axis 2 accounted for 12.4% of the variance in phospholipid fatty acid data, 25.2% of the variance in the phospholipid fatty acid -environment relationship and had a phospholipid fatty acid -environment correlation of 0.852 (Table 5.6B). Axis 3 and Axis 4 accounted for 5.1% and 2.5% of the variance in phospholipid fatty acid data, respectively (Table 5.6B).

Figure 5.4 shows the separation of samples by environmental factors and fatty acids as related to the axes from the Redundancy Analysis. PLFA profiles from UL soils loaded higher on PC2 than profiles from FK soils (Figure 5.4). UL soils had higher amounts

Table 5.5. Fatty acids used in principal components analysis of PLFA profiles. I describe fatty acids using standard nomenclature where the total number of carbon atoms appears before the colon and the total number of C-C bonds appears after it. Cyclo-propane analogs are indicated by "cyclo," and the location of the epoxy bond is indicated by a "c" followed by two numbers. If the cis or trans configuration is unknown, the word "at" is used. The number following "cis," "trans," or "at" indicates the location of the double bond in relation to the carboxyl end of the molecule. Fatty acids with the same retention time are grouped as "sum in feature" and given a unique number designation.

Phospholipid Fatty Acids (Full Experiment)	Phospholipid Fatty Acids (Exclosure subset)
C9 Dicarboxylic acid	C9 Dicarboxylic acid
14:0	14:0
15:0 iso	15:0 iso
15:0 anteiso	15:0 anteiso
15:0	15:0
16:0 iso	16:0 iso
16:1 cis 7	16:1 cis 7
16:1 cis 9	16:1 cis 9
16:1 cis 11	16:1 cis 11
16:0	16:0
iso 17:1 G	iso 17:1 G
17:0 iso	17:0 iso
17:0 anteiso	17:0 anteiso
17:0 cyclo	17:0 cyclo
16:1 2OH	17:0
18:1 trans 9 alcohol	16:1 2OH
18:2 cis 12	18:2 cis 12
18:1 cis 9	18:1 cis 9
18:1 cis 13	18:1 cis 13
18:0	18:0
19:0 cyclo c11-12	19:1 trans 11
20:0	19:0 cyclo c11-12
19:0 cyclo 11-12 2OH	20:4 cis 14
22:0	20:0
22:0 2OH	19:0 cyclo 11-12 2OH
24:0	22:0
23:0 2OH	22:0 2OH
Coprostane	24:0
Unknown 25.339	23:0 2OH
Cholesteryl-palmitate	Coprostane
summed feature 8: 18:1 trans 9	Unknown 25.339
	Cholesteryl-palmitate
	summed feature 8: 18:1 trans 9
	summed feature 12:

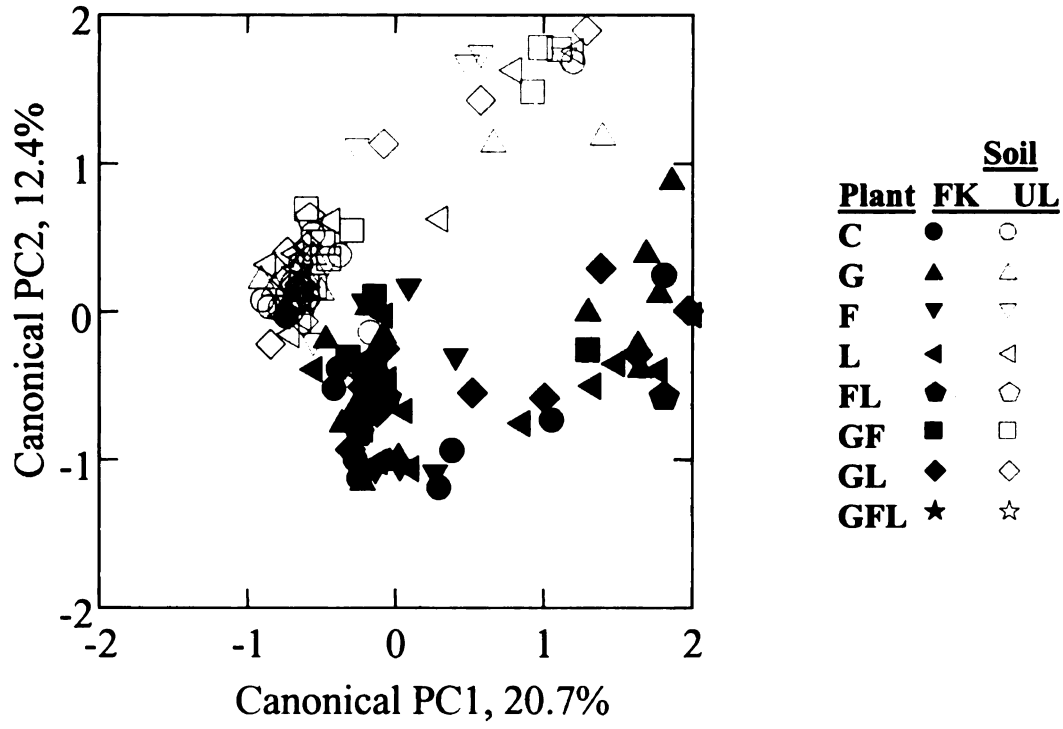

Figure 5.4. Distance-based Redundancy Analysis of Phospholipid Fatty Acid profiles, investigating the effects of soil origin, plant, and time effects on the structure of the soil microbial community. Canonical principal component plot for the full RDA. The PLFA profiles are distinguished between soil microbial communities from FK soil (solid symbols) and UL soil (open symbols). Soil microbial communities in soils with different plant communities are indicated by symbols: circles. controls; triangles, monocultures; four or five-sided polygons, mixtures. Labels for phospholipids are listed in Table 5.4. Significance values from the RDA are listed in Table 5.5A.

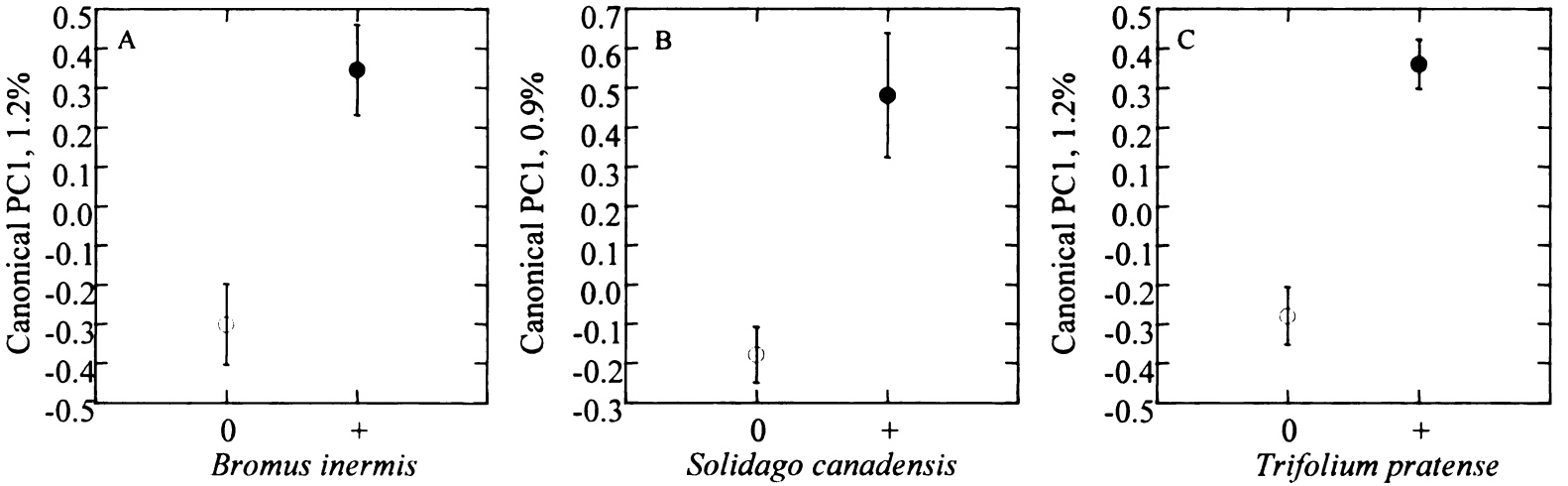

Figure 5.5. (A-C) Partial canonical principal component plots derived from the redundancy analysis of PLFA profiles. (A) *Bromus inermis* axis (1 of 1) with soil origin, replicate, *Solidago* and *Trifolium* treatments, and time partialled out. (B) *Solidago canadensis* axis (1 of 1) with soil origin, replicate, *Bromus* and *Trifolium* treatments, and time partialled out. (C) *Trifolium pratense* axis (1 of 1) with soil origin, replicate, *Bromus* and *Solidago* treatments, and time partialled out. Labels for phospholipids are listed in Table 5.4. Significance values from the RDA are listed in Table 5.5A.

Table 5.6. Effect of soil origin, time, replicate, and presence of *Bromus inermis* (BROIN), *Solidago canadensis* (SOOCA), and *Trifolium pratense* (TRFPR) on the soil microbial community as detected by Distance-Based Redundancy Analysis (db-RDA) of PLFA profiles. (A) Significance values for the permutation tests on the environmental factors of the RDA. (B) Variance explained by species data and species-environment correlations for the RDA.

(A)

Factor	Lambda	F-stat	p-value	% variance
Soil	0.12	24.4	0.001	12.2
BROIN	0.01	2.3	0.017	1.1
SOOCA	0.01	1.9	0.050	0.9
TRFPR	0.01	2.5	0.010	1.2
Time	0.10	20.4	0.001	10.3
Rep	0.05	10.6	0.001	5.3
Sum	0.50	2.7	0.001	

(B)

Axis	Eigenvalue	Species-Environment Correlation	Cum % Variance of Species Data	Cum % Variance of Species-Environment
1	0.207	0.853	20.7	41.1
2	0.124	0.852	33.1	66.3
3	0.051	0.761	38.2	76.5
4	0.025	0.589	40.6	81.4

of 18:1 cis 13, 16:1 cis 11, and 18:2 cis 12, indicating a higher fungal to bacterial ratio and more gram-negative bacteria (Harwood and Russell 1984). Partial canonical principal components derived from the redundancy analysis of PLFA profiles revealed that soil microbial communities grown under *Bromus inermis* had higher amounts of 16:1 cis 9, 16:1 cis 11, 18:1 cis 9, and summed in feature 8 (Figure 5.5A).

Communities grown in the presence of *Solidago canadensis* had higher 18:1 cis 13, 16:1 cis 7, 16:1 cis 11, and summed in feature 8 (Figure 5.5B), while communities grown in the presence of *Trifolium pratense* had higher 18:1 cis 13, summed in feature 8, 16:0 iso, iso 17:1 G, 16:1 cis 9, 17:0 cyclo, and 18:1 cis 9 (Figure 5.5C). This indicates that the microbial communities under all three plants had a higher proportion of gram-negative bacteria than microbial communities in the no-plant controls. In addition, microbial communities grown in the presence of *Trifolium pratense* had a higher proportion of gram-positive bacteria than soil microbial communities not exposed to *Trifolium pratense*. Overall there were few differences in PLFA profiles among replicates (within time) or time.

A Comparison of the Microbial Communities within and outside the Root Exclosures

I used root exclosures to exclude rhizosphere effects and create "bulk" soil in these pots. I compared the structure of the soil microbial community of the "bulk" soil to that of the rhizosphere soil using PLFA profiles. The RDA of the PLFA profiles showed no

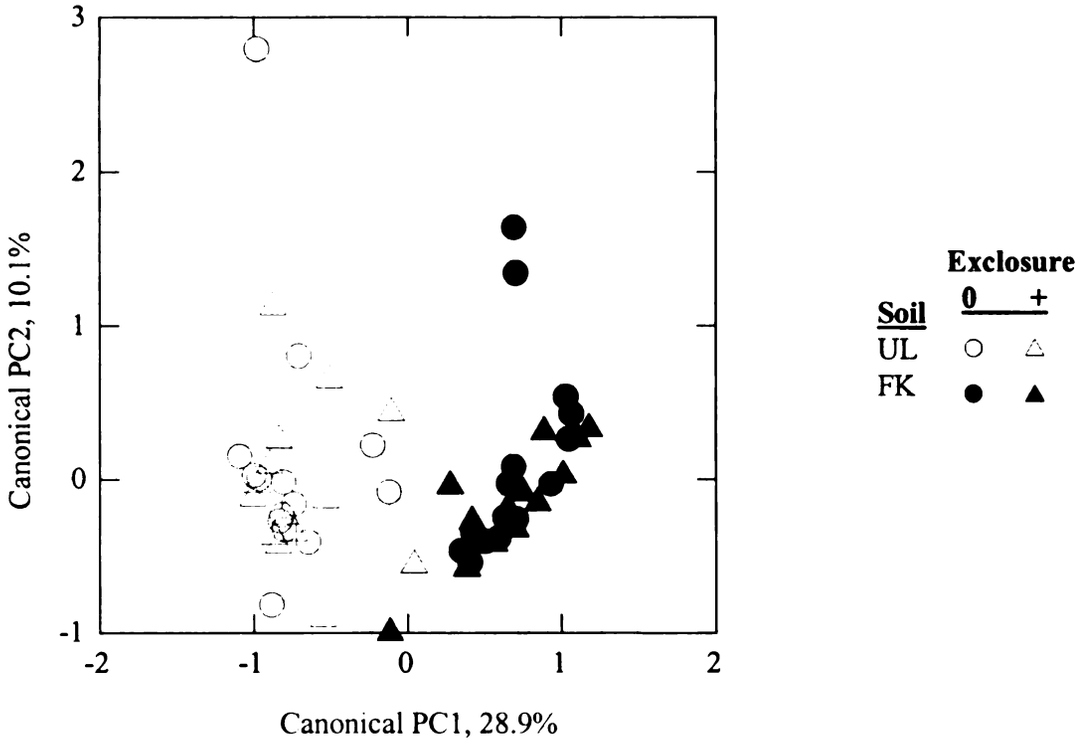

Figure 5.6. Distance-based Redundancy Analysis of Phospholipid Fatty Acid profiles, investigating the effects of exclosure, soil origin, plant, and time effects on the structure of the soil microbial community. Canonical principal component plot for the full RDA. The PLFA profiles are distinguished between soil microbial communities from FK soil (solid symbols) and UL soil (open symbols).

Table 5.7. Effect of exclosure, soil origin, time, and presence of *Bromus inermis* (BROIN), *Solidago canadensis* (SOOCA), and *Trifolium pratense* (TRFPR) on the soil microbial community as detected by Distance-Based Redundancy Analysis (db-RDA) of PLFA profiles. (A) Significance values for the permutation tests on the environmental factors of the RDA. (B) Variance explained by species data and species-environment correlations for the RDA.

(A)

Factor	Lambda	F-stat	p-value	% variance
Exclosure	0.02	1.4	0.110	1.8
Soil	0.25	20.0	0.001	24.8
BROIN	0.02	1.8	0.060	2.2
SOOCA	0.01	0.9	0.520	1.1
TRFPR	0.03	2.6	0.002	3.3
Time	0.02	1.6	0.060	2.0
Sum	0.79	2.0	0.001	

(B)

Axis	Eigenvalue	Species-Environment Correlation	Cum % Variance of Species Data	Cum % Variance of Species-Environment
1	0.289	0.976	28.9	36.8
2	0.101	0.920	39.0	49.6
3	0.085	0.898	47.5	60.4
4	0.066	0.907	54.1	68.8

effect of root exclosure on the structure of the soil microbial community. However, like RDA of the PLFA profiles for the whole experiment (above), the RDA for the PLFA profiles of the soil microbial communities within and outside the root exclosures also revealed variation between sites and detected an effect of the presence of *Trifolium pratense* on the soil microbial community (Table 5.7, Figure 5.6). Axis 1 accounted for 28.9% of the variance in phospholipid fatty acid data, while Axis 2 accounted for 10.1% of the variance in phospholipid fatty acid data (Table 5.6B). Soil origin accounted for 25% of the variation in PLFA profiles (Figure 5.6, $p < 0.001$, $F = 20.0$), while *Trifolium* pratense accounted for 3% of the variation ($p < 0.01$, $F = 2.6$). However, there was no effect of root exclosure on PLFA patterns (Figure 5.6), indicating that resources that had influenced the microbial community in these pots were able to travel through the soil and away from the roots. Differences detected among plant species were not due to direct contact with the roots.

Discussion

I expected to find that plant species had unique effects on the structure and function of soil microbial communities and that the effects of different plant species on soil microbial community structure and function are non-additive. I found that plant species had unique effects on soil microbial community structure (as indicated by PLFA) and some soil processes like total inorganic Nitrogen pools, N-mineralization and nitrification rates, and microbial respiration. However, I did not find additive effects of plant diversity, and soil effects were much stronger than individual plant effects on the

soil microbial community. This study suggests the origin of the soil and the presence of a plant both independently influence the structure and functioning of the soil microbial community. Most previous studies have been unable to distinguish between the effects of plants and the effects of soil origin on the structure of the soil microbial community.

As I saw in the previous chapter, soil process rates were higher and nitrogen pools were lower in the higher fertility soil (FK). However, because of the longer time frame during which these experiments were conducted (4 months in Chapter 4 versus 16 months in this study), Time did significantly affect soil and microbial process rates. The presence of a plant significantly affected soil and microbial processes and soil microbial community structure. In addition, the effects of different plant species on soil microbial community structure and function were non-additive. Process rates from mixtures could not be determined by summing process rates from monocultures of the plant species included in the mixtures. Finally, contrary to our hypothesis, soil microbial communities from the root exclosures did not differ structurally (as measured by PLFA) from soil microbial communities in soil closely associated with plant roots.

The Effects of Soil Origin on Soil and Microbial Processes and Soil Microbial Community Structure

In this study, soil origin had a significant influence on soil properties controlled by the soil microbial community and on the structure of the soil microbial community itself.

In fact, soil origin explained more variation in soil microbial community structure (PLFA profiles) than any of the other explanatory variables. This suggests that the history of the soil and plant community plays a major role in structuring the soil microbial community and, consequently, influences ecosystem functioning through the soil microbial community. Many studies have shown site or soil characteristics to be an important influence on soil microbial processes and structure. Zelles *et al.* (1992) differentiated among eight agricultural management treatments using PLFA. Zelles et al. (1995) distinguished among three different soils in farmland and grassland using PLFA. Bossio *et al.* (1998) determined that soil type was the most important environmental factor in structuring the soil microbial communities of sustainable agriculture systems in California. Groffman *et al.* (1996) measured microbial biomass and activity and nitrogen transformation rates of soil taken from a range of old-field sites. As with our study, Groffman *et al.* (1996) concluded that the main driver for microbial biomass and activity was soil type.

Plant Effects on Soil and Microbial Processes and Soil Microbial Community Structure

As I saw in Chapter 2 (Broughton and Gross 2000), Chapter 3 (Broughton and Gross 2001), and Chapter 4, in this study, plants can have significant effects on soil and microbial processes and soil microbial community structure. Plant effects are complex and include effects that will be mediated through diversity, species composition, and individual plant species effects. This study cannot distinguish among the effects of

species diversity, functional group diversity, and plant community composition as aspects of the overall plant effect because each functional group is represented by only one species. However, I can address whether species differ in their effects on soil and microbial characteristics, whether plant species effects are detectable in mixtures, and whether these effects are enhanced by diversity. Which component or components (diversity, composition, individual plant species) are most important influencing the soil microbial community can be addressed by other studies like the BIODEPTH experiment in Chapter 3.

The results from this study do not support the hypothesis that increased plant species diversity leads to increased soil and microbial processes because monocultures differed, but mixtures did not. The mixtures did not have higher process rates than the monocultures. Similarly, Wardle (Wardle *et al.* 1999, Wardle *et al.* 2000, Wardle and Nicholson 1996) has consistently shown no relationship between diversity and ecosystem function in a series of plant removal experiments in New Zealand perennial grasslands. Symstad *et al.* (1998) also found no relationship between plant diversity and ecosystem functions (other than productivity) in a plant removal study in a North American grassland.

However, other experiments investigating the relationship between plant diversity and ecosystem function have shown variation in the relationship between plant diversity and ecosystem function across habitats. In Chapter 2, Broughton and Gross (2000) found that there was a significant effect of plant species diversity on the respiration or

biomass of the soil microbial community. However, in that study, changes in diversity were correlated with changes in edaphic variables. My work at the Silwood, England BIODEPTH site (Chapter 3) also showed a significant relationship between plant diversity and two measures of microbial community structure (CLPP and PLFA). Similarly, results from the Swiss BIODEPTH experiment have shown positive relationships between plant diversity and plant biomass (Spehn *et al.* 2000a, Spehn *et al.* 2000b), soil microbial respiration and functional diversity (Stephan *et al.* 2000), microbial biomass (Spehn *et al.* 2000a), and earthworm population density (Spehn *et al.* 2000a).

In Chapter 4, I saw that soil effects alone can influence soil and microbial processes, but these effects could be mediated by plants. In this study, the plant effect on soil and microbial processes seemed to be limited only to the presence of a plant: the identity of the plant mattered very little for soil processes in this study. The exception was *Trifolium pratense*, which did affect inorganic nitrogen pools and nitrogen process rates. In addition, I did find small differences among the plant treatments in their effects on soil microbial community structure. At the Swiss BIODEPTH site, Stephan *et al.* (2000) also detected a legume effect on soil processes. *Trifolium repens* significantly increased diversity and activity of catabolic profiles of the soil microbial community (Stephan *et al.* 2000). Many studies have shown plant composition effects on ecosystem functioning. Symstad *et al.* (1998) found plant composition effects on productivity and nitrogen retention, and Wardle *et al.* (1999) observed plant composition effects on PLFA patterns in the soils from a plant removal experiment in

New Zealand grasslands. Hector *et al.* (2000) detected a relationship between the species composition of litter and the decomposition rate at the Silwood Park, England BIODEPTH site. Hooper and Vitousek (1998) determined that plant community composition accounted for much more of the variation in nutrient cycling processes on serpentine soil in California than just plant functional group diversity. Broughton and Gross found some evidence to support this view in the relationships between plant composition and microbial respiration and soil PLFA patterns at the Silwood Park, England BIODEPTH site (Chapter 3).

Plant community composition should influence the soil microbial community through inputs of carbon into the soil. The quality and/or quantity of the carbon available to the microorganisms should influence which microorganisms thrive in a particular environment: therefore, the identity of the plant species providing that carbon should influence how ecosystem functions change (Paul and Clark 1996). Plants provide carbon to the soil in two ways: (1) litter and (2) root exudation. Hector *et al.* (2000) found large effects of plant litter composition on litter chemistry and decomposition rate at the Silwood Park, BIODEPTH site. However, this study did not last long enough to test litter effects. All differences in soil and microbial processes and soil microbial community structure among plant treatments must have been driven by differences in root exudation and root turnover. A recent study by Hamilton and Frank (2001) showed that herbivory could cause plants to stimulate rhizospheric microbial communities to increase nitrogen cycling and make nitrogen more available to the plants.

Several studies have detected differences among the rhizosphere soil microbial communities from different plant species. Grayston and Campbell (1996) distinguished between the rhizosphere of hybrid larch (*Larix eurolepis*) and Sitka spruce (*Picea sitchensis*) trees using CLPP at a woodland site and two plantations. Garland (1996) and Westover *et al.* (1997) also were able to differentiate the rhizosphere communities of several herbaceous plant species both in the field and greenhouse using Biolog. Miethling *et al.* (2000) used CLPP and PLFA profiles to distinguish among soil microbial communities from different fields planted with both alfalfa *(Medicago sativa*) and rye *(Secale cereale*). Miethling *et al.* (2000) determined that plant species identity was the most important factor in determining microbial community characteristics in the rhizosphere. Bachmann and Kinzel (1992) showed that plant species exude different amounts of organic metabolites, indicating that individual plants might have quite different effects on the soil microbial community. These studies suggest that plant species differences in root exudates may be important in distinguishing the composition of the rhizosphere communities. The results from my study also indicate that it is possible to distinguish among soil microbial communities grown in the presence of different plant species, as I could distinguish among the plant treatments with PLFA profiles.

Plant species effects on soil microbial processes are often detected in greenhouse studies; however, these effects are much more difficult to find in the field. Buckley and Schmidt (2001) found that there was little difference between the soil microbial

communities of a continuously tilled agricultural site and a companion successional site in southwestern Michigan that had different plant communities for 12 years. Plant species effects on soil microbial processes may take a long time to manifest themselves (Buckley and Schmidt 2001, Broughton and Gross 2000), despite the possibility that plant species may need only to change the process rates of a small proportion of the soil organic matter to have large effects on soil processes (Wedin and Pastor 1993).

Implications of the Similarity of Soil Microbial Communities in the Root Exclosures and the Rhizosphere Soil

In this experiment, all of the soil in the pot was available to the plant roots, except the soil in the exclosure in the center. Root production was high in the experiment. Most of the plants in the experiment were root-bound by harvest time. The 20-micron mesh of the exclosure allowed nutrients, water, microorganisms, and mycorrhizae to pass through, but not plant roots. Consequently, there was no direct "contact" effect of plants on the soil and the soil microbial communities inside the root exclosure. In effect, the soil outside the exclosure was entirely rhizosphere soil, while the soil inside the exclosure was the equivalent of bulk soil. However, I could not detect any differences (in PLFA profiles) in the soil microbial communities in the two different types of soil (rhizosphere and bulk). This implies that the soil microbial communities in the rhizosphere and bulk soils were not significantly different in structure. This suggests that root exudates that can influence soil microbial community structure moved freely from the soil near the plant roots to the soil within the root exclosure.

This effect implies that in field settings I often do not find plant effects in the bulk soil because the resources (exudates) are used by rhizosphere microorganisms before the resources have a chance to migrate away from the roots.

The soil microbial community plays a crucial role in the flow of nutrients and energy through the ecosystem. Changes in ecosystem function are intimately tied to the composition and activity of the soil microbial community. This study has provided evidence that soil is the most important factor influencing soil microbial communities, but the extant plant community composition can also influence soil microbial community structure. Better understanding the role microorganisms play in the overall functioning of the ecosystem can only improve our ability to predict how these ecosystems will change in the future under the influences of changing vegetation cover, agricultural practices, invasive species, and other dynamic drivers of the plant-soil ecosystem.

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CHAPTER 6

SUMMARY

From the collection of field surveys and manipulative experiments in the previous chapters I can make several conclusions. This dissertation suggests the origin of the soil and the presence of a plant both influence the structure and functioning of the soil microbial community. However, plant effects were often not as strong as I had expected and there were other factors influencing the structure and function of the soil microbial community.

First, I found that legacy effects can last for years. The disturbance caused during site preparation at the Silwood Park BIODEPTH site was still influencing the soil microbial community four years later (Chapter 3).

Plant effects on the soil microbial community were often the result of plant biomass. In Chapter 2, plant productivity had significant effects on soil microbial respiration. In Chapter 3 many plant effects on soil microbial community processes were associated with larger amounts of plant matter rather than the diversity of the plant community.

As I saw in Chapter 2 (Broughton and Gross 2000), Chapter 3 (Broughton and Gross 2001), Chapter 4, and Chapter 5, plants can have significant effects on soil and microbial processes and soil microbial community structure. Plant effects are complex and include effects that will be mediated through productivity (Chapters 2 and 3),

diversity (Chapter 3), species composition (Chapters 3 and 5), and individual plant species effects (Chapters 4 and 5).

In Chapter 4, I saw that soil effects alone can influence soil and microbial processes, but these effects could be mediated by plants. In Chapter 5, the plant effect on soil and microbial processes seemed to be limited only to the presence of a plant: the identity of the plant mattered very little soil processes in this study. The exception was *Trifolium pratense*, which did affect inorganic nitrogen pools and nitrogen process rates. In addition, I did find small differences among the plant treatments in their effects on soil microbial community structure. However, these unique effects of plant species did not seem to be additive in mixtures. Finally, root exclosures had no effect on the soil microbial community structure, indicating plant resources can migrate away from the roots. The large number of findings in which plant effects are limited to rhizosphere soils in the field imply that these plant resources are used before they have the opportunity to migrate away from the roots in the field.

The soil microbial community is a complex part of the old-field ecosystem that controls many important ecological processes. A multitude of factors influence the structure and function of this community. Understanding the roles each aspect of the community plays in structuring the soil microbial community will allow better understanding of the effects of global change on these important processes.