AN ABSTRACT OF THE THESIS OF

Justin B. Brant for the degree of Master of Science in Soil Science presented on March 10, 2005.

Title: Litter Controls of Microbial Community Composition and Function in Forest Soils.

Abstract approved:

Redacted for privacy

David D. Myrold

Redacted for privacy

Elizabeth W. Sulzman

Most carbon (C) transformations in soil are carried out by a diverse and complex soil microbial community. The size and composition of the soil microbial community is determined by poorly understood interactions between the quantity and chemical composition of plant inputs, as well as climate. Given the metabolic diversity of soil microorganisms and functional overlap among distinct taxonomic groups, changes in microbial community composition do not necessarily lead to changes in microbial community function at the ecosystem scale. The objective of this study was to investigate the effects of long-term manipulations of above- and belowground plant litter inputs to soil on the composition and function of the soil microbial community in forest ecosystems, as part of a larger detritus input and removal treatment experiment.

In the first part of my study, soils from three very different forest ecosystems subjected to the same long-term C input manipulations were analyzed using phospholipid fatty acid (PLFA) analysis to determine if there were changes in microbial community composition in response to litter input manipulations. This experiment also allowed me to examine if the observed changes were consistant across the three ecosystems sampled. In all three forest ecosystems, root exclusion led to changes in microbial community structure, whereas wood and litter input additions and exclusion did not change the microbial community composition. The soil without roots had a lower fungal:bacterial ratio; in addition, changes were found in the bacterial community, especially actinomycetes, even after accounting for the potential loss of mycorrhizal fungi due to root exclusion. Seasonal differences in the PLFA profile at one site were greater than any

of the treatment differences, with the taxonomic biomarkers responsible for treatment differences varying by sampling date, underlining the importance of seasonal sampling.

In the second part of my study soils from an old-growth Douglas-fir (*Pseudotsuga menziesii*) - western hemlock (*Tsuga heterophylla*) forest in the Oregon Cascades that has received seven years of either wood addition or root and litter input exclusion were incubated with 50 μg C g^{-1} soil universally ^{13}C-labeled glucose, glutamate, oxalate, and phenol in a 14-day lab incubation. Changes in the rate and mechanism of substrate degradation were examined by following the ^{13}C tracer into microbial respiration, as well as looking at incorporation of ^{13}C into microbial biomass and PLFAs. Utilization of the four added substrates varied in soil of each litter manipulation treatment. Glucose and glutamate respiration rates were similar in soils from all three litter treatments, and were readily incorporated into all PLFA biomarkers. Higher rates of oxalate and phenol respiration were found in the soils with added wood and lower rates in the soils with litter and root exclusion, compared with the control soil. Phenol was utilized primarily by fungi, with little incorporation into any other PLFA biomarkers. The addition of each of the four substrates led to the enhanced degradation of soil organic matter (priming) in soils of all three litter treatments, and was greater following the addition of phenol and oxalate. Of the three litter teatments, priming was greatest in the soils with litter excluded. These results demonstrate that altering plant inputs to soil can lead to changes in microbial utilization of C compounds. In appears that many of the obseved C utilization differences are a result of changes in the size and composition of the fungal community.

Litter Controls of Microbial Community Composition and Function in Forest Soils

by

Justin B. Brant

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented March 10, 2005
Commencement June 2005

Master of Science thesis of Justin B. Brant presented on March 10, 2005.

APPROVED:

Redacted for privacy

Co-Major Professor, representing Soil Science

Redacted for privacy

Co-Major Professor, representing Soil Science

Redacted for privacy

Head of the Department of Crop and Soil Science

Redacted for privacy

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Redacted for privacy

Justin B. Brant, Author

ACKNOWLEDGEMENTS

First I would like to thank my co-advisors, David Myrold and Elizabeth Sulzman for being so generous with their time, as well as all their help, guidance, and patience. I would also like to thank Kate Lajtha for serving on my committee and providing help and insight from the beginning.

I would like to thank Rockie Yarwood for putting up with my questions and complaints, fixing mechanical problems, and early morning samplings. Thank you to Stacie Kageyama, Stephanie Boyle, Laurel Kluber, Jen Hooke, Heath Keirstead, and Lisa Brutcher for help with lab incubations, they certainly would not have been possible without them.

Thanks to Jennifer Moore for all her help with data analysis as well as a place to vent. Susan Crow for help with statistics and data acquisition. All of the DIRT crew for good times in the field and help around the lab.

I would like to thank my parents. They may not understand what I'm doing, but they have always been supportive of me. Finally, I'd like to thank Laila Parker for making sure I didn't work too hard, as well as love and too many other things to list.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

Litter Controls of Microbial Community Composition and Function in Forest Soils

Chapter 1:
General Introduction

Justin B. Brant

Introduction

Soil has been described as the 'black box' of the global C cycle. One of the difficulties in studying soil C dynamics in that most processes are carried out by a diverse and complex soil microbial community. Given the metabolic diversity of most taxonomic groups, and the large quantity of unculturable microbes in soil, it is difficult to generalize about the effects of microbial community composition on C cycling at the ecosystem scale. The goal of this thesis was to examine the role of C input quality and quantity on microbial community structure and function in forest ecosystems. This goal was addressed using two different experiments. First, the microbial community structure in soils from three very different forest ecosystems subjected to the same long-term C input manipulations were analyzed using PLFA analysis to determine if similar changes in community composition result from the same chronic C input manipulations. Second, soils from one of the above sites were incubated with ^{13}C-labeled substrates of differing degradability in order to see if microbial community changes resulting from chronic C input manipulation result in changes in belowground C utilization.

The Role of Soils in the Global Carbon Cycle

Soils are a key component of the global C cycle. It is estimated that approximately 1500 Pg of C is stored in soils, compared to 750 Pg in the atmosphere (Schlesinger, 1997). Fluxes from soils to the atmosphere through heterotrophic decomposition (R_h) and rhizosphere respiration account for 70% of the total C exchange between terrestrial ecosystems and the atmosphere, ten-fold greater than fossil-fuel burning and deforestation combined (Schimel et al., 2000). Given the size of the soil C pool and the magnitude of fluxes between soil and the atmosphere, even small changes to total fluxes could significantly impact atmospheric CO_2 concentration.

The ability of an ecosystem to store or release C is known as net ecosystem production (NEP). It is the difference between net primary production (NPP) and R_h. Carbon enters the soil through litterfall, root turnover, and root rhizodeposition, where

it is either respired as CO_2 as a byproduct of microbial metabolism, incorporated into microbial biomass, or retained in the soil as soil organic matter (SOM) (Post et al., 1990). Most C models generally represent the soil as two different C pools, one that cycles rapidly and one that is more recalcitrant. Easily decomposable compounds such as carbohydrates and lipids are part of the active pool, which has a turnover time of approximately one to five years. More recalcitrant compounds such as lignin are part of a relatively inactive pool that turns over in hundreds to thousands of years (Post et al., 1990).

In the next few centuries atmospheric CO_2 concentrations are expected to double, and the global average temperature is expected to increase between 1.8 and 5.8°C (IPCC, 2001). Increased temperature and atmospheric CO_2 concentration are expected to have both direct and indirect effects on belowground C cycling. Understanding the mechanisms controlling belowground C processes are important in order to predict whether soils will become a source, sink, or remain neutral C stores in the future (Pendall et al., 2004).

The major direct effect of increased CO_2 concentration is an increase in NPP through CO_2 fertilization, which should help mitigate the increasing atmospheric CO_2 concentration (Schlesinger, 1997). On the other hand, raising temperature could significantly increase R_h, which would have the opposite effect, increasing C release to the atmosphere. It has been suggested that these two effects may counteract one another, leading to little net change in soil C stocks (Kirschbaum, 2000). However, other indirect effects of global change, mediated through changes in both water and nutrient cycling, make the picture much more complicated. These complex interactions could lead to either increased sequestration or release of C in the future (Pendall et al., 2004).

Priming

The priming effect is a short-term change in the turnover of native SOM caused by the addition of fertilizer or organic substances (Kuzyakov et al., 2000). Additions can lead to either an increase (positive priming) or a decrease (negative

priming) in SOM decomposition compared to unamended soil. Positive priming of SOM has been shown to occur in laboratory incubations with the addition of various simple organic compounds (Hamer and Marschner, 2002), plant litter (Waldrop and Firestone, 2004), or root inputs to soil (Kuzyakov and Cheng, 2001). A recent study has also shown priming as a result of seven years of litter addition in the field (Sulzman et al., 2005).

Little is known about the mechanisms behind priming from the addition of organic substrates, although the process is likely microbially mediated (Kuzyakov et al., 2000). It is hypothesized that the low quality of native SOM limits the amount of energy available to soil microorganisms. The addition of a more labile substrate could result in an increase in overall microbial biomass or activity, which in turn has the ability to mineralize a larger amount of native SOM (Kuzyakov et al., 2000). However, a recent review of the subject found that the addition of easily decomposable substrates such as glucose and fructose induced a much smaller priming effect than the addition of more complex substrates, such as cellulose or plant material (Fontaine et al., 2003). This result suggests a much more complicated mechanism.

Multiple mechanisms for the priming effect have been proposed, although there is little evidence supporting any of them. Fontaine et al. (2003) suggest that priming could result from competition between microbes that employ either an r- or K-growth strategy. The soil microbial community contains both r-strategists, which grow rapidly on labile C sources but are unable to degrade more recalcitrant SOM, and K-strategists, which grow more slowly and are able to use a wider range of C sources. The addition of complex substrates stimulates the growth of K-strategists, unlike simple substrates that would only stimulate r-strategists. The K-strategists possess the ability to degrade complex SOM, so stimulating this part of the community increases the decomposition of native SOM increases. Nutrient poor soils tend to show a larger priming effect than nutrient rich soils, even with the addition of easily degradable substrates (Fontaine et al., 2003). This finding further supports the K-strategist mechanism, as the lack of other nutrients should limit the speed of r-strategist's growth, allowing K-strategists to better compete for added C.

Other researchers hypothesize that the priming effect in response to C addition is caused by the turnover of microbial biomass C and the respiration of endogenous C, rather than the decomposition of native SOM (Bell et al., 2003; Dalenberg and Jager, 1989; De Nobili et al., 2001; Wu et al., 1993). Taking this theory a little further, De Nobili et al. (2001) hypothesized that the soil microbial biomass is in a resting state most of the time, but keeps itself in a state of 'metabolic alertness' to respond to the presence of any easily degradable compounds. They go on to suggest that the addition of low molecular weight trigger molecules can activate the resting biomass to metabolize the added substrate. This, in turn, leads to the turnover of microbial biomass as well as endogenous C from the now active cells, which results in much of the observed priming effect.

Aboveground Input Decomposition and Manipulation

Litter decomposition is a process involving many different members of the soil microbial community. When litter enters the soil, simple compounds such as water-soluble sugars and amino acids are degraded first. Successive stages of decay involve the metabolism of progressively more complex litter components. The substrate remaining in the last stages of decomposition is high in lignin and other complex and aromatic compounds. Only a small portion of the soil microbial community, such as fungi and actinomycetes, produce the enzymes necessary to degrade these compounds (Wagner and Wolf, 1999). Litter decomposition is often modeled using a first-order decay equation (Wagner and Wolf, 1999). The rate of decay is related to the quality of litter inputs, which can be assessed by the litter C:N or lignin:N ratios.

Ten years of doubling litter inputs in a deciduous forest appeared to cause increased decomposition of more recalcitrant SOM (Nadelhoffer et al., 2004). Parker et al. (2002) hypothesized that litter quality had more of an effect than quantity on decomposition rates. As expected, they found that litter with a high C:N ratio decomposed more slowly than litter with a low C:N ratio. In the same study, soils at high C:N ratio sites had larger pools of labile C compared to sites with low C:N ratio

inputs, regardless of the quantity of litter input. This suggests faster decomposition of the high quality plant material at these sites, irrespective of quantity.

Adding litter to soil increases the quantity of DOC and DON leaving the organic layer (Lajtha et al., 2005; Nadelhoffer et al., 2004; Park and Matzner, 2003), but it does not appear to alter the chemistry of the soil solution (Lajtha et al., 2005). The amount of DOC leaving the litter layer does not appear to correspond with an increase in DOC losses from the system, suggesting that the added C is either processed by microbes or physically protected within the soil.

Doubling litter inputs has also been shown to increase the fungal biomass within the organic layer, although both fungal and bacterial biomass in the mineral soil was found to be unchanged after five years of litter manipulation (Nadelhoffer et al., 2004).

Belowground Input Decomposition and Manipulation

Carbon enters the soil from two major belowground sources, rhizodeposition and root turnover. Plant roots secrete both low- and high-molecular-weight C-containing molecules into the rhizosphere, as well as ions and water (Bertin et al., 2003). Almost all classes of organic compounds are released in rhizodeposition including carbohydrates, amino acids, aliphatic acids, aromatic acids, phenolics, fatty acids, and sterols (Bertin et al., 2003). Root exudates are believed to be involved in the regulation of internal processes, such as respiration, as well as facilitating plant nutrient acquisition.

It is estimated that plants allocate 20-50% of their photosynthetically fixed C belowground (Kuzyakov and Domanski, 2000). This quantity varies by plant species, soil texture, growth stage, and soil nutrient status. Rhizodeposition provides a relatively constant source of easily decomposable plant compounds, which appear to drive belowground C dynamics (Kuzyakov and Domanski, 2000). In a mixed deciduous forest with relatively high N input, it was estimated that 30% of the total soil CO_2 efflux was derived from the decomposition of belowground litter, with over 60% of soil CO_2 efflux coming from all belowground sources combined (root

respiration and belowground litter) (Bowden et al., 1993). In a coniferous forest with much lower N inputs, belowground litter decomposition was estimated to be 49-65% of total soil CO_2 efflux, with total belowground sources accounting for over 70% of total soil CO_2 efflux (Sulzman et al., 2005). A large-scale tree-girdling experiment, which stopped the flow of recent photosynthate to the roots without disrupting the root system, found a 37% decrease in soil CO_2 efflux five days after girdling and a 54% decrease after two months, suggesting that recent photosynthate is driving soil processes in this system (Högberg et al., 2001).

The other major belowground plant C input to soil is the turnover of roots. The decomposition of coarse roots can be modeled using similar chemical parameters to those used to predict aboveground litter decomposition rates, such as C:N or lignin:N ratio (Silver and Miya, 2001). This relationship does not appear to hold true for fine roots, which are frequently colonized by mycorrhizal fungal. Langley and Hungate (2003) hypothesized that mycorrhizal status could substantially influence fine root decomposition and soil C cycling rates, although further work is necessary to explore this theory.

Root exclusion is one method that has been used to study the effects of roots on soil properties and dynamics. Root trenching is the most effective method of root exclusion in forest ecosystems. Root trenching involves severing live roots by digging down below the rooting zone and placing impenetrable barriers in the trenches to prevent the ingrowth of new roots. Trenched plots are then compared with an untrenched control to examine root effects. One unintentional effect of root trenching is an increase in soil water content that occurs when transpiration is stopped (Hart and Sollins, 1998; Lavoie and Bradley, 2003; Ross et al., 2001; Sulzman et al., 2005).

The effects of root exclusion on soil C and N dynamics vary by tree species and time since trenching. Twenty-seven months after trenching in a Monterey pine plantation, the quantity of extractable C and microbial biomass C was reduced by 40% and 28%, respectively (Ross et al., 2001). However, biomass C and total C was not changed 13 years after trenching in an old-growth coniferous forest (Hart and Sollins, 1998).

Preventing plant uptake of N leads to large changes in N cycling in trenched plots. Nitrogen pool size, DON concentration, mineralizable N, and potential nitrification rates were increased by trenching in various hardwood and coniferous ecosystems (Hart and Sollins, 1998; Lajtha et al., 2005; Lavoie and Bradley, 2003; Ross et al., 2001). Decreased NO_3^- immobilization has also been reported for an old-growth Douglas-fir forest (Hart and Sollins, 1998).

Despite the fact that microbial biomass C remains relatively stable in the mineral soil after trenching in high C coniferous forest soils, soil CO_2 efflux rates and the amount of easily mineralizable C were reduced in several studies involving trenching (Bowden et al., 1993; Nadelhoffer et al., 2004; Rey et al., 2002; Ross et al., 2001; Sulzman et al., 2005). The effect of trenching on the soil microbial community also varies across ecosystems (Hart and Sollins, 1998; Siira-Pietikainen et al., 2001, 2003). In a mixed deciduous forest trenching caused no change in total fungal and bacterial biomass in either the forest floor or mineral soil, but active bacterial biomass decreased in trenched plots (Nadelhoffer et al., 2004). In a high latitude coniferous forest root trenching was shown to decrease soil fungal populations by 40%, as well as altering soil bacterial community structure in both the organic layer and mineral soil (Siira-Pietikainen et al., 2001, 2003). These studies also found that trenching led to changes in the soil microfauna community composition affecting organisms such as collembolans and protozoa.

Soil Microbial Community

Soil microbial ecosystems are some of the most diverse on earth. Traditionally, the analysis of soil microbial communities has relied on culturing techniques using a variety of culture media designed to maximize the recovery of different microbial species. Unfortunately, it has been estimated that less than 0.1% of the microorganisms found in typical soils are culturable using current culturing techniques, begging for alternative methods of analysis (Atlas and Bartha, 1998).

The soil microbial community is responsible for performing most of the chemical transformations involved in biogeochemical cycles. However, the question

of whether the diversity and composition of the microbial community relates to ecosystem function is hard to answer, given the complex interactions between soil microbes and the diverse metabolic capabilities of microbes within the same taxonomic group. At the ecosystem scale community structure probably does not affect ecosystem function, although at smaller scales such as litter decomposition, community structure may have an important effect on function (Schimel, 1995).

Most plant litter is composed of both labile and recalcitrant C compounds. A broad range of organisms are able to break down the labile components (protein, lipids, and simple carbohydrates). More recalcitrant polymeric compounds such as lignin and cellulose are broken down into smaller units by exoenzymes. Only a relatively small group of microorganisms, such as fungi and actinomycetes, produce these enzymes. Fungi are especially important in litter decomposition as they are responsible for nearly all extracellular enzyme production necessary to degrade recalcitrant SOM such as lignin-derived compounds (Schimel, 1995). They are also very important in the degradation of aboveground litter as they are able to colonize surface litter and translocate C and nutrients into the mineral soil (Frey et al., 2003).

Structurally distinct microbial communities can have virtually identical C utilization patterns as a result of the functional diversity of soil microorganisms (Buyer and Drinkwater, 1997; Waldrop et al., 2000). Soil ecosystems are thought to be functionally redundant, meaning the loss of one specific organism will generally not significantly alter decomposition rates (Ekschmitt and Griffiths, 1998). The large diversity of the soil microbial community makes studies relating biodiversity to ecosystem function very difficult. One such study found a positive relationship between fungal diversity and decomposition rate at only the species-poor end of fungal diversity, suggesting significant redundancy in this community (Setala and McLean, 2004). Unfortunately, many diversity studies in soil have been performed under laboratory conditions, where decomposition rates can differ significantly from *in situ* rates, which are regulated by nutrient availability and competition (Schimel, 1995).

The Effects of Disturbance on Microbial Communities

Both human-induced and natural disturbances can lead to changes in microbial community structure and function. These include the introduction of non-native plant species (Kourtev et al., 2002), tree harvesting (Chanasyk et al., 2003), and plant succession (Merila et al., 2002). Disturbance affects the soil microbial community in many ways including altering the quantity and quality of C inputs or physically disturbing the soil. Along with these direct effects, indirect effects such as changes in soil temperature and moisture can have profound and long-term effects on soil processes (Chanasyk et al., 2003). Younger forest stands generally have higher soil temperatures given the lack of tree cover (Griffiths and Swanson, 2001). Tree removal has also been shown to change soil moisture content, but these changes appear to be influenced more by site without any clear patterns emerging (Griffiths and Swanson, 2001). These indirect effects can persist for 40 years or longer, and can lead to significant changes in decomposition and nutrient cycling, such as increased denitrification where lack of transpiration leads to saturated soils. Altering the microclimate can also change the intensity and number of drying-rewetting events and other stresses on the microbial community, which can in turn alter the microbial community structure, as well as C and N cycling (Fierer and Schimel, 2002).

Phospholipid Fatty Acid Analysis

Phospholipid fatty acid analysis (PLFA) is one method that provides information about the soil microbial community composition *in situ*. Phospholipids are essential membrane components of all living cells. They degrade rapidly upon cell death and are not found in storage products. Under conditions in which most naturally occurring communities exist, phospholipids make up a relatively constant proportion of cell biomass (Zelles, 1999). Although phospholipids are found in all living organisms, unique fatty acids are indicative of specific groups of microorganisms. The presence and abundance of these signature fatty acids in soils reveals the presence and abundance of particular organisms or groups of organisms in which those signatures can be found (Zelles, 1999).

Eukaryotes and eubacteria have very different PLFA composition (Zelles, 1999). Pure culture studies have shown that a few PLFAs are very common in eukaryotic organisms such as fungi, but rare in bacteria. Unfortunately, these PLFAs are also common in some plants. Fungi and other eukaryotic organisms use neutral lipids (NLFA) such as triglycerols for energy storage, whereas bacteria do not. Recently, it has been shown that the ratio of NLFA/PLFA for certain fungal biomarkers can give an indication of fungal nutrient status or physiological state (Bååth, 2003).

Despite the utility of this method for looking at small differences in microbial community composition, there are some important limitations. First, even though specific fatty acids can be correlated with certain groups of organisms, they are not unique to that group under all conditions (Zelles, 1999). Second, microorganisms change their PLFA profile under different nutrient and environmental conditions, making it impossible to tell whether changes in a PLFA profile result from changes in community composition or physiological changes in a static community (Bossio and Scow, 1998). This method is also not able to characterize microorganisms to the species level in complex communities, as it only indicates the presence and dominance of broader groups of organisms (Hill et al., 2000).

Linking Microbial Community Structure and Function

There are multiple methods that attempt to link microbial community structure with function in soil ecosystems. The simplest method has been to combine substrate induced respiration (SIR) or enzyme assays with either PLFA or DNA analysis (Kourtev et al., 2002; Waldrop et al., 2000). A correlation between PLFA biomarkers and specific enzymes or substrate degradation gives information about which components of the microbial community are responsible for that function. This method only yields indirect evidence that specific ecosystem processes are being performed by different parts of the microbial community.

The most promising new method for connecting microbial function with specific organisms or groups of organisms involves using C isotopes in conjunction

with PLFA analysis (Burke et al., 2003; Hanson et al., 1999; Johnsen et al., 2002) and DNA analysis (Manefield et al., 2002; Padmanabhan et al., 2003). By using isotopically labeled substrates or isotopic differences between C_3 and C_4 plants in conjunction with DNA or PLFA analysis, it is possible to see which member or members of a complex microbial community are involved in the metabolism of that specific substrate. Both ^{13}C- and ^{14}C-labeled substrates have been used to look at functional differences between microbial communities (DeForest et al., 2004; Hanson et al., 1999; Waldrop and Firestone, 2004) as well as partitioning soil respiration into root and heterotrophic components (Högberg and Ekblad, 1996; Kuzyakov and Cheng, 2001; Kuzyakov et al., 2001). Natural abundance differences in ^{13}C composition have also been used in conjunction with PLFA analysis.

The use of isotopes in both tracer and natural abundance studies of the soil microbial community is based on the idea that "you are what you eat", assuming that a microbe's isotopic signature should be similar to that of the substrates it is utilizing. Unfortunately, pure culture laboratory incubations with simple substrates demonstrated a wide range of fractionations of ^{13}C in the biosynthesis pathway of a single PLFA, dependent on substrate, microbial species, and physiological state (Abraham et al., 1998). However, it appears that when a complex microbial community utilizes a complex mixture of substrates in aerobic soils, the different individual fractionations associated with the pathway for synthesizing each PLFA tend to cancel each other out and the mean ^{13}C-PLFA values are good indicators of the mean ^{13}C value of the substrate (Burke et al., 2003; Cifuentes and Salata, 2001).

The first studies using ^{13}C-PLFA analysis in soils were incubation experiments in specific soils known to have microbial communities that could degrade a certain xenobiotic compound such as toluene or phenanthrene (Hanson et al., 1999; Johnsen et al., 2002). The soils were incubated with ^{13}C-labeled substrates, and the small portion of the microbial community associated with the metabolism of the specific compound could be identified by looking at which lipid biomarkers incorporated the ^{13}C label.

A few studies published recently have examined the degradation of ^{13}C-labeled substrates in order to examine differential processing of soil C inputs (DeForest et al., 2004; Phillips et al., 2002; Waldrop and Firestone, 2004; Waldrop et al., 2004). These studies have added substrates to soil that resemble different components of plant inputs such as simple sugars, cellobiose, and phenolic compounds. By adding these compounds to soils in the laboratory, and tracking their incorporation into microbial PLFAs, it is possible to visualize how distinct microbial communities process different C compounds. In addition, by looking at the incorporation of ^{13}C into lipid biomarkers it is possible to determine which parts of the microbial community are involved in the decomposition of specific organic substrates.

It is more difficult to use natural abundance differences in PLFA analysis because of the small expected differences in the $\delta^{13}C$ of specific PLFAs could be larger than the fractionation between that specific PLFA and the substrate utilized (Waldrop and Firestone, 2004). There are a few studies that use isotopic differences from a shift between C_3 and C_4 vegetation to examine microbial utilization of old and new C (Burke et al., 2003; Waldrop and Firestone, 2004). In these studies, the analysis of individual lipids gives an idea of the relative age of C utilized by different components of the microbial community, although quantitative analysis of percentages is not possible given the fractionation issues previously described. By assuming these individual fractionations cancel out it is possible to determine the turnover time of various SOM pools.

Objectives

The objective of this thesis was to examine how the quality and quantity of plant C inputs to soil controls soil microbial community structure and function. In chapter two, PLFA analysis was used to examine changes in microbial community structure in response to long-term C input manipulation in three forest ecosystems that vary with respect to tree species, C background, N deposition rate, and length of manipulation. It was hypothesized that root exclusion and wood additions would have the most profound effects on the composition of the soil microbial community. These

effects were expected to be greater at the sites where the manipulations have been established for a longer period of time, as well as at the sites with smaller soil C stocks.

In chapter three, soils from one of the above sites that had received seven years of coarse woody debris additions, root and litter exclusion, or normal plant inputs were incubated with a variety of simple ^{13}C-labeled substrates. Microbial groups involved in the degradation of these compounds were identified by following the ^{13}C-label into microbial PLFAs. Evidence of a priming effect induced by substrate addition, as well the efficiency of microbial utilization of these substrates was also examined to explore differential utilization of C compounds. It was hypothesized that changes in the structure of the soil microbial community would lead to changes in both the rate and mechanism of microbial utilization of recalcitrant C compounds, while having little effect on the mineralization of labile compounds.

References

Abraham, W., C. Hesse, and O. Pelz. 1998. Ratios of carbon isotopes in microbial lipids as an indicator of substrate usage. Applied and Environmental Microbiology 64:4202-4209.

Atlas, R.M., and R. Bartha. 1998. Microbial Ecology: Fundamentals and Applications Benjamin/Cummings, Redwood City, CA.

Bååth, E. 2003. The use of neutral lipid fatty acids to indicate the physiological conditions of soil fungi. Microbial Ecology 45:373-383.

Bell, J.M., J.L. Smith, V.L. Bailey, and H. Bolton. 2003. Priming effect and C storage in semi-arid no-till spring crop rotations. Biology And Fertility Of Soils 37:237-244.

Bertin, C., X.H. Yang, and L.A. Weston. 2003. The role of root exudates and allelochemicals in the rhizosphere. Plant and Soil 256:67-83.

Bossio, D.A., and K.M. Scow. 1998. Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patters. Microbial Ecology 35:265-278.

Bowden, R.D., K.J. Nadelhoffer, R.D. Boone, J.M. Melillo, and J.B. Garrison. 1993. Contributions of aboveground litter, belowground litter, and root respiration to total soil respiration in a temperature mixed hardwood forest. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 23:1402-1407.

Burke, R., M. Molina, J. Cox, L. Osher, and M. Piccolo. 2003. Stable carbon isotope ratio and composition of microbial fatty acids in tropical soil. J Environmental Quality 32:198-206.

Buyer, J.S., and L.E. Drinkwater. 1997. Comparison of substrate utilization assay and fatty acid analysis of soil microbial communities. Journal of Microbiological Methods 30:3-11.

Chanasyk, D.S., I.R. Whitson, E. Mapfumo, J.M. Burke, and E.E. Prepas. 2003. The impacts of forest harvest and wildfire on soils and hydrology in temperate forests: A baseline to develop hypotheses for the Boreal Plain. Journal Of Environmental Engineering And Science 2:S51-S62.

Cifuentes, L.A., and G.G. Salata. 2001. Significance of carbon isotope discrimination between bulk carbon and extracted phospholipid fatty acids in selected terrestrial and marine environments. Organic Geochemistry 32:613-621.

Dalenberg, J.W., and G. Jager. 1989. Priming effect of some organic additions to C-14-labeled soil. Soil Biology & Biochemistry 21:443-448.

De Nobili, M., M. Contin, C. Mondini, and P.C. Brookes. 2001. Soil microbial biomass in triggered into activity by trace amounts of substrate. Soil Biology & Biochemistry 33:1163-1170.

DeForest, J.L., D.R. Zak, K.S. Pregitzer, and A.J. Burton. 2004. Atmospheric nitrate deposition and the microbial degradation of cellobiose and vanillin in a northern hardwood forest. Soil Biology & Biochemistry 36:965-971.

Ekschmitt, K., and B.S. Griffiths. 1998. Soil biodiversity and its implications for ecosystem functioning in a heterogeneous and variable environment. Applied Soil Ecology 10:201-215.

Fierer, N., and J.P. Schimel. 2002. Effects of drying-rewetting frequency of soil carbon and nitrogen transformations. Soil Biology & Biochemistry 34:777-787.

Fontaine, S., A. Mariotti, and L. Abbadie. 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology & Biochemistry 35:837-843.

Frey, S.D., J. Six, and E.T. Elliott. 2003. Reciprocal transfer of carbon and nitrogen by decomposer fungi at the soil-litter interface. Soil Biology & Biochemistry 35:1001-1004.

Griffiths, R.P., and A.K. Swanson. 2001. Forest soil characteristics in a chronosequence of harvested Douglas-fir forests. Canadian Journal Of Forest Research-Revue Canadienne De Recherche Forestiere 31:1871-1879.

Hamer, U., and B. Marschner. 2002. Priming effects of sugars, amino acids, organic acids and catechol on the mineralization of lignin and peat. Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde 165:261-268.

Hanson, J., J. Macalady, D. Harris, and K. Scow. 1999. Linking toluene degradation with specific microbial populations in soil. Applied and Environmental Microbiology 65:5403-5408.

Hart, S.C., and P. Sollins. 1998. Soil carbon and nitrogen pools and processes in an old-growth conifer forest 13 years after trenching. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 28:1261-1265.

Hill, G.T., N.A. Mitkowski, L. Aldrich-Wolfe, L.R. Emele, D.D. Jurkonie, A. Ficke, S. Maldonado-Ramirez, S.T. Lynch, and E.B. Nelson. 2000. Methods for assessing the composition and diversity of soil microbial communities. Applied Soil Ecology 15:25-36.

Högberg, P., and A. Ekblad. 1996. Substrate-induced respiration measured in situ in a C_3-plant ecosystem using additions of C_4-sucrose. Soil Biology & Biochemistry 28:1131-1138.

Högberg, P., A. Nordgren, N. Buchmann, A.F.S. Taylor, A. Ekblad, M.N. Högberg, G. Nyberg, M. Ottosson-Löfvenius, and D.J. Read. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411:789-792.

IPCC. 2001. Summary for policymakers, *In* R. Watson, ed. Climate change 2001: the scientific basis. Cambridge University Press, Cambridge, UK.

Johnsen, A.R., A. Winding, U. Karlson, and P. Roslev. 2002. Linking of microorganisms to phenanthrene metabolism in soil by analysis of C-13-labeled cell lipids. Applied and Environmental Microbiology 68:6106-6113.

Kirschbaum, M.U.F. 2000. Will changes in soil organic carbon act as a positive or negative feedback on global warming? Biogeochemistry 48:21-51.

Kourtev, P.S., J.G. Ehrenfeld, and M. Haggblom. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology 83:3152-3166.

Kuzyakov, Y., and G. Domanski. 2000. Carbon input by plants into the soil. Review. Journal of Plant Nutrition and Soil Science Zeitschrift Fur Pflanzenernahrung und Bodenkunde 163:421-431.

Kuzyakov, Y., and W. Cheng. 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology & Biochemistry 33:1915-1925.

Kuzyakov, Y., J.K. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. Soil Biology & Biochemistry 32:1485-1498.

Kuzyakov, Y., H. Ehrensberger, and K. Stahr. 2001. Carbon partitioning and below-ground translocation by Lolium perenne. Soil Biology & Biochemistry 33:61-74.

Lajtha, K., S. Crow, Y. Yano, S.S. Kaushal, E.W. Sulzman, and J.D.H. Spears. 2005. Detrital controls on soil solution N and dissolved organic matter in soils: a field experiment. Biogeochemistry (In review).

Langley, J.A., and B.A. Hungate. 2003. Mycorrhizal controls on belowground litter quality. Ecology 84:2302-2312.

Lavoie, M., and R.L. Bradley. 2003. Short-term increases in relative nitrification rates due to trenching in forest floor and mineral soil horizons of different forest types. Plant and Soil 252:367-384.

Manefield, M., A.S. Whiteley, R.I. Griffiths, and M.J. Bailey. 2002. RNA stable isotope probing, a novel means of linking microbial community function to Phylogeny. Applied and Environmental Microbiology 68:5367-5373.

Merila, P., R. Strommer, and H. Fritze. 2002. Soil microbial activity and community structure along a primary succession transect on the land-uplift coast in western Finland. Soil Biology & Biochemistry 34:1647-1654.

Nadelhoffer, K.J., R.D. Boone, R.D. Bowden, J.D. Canary, J. Kaye, P. Micks, A. Ricca, J.A. Aitkenhead, K. Lajtha, and W.H. McDowell. 2004. The DIRT Experiment: Litter and Root Influences on Forest Soil Organic Matter Stocks and Function, *In* D. Foster and J. Aber, eds. Forest Landscape Dynamics in New England: Ecosystem Structure and Function as a Consequence of 5000 years of Change. Oxford University Press, New York.

Padmanabhan, P., S. Padmanabhan, C. DeRito, A. Gray, D. Gannon, J. Snape, C. Tsai, W. Park, C. Jeon, and E. Madsen. 2003. Respiration of ^{13}C-labeled substrates added to soil in the field and subsequent 16S rRNA gene analysis of ^{13}C-labeled soil DNA. Applied and Environmental Microbiology 69:1614-1622.

Park, J.H., and E. Matzner. 2003. Controls on the release of dissolved organic carbon and nitrogen from a deciduous forest floor investigated by manipulations of aboveground litter inputs and water flux. Biogeochemistry 66:265-286.

Parker, J.L., I.J. Fernandex, L.E. Rustad, and S.A. Norton. 2002. Soil organic matter fractions in experimental forested watersheds. Water Air and Soil Pollution 138:101-121.

Pendall, E., S. Bridgham, P.J. Hanson, B.A. Hungate, D.W. Kicklighter, D.W. Johnson, B.E. Law, Y.Q. Luo, J.P. Megonigal, M. Olsrun, M.G. Ryan, and S.Q. Wan. 2004. Below-ground process responses to elevated CO_2 and temperature:

a discussion of observations, measurement methods, and models. New Phytologist 162:311-322.

Phillips, R.L., D.R. Zak, W.E. Holmes, and D.C. White. 2002. Microbial community composition and function beneath temperate trees exposed to elevated atmospheric carbon dioxide and ozone. Oecologia 131:236-244.

Post, W.M., T.-H. Peng, W.R. Emanual, A.W. King, V.H. Dale, and D.L. DeAngelis. 1990. The global carbon cycle. American Scientist 78:310-326.

Rey, A., E. Pegoraro, V. Tedeschi, I. De Parri, P.G. Jarvis, and R. Valentini. 2002. Annual variation in soil respiration and its components in a coppice oak forest in Central Italy. Global Change Biology 8:851-866.

Ross, D.J., N.A. Scott, K.R. Tate, N.J. Rodda, and J.A. Townsend. 2001. Root effects on soil carbon and nitrogen cycling in a Pinus radiata D. Don plantation on a coastal sand. Australian Journal of Soil Research 39:1027-1039.

Schimel, D.S., J.M. Melillo, J. Tian, A.D. McGuire, D.W. Kicklighter, T.G.F. Kittel, N. Rosenbloom, S. Running, P. Thornton, D. Ojima, W. Parton, R. Kelly, M. Sykes, R.P. Neilson, and B. Rizzo. 2000. Contribution of increasing CO_2 and climate to carbon storage by ecosystems in the United States. Science 287:2004-2006.

Schimel, J.P. 1995. Ecosystem consequences of microbial diversity and community structure, *In* F. S. Chapin III and C. Korner, eds. Arctic and Alpine Biodiversity: Patterns, Causes and Ecosystem consequences, Vol. 113. Springer-Verlag, New York.

Schlesinger, W.H. 1997. Biogeochemistry: An Analysis of Global Change Academic Press, San Diego, Ca.

Setala, H., and M.A. McLean. 2004. Decompostion rate of organic substrates in relation to the species diversity of soil saprophytic fungi. Oecologia 139:98-107.

Siira-Pietikainen, A., J. Haimi, and H. Fritze. 2003. Organisms, decomposition, and growth of pine seedlings in boreal forest soil affected by sod cutting and trenching. Biology and Fertility of Soils 37:163-174.

Siira-Pietikainen, A., J. Haimi, A. Kanninen, J. Pietikainen, and H. Fritze. 2001. Responses of decomposer community to root-isolation and addition of slash. Soil Biology & Biochemistry 33:1993-2004.

Silver, W.L., and R.K. Miya. 2001. Global patterns in root decomposition: comparisons of climate and litter quality effects. Oecologia 129:407-419.

Sulzman, E.W., J.B. Brant, R.D. Bowden, and K. Lajtha. 2005. Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO_2 efflux in an old growth coniferous forest. Biogeochemistry (In press).

Wagner, G.H., and D.C. Wolf. 1999. Carbon transformations and soil organic matter formation, *In* D. M. Sylvia, et al., eds. Principles and Applications of Soil Microbiology. Prentice Hall, Upper Saddle River, NJ.

Waldrop, M.P., and M.K. Firestone. 2004. Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities. Oecologia 138:275-284.

Waldrop, M.P., and M.K. Firestone. 2004. Altered utilization patterns of young and old soil C by microorganisms caused by temperature shifts and N additions. Biogeochemistry 67:235-248.

Waldrop, M.P., T.C. Balser, and M.K. Firestone. 2000. Linking microbial community composition to function in a tropical soil. Soil Biology & Biochemistry 32:1837-1846.

Waldrop, M.P., D.R. Zak, and R.L. Sinsabaugh. 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biology & Biochemistry 36:1443-1451.

Wu, J.G., P.C. Brookes, and D.S. Jenkinson. 1993. Formation and destruction of microbial biomass during the decomposition of glucose and ryegrass in soil. Soil Biology & Biochemistry 25:1435-1441.

Zelles, L. 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review. Biology & Fertility of Soils 29:111-129.

Chapter 2:
Root Controls on Soil Microbial Community Structure in Forest Soils

Justin B. Brant

Abstract

I assessed microbial community composition as a function of altered above- and belowground C inputs to soil in forest ecosystems of Oregon, Pennsylvania, and Hungary as part of a larger Detritus Input and Removal Treatment (DIRT) experiment. DIRT plots, which include root trenching, aboveground litter exclusion, and doubling of litter inputs, have been established in forested ecosystems in the US and Europe that vary with respect to dominant tree species, C background, N deposition rate, and soil type. These treatments provide a unique opportunity to study the feedbacks and processes controlling soil organic matter (SOM) transformations. This study used phospholipid fatty-acid (PLFA) analysis to examine changes in the soil microbial community size and composition in the mineral soil (0-10 cm) as a result of the DIRT treatments. At all sites the PLFA profiles from the plots without roots were significantly different than all other treatments, even after accounting for the loss of mycorrhizal fungi after root trenching. PLFA analysis showed that the rootless plots generally had larger quantities of actinomycete biomarkers and lower amounts of fungal biomarkers. At one of the sites, in an old-growth coniferous forest, seasonal changes in PLFA profiles were also examined. Seasonal differences in soil microbial community composition were greater than treatment differences. Throughout the year, treatments without roots continued to have a different microbial community composition than the treatments with roots, although the specific PLFA biomarkers responsible for these differences varied by season. These data provide direct evidence that root C inputs control microbial community composition in forest soils.

Introduction

Soils are the largest terrestrial sink for organic carbon (C), storing approximately twice as much C as the atmosphere (Schimel, 1995). Carbon storage in soil is mediated by microbes that use plant primary production from above- and belowground litter and soil organic matter (SOM) as their sources of C. In turn, the size and composition of the soil microbial community is controlled through complex interactions with plants (Bohlen et al., 2001; Butler et al., 2004; Zak et al., 2000), and is a function of net primary production, plant C allocation, rhizosphere activity, and litter substrate quality (Fisk and Fahey, 2001; Myers et al., 2001; Smith and Paul, 1990).

Altering root and litter inputs to soil is one method to examine the feedbacks between plants, microbes, and SOM (Nadelhoffer et al., 2004). Litter and root exclusion has been shown to decrease heterotrophic soil CO_2 efflux (Boone et al., 1998; Bowden et al., 1993; Fisk and Fahey, 2001; Lavigne et al., 2004; Li et al., 2004; Rey et al., 2002; Sulzman et al., 2005), although these changes do not appear to alter the size of microbial biomass in the mineral soil of forest ecosystems (Brant et al., 2004; Fisk and Fahey, 2001; Nadelhoffer et al., 2004; Siira-Pietikainen et al., 2001, 2003). Root exclusion has been shown to decrease fungal biomass and alter bacterial community structure in the organic layer (Siira-Pietikainen et al., 2001, 2003; Subke et al., 2004), with no changes in the mineral soil. Spears et al. (2003) found that the addition of coarse woody debris (CWD) to coniferous forests did not alter microbial biomass in the mineral soil. Similarly, the addition of aboveground litter did not affect either bacterial or fungal biomass in the mineral soil (Nadelhoffer et al., 2004), although an increase in fungal biomass (Nadelhoffer et al., 2004) and microbial C (Subke et al., 2004) has been found in the organic layer.

In this study, I used soils of three forest ecosystems from plots with chronically altered C inputs via root and litter exclusion as well as litter and CWD addition. The forest ecosystems vary with respect to dominant tree species, C background, nitrogen (N) deposition rates, and soil type. These long-term manipulations have been shown to alter soil CO_2 efflux (Bowden et al., 1993; Sulzman et al., 2005), dissolved organic

carbon (DOC) and dissolved organic nitrogen (DON) concentration (Lajtha et al., 2005; Nadelhoffer et al., 2004), and soil enzyme activities (Caldwell, unpublished data), although C and N concentrations in the mineral soil have remained unchanged (Keirstead, 2004; Nadelhoffer et al., 2004). At the three forest sites, soil microbial biomass and community composition were assessed using phospholipid fatty-acid (PLFA) analysis. The objective of this study was to determine whether long-term manipulation of the nature and quantity of C inputs have altered the microbial community in three very different forest ecosystems. I also examined whether changes in the soil microbial community were consistent across vastly different forest ecosystems. At one of the three sites, seasonal dynamics in the microbial community were examined.

Materials and Methods

Study Framework

The three sites in this study are part of a long-term inter-site project (Detritus Input and Removal Treatments, DIRT), designed to assess how rates and sources of plant inputs control accumulation and dynamics of SOM and nutrients in forest soils. The original DIRT treatments, designed by the late Dr. Francis Hole at the University of Wisconsin Arboretum in 1956, consist of chronic alterations of plant inputs to forest soils by transferring surface litter between permanent plots. A more detailed description of the DIRT study design can be found in Nadelhoffer et al. (2004). The current DIRT network now includes five sites, four located in deciduous forests and one in a coniferous forest.

Site Descriptions

Plant litter inputs have been manipulated at the DIRT plots in the H.J. Andrews Experimental Forest (HJA) in Oregon (44°15'N, 122°10'W, 531 m elevation) since 1997 (Table 2.1). Mean annual temperature (MAT) at the headquarters site of HJA is 8.7 °C (1973-2002) and mean annual precipitation (MAP) over the same period is 2370 mm, which falls mostly as rain. In general, over 70% of the precipitation occurs

during a "wet season", between November and March. Nitrogen deposition to this area is 1.6 kg N $ha^{-1}y^{-1}$ (Vanderbilt et al., 2003). The DIRT site was established in an undisturbed old-growth Douglas-fir (*Pseudotsuga menziesii*) - western hemlock (*Tsuga heterophylla*) stand. Other important tree species at the site include western red cedar (*Thuja plicata*) and vine maple (*Acer circinatum*). Soils are derived from volcanic parent materials and have been classified as coarse loamy mixed mesic Typic Hapludands (Dixon, 2003). Soil temperature and moisture data were collected at the Primet meteorological station located at the HJA Headquarters site (HJA LTER, 2005).

The DIRT sites at Bousson Experimental Forest (BOU) in Pennsylvania (41° 35.99'N, 80° 2.53'W, 381 m) were established in 1991. The MAT is 8.3°C and the MAP is 1050 mm. Atmospheric N deposition at BOU is ~13.0 kg N $ha^{-1}y^{-1}$ (Holland et al., 2004). The site is in an approximately 80-year-old mixed deciduous forest dominated by black cherry (*Prunus serotina* Ehrh.) and sugar maple (*Acer saccharum* Marsh). Soils are Alfisols, consisting of poorly drained sandy-loams of the Cambridge series.

The newest DIRT site included in this study was established in 2000 at Síkfőkút Forest (SIK) in Eger, Hungary (47° 90'N, 20° 46'E, 330 m). This is the driest and warmest site, with a MAT of 10°C and a MAP of 550 mm. This site has very

Table 2.1. Environmental characteristics of the three sites included in this study.

	SIK	**BOU**	**HJA**
Dominant Tree Species	*Quercus petraea/ Quercus cerris*	*Acer saccharum/ Prunus serotina*	*Pseudotsuga menziesii/ Tsuga heterophylla*
MAT (°C)	10	8.3	7.9
MAP (cm)	55	105	237
N deposition (kg $ha^{-1}y^{-1}$)	15	13	1.6
Soil Order	Alfisol	Alfisol	Andisol
Manipulation Started	2000	1991	1997
% Carbon	4.2	6.3	9.3
% Nitrogen	0.3	0.5	0.3
C:N ratio	15.8	14.2	30.1

high N deposition with an annual rate of ~15 kg N $ha^{-1}y^{-1}$ (Holland et al., 2004). The plots are located in a mature temperate deciduous oak forest dominated by sessile oak (*Quercus petraea*) and turkey oak (*Quercus cerris*). The soils are brown forest soils with Cambisol features. The most similar US Taxonomic soil order is Alfisols.

Experimental Manipulations

There are six litter input/exclusion treatments at HJA and SIK, with five treatments at BOU (three replicates per treatment, Table 2.2). Plots vary in size from site to site dependent on site characteristics. Individual plots at BOU are 3 m x 3 m, SIK has 10 m x 10 m plots, and HJA has 10 m x 15 m plots given the large heterogeneity of this site. On No Litter (NL) and No Input (NI) plots, litter is excluded with 1-mm-mesh screens. Aboveground litter that is swept off NL plots is added to Double Litter (DL) plots. Root growth is prevented in the No Roots (NR) plots by trenching to 1 m followed by insertion of impenetrable barriers. The NI treatment combines the NR and NL treatments. Wood inputs are doubled in Double Wood (DW; HJA and SIK only) plots by the addition of chipped whole logs. Control (CO) plots with no inputs manipulation are also found at all sites.

Table 2.2. Summary of the DIRT treatments

Treatment	Method
Control	Normal litter inputs
No Litter	Aboveground inputs are excluded
Double Litter	Aboveground leaf/needle inputs are doubled by adding litter removed from NO LITTER plots
Double Wood	Aboveground wood inputs are doubled by adding large shredded wood pieces based on measure input rates of CWD (HJA and SIK only)
No Roots	Roots are excluded by trenching to 1 m and the insertion of impenetrable barriers
No Inputs	Aboveground inputs excluded as in NO LITTER plots Belowground inputs excluded as in NO ROOTS plots

PLFA Analysis and Extraction

Seven to ten 0-10 cm soil cores from each plot were collected using stratified random sampling. The cores were then combined to yield one composite sample per plot. Soils were passed through a 2-mm sieve and stored at 4°C until PLFA extraction within 3 d of sampling. BOU was sampled in May 2003 and SIK was sampled in October 2003. HJA was sampled in July 2003, November 2003, and April 2004.

PLFAs were extracted from 2 g of soil using the modified Bligh and Dyer method (Bligh and Dyer, 1959; White and Ringelberg, 1998). Briefly, soils were incubated in a 2:1:0.8 solution of methanol, chloroform, and phosphate buffer. The soil was then filtered and the chloroform phase separated. Phospholipids were separated from glycolipids and neutral lipids using solid phase extraction columns. Phospholipids were saponified and methylated to fatty-acid methyl esters (FAME). FAMEs were run on an Agilent 6890 gas chromatograph (GC) equipped with a flame ionization detector (FID) and an Ultra-2 column. Peaks were identified based on comparing retention times with known standards. Concentration of each PLFA was obtained by comparing peak areas with a 13:0 standard curve.

Standard nomenclature was used to describe PLFAs. The number before the colon refers to the total number of C atoms, while the number following the colon refers to the number of double bonds. The location of any double bonds in the fatty acid molecule is indicated by the number after the 'ω'. The notations: "Me," "OH," "cy," "i," and "a" refer to methyl group, hydroxy, cyclopropane groups, and iso- and anteiso-branched fatty acids, respectively.

Total extractable PLFAs were used as an indicator of living biomass. This method has been found to be proportional to other microbial biomass measures (Fierer et al., 2003; Fritze et al., 2000). The total bacterial biomass was calculated by summing the i15:0, a15:0, i16:0, 16:1ω9, 16:1ω5, 17:1ω9, i17:0, a17:0, cy17:0, 18:1ω7, and cy19:0 PLFAs, which are mostly of bacterial origin (Blume et al., 2002; Hill et al., 2000). Specific PLFA markers were used to quantify the relative abundance of various taxonomic groups in a manner similar to Fierer et al. (2003). Gram-positive bacteria were identified by summing i14:0, i15:0, a15:0, i16:0, i17:0,

and a17:0, while gram-negative bacteria were identified by summing cy17:0, 16:1ω9c, 17:1ω9c, and 18:1ω7c. The 18:2ω6c PLFA was used as a marker for fungi. Actinomycetes were identified using the 10Me 16:0, 10Me 17:0, and 10Me 18:0 PLFAs; while 20:4ω6 and 20:2ω6 were used as biomarkers for protozoa.

Statistical Analysis

PLFA relative abundances (mol %) for all 40 PLFAs identified were used to examine community differences among treatments. Microbial community structure was examined by non-metric multidimensional scaling (NMS: Kruskal, 1964; Mather, 1976) using the PC-ORD software package (McCune and Mefford, 1999). The medium setting of 'autopilot' mode in PC-ORD was used, which attempts to find a stable solution from a random starting position (McCune and Mefford, 1999). This method uses a maximum of 200 iterations in 15 runs with real data. The final dimensionality is determined by comparing the final stress values among the best solution for each axis. Significance of axes is assessed by comparing observed final stress with the final stress in 30 runs of randomized data using a Monte Carlo test (McCune and Grace, 2002). Final ordinations were rotated to maximize environmental variables represented and separation of treatments on axes 1 and 2. Summed abundances for taxonomic biomarkers were used as overlays in a joint plot to visualize correlations between the ordination and the relative abundance of specific taxonomic groups. In a joint plot the length and direction of each vector is related to correlations with the original PLFA data (McCune and Grace, 2002).

Statistical differences between PLFA profiles was assessed using Multi-response Permutation Procedures (MRPP: Mielke Jr., 1984; Mielke Jr. and Berry, 2001) in PC-ORD. MRPP is a nonparametric procedure similar to MANOVA for testing the hypothesis of no difference between two or more preexisting groups (McCune and Grace, 2002). MRPP yields a p-value to evaluate how likely it is that an observed difference is due to chance, as well as the chance-corrected within-group agreement (A), which describes within-group homogeneity compared to random expectation (McCune and Grace, 2002). An A-value equal to 1 is found when all

items within a group are identical, when heterogeneity within groups equals expectation by chance, A = 0. Results with statistically significant p-values, and very small A-value (A < 0.02) can result from a large sample size (McCune and Grace, 2002). In instances where there were no significant differences among plots with roots ($p > 0.1$) these treatments were combined and compared with the rootless treatments in a two-way analysis.

Indicator species analysis (ISA) was used to look at the whether specific PLFAs were indicative of certain treatments (Dufrene and Legendre, 1997). This method looks at the abundance and frequency of different species markers within specific pre-determined groups (McCune and Grace, 2002). ISA was also performed in PC-ORD using a Monte Carlo test with 10,000 randomizations to test for statistical significance. ISA yields an indicator value and a p-value. Indicator values show the percent of perfect indications for that marker, with a value of 100 a perfect indictor. The p-value gives an idea of the significance of getting an indicator value greater than or equal to the observed value using a Monte Carlo test.

The statistical significance of summed taxonomic group differences between treatment and season at HJA, as well as their interaction, were analyzed with repeated measures analysis of variance (ANOVA) using PROC MIXED (SAS version 8.1, SAS Institute, Inc). In instances where the interaction between treatment and year was not significant ($p > 0.05$), the interaction was removed and the analysis was repeated using data from all three sampling dates. When ANOVA resulted in a p-value < 0.05, pre-planned comparisons between the six treatments were made using orthogonal contrasts. At BOU and SIK, where data were from only one sampling date, the same comparisons were made with ANOVA using the Tukey-Kramer procedure to correct for family-wise error rates (Splus version 6.1, Insightful Corp., Seattle, WA).

Results

There is moderate evidence of a difference in soil water content at the time of sampling among treatments at BOU (ANOVA; $p = 0.05$), although none of the multiple comparisons between treatments were significant ($p > 0.1$) (Table 2.3). At

SIK, there was a difference in soil water content at the time of sampling (ANOVA; $p < 0.001$), with the rootless (NR and NI) treatments being wetter than the CO, DL, and DW treatments. At HJA, the NI and NR plots are generally wetter than the other treatments. This difference is most pronounced in July, with virtually no difference among treatments in either November or April. However, when all the dates are analyzed together, there is only suggestive evidence of a difference in soil water content among treatments ($p = 0.07$) over all three sampling dates. There was a significant difference in soil water content by sampling date, with the July sampling being drier than either of the other two dates.

Microbial biomass, as determined by total extractable PLFA, was relatively constant by treatment at all three sites (Table 2.3). The only significant differences in biomass between treatments were found at SIK (ANOVA; $p = 0.05$). At that site there is some evidence that the DW plots have a larger biomass than the NR plots ($p < 0.05$) and suggestive evidence that the DW plots have a larger biomass than the NI plots ($p < 0.1$). At HJA, there were no treatment effects on microbial biomass, although there were seasonal trends (Table 2.3; $p < 0.0001$). There was no evidence of a difference in bacterial biomass ($p > 0.05$) at any of the sites (Table 2.3).

A NMS of the BOU PLFA data suggests that the treatments without roots had different microbial communities than all the other treatments (Figure 2.1). There is no evidence of a difference in the PLFA profiles among any of the three treatments with roots (CO, DL, and NL) (MRPP; $p = 0.49$). When these treatments are grouped together and compared with the two rootless treatments there is convincing evidence of a difference in PLFA profiles between the root and rootless treatments (MRPP; $p = 0.002$, $A = 0.14$). Removing the fungal biomarker from the analysis has little effect on the final NMS solution (data not shown) and actually increases the observed treatment effects (MRPP; $p = 0.002$, $A = 0.17$), indicating that differences in the bacterial community are driving these treatment differences. An examination of taxonomic markers from the joint plot suggests that the rootless plots tend to have higher concentrations of the actinomycete biomarkers and a lower concentration of the gram-

Table 2.3. Total microbial and bacterial biomass, as estimated by summing extractable PLFAs, and soil water content at the time of sampling at all three DIRT sites. Letters indicate significant difference (ANOVA; $p < 0.05$) of treatments within one site. Numbers are means (n=3), with one standard error in parentheses.

Site	Treatment	Microbial Biomass (nmol PLFA g^{-1} soil)	Bacterial PLFA (nmol g^{-1} soil)	Water Content (g H_2O g^{-1} soil)
Bousson (BOU)	Double Litter (DL)	61.9 (17.9)	31.3 (10.0)	0.53 (0.04)
	Control (CO)	119.9 (66.7)	63.9 (36.4)	0.57 (0.04)
	No Litter (NL)	114.2 (19.6)	58.7 (11.2)	0.59 (0.02)
	No Roots (NR)	93.8 (27.9)	48.1 (17.2)	0.67 (0.03)
	No Inputs (NI)	65.4 (16.1)	30.6 (7.6)	0.65 (0.02)
Síkfőkút (SIK)	Double Wood (DW)	79.0 (13.9)[b]	40.9 (7.9)	0.30 (0.01)[a]
	Double Litter (DL)	59.3 (15.3)[ab]	31.4 (8.5)	0.26 (0.01)[a]
	Control (CO)	50.8 (6.0)[ab]	26.1 (3.2)	0.27 (0.02)[a]
	No Litter (NL)	57.6 (5.4)[ab]	29.8 (2.8)	0.31 (0.01)[ab]
	No Roots (NR)	34.2 (5.5)[a]	17.9 (2.8)	0.36 (0.01)[b]
	No Inputs (NI)	38.5 (2.5)[ab]	20.5 (1.9)	0.35 (0.01)[b]
H.J. Andrews (HJA)				
April	Double Wood (DW)	90.5 (22.5)	45.1 (11.5)	0.48 (0.02)
	Double Litter (DL)	92.5 (21.1)	45.8 (9.4)	0.47 (0.01)
	Control (CO)	92.0 (11.1)	45.2 (4.4)	0.49 (0.01)
	No Litter (NL)	73.2 (18.1)	35.6 (7.4)	0.47 (0.02)
	No Roots (NR)	86.7 (21.1)	47.5 (11.8)	0.51 (0.02)
	No Inputs (NI)	78.0 (13.0)	42.4 (7.0)	0.51 (0.03)
July	Double Wood (DW)	67.0 (5.2)	32.3 (1.0)	0.30 (0.02)
	Double Litter (DL)	42.9 (3.0)	20.6 (2.3)	0.31 (0.01)
	Control (CO)	39.4 (6.6)	18.3 (3.1)	0.32 (0.01)
	No Litter (NL)	44.8 (2.5)	22.0 (0.8)	0.34 (0.01)
	No Roots (NR)	44.9 (2.0)	22.8 (1.1)	0.41 (0.02)
	No Inputs (NI)	52.2 (1.9)	25.9 (0.9)	0.40 (0.02)
November	Double Wood (DW)	37.5 (3.9)	18.4 (2.4)	0.43 (0.04)
	Double Litter (DL)	23.3 (4.9)	11.0 (2.8)	0.46 (0.06)
	Control (CO)	35.6 (6.9)	17.7 (3.8)	0.49 (0.03)
	No Litter (NL)	19.8 (4.9)	9.3 (2.3)	0.45 (0.02)
	No Roots (NR)	28.3 (2.3)	14.5 (1.2)	0.49 (0.02)
	No Inputs (NI)	30.2 (3.5)	15.2 (2.0)	0.53 (0.07)

negative biomarkers (Figure 2.1), although these differences are not statistically different (Table 2.4).

A NMS of the PLFA profile at SIK shows a similar trend as that seen with the BOU data, with the plots without roots appearing to have a different PLFA profile than the plots with roots (Figure 2.2). Again there is no difference in PLFAs among the treatments with roots (MRPP; $p = 0.34$). When these treatments are compared with the rootless treatments there is moderate evidence of a difference in PLFA profiles (MRPP; $p = 0.03$, $A = 0.05$). However, if the 18:2ω6,9 PLFA is removed from the analysis there is no evidence of a treatment difference between bacterial communities (MRPP; $p = 0.73$). From the joint plot (Figure 2) rootless plots have larger concentrations of the actinomycete biomarker and significantly lower relative

Figure 2.1. NMS of PLFA relative abundance at BOU (final stress = 9.7, instability = 0.00006) from a 2-D solution. Symbols are mean loading scores for each treatment (n=3) with error bars showing plus and minus one standard error. R^2 axis 1 = 0.76, axis 2 = 0.14. Vectors are based on summed abundances of specific PLFAs. The length of a vector is proportional to the correlation between that variable and the NMS axes.

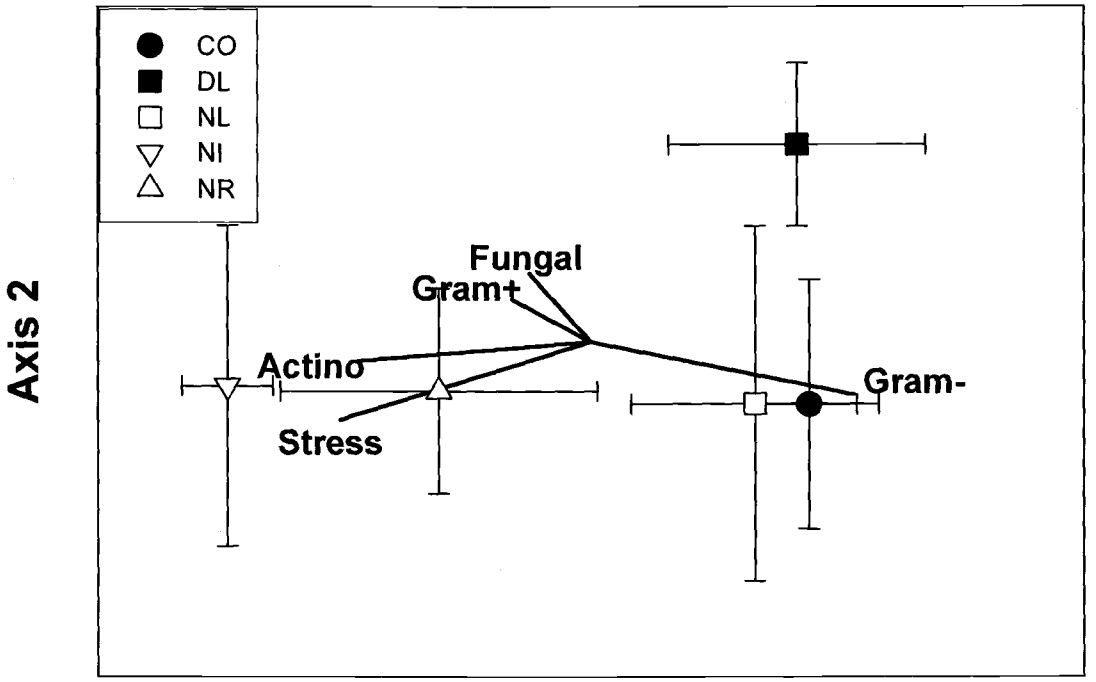


Figure 2.2. NMS of PLFA relative abundance at SIK (final stress = 6.7, instability = 0.00008). This figure shows the first two axes of a three dimensional solution (R^2 axis 1 = 0.59, axis 2 = 0.19, axis 3 = 0.16) Symbols are mean loading scores (n=3) for each treatment with error bars plus and minus one standard error. Vectors are based on summed abundances of specific PLFAs.

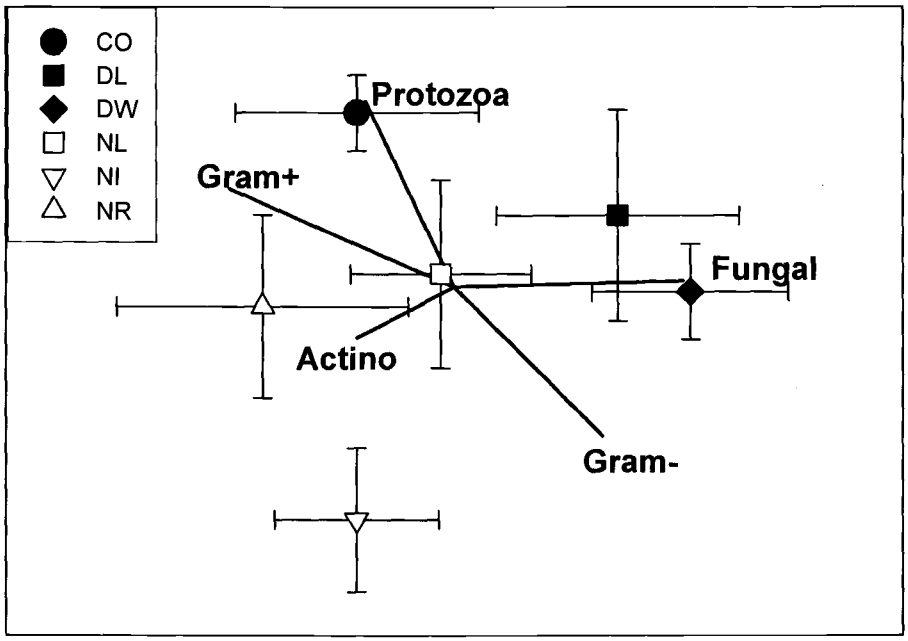
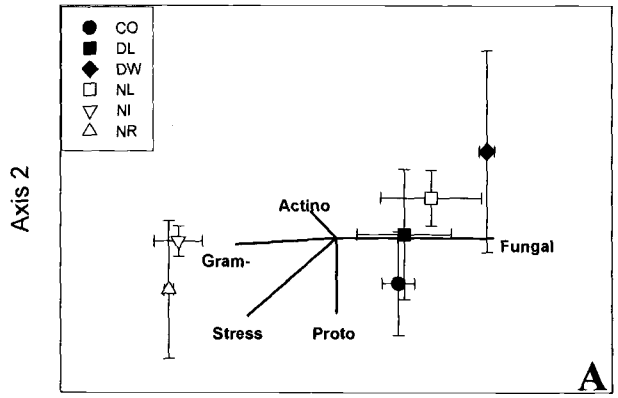
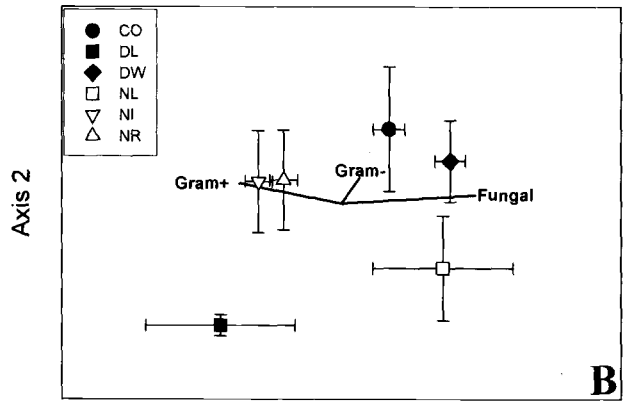


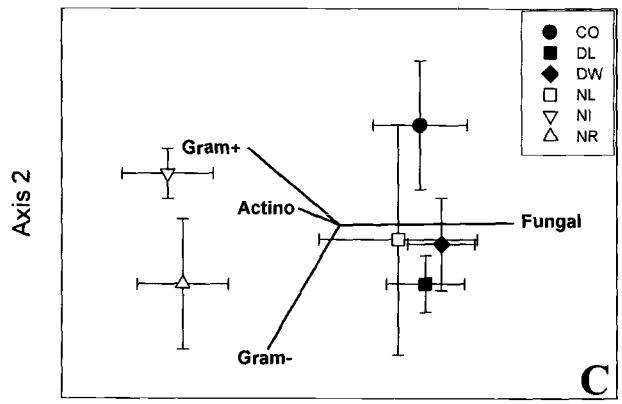
Table 2.4. Treatment differences in taxonomic groups, as evidenced by the abundance of specific PLFA biomarkers. Numbers show mean relative abundance (n=3) with standard error in parentheses. Letters denote statistical differences (ANOVA; $p < 0.05$) between treatments.

Treatment	Gram-positive bacteria	Gram-negative bacteria	Actino	Protozoa	Fungi	F:B Ratio	17:0cy, 19:0 cy: precursors
BOU							
DL	16.7 (0.5)	17.9 (1.0)	9.2 (0.3)	1.6 (0.3)	3.3 (0.7)	0.07 (0.02)	0.90 (0.03)^{a}
CO	17.8 (0.1)	18.3 (0.4)	9.3 (0.3)	1.1 (0.3)	2.9 (0.2)	0.06 (0.01)	0.99 (0.12)^{ab}
NL	16.0 (0.7)	17.9 (1.3)	8.7 (0.6)	1.2 (0.1)	4.1 (0.4)	0.08 (0.01)	1.01 (0.16)^{ab}
NR	18.7 (0.6)	14.6 (2.2)	9.7 (0.9)	1.3 (0.1)	2.5 (0.5)	0.05 (0.01)	1.26 (0.14)^{ab}
NI	18.4 (1.3)	11.9 (0.9)	10.3 (0.1)	1.3 (0.1)	4.0 (0.3)	0.09 (0.01)	1.42 (0.07)^{b}
SIK							
DW	22.2 (1.0)	17.7 (1.0)	10.0 (0.3)	0.56 (0.2)^{ab}	3.6 (0.3)^{a}	0.07 (0.01)^{a}	0.77 (0.04)
DL	23.7 (1.0)	17.2 (1.0)	10.2 (0.3)	0.66 (0.2)^{ab}	3.8 (0.8)^{a}	0.08 (0.01)^{a}	0.74 (0.03)
CO	25.1 (0.1)	15.5 (0.6)	10.8 (0.1)	1.56 (0.4)^{a}	2.5 (0.5)^{ab}	0.05 (0.01)^{ab}	0.94 (0.08)
NL	24.7 (1.3)	16.2 (0.8)	11.0 (0.2)	0.66 (0.3)^{ab}	2.6 (0.2)^{ab}	0.05 (0.01)^{ab}	0.89 (0.03)
NR	25.3 (1.8)	16.4 (1.1)	10.9 (0.3)	1.07 (0.1)^{ab}	1.2 (0.2)^{b}	0.02 (0.01)^{b}	0.91 (0.09)
NI	24.3 (0.7)	16.5 (1.0)	11.5 (0.4)	0.23 (0.1)^{b}	1.2 (0.2)^{b}	0.02 (0.01)^{b}	0.89 (0.04)

Figure 2.3. NMS of PLFA relative abundances for HJA treatments from A) July 2003 (stress 12.1, instability 0.00009, R^2 axis 1 = 0.43, axis 2 = 0.42), B) November 2003 (stress 11.32, instability 0.00005, R^2 axis 1 = 0.20, axis 2 = 0.68), and C) April 2004 (stress 6.05, instability 0.00008, R^2 axis 1 = 0.63, axis 2 = 0.19, axis 3 = 0.14). Symbols show the average loading scores for each treatment (n=3) plus and minus one S.E.







abundance of fungi, a lower fungal:bacterial ratio, and fewer protozoa than the treatments with roots (Table 4 and Figure 2), although only protozoan abundance shows a significant treatment effect (ANOVA; $p = 0.03$).

At HJA, the PLFA profiles vary by both treatment and season (Figure 2.3). On all sampling dates, there was no evidence of a difference in PLFA profiles among any of the plots with roots (MRPP; $p > 0.25$). In the April and July data there is overwhelming evidence that the rootless plots have a different PLFA profile than all other plots (MRPP; $p < .0001$, $A = 0.15$ and 0.13, respectively). In the November data there is moderate evidence that the rootless plots were different from all other plots (MRPP; $p=0.04$, $A=0.05$). If the fungal biomarker is removed, the bacterial PLFA profiles of the rootless plots are still significantly different from all other plots in April and July (MRPP; $p < 0.05$, $A = 0.03$ and 0.06, respectively), although the differences are much reduced. In November, there is no evidence of a treatment effect on the bacterial communities (MRPP; $p = 0.41$). The rootless plots have a lower abundance of fungi regardless of season (ANOVA; $p < 0.05$), although the portions of the bacterial community driving the treatment differences vary by sampling date. The rootless plots generally have a larger abundance of actinomycetes and gram-positive bacteria (Figure 2.3), although these differences are never significant (Table 2.5). The rootless plots also have a larger abundance of gram-negative bacteria during April and July, with no difference in November. The ratio of cyclopropyl PLFAs to their precursors is larger in the rootless plots during April and July (Table 2.5).

Table 2.5. Mean soil temperature and soil water content at the Primet meteorological station for the 14 days prior to each sampling date at HJA.

Sampling Date	Mean Soil Temperature (°C)	Mean Soil Water Content (% H_2O filled pore space)
July 2003	20.0	0.08
November 2003	7.22	0.22
April 2004	10.9	0.21

Seasonal changes in microbial community at HJA

At the three sampling dates at HJA the soils at the DIRT sites were at different water contents with the soils much wetter in the April and November sampling dates than in July, although there were no significant differences in water content among the different litter treatments (Table 2.3). At a nearby meteorological station the average soil moisture content in the 14 days preceding sampling was also much higher before the April and November sampling dates than before the July sampling date (Table 2.5). In addition, the average soil temperature in the 14 days preceding sampling was highest in July and lowest in November (Table 2.5).

In general at HJA, the seasonal differences in microbial community structure were larger than the treatment differences, as all the taxonomic groups showed significant changes by season (ANOVA; $p < 0.05$), whereas only the fungi and fungal:bacterial ratio showed any evidence ($p < 0.003$) of differences by treatment (Table 2.6). The total microbial biomass was also statistically different at all sampling

Table 2.6. Treatment differences in the abundance of taxonomic biomarkers over the three sampling dates at HJA. Numbers are mean PLFA relative abundance (n=3) with S.E. in parentheses.

Season	Gram-positive bacteria	Gram-negative bacteria	Actino	Protozoa	Fungi	F:B Ratio	17:0cy, 19:0 cy: precursors
April							
DW	13.1 (0.6)	23.0 (0.5)	4.8 (0.3)	0.65 (0.11)	7.9 (0.5)[a]	0.16 (0.01)[a]	0.66 (0.05)
DL	13.7 (0.1)	23.7 (1.3)	5.5 (0.7)	0.53 (0.13)	7.9 (0.9)[a]	0.16 (0.02)[a]	0.62 (0.04)
CO	15.0 (1.0)	22.6 (0.4)	5.8 (0.2)	0.61 (0.13)	7.9 (0.9)[a]	0.16 (0.02)[a]	0.58 (0.03)
NL	13.9 (0.4)	23.2 (1.5)	5.3 (0.2)	0.64 (0.04)	6.7 (1.4)[a]	0.14 (0.03)[a]	0.59 (0.04)
NR	15.3 (0.9)	24.7 (0.5)	5.5 (0.6)	0.70 (0.16)	2.7 (0.4)[b]	0.05 (0.01)[b]	0.69 (0.03)
NI	16.9 (1.2)	23.5 (0.5)	5.9 (0.4)	0.66 (0.10)	2.6 (0.2)[b]	0.05 (0.01)[b]	0.68 (0.02)
July							
DW	12.5 (1.7)	21.0 (1.6)	6.1 (0.3)	0.58 (0.7)	7.4 (1.1)[a]	0.16 (0.03)[a]	0.69 (0.07)
DL	12.6 (0.3)	21.7 (1.8)	5.9 (0.2)	0.90 (0.18)	7.1 (0.7)[a]	0.15 (0.02)[a]	0.77 (0.02)
CO	13.5 (0.6)	20.7 (0.8)	6.3 (0.2)	1.49 (0.22)	6.3 (0.5)[a]	0.14 (0.01)[a]	0.75 (0.07)
NL	13.8 (1.4)	21.9 (0.9)	6.0 (0.1)	0.78 (0.15)	6.8 (1.3)[a]	0.14 (0.03)[a]	0.68 (0.07)
NR	14.0 (0.9)	21.8 (0.6)	6.3 (0.9)	1.05 (0.34)	2.8 (0.1)[b]	0.06 (0.001)[b]	0.91 (0.07)
NI	14.3 (0.1)	21.8 (0.3)	6.6 (0.4)	0.80 (0.14)	2.7 (0.2)[b]	0.05 (0.005)[b]	0.88 (0.02)
November							
DW	14.3 (0.4)	20.9 (1.8)	5.8 (0.3)	1.1 (0.2)	6.7 (0.4)[a]	0.14 (0.01)[a]	0.82 (0.09)
DL	15.4 (1.4)	18.2 (1.3)	6.9 (0.9)	2.1 (0.3)	4.5 (0.1)[a]	0.09 (0.01)[a]	0.95 (0.12)
CO	15.1 (0.5)	21.3 (1.3)	6.7 (0.4)	1.1 (0.5)	5.4 (0.5)[a]	0.11 (0.01)[a]	0.74 (0.09)
NL	12.0 (1.4)	20.5 (0.8)	6.7 (0.1)	2.3 (1.0)	5.2 (1.3)[a]	0.11 (0.03)[a]	0.87 (0.06)
NR	15.5 (0.5)	21.3 (1.3)	6.0 (0.5)	1.3 (0.4)	2.5 (0.2)[b]	0.05 (0.01)[b]	0.86 (0.07)
NI	15.9 (1.2)	20.4 (0.7)	6.9 (0.4)	1.7 (0.5)	2.4 (0.3)[b]	0.05 (0.01)[b]	0.87 (0.10)

Lipid	Indicator value (IV)	p-value
15:1i	43.9	0.07
16:1 ω 9	36.9	0.04
16:1 ω 7	39.0	0.08
17:1a	35.6	0.03
17:0	36.9	0.04
16:0 3OH	45.0	0.03
18:3 ω 6c	48.0	0.07
18:1 ω 7	35.8	0.1
18:2 ω 6,9	40.4	0.08
18:0	42.5	0.03

Table 2.7. Indicator species analysis for the Control plots over the three sampling dates at HJA. P-value is based on a Monte Carlo test of the data with 10,000 randomizations. Only PLFAs with a p-value ≤ 0.1 are shown.

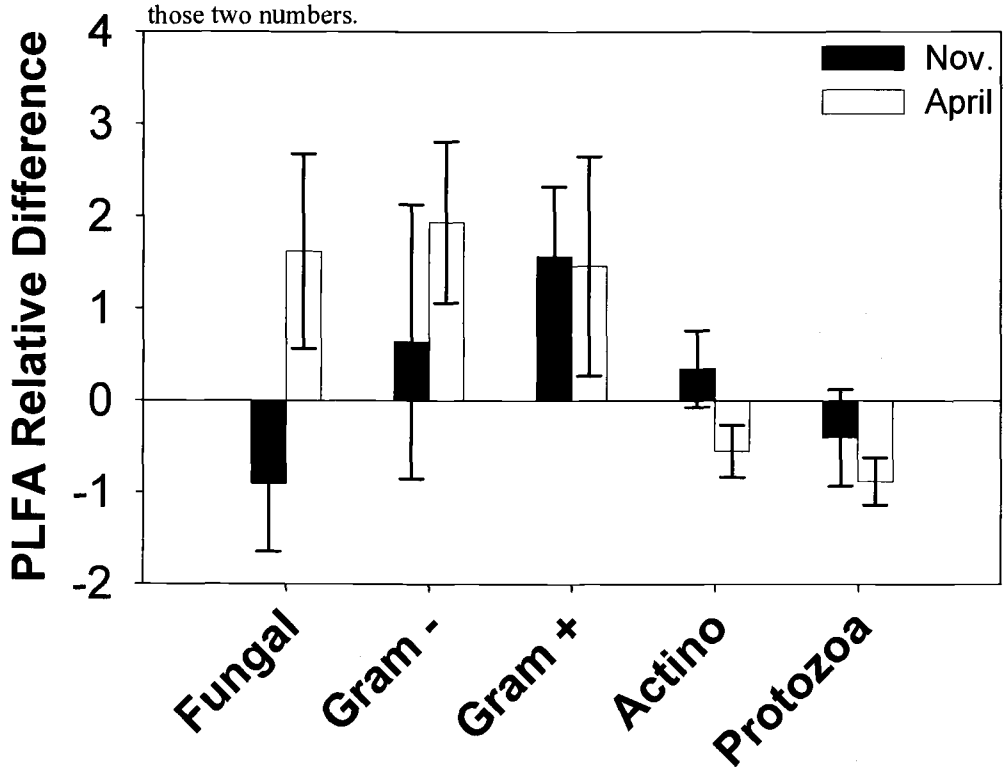
dates (ANOVA; $p < 0.05$), with the lowest biomass in November and the highest biomass in April (Table 2.3). The bacterial biomass showed a similar trend, with significant evidence of a difference between April and the other two dates (ANOVA; $p < 0.05$) and suggestive evidence of a difference in bacterial biomass in November and July (ANOVA; $p < 0.1$).

There is no evidence of an interaction between season and treatment (ANOVA; $p > 0.05$) for any of the taxonomic groups. Thus, I examined the seasonal changes in the relative abundance of specific PLFAs using ISA in control plots as an example (Table 2.7). The seasonal changes were found to be similar in all other treatments (data not shown), except that the 18:2ω6,9 remained relatively constant over the year in the rootless plots (Table 2.6). In the control plots, abundances of monounsaturated and polyunsaturated lipids varied the most among the three sampling date (Table 2.7). In addition, some very low abundance branched and saturated PLFAs are significantly different by season.

I also looked at seasonal changes in the relative abundance of microbial groups, using the control plots as an example (Figure 2.4). The fungal biomarker and the fungal:bacterial ratio showed a significantly ($p < 0.05$) lower relative abundance in November and a significantly higher relative abundance in April when compared with July. Gram-positive markers were significantly ($p < 0.05$) lower in July than either November or April, whereas the relative abundance of the gram-negative biomarkers

was significantly higher ($p < 0.05$) in April than July, with no difference between the relative abundance in November and either of the two other sampling dates. Conversely, the actinomycete markers were significantly lower in the April sampling than either the July or November sampling. The protozoan markers were significantly higher ($p < 0.05$) in the July sampling than in the April sampling, with no differences between November and either of the other two sampling dates. Finally, the ratio of cyclopropyl PLFAs to their precursors was significantly lower in April than either July or November (data not shown).

Figure 2.4. Differences in the relative abundance (mol %) of PLFA taxonomic biomarker at the three HJA sampling date. The bars show the difference in relative abundance between either the November or April sampling date, and the July sampling date. Error bars show the S.E. of the differences between those two numbers.



Discussion

Effects of C input manipulation on the microbial community

Both total microbial biomass and bacterial biomass, measured as total PLFA, were not affected by manipulation of C inputs at any of our sites (Table 2.3). These results are consistent with other studies with similar C input manipulations where no change in either microbial or bacterial biomass has been found in the mineral soil (Fisk and Fahey, 2001; Nadelhoffer et al., 2004; Siira-Pietikainen et al., 2001, 2003; Spears et al., 2003).

PLFA analyses at the DIRT plots shows that belowground C inputs exert more influence on the soil microbial community than aboveground inputs in three very different forest ecosystems (Figures 2.1, 2.2, and 2.3). The small influence of chronic aboveground C inputs is somewhat surprising. Litter and wood additions have been shown to lead to an increase in DOM fluxes from the organic horizon to the mineral soil after at least 5 years of manipulation (Lajtha et al., 2005; Nadelhoffer et al., 2004). Treatment differences in dissolved organic matter (DOM) concentration entering the soil disappear between 0 and 30 cm, except in DW plots where the treatment differences disappear between 30 and 100 cm (Lajtha et al., 2005; Nadelhoffer et al., 2004). Although the quantity of DOC entering the mineral soil was different between treatments, the chemistry of the soil solution leaving the organic horizon in the CO, DW, and DL treatments was the same (Yano et al., 2005), suggesting microbial degradation of labile C before soil solution enters the mineral soil (Lajtha et al., 2005).

However, root exclusion by trenching has significantly altered the microbial community structure in the mineral soil at all three sites (Figure 2.1, 2.2, and 2.3). Theses changes in community structure go beyond the loss of mycorrhizal fungi and include changes in the bacterial community at the two sites where the treatments have been in place for at least 5 years. The only other studies to look at the effects of trenching on microbial community structure concentrated on the organic horizon. Siira-Peitikainen et al. (2001, 2003) found a decrease in the relative abundance of fungi, as well as a change in the bacterial community composition after only three years of treatment.

Under ectomycorrhizal tree species, root trenching has been shown to significantly decrease the population size, species richness, and root colonization of ectomycorrhizal fungi 16 months after trenching (Simard et al., 1997). The loss of ectomycorrhizal fungi appears to contribute to most of the observed differences in PLFA profiles at HJA and SIK. On the other hand, the rootless plots at BOU have a larger abundance of the fungal biomarker than the other treatments (Table 2.4). Sugar maple form only arbuscular mycorrhizal (AM) associations. AM fungi do not generally have a large portion of the standard 18:2ω6,9 fungal biomarker, and the 16:1ω5 PLFA has been used as a biomarker for this group (Olsson and Johansen, 2000). At BOU there was some evidence of a smaller relative abundance of the 16:1ω5 biomarker in the rootless plots (ISA; $p = 0.06$). The change in the relative abundance of the fungal biomarker at BOU suggests an increase in the population of saprophytic fungi for which there is no PLFA biomarker in the rootless plots. This is expected as these fungi are considered important in the degradation of more complex C substrates (Entry et al., 1991). Conversely, studies where root inputs and growth are increased by exposure to levels of atmospheric CO_2 above ambient show an increase in the relative abundance of fungi after 2.5 years in a deciduous forest ecosystem (Zak et al., 2000) and after 4 years in a model beech-spruce ecosystem (Wiemken et al., 2001), although neither study found any changes in bacterial community structure. Any changes in the abundance and composition of mycorrhizal fungi could have a large influence on soil C processing rates and C input quality (Langley and Hungate, 2003).

At SIK, BOU, and to a lesser extent HJA, the actinomycete biomarkers tend to have larger relative abundances in the rootless treatments (Figures 2.1, 2.2, and 2.3), although none of these changes is significant. Actinomycetes are filamentous bacteria that have traditionally been associated with the degradation of more recalcitrant C compounds such at chitin and cellulose (McCarthy and Williams, 1992). Actinomycetes have been shown to increase in relative abundance with lower resource availability either due to C addition in the form of synthetic root exudates (Griffiths et al., 1999) or an increase in soil depth (Fierer et al., 2003). These members of the soil

microbial community might be filling the niche decomposing more recalcitrant C compounds usually filled by mycorrhizal fungi in the plots without roots; however, more research into functional differences in the rootless plots is necessary to determine if this is the case.

The ratio of cyclo PLFAs to their precursor PLFAs has been used as an indicator of microbial community stress (Bossio and Scow, 1998). This ratio is higher in the rootless plots at BOU, implying that the lack of labile root inputs puts physiological stress on the microbial community (Table 2.4). This ratio is also larger in the rootless plots at HJA during July, although the difference is not statistically significant (Figure 2.3A). Various studies have found that C limitation can lead to an increase in microbial community stress as determined by this ratio (Bossio and Scow, 1998; Fierer et al., 2003; Kieft et al., 1997; Lundquist et al., 1999; Macdonald et al., 2004).

Soil water content in the rootless plots is generally higher than in the plots with roots because of the loss of plant transpiration (Table 2.3). At HJA, the NI and NR plots are significantly wetter than all other plots during the both the dry (June-September) and the wet season (November-March) (Sulzman et al., 2005). These differences in water content cannot be ruled out as a major cause of microbial community changes in response to root trenching, as moisture content has been shown to have a major effect on microbial community structure (Kieft et al., 1997; Schimel et al., 1999; Wilkinson et al., 2002). During the summer, changes in soil water content would be expected to have the largest effects on the microbial community, yet it appears that the bacterial community in the rootless plots is under more physiological stress even though these plots are wetter as evidenced by a larger ratio of cyclopropyl PLFAs to their precursors in the rootless plots. As a result, it appears that C limitation plays a major role in the observed treatment differences, regardless of water content.

Seasonal dynamics at HJA

Seasonal changes in the soil microbial community were more pronounced than the effects of the DIRT treatments at HJA (Table 2.6). Similar results have been

found in other studies that look at soils under the same plant species (Blume et al., 2002; Bossio et al., 1998; Myers et al., 2001). Microbial biomass changed over the course of the year, with the highest biomass in April, the period of peak photosynthesis (Waring and Franklin, 1979), and the lowest values in November, when the soils were fairly saturated and cold. Seasonal changes in microbial biomass have been reported in other studies in forest ecosystems, although the season of high biomass varies by climate and tree physiology (Bohlen et al., 2001; Litton et al., 2003; Myers et al., 2001).

The relative abundance of fungi was significantly lower in November than either April or July (Figure 2.4). Wallander et al. (2001) found that mycorrhizal biomass in a coniferous forest generally followed fine root production, with high biomass during periods of high fine root production and low biomass during periods of low fine root production. In November, mycorrhizal biomass would be expected to decrease, as fine root production in Douglas-fir forests is low during the winter (Tingey et al., 2005). Conversely, when the abundance of fungi was low, the relative abundance of actinomycetes increased (Figure 2.4). This could be further evidence that fungi and actinomycetes are competing for similar resources in this ecosystem.

The bacterial community also showed pronounced seasonal changes (Table 2.6). The monounsaturated PLFAs were most variable throughout the year (Table 2.7). These lipids are generally associated with gram-negative bacteria and might be expected to be sensitive to seasonal changes in labile C availability and climate.

The rootless plots are significantly different than all other treatments at all three sampling dates (Figure 2.3), although the specific PLFAs and microbial groups that are different in the rootless plots changes throughout the year. The fungal:bacterial ratio is lower in November, implying a shift from fungal to bacterial dominance (Table 2.6). These changes are probably due to changes in the abundance of mycorrhizal fungi, as no changes are seen in either of the treatments without roots (data not shown). At the point of lowest fungal activity (Nov), there is no significant treatment effect on bacterial community structure. Soil water content can be ruled out as the cause of these treatment effects as there is no evidence of a difference in

moisture content of the sampled soils between the April and November sampling dates (ANOVA; $p = 0.77$), as well as no treatment effect on moisture content on either of these dates (Table 2.3). Thus, it appears that root inputs are driving bacterial community structure in the mineral soil in this ecosystem.

Conclusions

In the mineral soil of three distinct forest ecosystems root inputs were found to control soil microbial community structure. Changes were found in the bacterial community, especially actinomycetes, after accounting for the loss of mycorrhizal fungi following root exclusion. Additionally, seasonal differences in the PLFA profiles at HJA were greater than any of the treatment differences, with the taxonomic biomarkers responsible for treatment differences varying by season. This underscores the importance of seasonal sampling in any study looking at changes in microbial community composition in response to field experiments.

These data provide evidence that root C inputs exert a large control on soil microbial community composition in forest soils. The lack of an effect of aboveground input manipulation was surprising, especially after 13 years of manipulation at BOU. Continued monitoring of these permanent plots could further our understanding of the litter controls on soil microbial community composition. Future research is needed into the mechanisms of these controls, as well determining if compositional changes in soil microbial community structure lead to changes in ecosystem function and C utilization rates.

References

Bligh, E.G., and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911-917.

Blume, E., M. Bischoff, J.M. Reichert, T. Moorman, A. Konopka, and R.F. Turco. 2002. Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. Applied Soil Ecology 20:171-181.

Bohlen, P.J., P.M. Groffman, C.T. Driscoll, T.J. Fahey, and T.G. Siccama. 2001. Plant-soil-microbial interactions in a northern hardwood forest. Ecology 82:965-978.

Boone, R.D., K.J. Nadelhoffer, J.D. Canary, and J. Kaye. 1998. Roots exert a strong influence on the temperature sensitivity of soil respiration. Nature 396:570-572.

Bossio, D.A., and K.M. Scow. 1998. Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patters. Microbial Ecology 35:265-278.

Bossio, D.A., K.M. Scow, N. Gunapala, and K.J. Graham. 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microbial Ecology 36:1-12.

Bowden, R.D., K.J. Nadelhoffer, R.D. Boone, J.M. Melillo, and J.B. Garrison. 1993. Contributions of aboveground litter, belowground litter, and root respiration to total soil respiration in a temperature mixed hardwood forest. Canadian Journal of Forest Research 23:1402-1407.

Brant, J.B., E.W. Sulzman, and D.D. Myrold. 2004. Root influences on microbial community composition in forest soils Ecology Society of America, Portland, OR.

Butler, J.L., P.J. Bottomley, S.M. Griffith, and D.D. Myrold. 2004. Distribution and turnover of recently fixed photosynthate in ryegrass rhizospheres. Soil Biology & Biochemistry 36:371-382.

Dixon, J.J. 2003. Applying GIS to soil-geomorphic landscape mapping in the Lookout Creek valley, Western Cascades, Oregon. M.S. Thesis, Oregon State University, Corvallis, OR.

Dufrene, M., and P. Legendre. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67:345-366.

Entry, J.A., P.K. Donnelly, and K. Cromack. 1991. Influence of Ectomycorrhizal Mat Soils on Lignin and Cellulose Degradation. Biology and Fertility of Soils 11:75-78.

Fierer, N., J.P. Schimel, and P.A. Holden. 2003. Variation in microbial community composition through two soil depth profiles. Soil Biology & Biochemistry 35:167-176.

Fisk, M.C., and T.J. Fahey. 2001. Microbial biomass and nitrogen cycling responses to fertilization and litter removal in young northern hardwood forests. Biogeochemistry 53:201-223.

Fritze, H., J. Pietikainen, and T. Pennanen. 2000. Distribution of microbial biomass and phospholipid fatty acids in Podzol profiles under coniferous forest. European Journal of Soil Science 51:565-573.

Griffiths, B.S., K. Ritz, N. Ebblewhite, and G. Dobson. 1999. Soil microbial community structure: Effects of substrate loading rates. Soil Biology & Biochemistry 31:145-153.

Hill, G.T., N.A. Mitkowski, L. Aldrich-Wolfe, L.R. Emele, D.D. Jurkonie, A. Ficke, S. Maldonado-Ramirez, S.T. Lynch, and E.B. Nelson. 2000. Methods for assessing the composition and diversity of soil microbial communities. Applied Soil Ecology 15:25-36.

HJA LTER. 2005. Primet soil temperature and moisture data set. Available on-line [http://www.fsl.orst.edu/lter/data.cfm?topnav=8] from the Forest Science Data Base, a partnership between the Department of Forest Science, Oregon State University, and the U.S. Forest Service Pacific Northwest Research Station. Corvallis, OR.

Holland, E.A., B.H. Braswell, J.M. Sulzman, and J.-F. Lamarque. 2004. Nitrogen deposition onto the United States and Western Europe. Data set. Available on-line [http://www.daac.ornl.gov] from Oak Ridge National Laboratory Distributed Active Archive Center. Oak Ridge, TN.

Keirstead, H. 2004. Quantifying C and N contents and isotopic signatures of SOM pools in the H.J. Andrews DIRT plots. M.S. Thesis, Oregon State University, Corvallis, OR.

Kieft, T.L., E. Wilch, K. Oconnor, D.B. Ringelberg, and D.C. White. 1997. Survival and phospholipid fatty acid profiles of surface and subsurface bacteria in natural sediment microcosms. Applied and Environmental Microbiology 63:1531-1542.

Kruskal, J.B. 1964. Nonmetric multidimensional scaling: a numerical method. Psychometrika 29:115-129.

Lajtha, K., S. Crow, Y. Yano, S.S. Kaushal, E.W. Sulzman, and J.D.H. Spears. 2005. Detrital controls on soil solution N and dissolved organic matter in soils: a field experiment. Biogeochemistry (In review).

Langley, J.A., and B.A. Hungate. 2003. Mycorrhizal controls on belowground litter quality. Ecology 84:2302-2312.

Lavigne, M.B., R.J. Foster, and G. Goodine. 2004. Seasonal and annual changes in soil respiration in relation to soil temperature, water potential and trenching. Tree Physiology 24:415-424.

Li, Y.Q., M. Xu, O.J. Sun, and W.C. Cui. 2004. Effects of root and litter exclusion on soil CO_2 efflux and microbial biomass in wet tropical forests. Soil Biology & Biochemistry 36:2111-2114.

Litton, C.M., M.G. Ryan, D.H. Knight, and P.D. Stahl. 2003. Soil-surface carbon dioxide efflux and microbial biomass in relation to tree density 13 years after a stand replacing fire in a lodgepole pine ecosystem. Global Change Biology 9:680-696.

Lundquist, E.J., K.M. Scow, L.E. Jackson, S.L. Uesugi, and C.R. Johnson. 1999. Rapid response of soil microbial communities from conventional low input, and organic farming systems to a wet/dry cycle. Soil Biology & Biochemistry 31:1661-1675.

Macdonald, L.M., E. Paterson, L.A. Dawson, and A.J.S. McDonald. 2004. Short-term effects of defoliation on the soil microbial community associated with two contrasting Lolium perenne cultivars. Soil Biology & Biochemistry 36:489-498.

Mather, P.M. 1976. Computation methods of multivariate analysis in physical geography J. Wiley and Sons, London.

McCarthy, A.J., and S.T. Williams. 1992. Actinomycetes as agents of biodegradation in the environment - a review. Gene 115:189-192.

McCune, B., and M.J. Mefford. 1999. Multivariate analysis on the PC-ORD system. Version 4 MjM Software, Gleneden Beach, Oregon.

McCune, B., and J.B. Grace. 2002. Analysis of Ecological Communities MjM Software Design, Gleneden Beach, Oregon.

Mielke Jr., P.W. 1984. Meteorological applications of permutation techniques based on distance functions, *In* P. R. Krishnaiah and P. K. Sen, eds. Handbook of Statistics, Vol. 4. Elsevier Science Publishers.

Mielke Jr., P.W., and K.J. Berry. 2001. Permutation Methods: A Distance Function Approach.

Myers, R.T., D.R. Zak, D.C. White, and A. Peacock. 2001. Landscape-level Patterns of microbial community composition and substrate use in upland forest ecosystems. Soil Science Society of America Journal 65:359-367.

Nadelhoffer, K.J., R.D. Boone, R.D. Bowden, J.D. Canary, J. Kaye, P. Micks, A. Ricca, J.A. Aitkenhead, K. Lajtha, and W.H. McDowell. 2004. The DIRT Experiment: Litter and Root Influences on Forest Soil Organic Matter Stocks and Function, *In* D. Foster and J. Aber, eds. Forest Landscape Dynamics in New England: Ecosystem Structure and Function as a Consequence of 5000 years of Change. Oxford University Press, New York.

Olsson, P.A., and A. Johansen. 2000. Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. Mycological Research 104:429-434.

Rey, A., E. Pegoraro, V. Tedeschi, I. De Parri, P.G. Jarvis, and R. Valentini. 2002. Annual variation in soil respiration and its components in a coppice oak forest in Central Italy. Global Change Biology 8:851-866.

Schimel, D.S. 1995. Terrestrial ecosystems and the carbon cycle. Global Change Biology 1:77-91.

Schimel, J.P., J.M. Gulledge, J.S. Clein-Curley, J.E. Lindstrom, and J.F. Baddock. 1999. Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. Soil Biology & Biochemistry 31:831-838.

Siira-Pietikainen, A., J. Haimi, and H. Fritze. 2003. Organisms, decomposition, and growth of pine seedlings in boreal forest soil affected by sod cutting and trenching. Biology and Fertility of Soils 37:163-174.

Siira-Pietikainen, A., J. Haimi, A. Kanninen, J. Pietikainen, and H. Fritze. 2001. Responses of decomposer community to root-isolation and addition of slash. Soil Biology & Biochemistry 33:1993-2004.

Simard, S.W., D.A. Perry, J.E. Smith, and R. Molina. 1997. Effects of soil trenching on occurrence of ectomycorrhizas on Pseudotsuga menziesii seedlings grown in mature forests of Betula papyrifera and Pseudotsuga menziesii. New Phytologist 136:327-340.

Smith, J.L., and E.A. Paul. 1990. The significance of soil microbial biomass estimations, p. 357-393, *In* J. Bollag and G. Stotsky, eds. Soil Biochemistry, Vol. 8. Mercel Dekker, New York, New York.

Spears, J.D.H., S.M. Holub, M.E. Harmon, and K. Lajtha. 2003. The influence of decomposing logs on soil biology and nutrient cycling in an old-growth mixed coniferous forest in Oregon, USA. Canadian Journal of Forest Research 33:2193-2201.

Subke, J.A., V. Hahn, G. Battipaglia, S. Linder, N. Buchmann, and M.F. Cotrufo. 2004. Feedback interactions between needle litter decomposition and rhizosphere activity. Oecologia 139:551-559.

Sulzman, E.W., J.B. Brant, R.D. Bowden, and K. Lajtha. 2005. Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO_2 efflux in an old growth coniferous forest. Biogeochemistry (In press).

Tingey, D.T., D.L. Phillips, M.G. Johnson, P.T. Rygiewicz, P.A. Beedlow, and W.E. Hogsett. 2005. Estimates of Douglas-fir fine root production and mortality from minirhizotrons. Forest Ecology and Management 204:359-370.

Vanderbilt, K.L., K. Lajtha, and F.J. Swanson. 2003. Biogeochemistry of unpolluted forest watersheds in the Oregon Cascades: temporal patterns of precipitation and stream nitrogen fluxes. Biogeochemistry 62:87-117.

Wallander, H., L.-O. Nilsson, D. Hagerberg, and E. Bååth. 2001. Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. New Phytologist 151:753-760.

Waring, R.H., and J.F. Franklin. 1979. Evergreen coniferous forests of the Pacific Northwest. Science 204:1380-1386.

White, D.C., and D.B. Ringelberg. 1998. Signature lipid biomarker analysis, p. 255-272, *In* R. S. Burlage, et al., eds. Techniques in Microbial Ecology. Oxford University Press, New York, NY.

Wiemken, V., E. Laczko, K. Ineichen, and T. Boller. 2001. Effects of elevated carbon dioxide and nitrogen fertilization on mycorrhizal fine roots and the soil microbial community in beech-spruce ecosystems on siliceous and calcareous soil. Microbial Ecology 42:126-135.

Wilkinson, S.C., J.M. Anderson, S.P. Scardelis, M. Tisiafouli, A. Taylor, and V. Wolter. 2002. PLFA profiles of microbial communities in decomposing conifer litters subject to moisture stress. Soil Biology & Biochemistry 34:189-200.

Yano, Y., K. Lajtha, P. Sollins, and B.A. Caldwell. 2005. Chemistry and dynamics of dissolved organic matter in a temperate coniferous forest on andic soils: effects of litter quality. Ecosystems (In Press).

Zak, D.R., K.S. Pregitzer, P.S. Curtis, and W.E. Holmes. 2000. Atmospheric CO_2 and the composition and function of soil microbial communities. Ecological Applications 10:47-59.

Chapter 3:
Microbial Community Utilization of Added Carbon Substrates in Response to Long-Term Carbon Input Manipulation

Justin B. Brant

Abstract

The chemical composition and quantity of plant inputs to soil are primary factors controlling the size and structure of the soil microbial community. Little is known about how changes in the composition of the soil microbial community can affect decomposition rates and other ecosystem functions. This study examined the degradation of universally ^{13}C-labeled glucose, glutamate, oxalate, and phenol in soil from an old-growth Douglas-fir (*Pseudotsuga menziesii*) - western hemlock (*Tsuga heterophylla*) forest in the Oregon Cascades that has experienced seven years of chronic C input manipulation. The soils used in this experiment were part of a larger Detritus Input and Removal Treatment (DIRT) experiment and have received normal C inputs, doubled wood inputs, or root and litter input exclusion (no inputs). The doubled wood soil had a higher fungal:bacterial ratio, while the no inputs soil had a lower fungal:bacterial ratio, than the control soil. Differences in the utilization of the added compounds were assessed by following the ^{13}C tracer into microbial respiration and microbial biomass. In addition, ^{13}C-phospholipid fatty-acid (PLFA) analysis was used to examine differential microbial utilization of the substrates. Utilization of the glucose and glutamate were similar in all three soils. The microbial community in the double wood soil respired more added phenol and oxalate, while microbes in the no inputs soil respired less added phenol and oxalate, than the control soil. Phenol was primarily degraded by fungi, with more incorporation into fungal PLFAs in the double wood soil. There was also significantly more incorporation of phenol into microbial and fungal biomass in the double wood soil. The addition of all four substrates lead to the enhanced degradation of soil organic matter (priming) in all three soils, and was greater following the addition of more complex compounds (phenol and oxalate) and in the no inputs soil. These results demonstrate that altering plant inputs to soil can lead to changes in microbial utilization of more complex C compounds. In appears that many of these changes are the result of alteration in the size and composition of the fungal community.

Introduction

The size and composition of the soil microbial community is a function of net primary production, plant carbon (C) allocation, rhizosphere activity, and litter substrate quality (Fisk and Fahey, 2001; Myers et al., 2001; Smith and Paul, 1990), and is controlled through complex interactions with plants (Bohlen et al., 2001; Butler et al., 2004; Zak et al., 2000). Changes in atmospheric CO_2 concentration and nitrogen (N) deposition rates alter both the quality and quantity of above- and belowground plant litter inputs to soil (Aber et al., 1993; Canadell et al., 1996; Mooney et al., 1999), which in turn can affect belowground microbial community structure and function (Frey et al., 2004; Phillips et al., 2002; Waldrop et al., 2004). Understanding the mechanisms controlling belowground C processes is useful in predicting future changes in soil C stores in response to climate and land-use change (Pendall et al., 2004).

Altering root and coarse woody debris (CWD) inputs to soil is one method to examine the feedbacks between plants, microbes, and soil organic matter (SOM) (Nadelhoffer et al., 2004). In a Douglas-fir forest seven years of CWD additions and litter and root exclusion have produced significant changes in annual soil CO_2 efflux (Sulzman et al., 2005). Similar results have been reported for a deciduous forest (Bowden et al., 1993). In contrast, in a year-long incubation of mineral soil, doubling CWD inputs did not affect respiration rates in either bulk soil, light fraction, or heavy fraction, while litter and root exclusion decreased cumulative C loss from all three fractions (Crow and Lajtha, 2004). Soil solution chemistry is also altered by changes in detrital inputs. Lajtha et al. (2005) showed that seven y of CWD addition caused an increase in dissolved organic matter (DOM) fluxes from the organic layer to the mineral soil. These differences remained at 30 cm, but had disappeared by 100 cm (Lajtha et al., 2005). Although the quantity of DOM entering the mineral soil is higher following the addition of CWD, the chemistry of the soil solution leaving the organic horizon is the same, suggesting microbial degradation of labile components of CWD-C before soil solution enters the mineral soil (Lajtha et al., 2005; Yano et al.,

2005). Neither the addition of CWD nor root and litter exclusion altered the size of the microbial community in the mineral soil (Chapter 2; Spears et al., 2003). Root and litter exclusion did alter the microbial community composition, leading to a loss in fungal biomass and a change in the soil bacterial community structure (Chapter 2).

The activity of the soil microbial community, as defined by soil respiration, can be effectively modeled using soil temperature and moisture without taking the structure of the soil microbial community into account (Andren et al., 1999). However, the rate of litter decomposition is likely to respond to changes in microbial community composition at smaller scales (Schimel, 1995). Litter is composed of a complex mixture of compounds including recalcitrant polymeric compounds such as lignin and cellulose that are broken down into smaller units by exoenzymes. Only a relatively small group of microorganisms, such as fungi and actinomycetes, produce these enzymes. Fungi are especially important in litter decomposition as they are responsible for nearly all of the extracellular enzyme production necessary to degrade recalcitrant SOM such as lignin-derived compounds (Wagner and Wolf, 1999). Changes in the composition of the ligninolytic portion of the community are likely to have an effect on overall community function (Schimel and Gulledge, 1998). However, structurally distinct microbial communities can have virtually identical C utilization patterns (Buyer and Drinkwater, 1997; Waldrop et al., 2000) as a result of the functional diversity of soil microorganisms and the functionally redundancy of soil ecosystems (Ekschmitt and Griffiths, 1998).

Some recently developed techniques allow examination of the linkages between soil microbial community structure and function. One technique involves the addition of ^{13}C-labeled substrates that resemble different components of plant inputs such as simple sugars, cellobiose, and phenolic compounds. By adding these compounds to soils in the laboratory, and tracking their incorporation into microbial phospholipid fatty acids (PLFA), it is possible to visualize how distinct microbial communities process different C compounds (DeForest et al., 2004; Phillips et al., 2002; Waldrop and Firestone, 2004; Waldrop et al., 2004). In addition, by looking at

the incorporation of ^{13}C into lipid biomarkers it is possible to determine which parts of the microbial community are involved in the decomposition of specific organic substrates.

The addition of both simple and complex organic substrates to soil has been shown to result in a short-term change in the turnover of native SOM, termed the 'priming effect' (Kuzyakov et al., 2000). Additions can lead to either an increase (positive priming) or a decrease (negative priming) in SOM decomposition compared to unamended soil. The priming effect has long been observed, though little is known about its underlying mechanisms. A recent review found a larger priming effect in nutrient poor soils as well as following the addition of more complex substrates (Fontaine et al., 2003). Positive priming of SOM has been shown to occur in laboratory incubations with the addition of various simple organic compounds, plant litter, and root rhizodeposition in a range of soils (Hamer and Marschner, 2002, 2005; Kuzyakov and Cheng, 2001; Waldrop and Firestone, 2004). One recent study has also shown priming as a result of 7 y of litter addition to forest soils at a field scale (Sulzman et al., 2005).

The objective of this study was to determine whether 7 y of CWD addition and root and litter exclusion has altered the ability of the soil microbial community in an old-growth Douglas-fir forest to mineralize a variety of ^{13}C-labeled substrates (glucose, glutamate, oxalate, and phenol). The added substrates are products of plant litter degradation, components of root exudates, or produced by fungi. CWD additions have to lead to an increase in the size of the fungal community, while root exclusion has decreased the size of the fungal community. It was hypothesized that these changes in fungal:bacterial ratio would, in turn, alter the utilization of more complex C compounds such as phenol, while the organisms in all litter treatments would be able utilize simple compounds, such as glucose and glutamate in a similar manner. It was also hypothesized that the addition of C compounds would lead to a positive priming effect. The priming effect was expected to be larger in the no inputs soil as a result of lower quality SOM. Differences in microbial utilization of C compounds were

assessed by following the ^{13}C tracer into microbial biomass and microbial respiration, as well examining differences in growth yield efficiency. In addition, microbial groups involved in the degradation of these compounds were identified by following the ^{13}C-label into microbial PLFAs.

Materials and Methods

Study Site

Plant litter inputs have been manipulated at the Detritus Input and Removal Treatment (DIRT) plots in the H.J. Andrews Experimental Forest (HJA) in Oregon (44°15'N, 122°10'W, 531 m elevation) since 1997. Mean annual temperature at the headquarters site of HJA is 8.7 °C (1973-2002) and mean annual precipitation over the same period is 2370 mm y^{-1}, mostly as rain. In general, over 70 % of the precipitation occurs during a "wet season", between November and March. Nitrogen deposition to this area is ~1.6 kg N $ha^{-1}y^{-1}$ (Vanderbilt et al., 2003). The DIRT site was established in an undisturbed old-growth Douglas-fir (*Pseudotsuga menziesii*) - western hemlock (*Tsuga heterophylla*) stand. Other important tree species at the site include western red cedar (*Thuja plicata*) and vine maple (*Acer circinatum*). Soils are derived from volcanic parent materials and have been classified as coarse loamy mixed mesic Typic Hapludands (Dixon, 2003).

In 1997 the three litter manipulation treatments used in this study were established as part of a larger inter-site comparison looking at plant controls on SOM formation and transformation over decadal time-scales (Nadelhoffer et al., 2004). The plots at this site are 10 m x 15 m, with three replicates of each treatment. To double the mass of woody debris in the forest floor of Double Wood (DW) plots, a mix of extremely decomposed woody debris and the chips of large pieces of intact Douglas-fir, with a ratio of decomposed woody debris to intact woody debris of 4:1, are added every other year. The rate of addition is 576 g C m^{-2} y^{-1}. Above- and belowground litter inputs are excluded in the No Inputs (NI) plots. Belowground inputs are excluded by trenching the perimeter to 1 m depth, inserting a 10 mil (0.025 cm) thick

polyethylene sheet along the bottom and sides of the trench, then back-filling the trenches. Aboveground litter inputs are excluded with 1-mm mesh screens. The Control (CO) plots have received normal plant inputs.

Soil Incubations

Zero to 10 cm soil cores were collected from the three replicates of each treatment in September 2003. The soil cores were then composited by treatment to yield one sample for each treatment. The soils were brought back to the laboratory, sieved to 2 mm and stored at 4°C until use. Before the incubations began the soils were brought up to 50% (-33 kPa) gravimetric water content (GWC) and pre-incubated at 22°C for 5 d.

One of four substrates was added to twelve replicates of each of the three soils, for a total of 48 samples. Fifty μg C g^{-1} soil (approx. 10% of the microbial biomass C) of universally ^{13}C-labeled glucose, glutamate, oxalate, and phenol were added to each 25 g sample of soil in 2 mL of deionized H_2O. Ninety-nine atom% ^{13}C substrates were diluted with unlabeled compounds, such that 22 atom% ^{13}C compounds were added to soils. Two mL of deionized H_2O was added to a second set of samples as a control. The soils were incubated at 22°C for 14 d in 0.5-L mason jars covered with Parafilm (Pechiney Plastic Packaging, Chicago, IL) to prevent drying. Respiration was measured every 6 h for the first 3 d, and then 5, 7, 10, and 14 d after substrate addition. During the first 3 d of the incubation, jars were capped for 6 h with airtight lids fitted with a septum; for all subsequent samplings, the jars were capped for approximately 24 h prior to gas sampling. Respiration was measured on three randomly selected replicates of each treatment, as well as the unamended control. Two gas samples were collected from each replicate using airtight plastic syringes (Becton-Dickinson, Franklin Lakes, NJ), and injected into pre-evacuated 10-mL and 3-mL Vacutainers (Becton-Dickinson, Franklin Lakes, NJ). The 3-mL sample was analyzed for total CO_2 concentration by gas chromatography (Carle Series 100 Analytical GC, Loveland, CO), and the 10-mL sample was analyzed for $\delta^{13}C$ using a

PDZ Europa 20/20 isotope ratio mass spectrometer (IRMS; Cheshire, UK). Three randomly selected replicates of each treatment were destructively sampled at 2, 4, 8, and 14 d for microbial biomass carbon (MBC) and PLFA analysis.

Soil respiration

The percent CO_2-C coming from the added substrate was calculated as in Waldrop and Firestone (2004):

$$\%C_{sub} = \left[\frac{(\delta_C - \delta_T)}{(\delta_C - \delta_S)}\right] \times 100 \quad (1)$$

where δ_C is the $\delta^{13}C$ value of the respired CO_2 from the control (no added substrate) soils, δ_T is the $\delta^{13}C$ respired CO_2 in the treated soils, and δ_S is the $\delta^{13}C$ of the label substrate. The increase in SOM-C utilization following substrate addition (priming) was calculated as the increase in total soil respiration following substrate addition minus the amount of C respired from the added substrate; this was expressed as a percent where 100% is represents a doubling of SOM-C respiration.

^{13}C-CO_2 evolution kinetics were modeled with single- and double-exponential models using experimental curve-fitting software (SigmaPlot, Systat Software Inc., Point Richmond, CA). Equations for the two models were as follows:

Single Exponential Model: $CO_2 - C = A_1\left(1 - e^{-k_1 t}\right)$ (2)

Double Exponential Model: $CO_2 - C = A_1\left(1 - e^{-k_1 t}\right) + A_2\left(1 - e^{-k_2 t}\right)$ (3)

Microbial Biomass

Soil was analyzed for MBC immediately following sampling. The fumigation-extraction procedure of Vance et al. (1987) was used, as modified by Bruulsema and Duxbury (1996) for ^{13}C analysis. Briefly, a 10 g (dry wt.) sample of soil was placed on a shaker for 1 h with 30 mL of 0.05 M K_2SO_4 and subsequently filtered through Whatman #40 filter papers. Another sample was fumigated for 24 h with ethanol-free chloroform and extracted in the same way. Three aliquots (0.75 mL) of each K_2SO_4 extract were pipetted onto acetone-rinsed tin squares (37 mm x 37 mm)

(Environmental Microanalysis, Manchester, MA), and dried at 60°C for 2 h after the addition of each aliquot. The tin squares containing the dried extracts were balled up and analyzed for total C and $\delta^{13}C$ using a PDZ Europa 20/20 IRMS. A K_C of 0.41 was used to convert chloroform flush C values into MBC (Vance et al., 1987). The following equation was used to determine the $\delta^{13}C$ value of MBC:

$$\delta^{13}C_{MBC} = \frac{\left(\delta^{13}C_{fum} \times C_{fum} - \delta^{13}C_{unfum} \times C_{unfum}\right)}{C_{fum} - C_{unfum}} \quad (4)$$

where C_{fum} and C_{unfum} refer to the mass of C extracted from the fumigated and the non-fumigated samples, respectively, and $\delta^{13}C_{fum}$ and $\delta^{13}C_{unfum}$ refer to their corresponding $\delta^{13}C$ values. The unfumigated samples were also used to look at the quantity and $\delta^{13}C$ of dissolved organic carbon (DOC). The percentage of MBC and DOC from the added substrate was calculated using equation (1), with the exception that the $\delta^{13}C$ of unamended SOM was assumed to be the $\delta^{13}C$ of preincubation SOM.

Microbial growth yield efficiency (GYE) is the fraction of substrate C assimilated by the microbial community that is incorporated into MBC versus being respired as a byproduct of energy production. GYE was calculated using one of the four techniques outlined by Frey et al. (2001):

$$GYE = \frac{dB_C}{\left(dB_C + \sum CO_2 - C\right)} \quad (5)$$

where dB_C is the amount of ^{13}C-substrate incorporated into MBC and ΣCO_2-C is the cumulative substrate lost during respiration. This method assumes that the sum of biomass C and cumulative substrate respired equals the total amount of substrate utilized. This method does not account for metabolite production or for biomass that is consumed by grazers (Frey et al., 2001).

PLFA Analysis

PLFAs were extracted immediately after sampling from 2 g (dry wt.) of soil using the modified Bligh and Dyer method (Bligh and Dyer, 1959; White and Ringelberg, 1998), in which soils were incubated in a 2:1:0.8 solution of methanol,

chloroform, and phosphate buffer. The soil was then filtered and the chloroform phase separated. Phospholipids were separated from glycolipids and neutral lipids using 3 mL Supelclean LC-Si solid phase extraction columns (Supelco Park, Bellefonte, PA). Phospholipids were then saponified and methylated to fatty-acid methyl esters (FAME). FAMEs were then run twice, first on an Agilent 6890 gas chromatograph (GC) equipped with a flame ionization detector (FID) and an HP Ultra-2 column for total PLFA community identification, and next on an Agilent 6890 GC equipped with an HP Innowax-2 column connected to a Europa ORCHID on-line combustion interface attached to a Europa 20/20 IRMS to measure the $\delta^{13}C$ of PLFAs. Peaks were identified based on comparing retention times with known standards. Concentration of each PLFA was obtained by comparing peak areas with a 13:0 FAME standard curve. The $\delta^{13}C$ of each individual lipid was corrected for the methyl group added during transesterification (Abraham et al., 1998).

Specific PLFA markers were used to quantify the relative abundance and $\delta^{13}C$ (weighted average) of various taxonomic groups. Gram-positive bacteria were identified by i15:0, a15:0, i16:0, i17:0, and a17:0; gram-negative bacteria were identified by cy17:0, cy19:0, and 18:1ω7c. Other taxonomic groups identified include fungi (18:2ω6c) and actinomycetes (10Me 16:0). The total amount of isotope incorporated into fungal PLFAs was divided by the amount of ^{13}C in bacterial PLFAs to produce a fungal:bacterial incorporation ratio (Waldrop et al., 2004). The relative substrate incorporation was calculated by multiplying the mol% of each PLFA taxonomic biomarker by the percent of each taxonomic group from the added substrate using equation (1).

Statistical Analysis

PLFA relative abundances (mol %) for all 40 PLFAs identified were used to examine community differences among treatments. Microbial community structure was examined by non-metric multidimensional scaling (NMS: Kruskal, 1964; Mather, 1976) using the PC-ORD (MjM software, Gleneden Beach, OR) software package

(McCune and Mefford, 1999). The medium setting of 'autopilot' mode in PC-ORD was used, which attempts to find a stable solution from a random starting position (McCune and Mefford, 1999). This method uses a maximum of 200 iterations in 15 runs with real data. The final dimensionality is then determined by comparing the final stress values among the best solution for each axis. Significance of axes is assessed by comparing observed final stress with the final stress in 30 runs of randomized data using a Monte Carlo test (McCune and Grace, 2002). Final ordinations were rotated to maximize environmental variables represented and separation of treatments on axes 1 and 2.

Statistical differences between whole PLFA profiles was assessed using multi-response permutation procedures (MRPP: Mielke Jr., 1984; Mielke Jr. and Berry, 2001) in PC-ORD. MRPP is a nonparametric procedure similar to MANOVA for testing the hypothesis of no difference between two or more preexisting groups (McCune and Grace, 2002). MRPP yields a p-value to evaluate how likely it is that an observed difference is due to chance, as well as a chance-corrected within-group agreement (A) which describes within-group homogeneity compared to random expectation (McCune and Grace, 2002).

The statistical significance of the effects of substrate and sampling day on PLFA taxonomic groups in each soil, as well as their interaction, was analyzed with repeated measures analysis of variance (ANOVA) using PROC MIXED (SAS version 8.1, SAS Institute, Inc). In instances where the interaction between treatment and day was not significant ($p > 0.05$), the interaction was removed and the analysis was repeated using data from all four sampling points. When ANOVA resulted in a p-value < 0.05, pre-planned comparisons between the four substrates were made using orthogonal contrasts using the Tukey-Kramer procedure to correct for family-wise error rates. Differences between the three soils over time following substrate addition were analyzed using the same method. When only day 2 data were examined, one-way ANOVA was used, using the Tukey-Kramer procedure to correct for family-wise error rates (Splus version 6.1, Insightful Corp.).

Results

Microbial Community Composition

None of the substrates affected MBC in any of the soils over the whole incubation (ANOVA; $p > 0.05$), although the NI soil had lower MBC than the CO and DW soils (Table 3.1). At all four sampling points the three soils had significantly different PLFA profiles (data not shown: MRPP; $p < 0.05$). The added substrates had no effect on the PLFA profile in any of the three different soils (MRPP; $p > 0.05$), except for the DW (MRPP; $p = 0.02$, $A = 0.20$) soil on day 2 and both the CO (MRPP; $p = 0.01$, $A = 0.27$) and DW (MRPP; $p < 0.001$, $A = 0.26$) soils on day 14 of the incubation. These changes were slight and there were no significant effects of the substrates on the relative abundance of any of the taxonomic markers over the course of the incubation (data not shown; ANOVA, $p > 0.05$). Figure 3.1 is an NMS showing the microbial community differences among the soils with data from all four substrates after 2 d, as it is representative of soil differences throughout the incubation. The three soils are distinct along Axis 2. The three soils differ in the relative abundance of fungi, with the DW soil having the largest and the NI soil the smallest population (Table 3.1). The same trend is also seen in the fungal:bacterial ratio between soils. The DW soil has the lowest abundance of the three different bacterial taxonomic markers compared with the other two soils (Table 3.1).

As there was no significant effect of any of the substrates on the MBC or the relative abundance of any of the taxonomic markers, the data from all four substrates was combined to look at changes in the microbial community over the 14-day incubation (Table 3.1). In the DW treatment, the relative abundance of fungi and gram-negative bacteria generally decreased throughout the incubation, while the relative abundance of gram-positive bacteria increased. The fungal:bacterial ratio also decreased over time. The relative abundance of actinomycetes fluctuated during the incubation with no particular pattern and the MBC stayed relatively constant over the incubation, although there was some decline in MBC between 8 and 14 d. Similarly, the MBC in the CO soil was inconsistent during the incubation, being high at 2 and 8

d, and low at 4 and 14 d (Table 3.1). Changes in microbial community structure followed a similar pattern to those in the DW soil, although there was less of a decline in the relative abundance of fungi and a larger increase in gram-positive bacteria (Table 3.1). In the NI soil, MBC as well as the relative abundance of fungi remained constant over the experiment. Similar trends were found in the gram-negative and gram-positive abundances as seen in the DW and CO soils, however the changes over time in gram-negative bacteria were not significant.

Table 3.1. Soil differences in summed relative abundance of taxonomic biomarkers and MBC over the 14-d incubation. Numbers show mean (n=12) with standard error in parentheses. Different letters indicate a significant difference (ANOVA; $p < 0.05$) in the relative abundance of a particular biomarker on one of the four sampling dates.

	Day			
	2	4	8	14
Double Wood				
Fungal (mol %)	5.15 (0.21)^{a}	4.93 (0.25)^{a}	4.51 (0.09)^{a}	4.09 (0.13)^{b}
Gram-negative (mol %)	21.41 (0.38)^{ab}	21.8 (0.25)^{a}	20.61 (0.18)^{b}	20.28 (0.27)^{b}
Gram-positive (mol %)	12.75 (1.02)^{a}	13.06 (0.86)^{a}	16.05 (0.43)^{b}	15.80 (0.68)^{b}
Actinomycetes (mol %)	6.59 (0.21)^{ab}	7.03 (0.18)^{ab}	6.59 (0.05)^{a}	7.03 (0.11)^{b}
Fungal:Bacterial ratio	0.106 (0.005)^{ab}	0.100 (0.006)^{a}	0.090 (0.002)^{a}	0.082 (0.002)^{b}
MBC (μg C g^{-1} soil)	329.1 (16.6)^{ab}	329.5 (24.2)^{ab}	374.1 (11.1)^{a}	279.6 (11.4)^{b}
Control				
Fungal (mol %)	3.68 (0.12)^{ab}	4.01 (0.20)^{a}	3.67 (0.10)^{b}	3.28 (0.14)^{b}
Gram-negative (mol %)	23.23 (0.48)^{a}	23.03 (0.09)^{a}	22.8 (0.37)^{ab}	21.86 (0.18)^{b}
Gram-positive (mol %)	15.49 (1.14)^{ab}	15.18 (0.40)^{a}	15.86 (0.67)^{a}	17.5 (0.46)^{b}
Actinomycetes (mol %)	8.12 (0.12)	7.95 (0.13)	8.19 (0.10)	8.3 (0.17)
Fungal:Bacterial ratio	0.071 (0.002)^{ab}	0.078 (0.004)^{a}	0.070 (0.002)^{b}	0.063 (0.003)^{b}
MBC (μg C g^{-1} soil)	344.6 (7.8)^{ab}	296.8 (20.6)^{bc}	375.8 (13.0)^{a}	298.4 (12.9)^{c}
No Inputs				
Fungal (mol %)	1.9 (0.06)	1.98 (0.08)	1.99 (0.21)	1.76 (0.07)
Gram-negative (mol %)	24.22 (0.41)	23.58 (0.27)	22.56 (0.36)	22.76 (0.26)
Gram-positive (mol %)	13.38 (0.93)^{a}	15.19 (0.68)^{b}	16.41 (0.54)^{b}	15.60 (0.54)^{b}
Actinomycetes (mol %)	7.43 (0.15)	7.21 (0.11)	7.37 (0.10)	7.46 (0.17)
Fungal:Bacterial ratio	0.035 (0.001)	0.037 (0.001)	0.038 (0.004)	0.033 (0.001)
MBC (μg C g^{-1} soil)	216.7 (17.3)	228.9 (13.7)	253.6 (18.7)	218.9 (6.3)

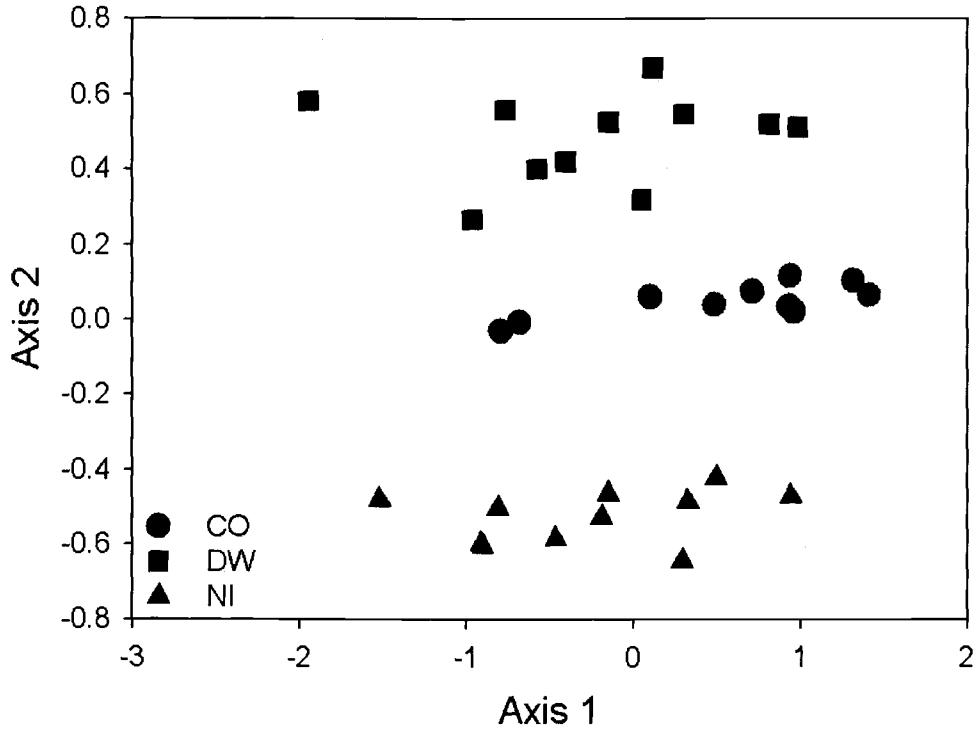


Figure 3.1. NMS of PLFA relative abundance on day 2 of the incubation (final stress = 2.56, instability = 0.00009). There was no difference in PLFA profiles by substrate, so all substrates are shown for each soil. The first 2 axes from a 3-D solution are shown. R^2 axis 1 = 0.80, axis 2 = 0.09, axis 3 = 0.05.

Substrate Utilization

The four substrates were respired by the microbial community at different rates (Figure 3.2). A large portion of the respiration of all substrates occurred in the first 2 d following substrate addition. The kinetics of substrate respiration was different for each of the four substrates (Table 3.2). Oxalate, phenol, and glutamate respiration were well explained using a single first-order exponential model. On the other hand, glucose respiration was poorly explained using a single first-order model ($R^2 < 0.70$) and a double exponential model was necessary to sufficiently explain the data (Table 3.2). In the glutamate, oxalate, and phenol addition at least 90% of the respired substrate was respired during the first 4 d of incubation (Figure 3.2). Contrary to the

hypothesis that simple substrates would be respired quickly, following glucose addition only 83% of the total substrate respired was respired after 4 d. ^{13}C-glucose continued to be respired at a lower rate throughout the incubation resulting in the need for a two-pool exponential model to explain the kinetics of glucose decomposition.

The amount of glucose respired differed among the three soils (Figure 3.2). During the 14-d incubation, more glucose was respired from the CO soil (17.5 μg g^{-1} soil) than either the DW (14.4 μg g^{-1} soil) or NI soil (12.6 μg g^{-1} soil). In all three soils most of the glucose was respired before 2 d. On day 2, the amount of substrate incorporated into MBC was very high in all three soils, although there was no difference among soils (ANOVA; $p > 0.05$, Table 3.3). The large quantity of substrate incorporated into MBC is shown by an especially high GYE during the first 2 d (Table 3.3). In all three soils, 70-80% of the substrate utilized by the microbial community was incorporated into cells. The specific substrate respiration rate (mg ^{13}C-CO_2 g^{-1} MBC h^{-1}) was fairly low for glucose given the high rate of substrate incorporation, with no differences among litter treatments (Table 3.3). The ratio of fungal:bacterial incorporation of ^{13}C-glucose into PLFAs was significantly higher in the DW soil than in either CO or NI soil (Table 3.3, ANOVA; $p < 0.05$). The quantity of ^{13}C-glucose recovered in the DOC pool after 2 d was significantly affected by the different litter treatments, with an increase in recovery in the DW (75%) and NI (113%) compared to the CO soil (ANOVA; $p < 0.05$).

Table 3.2. Coefficients from the single- and double-exponential models used to describe cumulative substrate respiration in Figure 3.2.

	Glucose			Glutamate			Oxalate			Phenol		
	DW	CO	NI	DW	CO	NI	DW	CO	NI	DW	CO	NI
A_1	8.508	12.00	8.302	19.27	19.37	17.55	31.56	29.15	26.70	7.191	6.111	4.583
k_1	5.216	4.696	4.042	1.507	1.712	1.045	0.997	0.668	0.475	0.759	0.792	0.486
A_2	6.281	5.995	4.646									
k_2	0.217	0.188	0.198									
R^2	0.999	0.999	0.999	0.957	0.949	0.972	0.961	0.951	0.943	0.931	0.942	0.954

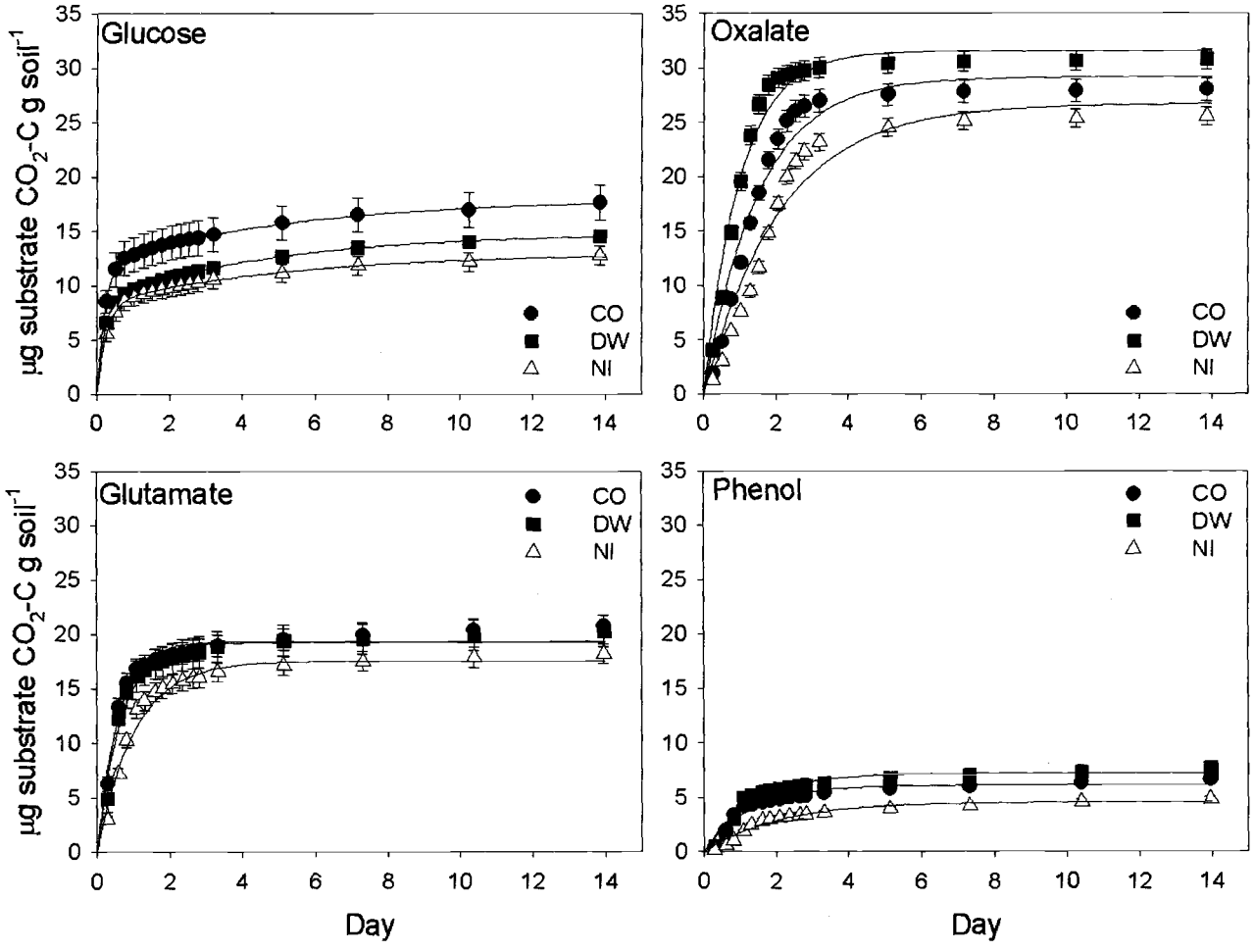


Figure 3.2. Cumulative substrate respiration for all four substrates. Each point represents the mean (n=3) amount of substrate respired at eh 16 sampling points during the incubation. Error bars are ± 1 S.E. of the mean.

The amount of ^{13}C-glutamate respired over the 14-d incubation was similar in all three soil treatments (Figure 3.2), with 17.5 μg ^{13}C g^{-1} soil respired from the NI soil compared to 20.8 μg ^{13}C g^{-1} soil from the CO soil and 20.4 μg ^{13}C g^{-1} soil from the DW soil. Approximately 90% of the glutamate respired was respired during the first 3 d of incubation in all three soils. The amount of ^{13}C-glutamate incorporated into MBC during the first 2 d was less than the amount of ^{13}C-glucose incorporated into MBC (Table 3.3). The CO (9.0 μg ^{13}C g^{-1} soil) and DW (8.6 μg ^{13}C g^{-1} soil) soils

Table 3.3. Incorporation of the four substrates into microbial pools and utilization parameters on day 2 of the incubation. Numbers are the mean (n=3) with standard errors in parentheses. Different letters denote a significant (ANOVA; $p < 0.05$) difference among the three soils within each substrate.

	DW	CO	NI
Glucose			
MBC from substrate (μg ^{13}C g^{-1} soil)	37.5 (7.6)	41.4 (2.9)	40.6 (1.1)
Substrate in DOC (μg ^{13}C g^{-1} soil)	2.86 (0.02)^a	1.63 (0.14)^b	3.48 (0.20)^a
Growth Yield Efficiency	0.78 (0.20)	0.79 (0.06)	0.83 (0.03)
Specific Substrate Respiration (mg ^{13}C-CO_2 g^{-1} MBC h^{-1})	0.130 (0.047)	0.089 (0.004)	0.110 (0.025)
Fungal:Bacterial ^{13}C Incorporation	0.35 (0.02)^a	0.24 (0.02)^b	0.22 (0.01)^b
Glutamate			
MBC from substrate (μg ^{13}C g^{-1} soil)	8.60 (0.80)^a	9.00 (0.16)^a	6.59 (0.26)^b
Substrate in DOC (μg ^{13}C g^{-1} soil)	0.56 (0.02)^a	0.38 (0.01)^b	0.52 (0.02)^a
Growth Yield Efficiency	0.33 (0.04)	0.33 (0.01)	0.30 (0.02)
Specific Substrate Respiration (mg ^{13}C-CO_2 g^{-1} MBC h^{-1})	0.125 (0.013)^a	0.110 (0.009)^a	0.235 (0.046)^b
Fungal:Bacterial ^{13}C Incorporation	0.26 (0.01)	0.25 (0.01)	0.23 (0.01)
Oxalate			
MBC from substrate (μg ^{13}C g^{-1} soil)	1.61 (0.21)^a	1.37 (0.28)^a	0.17 (0.09)^b
Substrate in DOC (μg ^{13}C g^{-1} soil)	0.41 (0.01)^a	0.48 (0.08)^a	2.36 (0.06)^b
Growth Yield Efficiency	0.055 (0.011)^a	0.053 (0.007)^a	0.010 (0.005)^b
Specific Substrate Respiration (mg ^{13}C-CO_2 g^{-1} MBC h^{-1})	0.328 (0.013)^a	0.990 (0.244)^b	2.15 (0.17)^c
Fungal:Bacterial ^{13}C Incorporation	0.20 (0.07)	0.24 (0.03)	0.18 (0.02)
Phenol			
MBC from substrate (μg ^{13}C g^{-1} soil)	4.71 (0.14)^a	3.29 (0.07)^b	2.07 (0.01)^c
Substrate in DOC (μg ^{13}C g^{-1} soil)	0.32 (0.01)^a	0.11 (0.01)^b	0.14 (0.01)^b
Growth Yield Efficiency	0.45 (0.03)	0.41 (0.01)	0.40 (0.02)
Specific Substrate Respiration (mg ^{13}C-CO_2 g^{-1} MBC h^{-1})	0.087 (0.010)^a	0.049 (0.008)^b	0.125 (0.003)^c
Fungal:Bacterial ^{13}C Incorporation	0.35 (0.01)^a	0.23 (0.01)^b	0.22 (0.02)^b

incorporated significantly more ^{13}C-glutamate into biomass than the NI soil (6.59 μg ^{13}C g^{-1} soil) (ANOVA; $p < 0.05$). Although less ^{13}C-glutamate was incorporated into biomass in the NI soils, there were soil differences in GYE. Similar to glucose, the amount of ^{13}C-glutamate recovered in DOC was significantly smaller in the CO soil than either NI or DW soil. Glutamate was incorporated into fungal and bacterial lipids at the same rate in all three soils. The specific substrate respiration rate for the added glutamate was significantly higher in the NI soil (ANOVA; $p < 0.05$), than in either the CO or DW soils.

More oxalate was recovered in microbial respiration than any of the other substrates (Figure 3.2). Oxalate also showed the largest differences in the amount of ^{13}C-substrate respired among the three soils. Microbes in the DW soil respired 31.9 μg ^{13}C g^{-1} soil, compared with 29.1 μg ^{13}C g^{-1} soil from the CO soil and 26.5 μg ^{13}C g^{-1} soil from the NI soil. Very little oxalate was incorporated into MBC (Table 3.3), and there was significantly less oxalate incorporated into MBC in the NI soil than in either the CO or DW soils (ANOVA; $p < 0.05$). The large difference in incorporation into biomass led to a significantly smaller GYE in NI soils compared with CO and DW. The amount of ^{13}C-oxalate recovered in DOC was significantly higher in the NI soils than in either the CO or DW soils (ANOVA; $p < 0.05$). The specific substrate respiration was highest in NI soil and lowest in DW soil at 2 d.

Less ^{13}C-phenol was respired over the14 d than the other three substrates (Figure 3.2). More ^{13}C was respired from the DW soil (7.7 μg ^{13}C g^{-1} soil), than either the CO (6.1 μg ^{13}C g^{-1} soil) or NI (4.5 μg ^{13}C g^{-1} soil) soil. Microbes in the DW soil respired more ^{13}C-phenol than either of the other treatments. There was significantly more ^{13}C incorporation into MBC of DW compared to the other treatments (ANOVA; $p < 0.05$) and more ^{13}C-phenol recovered in DOC of DW as compared to other treatments (Table 3.3). There was also a significantly higher ratio of ^{13}C incorporation into fungal lipids in the DW soils. Although the DW microbial community was able to incorporate more ^{13}C phenol into biomass there was no difference in GYE across litter treatments during the first 2 d of the incubation (Table 3.3). There was also a

significant difference in specific substrate respiration among all three soils on day 2 of the incubation (ANOVA; $p < 0.05$), with respiration highest in NI and lowest in CO soil.

Priming

The addition of all four substrates led to positive priming in all three soils (Figure 3.3). Cumulative priming is the total amount of excess SOM degraded over the whole incubation. Generally, cumulative priming was largest over the first 3 d and subsequently declined after that. In the glucose treatment, cumulative priming was largest in the CO soil over the first 3 d, with a maximum of 120%. After the first 3 d cumulative priming decreased exponentially in all soils; by the end of the incubation, priming in response to glucose addition was largest in the NI soil (18.6%), followed by CO (9.8%) and DW (9.8%). Similarly, following glutamate addition the priming effect was largest in the CO soil over the first 3 d. Cumulative priming decreased over the incubation, although the decrease was less rapid than in the glucose addition. By the end of the experiment, the CO (20.9%) and NI (21.1%) soils had a larger cumulative priming than DW (9.1%) soil. The shape of the cumulative priming curves following oxalate addition was similar with a peak in priming around 1d, followed by a decline. By the end of the incubation NI (30.1%) had a larger cumulative priming effect than CO (13.7%) or DW (4.9%) soil. Lastly, the shape of the cumulative priming curve following phenol addition was a bit different from that of the other treatments. In the CO soil, priming followed the exponential decline over the course of the incubation. However, in the DW and NI soils cumulative priming declined over the first 2 d and then began to increase until day 14. At day 14 the priming effect in the NI (26.4%) soil was larger than in CO (20.8%) and DW (13.3%) soils.

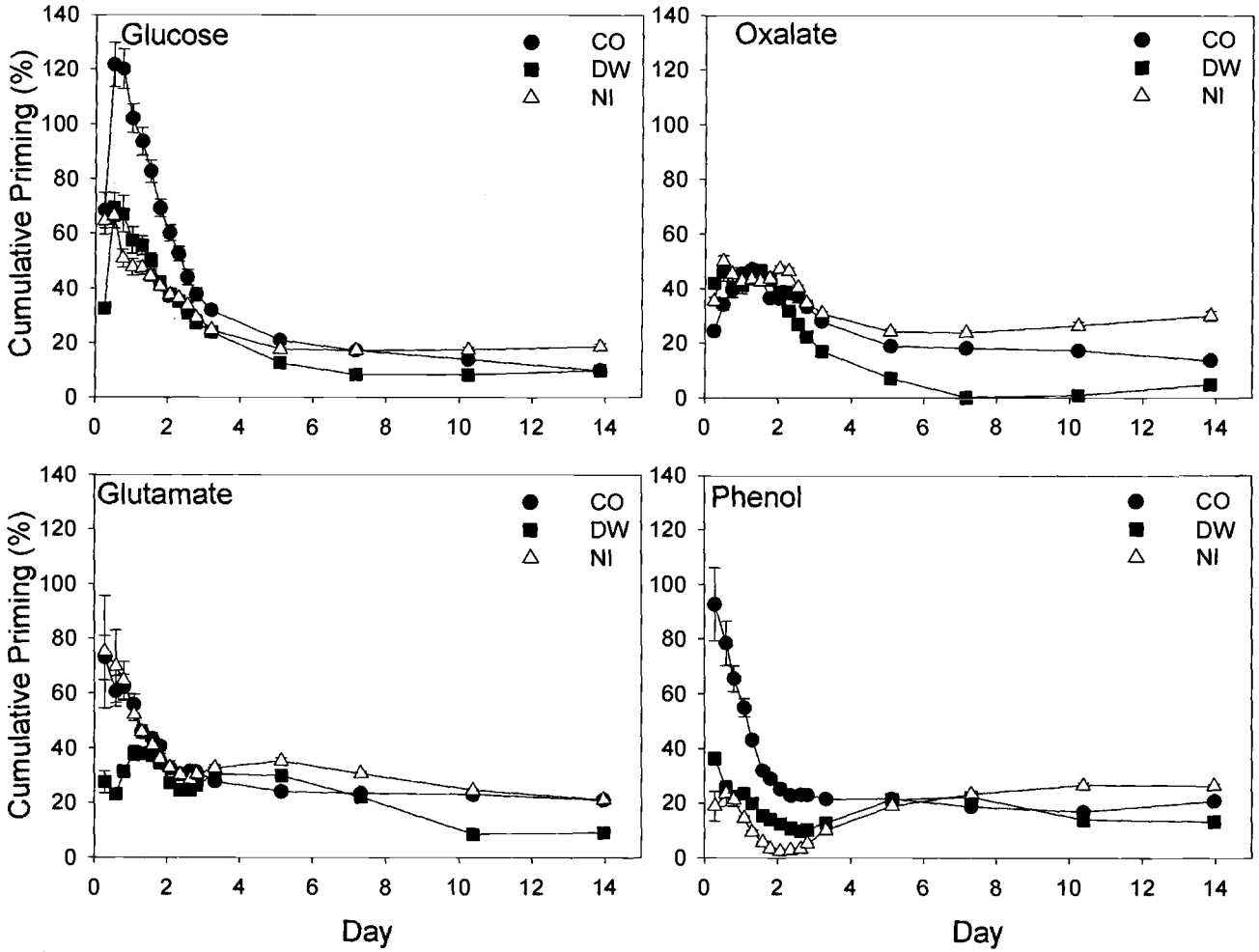


Figure 3.3. Cumulative priming over the 14-d incubation for glucose, glutamate, oxalate, and phenol additions. Symbols show the mean priming (n=3) with error bars ± 1 S.E.

^{13}C incorporation into PLFAs

Figure 3.4 shows the average $\delta^{13}C$ value for different taxonomic groups following glucose addition. In all three soils, all of the taxonomic biomarkers were well labeled by day 2, and there was little change in the $\delta^{13}C$ of these lipids during the 14 d incubation. There was a larger $\delta^{13}C$ for the fungal biomarker in the NI soil. These soils have a smaller fungal biomass (Table 3.1); the same amount of incorporation into a smaller biomass could yield a larger $\delta^{13}C$ value. In fact, when the $\delta^{13}C$ values of the taxonomic biomarkers are relativized by the mol%, there was more glucose incorporation into the fungal lipids in the DW soil than either the CO or NI soils (Figure 3.5). If the fungal value from the NI soil is ignored, the incorporation into the other taxonomic groups had a similar pattern and $\delta^{13}C$ value in the soils of all three litter treatments.

The pattern of ^{13}C-glutamate incorporation into lipids was somewhat different than glucose (Figure 3.6). The actinomycete biomarkers had a large $\delta^{13}C$ by day 2 of the incubation, and this value declined over the rest of the incubation. The gram-negative, gram-positive, and fungal biomarkers $\delta^{13}C$ remained relatively stable over the 14 d incubation, while the $\delta^{13}C$ of the cyclo lipids increased in all three soils. In the NI soil, the incorporation into actinomycete biomarkers was much higher than in the CO and DW soils by day 2. Even with the smaller MBC there was a higher relative incorporation into actinomycete lipids on day 2 of the incubation (Figure 3.5). Otherwise, the soils of the three treatments responded in a similar fashion, with the exception of higher incorporation into fungal lipids in the NI soil, followed by a decline over the incubation.

Following oxalate addition there was a much smaller amount of ^{13}C incorporation into lipids than with the addition of the other substrates (Figure 3.7). Moreover, the $\delta^{13}C$ of the three soils was the same, instead of higher in the NI soil as with the other substrates (Figures 3.4, 3.6, 3.8). In all three soils, the actinomycete biomarkers $\delta^{13}C$ declined over the incubation, while the $\delta^{13}C$ of the cyclo PLFAs increased. The $\delta^{13}C$ of the actinomycete markers was higher on day 2 in the CO and

NI soil, although these differences were gone by day 8. However, when ^{13}C incorporation is relativized by mol%, there was more incorporation into CO lipids than DW lipids on day 2 (Figure 3.5). The $\delta^{13}C$ of the other lipids biomarkers remained relatively stable over the incubation, with little difference between soils.

Incorporation of ^{13}C-phenol into lipids was much different than the other three substrates (Figure 3.8). There was fairly large incorporation into the fungal biomarkers, with little or no incorporation into any other lipids. Again, the $\delta^{13}C$ of the NI fungal biomarker was higher than in the soils of CO or DW treatments. When the relative incorporation into lipids is examined on day 2, there was more incorporation into the DW fungal community than into the CO soil, with intermediate incorporation in the NI soil (Figure 3.5). There was also less relative incorporation into the actinomycete biomarkers in the DW soil than in the CO and NI soils. The $\delta^{13}C$ of the lipid markers remained stable over the experiment, although there was a slight increase in the $\delta^{13}C$ of the cyclo PLFAs in all treatments.

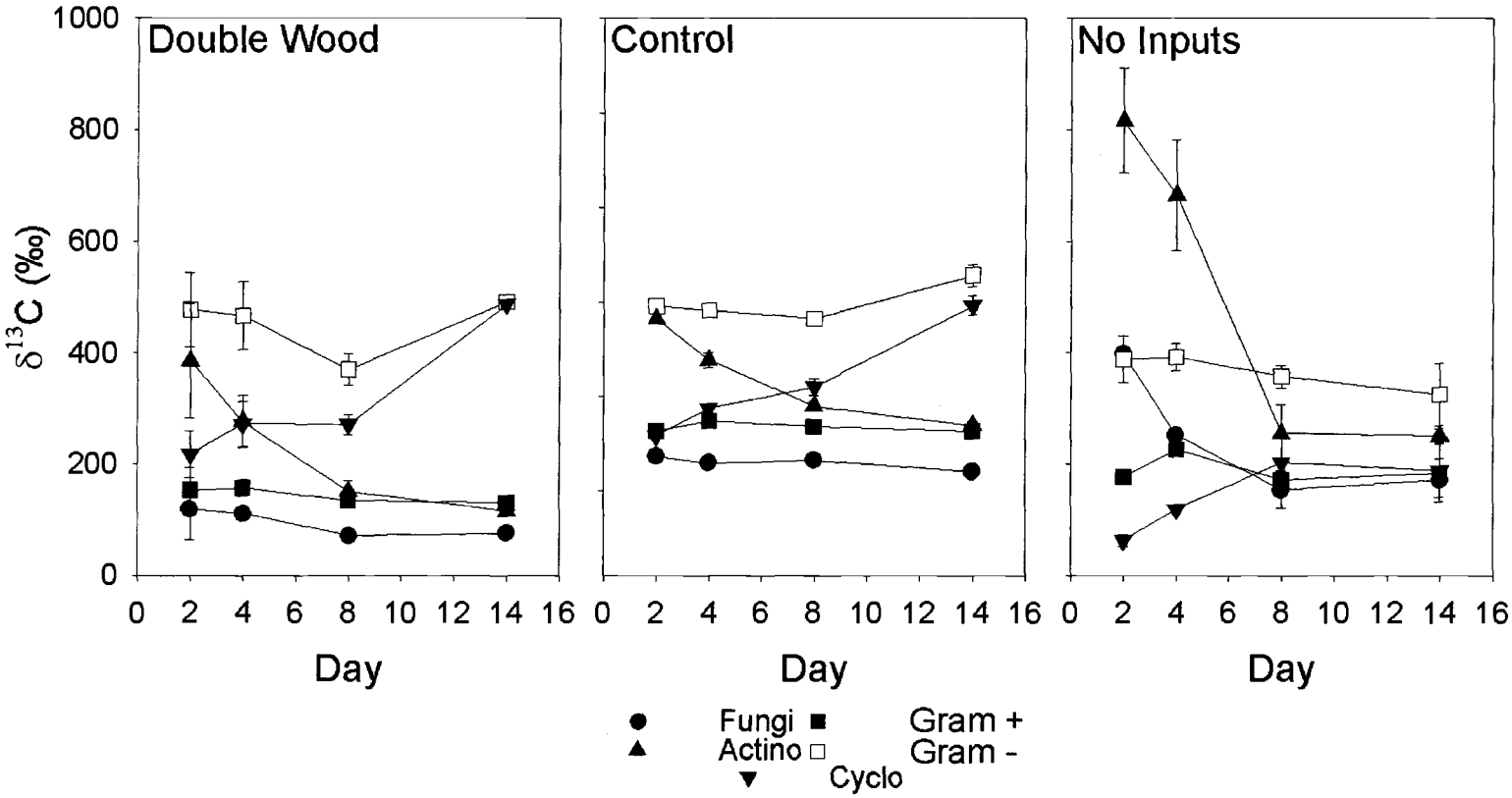


Figure 3.4. The $\delta^{13}C$ incorporation into lipid biomarkers over time in the three soils following glutcose addition. Each symbol is the weighted mean (n=3) $\delta^{13}C$ of five taxonomic biomarkers with error bars showing standard errors of the mean.

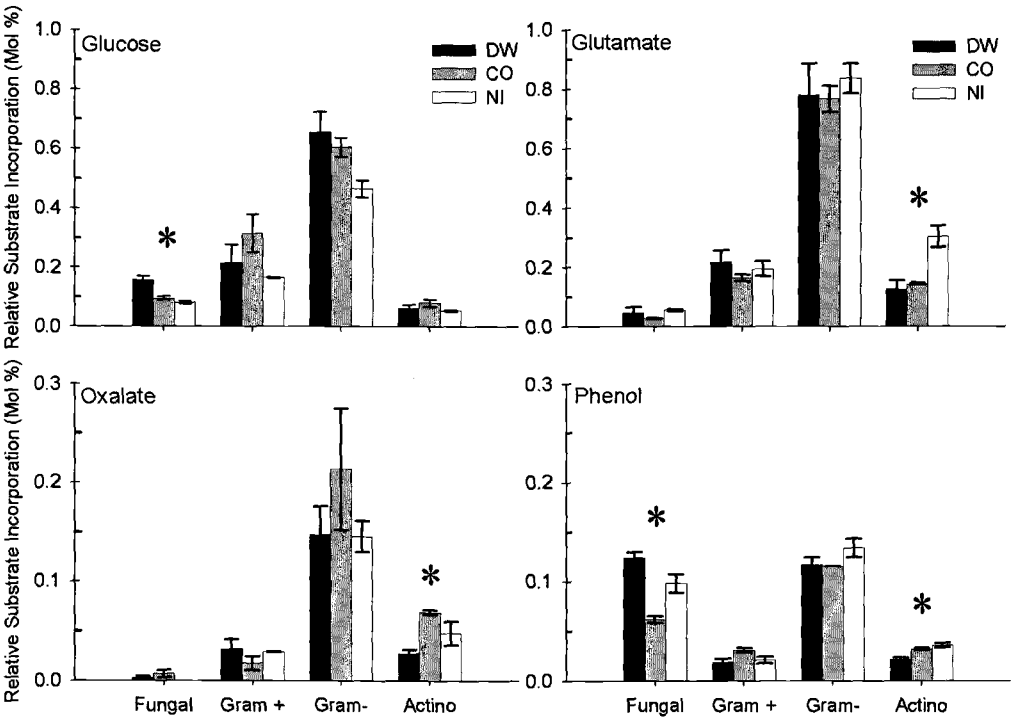


Figure 3.5. Relative $\delta^{13}C$ incorporation into PLFA biomarkers of day 2 of the incubation. Each bar is the mean (n=3) incorporation ± 1 standard error. Stars indicate a significant difference (ANOVA; $p < 0.05$) among the three soils for each substrate.

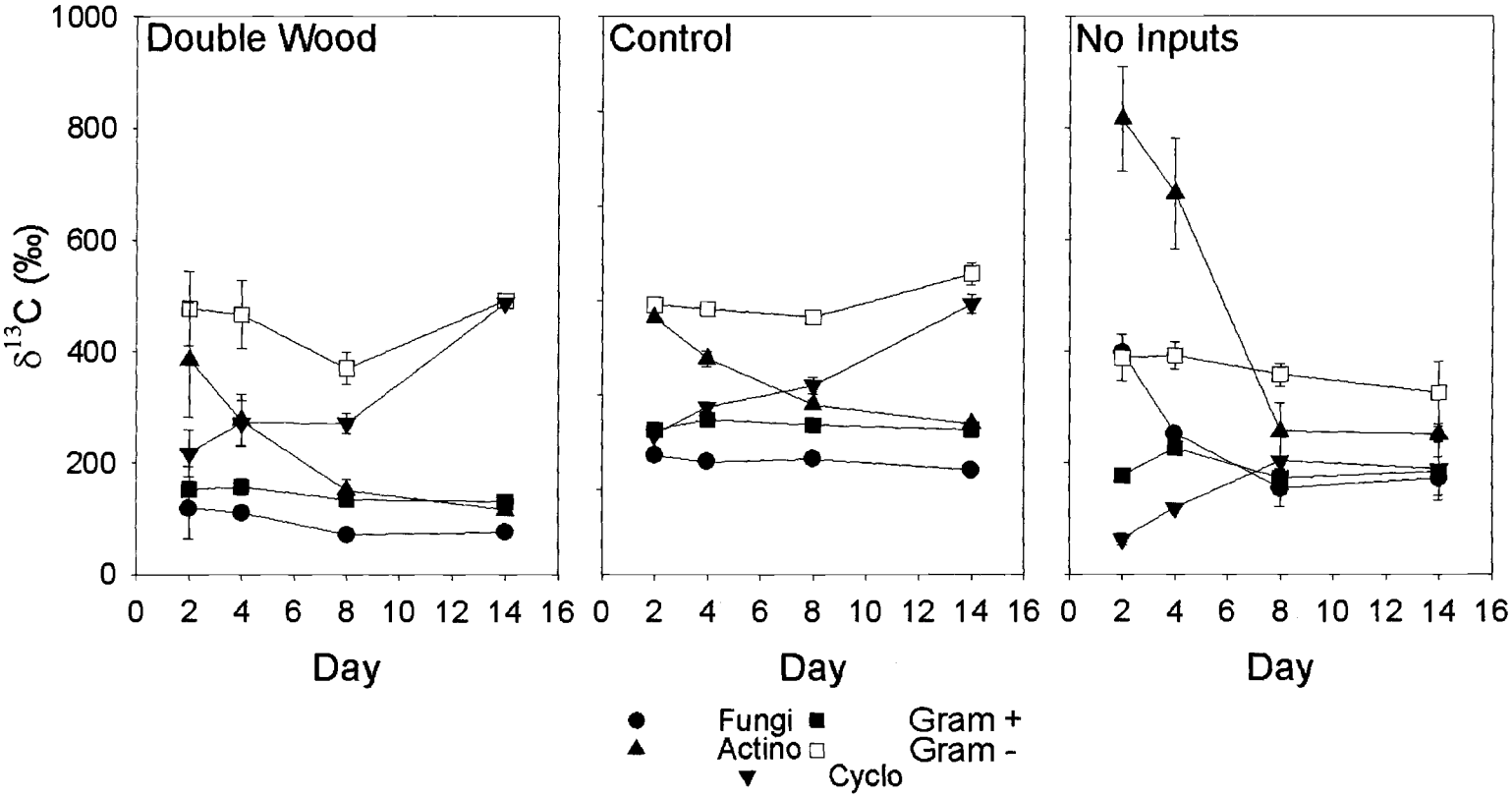


Figure 3.6. The $\delta^{13}C$ incorporation into lipid biomarkers over time in the three soils following glutamate addition. Each symbol is the weighted mean (n=3) $\delta^{13}C$ of five taxonomic biomarkers with error bars showing standard error of the mean.

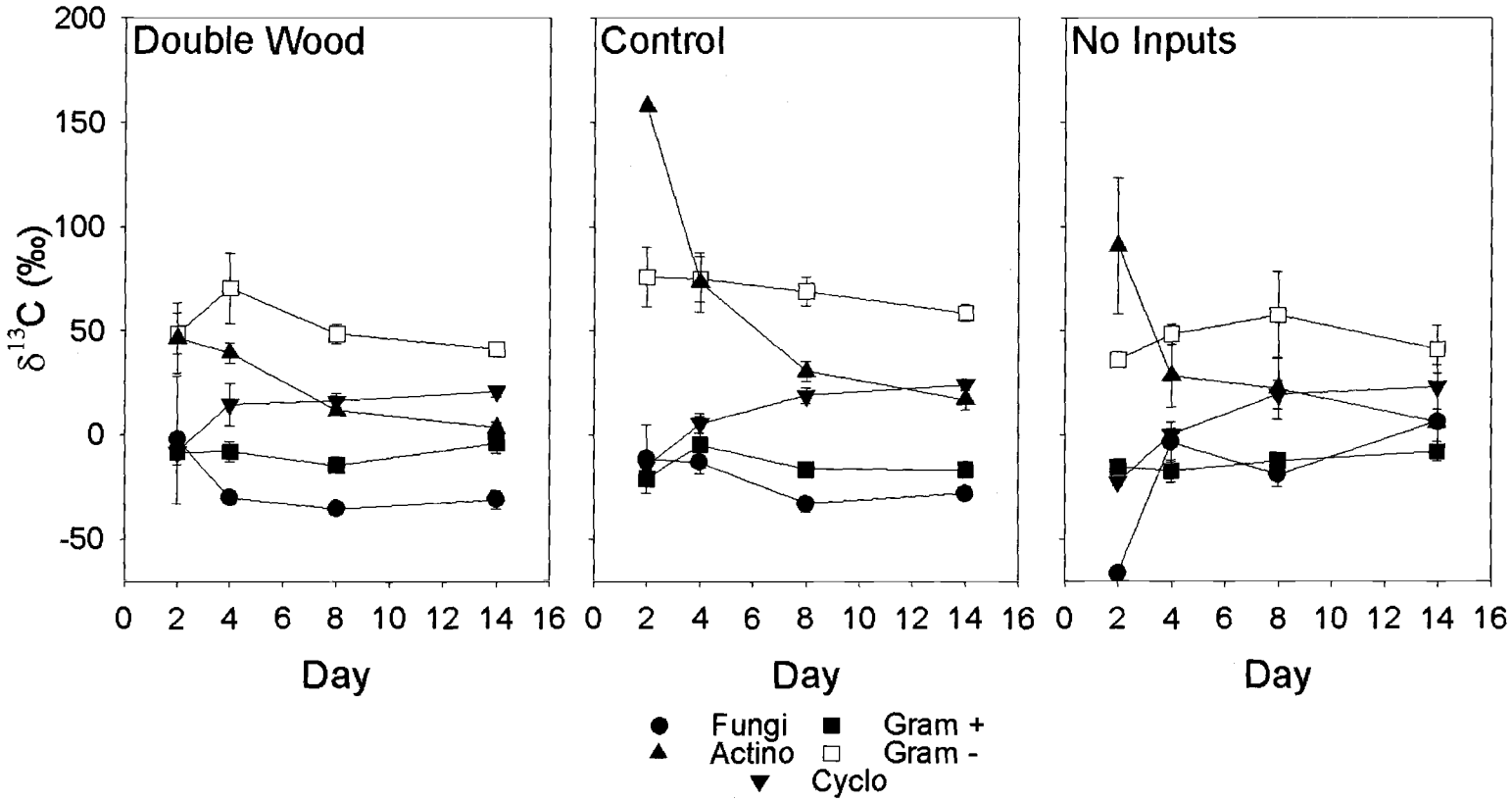


Figure 3.7. The $\delta^{13}C$ incorporation into lipid biomarkers over time in the three soils following oxalate addition. Each symbol is the weighted mean (n=3) $\delta^{13}C$ of five taxonomic biomarkers with error bars showing standard error of the mean.

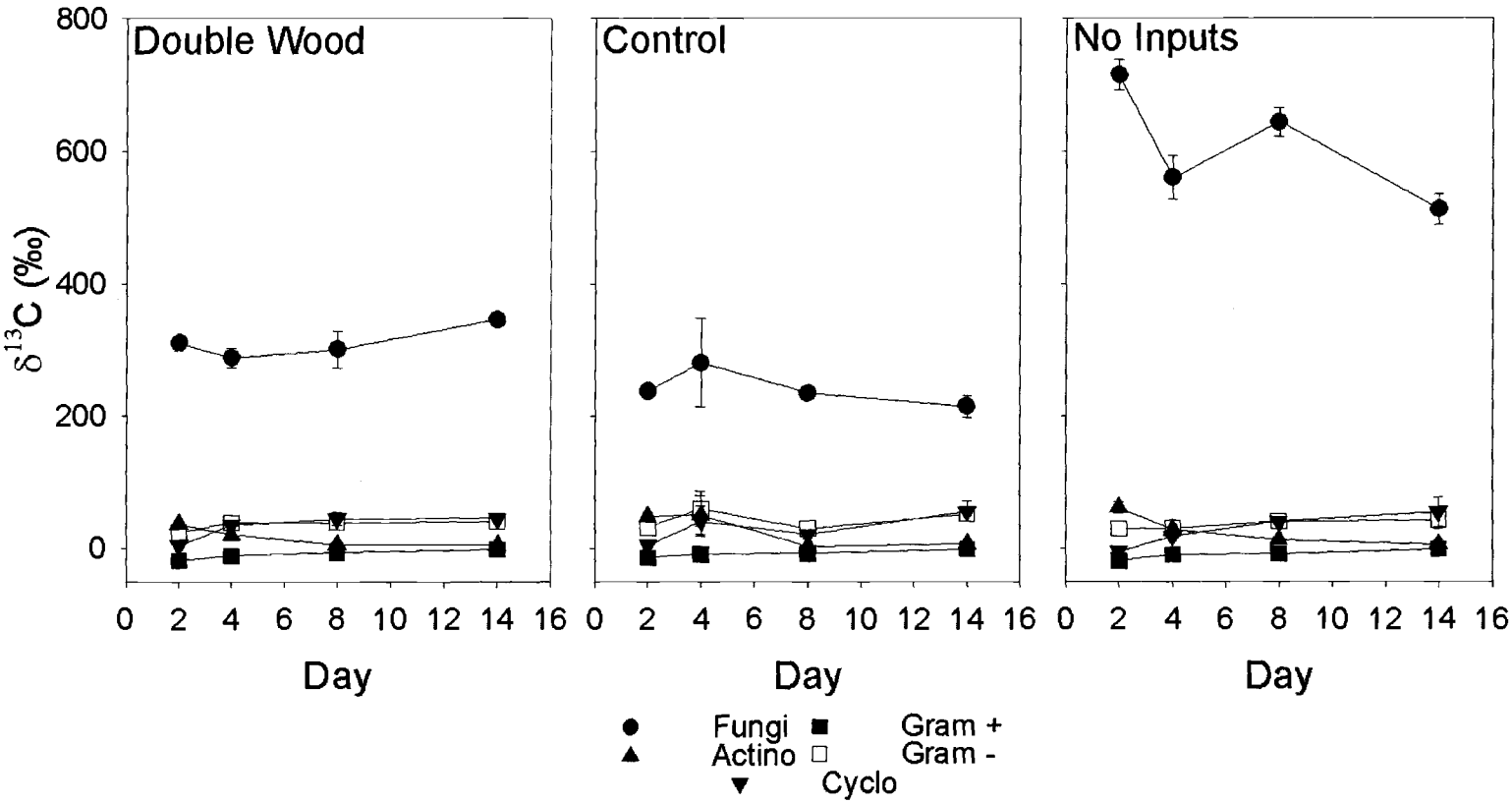


Figure 3.8. The $\delta^{13}C$ incorporation into lipid biomarkers over time in the three soils following phenol addition. Each symbol is the weighted mean (n=3) $\delta^{13}C$ of five taxonomic biomarkers with error bars showing standard

Discussion

Soil Microbial Community Dynamics

Substrate additions did not alter the PLFA profile or MBC in any of the three soils examined (Figure 3.1). I added a small quantity of C compared with most other studies looking at the response of the soil microbial community to substrate additions, in order to avoid a large increase in biomass and the selection of certain portions of the microbial community. In a study where a model root exudate was added to soil at different concentrations, no detectable changes in the soil PLFA profile were found until rates of addition exceeded 1000 μg C d^{-1}, well above the rate of addition in this study (Griffiths et al., 1999). The addition of a model root exudate consisting of simple sugars and amino acids at a rate of 100 μg C d^{-1} for 14 d double the rate of C addition used in this experiment, lead to detectable changes in the genetic profile of the bacterial community, but these methods are more sensitive than PLFA analysis (Baudoin et al., 2003). In soil from an oak woodland, the addition of 400 μg C g^{-1} soil of vanillin was found to change the PLFA profile, although no change was seen with similar addition rates of both simple and complex sugars (Waldrop and Firestone, 2004). A study using denaturing gradient gel electrophoresis (DGGE) found increases in specific portions of the bacterial community following addition of oxalate and glutamate, although there was no change in community composition with the addition of glucose (Falchini et al., 2003).

In all three of our soils, the gram-negative bacteria generally decreased over the 14 d incubation and the gram-positive bacteria increased (Table 3.1). Very few studies have reported changes in the microbial community over time following substrate addition. After 14 d Griffiths et al. (1999) found increases in the relative abundance of PLFAs from gram-positive bacteria, gram-negative bacteria, and actinomycetes, although each of these taxonomic groups responded differently to the substrate additions. In an oak soil Waldrop and Firestone (2004) found an increase in gram-positive and some gram-negative PLFAs 9 d after substrate addition; neither this

study nor Griffiths et al. (1999) reported changes over time following substrate addition.

Substrate Utilization

The percentage of added substrate respired during the incubation was consistent with other studies using similar substrates (Falchini et al., 2003; Hamer and Marschner, 2002, 2005; Shen and Bartha, 1996; Waldrop et al., 2004), although most of these studies added between 10 and 100 times the amount of C added in this study. The amount of added substrate respired differed by both soil and substrate. The respiration rate of all four substrates was lower in the NI soil, whereas the amount of ^{13}C respired from CO and DW soil varied by substrate (Figure 3.2). In both this study (data not shown) and the field (Sulzman et al., 2005), respiration rates was significantly lower from the NI soils, and significantly higher from the DW soils as compared to the CO soils. In the glucose and glutamate additions, more of the added substrate was respired from the CO soils than from the DW soil, although the difference between the two soils was insignificant in the glutamate treatment. All of the difference in the amount of ^{13}C-glucose respired from the CO and DW soils occurred in the first 12 h of incubation (3.0 μg ^{13}C g^{-1} soil at 12 h, 3.1 μg ^{13}C g^{-1} soil at 14 d), after which the two soils behaved similarly (Figure 3.2). With the two substrates of lower energy availability (oxalate and phenol), there was more ^{13}C-substrate respired from the DW soil than from the CO soil. The DW soil is more fungally dominated, and would be expected to have a larger community of wood-decomposing fungi (Table 3.1). White-rot fungi have the ability to degrade simple phenolic compounds (Bending and Read, 1997) and are major producers of oxalate in soil (Dutton and Evans, 1996). Thus, an increase in this type of fungi could lead to an increased oxalate production, and a greater ability of the microbial community to degrade these types of compounds.

The cumulative respiration of oxalate, phenol, and glutamate can be explained using a single first-order decay model. This type of model assumes a first-order

relationship between the amount of substrate remaining and the rate of substrate respiration, and has been used to characterize the decomposition of simple substrates (Wagner and Wolf, 1999). The ability of a single exponential model to explain the substrate respiration curves suggests that there is one easily mineralizable pool of substrate C following addition of these three substrates, most of which is respired during the first 3 d of incubation. Glucose, on the other hand, continues to be respired at a low rate throughout the incubation (Figure 3.2). In addition, the GYE following glucose addition is very high at day 2 of the incubation (Table 3.3) and decreases as the incubation progresses (data not shown). The GYE in our soils following glucose addition fall at the high end of the published values, but are not unreasonable (Frey et al., 2001). Nguyen and Guckert (2001) found that in soils with C limitation, glucose can be taken into the cell and stored rather than used for growth over the short-term. This stored glucose could then be utilized later in the incubation, leading to the appearance of multiple C pools derived from the added glucose.

Two days following the addition of all 4 substrates, the ratio of fungal to bacterial substrate incorporation was at least 0.2 in all three soils (Table 3.3). This number is greater than the fungal to bacterial ratio of the soils themselves (Table 3.1). In addition, there was no differences in fungal:bacterial ^{13}C-incorporation following glutamate and oxalate addition

On day 2 of the incubation, there was no difference in glucose incorporation into MBC in the three soils (Table 3.3). There was also no soil difference in GYE or specific substrate respiration, implying a similar ability of microbial communities in the three soils to utilize glucose. The microbes in the DW soil incorporated a larger percentage of ^{13}C-glucose into fungal lipids than in the other two treatments. Thus, the small observed differences in ^{13}C-respiration and microbial incorporation could be due to differences in microbial community structure, especially fungal:bacterial ratio. A higher fungal:bacterial ratio has been shown to lead to increased incorporation of cellobiose into fungal lipids, with little or no changes in the amount of cellobiose respired (Phillips et al., 2002). The distinct microbial communities in these three soils

had slight differences in their incorporation of glucose and small differences in glucose respiration, suggesting the functional equivalence of these microbial communities in degrading simple sugars (Waldrop and Firestone, 2004).

The amount of glutamate respired did not vary much among soils, with slightly less ^{13}C respired from the NI soil (Figure 3.2). Even though the soils are similar in terms of the amount of substrate respired, there is some evidence of differential processing in the NI soil. Less substrate was incorporated into MBC yet there was a higher relative respiration rate on day 2 (Table 3.3). Further, even though less of the substrate was incorporated into total MBC on day 2, there was more ^{13}C incorporated into actinomycete PLFAs in the NI soil (Figure 3.6).

Very little of the added oxalate was incorporated into either MBC (Table 3.3) or PLFAs (Figure 3.7), with less substrate incorporated in the MBC in the NI soil. Even though the GYE for the whole soil microbial community was very low after oxalate addition, some microorganisms were able to grow on oxalate as evidenced by incorporation of ^{13}C into microbial lipids. The taxonomic groups most able to use oxalate were gram-negative bacteria and actinomycetes. Most oxalate-degrading bacteria isolated from terrestrial ecosystems fall into these two broad categories (Sahin, 2003). The microbial community in the NI soil seemed to have trouble degrading oxalate when compared to those in CO and DW soils given the low substrate incorporation into MBC and high specific respiration rate (Table 3.3). Most oxalate in soil is either produced by fungi (Dutton and Evans, 1996) or excreted by plant roots (Bertin et al., 2003). The NI plots have a small fungal biomass as well as no plant inputs to soil and could have a smaller population of oxalate-degrading bacteria as a result.

It appears that only a small portion of the microbial population was able to utilize phenol, given the low respiration rate and small incorporation into MBC. However, the few microorganisms that were able to utilize the substrate seemed to have no problem incorporating phenol into microbial biomass given the GYE value of roughly 0.4 for this substrate. Phenol is toxic to most organisms in large quantities.

Following phenol addition, there was either a toxic effect or a delay in substrate utilization for enzyme production as evidenced by the S-shaped ^{13}C respiration curve, especially in the NI soil (Figure 3.2). It appears that if there was a toxic effect of phenol, it was fairly small and transient, as there was no difference in the PLFA profile or MBC by day 2 in the phenol addition experiment compared with the other substrates. Fungi were responsible for most of the phenol degradation in this ecosystem, as well as some gram-negative bacteria and actinomycetes (Figures 3.8, 3.5). White-rot fungi are the major phenol oxidase producers and phenol degraders in soil (Dix and Webster, 1995), although gram-negative bacteria, gram-positive bacteria, and actinomycetes with the ability to degrade phenol have been isolated from soils (Koutny et al., 2003; McCarthy and Williams, 1992).

Priming

All four of the added substrates induced positive priming in each of the three soils (Figure 3.3). Many other studies have shown positive priming in response to the addition of the same simple C compounds used in this study (e.g. Dalenberg and Jager, 1989; Falchini et al., 2003; Hamer and Marschner, 2002, 2005; Shen and Bartha, 1996; Waldrop and Firestone, 2004), although most of those have added significantly more C than was added in this study. One study that added simple C substrates in lower concentrations than in this study (11.3 to 34 μg C g soil^{-1}) found positive priming over 3 d following the addition of a dilute C solution (De Nobili et al., 2001). In the only study examining priming effects in response to phenol addition (Shen and Bartha, 1996), a large transient priming effect was found to occur during the first 15 d of incubation, similar to the results in this experiment. This effect was explained as the turnover of organisms killed by phenol addition, even at quantities below those added in this experiment. As said above, if there was a toxic effect of phenol it was undetectable at 2 d in either MBC or PLFA profiles and was probably small at the low concentration of phenol added in this experiment.

The largest priming effect was found during the first 2 d of the incubation, which was also the period of highest substrate respiration (Figures 3.2, 3.3). This result has been reported in other studies as well (Bell et al., 2003; Falchini et al., 2003; Hamer and Marschner, 2005). It has been hypothesized that the priming effect in response to C addition is caused by the turnover of microbial biomass C, rather than the decomposition of native SOM (Bell et al., 2003; Dalenberg and Jager, 1989; De Nobili et al., 2001; Wu et al., 1993). These studies have shown that the turnover of MBC is the source of positive priming following the addition of various simple C compounds and plant residue in quantities above and below those added in this study. Taking this theory a little further, De Nobili et al. (2001) hypothesized that the soil microbial biomass is in a resting state most of the time, but keeps itself in a state of 'metabolic alertness' to respond to the presence of any easily degradable compounds. They go on to suggest that the addition of low molecular weight trigger molecules can activate the resting biomass to metabolize the added substrate. This, in turn, leads to the turnover of microbial biomass as well as endogenous C from the now active cells, which results in much of the observed priming effect.

The largest positive priming effect was found in the NI soil (Figure 3.3). The NI soils are expected to have mostly very recalcitrant C as they have not received new C inputs for over 7 y. Fontaine et al. (2003) reported that nutrient-poor soils generally have a larger priming effect than nutrient-rich soil. The two compounds used in this study least available for microbial growth (oxalate and phenol) indeed induced a larger priming effect than the two more labile compounds as found in other studies, although these differences were fairly small.

Conclusions

Microbial utilization of all four substrates differed between all three litter treatments. These differences were most pronounced in the utilization of phenol and oxalate, where the amount of ^{13}C-respired from the DW soil was larger than the CO soil, and amount respired from the NI soil was smaller than the CO soil. In all three

soils most of the phenol incorporation was into the fungal PLFAs. Thus, it appears that the larger fungal community in the DW soil is able to degrade more of this complex substrate than the microbial communities of the other soils. It also appears that a larger portion of the fungal community in the DW soil is able to process phenol, as evidenced by an increase in the ratio of ^{13}C-fungal: ^{13}C-bacterial substrate incorporation, although more research is need into differences in fungal community structure in the DIRT plots, as both mycorrhizal and saprotrophic fungi have the same PLFA biomarkers and cannot be distinguished using this method.

Changes in atmospheric chemistry and N deposition could lead to long-term changes in the quality and quantity of plant inputs to soil. Understanding the structural and mechanistic changes altered plant inputs produce in the soil microbial community will allow us to better understand and predict how these changes might influence ecosystem functioning and soil C sequestration. Seven years of chronic changes in plant inputs to an old-growth forest have led to changes in the both the structure of the microbial community, as well as its ability to degrade a variety of C compounds. Many of these changes appear to be the result of a shift in the fungal community. More work is needed to assess how the functional changes observed in laboratory incubations translate into actual ecosystem functioning, given the highly mycorrhizal nature of this ecosystem.

References

Aber, J.D., A. Magill, R. Boone, J.M. Melillo, P. Steudler, and R. Bowden. 1993. Plant and soil responses to chronic nitrogen additions at the Harvard Forest, Massachusetts. Ecological Applications 3:156-166.

Abraham, W., C. Hesse, and O. Pelz. 1998. Ratios of carbon isotopes in microbial lipids as an indicator of substrate usage. Applied and Environmental Microbiology 64:4202-4209.

Andren, O., L. Brussaard, and M. Clarholm. 1999. Soil organism influence on ecosystem-level processes bypassing the ecological hierarchy? Applied Soil Ecology 11:177-188.

Baudoin, E., E. Benizri, and A. Guckert. 2003. Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. Soil Biology & Biochemistry 35:1183-1192.

Bell, J.M., J.L. Smith, V.L. Bailey, and H. Bolton. 2003. Priming effect and C storage in semi-arid no-till spring crop rotations. Biology and Fertility of Soils 37:237-244.

Bending, G.D., and D.J. Read. 1997. Lignin and soluble phenolic degradation by ectomycorrhizal and ericoid mycorrhizal fungi. Mycological Research 101:1348-1354.

Bertin, C., X.H. Yang, and L.A. Weston. 2003. The role of root exudates and allelochemicals in the rhizosphere. Plant and Soil 256:67-83.

Bligh, E.G., and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911-917.

Bohlen, P.J., P.M. Groffman, C.T. Driscoll, T.J. Fahey, and T.G. Siccama. 2001. Plant-soil-microbial interactions in a northern hardwood forest. Ecology 82:965-978.

Bowden, R.D., K.J. Nadelhoffer, R.D. Boone, J.M. Melillo, and J.B. Garrison. 1993. Contributions of aboveground litter, belowground litter, and root respiration to total soil respiration in a temperature mixed hardwood forest. Canadian Journal of Forest Research 23:1402-1407.

Bruulsema, T.W., and J.M. Duxbury. 1996. Simultaneous measurement of soil microbial nitrogen, carbon, and carbon isotope ratio. Soil Science Society of America Journal 60:1787-1791.

Butler, J.L., P.J. Bottomley, S.M. Griffith, and D.D. Myrold. 2004. Distribution and turnover of recently fixed photosynthate in ryegrass rhizospheres. Soil Biology & Biochemistry 36:371-382.

Buyer, J.S., and L.E. Drinkwater. 1997. Comparison of substrate utilization assay and fatty acid analysis of soil microbial communities. Journal of Microbiological Methods 30:3-11.

Canadell, J.G., L.F. Pitelka, and J.S.I. Ingram. 1996. The effects of elevated CO_2 on plant-soil carbon below-ground: a summary and synthesis. Plant and Soil 187:391-400.

Crow, S.E., and K. Lajtha. 2004. Nitrogen additions as a result of long-term root removal affects soil organic matter dynamics American Geophysical Union Annual Meeting, San Francisco, CA.

Dalenberg, J.W., and G. Jager. 1989. Priming effect of some organic additions to C-14-labeled soil. Soil Biology & Biochemistry 21:443-448.

De Nobili, M., M. Contin, C. Mondini, and P.C. Brookes. 2001. Soil microbial biomass in triggered into activity by trace amounts of substrate. Soil Biology & Biochemistry 33:1163-1170.

DeForest, J.L., D.R. Zak, K.S. Pregitzer, and A.J. Burton. 2004. Atmospheric nitrate deposition and the microbial degradation of cellobiose and vanillin in a northern hardwood forest. Soil Biology & Biochemistry 36:965-971.

Dix, N.J., and J. Webster. 1995. Fungal Ecology Chapman and Hall, London.

Dixon, J.J. 2003. Applying GIS to soil-geomorphic landscape mapping in the Lookout Creek valley, Western Cascades, Oregon. M.S. Thesis, Oregon State University, Corvallis, OR.

Dutton, M.V., and C.S. Evans. 1996. Oxalate production by fungi: its role in pathogenicity and ecology in the soil environment. Canadian Journal of Microbiology 42:881-895.

Ekschmitt, K., and B.S. Griffiths. 1998. Soil biodiversity and its implications for ecosystem functioning in a heterogeneous and variable environment. Applied Soil Ecology 10:201-215.

Falchini, L., N. Naumova, P.J. Kuikman, J. Bloem, and P. Nannipieri. 2003. CO_2 evolution and denaturing gradient gel electrophoresis profiles of bacterial communities in soil following addition of low molecular weight substrates to simulate root exudation. Soil Biology & Biochemistry 35:775-782.

Fisk, M.C., and T.J. Fahey. 2001. Microbial biomass and nitrogen cycling responses to fertilization and litter removal in young northern hardwood forests. Biogeochemistry 53:201-223.

Fontaine, S., A. Mariotti, and L. Abbadie. 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology & Biochemistry 35:837-843.

Frey, S.D., V.V.S.R. Gupta, E.T. Elliott, and K. Paustian. 2001. Protozoan grazing affects estimates of carbon utilization efficiency of the soil microbial community. Soil Biology & Biochemistry 33:1759-1768.

Frey, S.D., M. Knorr, J.L. Parrent, and R.T. Simpson. 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. Forest Ecology and Management 196:159-171.

Griffiths, B.S., K. Ritz, N. Ebblewhite, and G. Dobson. 1999. Soil microbial community structure: Effects of substrate loading rates. Soil Biology & Biochemistry 31:145-153.

Hamer, U., and B. Marschner. 2002. Priming effects of sugars, amino acids, organic acids and catechol on the mineralization of lignin and peat. Journal of Plant Nutrition and Soil Science 165:261-268.

Hamer, U., and B. Marschner. 2005. Priming effects in different soil types induced by fructose, alanine, oxalic acid and catechol additions. Soil Biology & Biochemistry 37:445-454.

Koutny, M., J. Ruzicka, and J. Chlachula. 2003. Screening for phenol-degrading bacteria in the pristine soils of south Siberia. Applied Soil Ecology 23:79-83.

Kruskal, J.B. 1964. Nonmetric multidimentional scaling: a numerical method. Psychometrika 29:115-129.

Kuzyakov, Y., and W. Cheng. 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology & Biochemistry 33:1915-1925.

Kuzyakov, Y., J.K. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. Soil Biology & Biochemistry 32:1485-1498.

Lajtha, K., S. Crow, Y. Yano, S.S. Kaushal, E.W. Sulzman, and J.D.H. Spears. 2005. Detrital controls on soil solution N and dissolved organic matter in soils: a field experiment. Biogeochemistry (In review).

Mather, P.M. 1976. Computation methods of multivariate analysis in physical geography J. Wiley and Sons, London.

McCarthy, A.J., and S.T. Williams. 1992. Actinomycetes as agents of biodegradation in the environment - a review. Gene 115:189-192.

McCune, B., and M.J. Mefford. 1999. Multivariate analysis on the PC-ORD system. Version 4 MjM Software, Gleneden Beach, Oregon.

McCune, B., and J.B. Grace. 2002. Analysis of Ecological Communities MjM Software Design, Gleneden Beach, Oregon.

Mielke Jr., P.W. 1984. Meteorological applications of permutation techniques based on distance functions, *In* P. R. Krishnaiah and P. K. Sen, eds. Handbook of Statistics, Vol. 4. Elsevier Science Publishers.

Mielke Jr., P.W., and K.J. Berry. 2001. Permutation Methods: A Distance Function Approach.

Mooney, H.A., J. Canadell, F.S. Chapin III, J.R. Ehleringer, C. Korner, R.E. McMurtrie, W.J. Parton, L.F. Pitelka, and E.D. Schulze. 1999. Ecosystem physiology responses to global change, p. 141-189, *In* B. H. Walker, et al., eds. The Terrestrial Biosphere: Global Change Implications for Natural and Managed Ecosystems. Cambridge University Press, Cambridge, UK.

Myers, R.T., D.R. Zak, D.C. White, and A. Peacock. 2001. Landscape-level Patterns of microbial community composition and substrate use in upland forest ecosystems. Soil Science Society of America Journal 65:359-367.

Nadelhoffer, K.J., R.D. Boone, R.D. Bowden, J.D. Canary, J. Kaye, P. Micks, A. Ricca, J.A. Aitkenhead, K. Lajtha, and W.H. McDowell. 2004. The DIRT Experiment: Litter and Root Influences on Forest Soil Organic Matter Stocks and Function, *In* D. Foster and J. Aber, eds. Forest Landscape Dynamics in New England: Ecosystem Structure and Function as a Consequence of 5000 years of Change. Oxford University Press, New York.

Nguyen, C., and A. Guckert. 2001. Short-term utilisation of C-14-[U]glucose by soil microorganisms in relation to carbon availability. Soil Biology & Biochemistry 33:53-60.

Pendall, E., S. Bridgham, P.J. Hanson, B.A. Hungate, D.W. Kicklighter, D.W. Johnson, B.E. Law, Y.Q. Luo, J.P. Megonigal, M. Olsrun, M.G. Ryan, and S.Q. Wan. 2004. Below-ground process responses to elevated CO_2 and temperature: a discussion of observations, measurement methods, and models. New Phytologist 162:311-322.

Phillips, R.L., D.R. Zak, W.E. Holmes, and D.C. White. 2002. Microbial community composition and function beneath temperate trees exposed to elevated atmospheric carbon dioxide and ozone. Oecologia 131:236-244.

Sahin, N. 2003. Oxalotrophic bacteria. Research In Microbiology 154:399-407.

Schimel, J.P. 1995. Ecosystem consequences of microbial diversity and community structure, *In* F. S. Chapin III and C. Korner, eds. Arctic and Alpine Biodiversity: Patterns, Causes and Ecosystem consequences, Vol. 113. Springer-Verlag, New York.

Schimel, J.P., and J. Gulledge. 1998. Microbial community structure and global trace gases. Global Change Biology 4:745-758.

Shen, J., and R. Bartha. 1996. Priming effect of substrate addition in soil-based biodegradation tests. Applied and Environmental Microbiology 62:1428-1430.

Smith, J.L., and E.A. Paul. 1990. The significance of soil microbial biomass estimations, p. 357-393, *In* J. Bollag and G. Stotsky, eds. Soil Biochemistry, Vol. 8. Mercel Dekker, New York, New York.

Spears, J.D.H., S.M. Holub, M.E. Harmon, and K. Lajtha. 2003. The influence of decomposing logs on soil biology and nutrient cycling in an old-growth mixed coniferous forest in Oregon, USA. Canadian Journal of Forest Research 33:2193-2201.

Sulzman, E.W., J.B. Brant, R.D. Bowden, and K. Lajtha. 2005. Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO_2 efflux in an old growth coniferous forest. Biogeochemistry (In press).

Vance, E.D., P.C. Brookes, and D.S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry 19:703-707.

Vanderbilt, K.L., K. Lajtha, and F.J. Swanson. 2003. Biogeochemistry of unpolluted forest watersheds in the Oregon Cascades: temporal patterns of precipitation and stream nitrogen fluxes. Biogeochemistry 62:87-117.

Wagner, G.H., and D.C. Wolf. 1999. Carbon transformations and soil organic matter formation, *In* D. M. Sylvia, et al., eds. Principles and Applications of Soil Microbiology. Prentice Hall, Upper Saddle River, NJ.

Waldrop, M.P., and M.K. Firestone. 2004. Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities. Oecologia 138:275-284.

Waldrop, M.P., and M.K. Firestone. 2004. Altered utilization patterns of young and old soil C by microorganisms caused by temperature shifts and N additions. Biogeochemistry 67:235-248.

Waldrop, M.P., T.C. Balser, and M.K. Firestone. 2000. Linking microbial community composition to function in a tropical soil. Soil Biology & Biochemistry 32:1837-1846.

Waldrop, M.P., D.R. Zak, and R.L. Sinsabaugh. 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biology & Biochemistry 36:1443-1451.

White, D.C., and D.B. Ringelberg. 1998. Signature lipid biomarker analysis, p. 255-272, *In* R. S. Burlage, et al., eds. Techniques in Microbial Ecology. Oxford University Press, New York, NY.

Wu, J.G., P.C. Brookes, and D.S. Jenkinson. 1993. Formation and destruction of microbial biomass during the decomposition of glucose and ryegrass in soil. Soil Biology & Biochemistry 25:1435-1441.

Yano, Y., K. Lajtha, P. Sollins, and B.A. Caldwell. 2005. Chemistry and dynamics of dissolved organic matter in a temperate coniferous forest on andic soils: effects of litter quality. Ecosystems (In Press).

Zak, D.R., K.S. Pregitzer, P.S. Curtis, and W.E. Holmes. 2000. Atmospheric CO_2 and the composition and function of soil microbial communities. Ecological Applications 10:47-59.

Chapter 4:
General Conclusion

Justin B. Brant

Soil has been described as the 'black box' of the global C cycle. Rates of C addition to and loss from the soil system can be measured and modeled, but little is known about the actual mechanisms of soil C dynamics as most processes are carried out by a diverse and complex soil microbial community. In addition, very little is known about the complex relationships between plant inputs and climate that control microbial community structure in the mineral soil. The goal of this thesis was to explore the role of plant input quantity and chemical composition on microbial community structure and function in forest ecosystems.

The first part of my research examined changes in microbial community structure in response to C input manipulation in three forest ecosystems. The key findings were:

- Root inputs control soil microbial community structure in the mineral soil from three distinct forest ecosystems
- Seasonal changes in microbial community structure at one site were larger than the treatment effects underscoring the need for seasonal sampling when examining changes in the soil microbial community in response to field manipulations.

The second experiment examined changes in microbial C utilization in response to the same long-term field-scale C manipulations at one of the sites used in the first experiment. The key findings from this experiment were:

- Long-term changes in C input affect both the rate and mechanisms of microbial degradation of C compounds. The effects were more pronounced following the addition of oxalate and phenol.
- Fungi are responsible for most phenol degradation in this soil.
- Changes in the fungal community were important drivers in the C utilization differences found in this experiment.

Soils are the largest terrestrial C sink, storing approximately twice as much C as the atmosphere. In addition, the decomposition of SOM is the largest flux from

terrestrial ecosystems to the atmosphere, approximately 10 times larger than fossil fuel burning and deforestation combined. Thus, small changes in the magnitude of fluxes into and out of soils could have significant effects on the atmospheric CO_2 concentration.

Additionally, the rate and chemical composition of plant inputs to soil is being altered as a result of increased atmospheric CO_2 concentration, changes in N deposition, and the altering of plant communities. In order to properly understand and predict how belowground processes and decomposition rates will respond to these environmental changes, it is important to understand the mechanisms controlling the composition and function of the soil microbial community responsible for these fluxes.

Future Research

The lack of an effect from aboveground litter manipulation, even after 13 years at Bousson, was surprising. Continued monitoring of these permanent treatment plots is necessary to determine when, and if, changes in the soil microbial community occur in response to altered aboveground inputs. In addition, further research is needed with regard to changes in fungal community structure following litter and wood addition. One of the major limitations of the PLFA technique is that both mycorrhizal and saprotrophic fungi have the same PLFA biomarker, thus changes in the composition of the fungal community will not be distinguished using this method.

In other studies, changes in the microbial community in the organic horizon were detected after only a few years of litter manipulation (Nadelhoffer et al., 2004; Siira-Pietikainen et al., 2003; Siira-Pietikainen et al., 2001; Subke et al., 2004). Further work at the DIRT plots is necessary to determine if differences in the soil CO_2 efflux rate at these sites (Bowden et al., 1993; Sulzman et al., 2005) could be a result of microbial activity and decomposition of aboveground C inputs before entering the mineral soil (Lajtha et al., 2005). In addition, more work is needed to examine the role of mycorrhizal fungi in the functioning of soil microbial

communities in forest ecosystems. Finally, further work is needed examining field-scale priming in response to root and litter inputs to soil, as this could have profound effects on predictions about long-term soil C stores in response to increasing atmospheric CO_2.

Bibliography

Aber, J.D., A. Magill, R. Boone, J.M. Melillo, P. Steudler, and R. Bowden. 1993. Plant and soil responses to chronic nitrogen additions at the Harvard Forest, Massachusetts. Ecological Applications 3:156-166.

Abraham, W., C. Hesse, and O. Pelz. 1998. Ratios of carbon isotopes in microbial lipids as an indicator of substrate usage. Applied and Environmental Microbiology 64:4202-4209.

Andren, O., L. Brussaard, and M. Clarholm. 1999. Soil organism influence on ecosystem-level processes bypassing the ecological hierarchy? Applied Soil Ecology 11:177-188.

Atlas, R.M., and R. Bartha. 1998. Microbial Ecology: Fundamentals and Applications Benjamin/Cummings, Redwood City, CA.

Bååth, E. 2003. The use of neutral lipid fatty acids to indicate the physiological conditions of soil fungi. Microbial Ecology 45:373-383.

Baudoin, E., E. Benizri, and A. Guckert. 2003. Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. Soil Biology & Biochemistry 35:1183-1192.

Bell, J.M., J.L. Smith, V.L. Bailey, and H. Bolton. 2003. Priming effect and C storage in semi-arid no-till spring crop rotations. Biology and Fertility of Soils 37:237-244.

Bending, G.D., and D.J. Read. 1997. Lignin and soluble phenolic degradation by ectomycorrhizal and ericoid mycorrhizal fungi. Mycological Research 101:1348-1354.

Bertin, C., X.H. Yang, and L.A. Weston. 2003. The role of root exudates and allelochemicals in the rhizosphere. Plant and Soil 256:67-83.

Bligh, E.G., and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911-917.

Blume, E., M. Bischoff, J.M. Reichert, T. Moorman, A. Konopka, and R.F. Turco. 2002. Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. Applied Soil Ecology 20:171-181.

Bohlen, P.J., P.M. Groffman, C.T. Driscoll, T.J. Fahey, and T.G. Siccama. 2001. Plant-soil-microbial interactions in a northern hardwood forest. Ecology 82:965-978.

Boone, R.D., K.J. Nadelhoffer, J.D. Canary, and J. Kaye. 1998. Roots exert a strong influence on the temperature sensitivity of soil respiration. Nature 396:570-572.

Bossio, D.A., and K.M. Scow. 1998. Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patters. Microbial Ecology 35:265-278.

Bossio, D.A., K.M. Scow, N. Gunapala, and K.J. Graham. 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microbial Ecology 36:1-12.

Bowden, R.D., K.J. Nadelhoffer, R.D. Boone, J.M. Melillo, and J.B. Garrison. 1993. Contributions of aboveground litter, belowground litter, and root respiration to total soil respiration in a temperature mixed hardwood forest. Canadian Journal of Forest Research 23:1402-1407.

Brant, J.B., E.W. Sulzman, and D.D. Myrold. 2004. Root influences on microbial community composition in forest soils Ecology Society of America, Portland, OR.

Bruulsema, T.W., and J.M. Duxbury. 1996. Simultaneous measurement of soil microbial nitrogen, carbon, and carbon isotope ratio. Soil Science Society of America Journal 60:1787-1791.

Burke, R., M. Molina, J. Cox, L. Osher, and M. Piccolo. 2003. Stable carbon isotope ratio and composition of microbial fatty acids in tropical soil. Journal of Environmental Quality 32:198-206.

Butler, J.L., P.J. Bottomley, S.M. Griffith, and D.D. Myrold. 2004. Distribution and turnover of recently fixed photosynthate in ryegrass rhizospheres. Soil Biology & Biochemistry 36:371-382.

Buyer, J.S., and L.E. Drinkwater. 1997. Comparison of substrate utilization assay and fatty acid analysis of soil microbial communities. Journal of Microbiological Methods 30:3-11.

Canadell, J.G., L.F. Pitelka, and J.S.I. Ingram. 1996. The effects of elevated CO_2 on plant-soil carbon below-ground: a summary and synthesis. Plant and Soil 187:391-400.

Chanasyk, D.S., I.R. Whitson, E. Mapfumo, J.M. Burke, and E.E. Prepas. 2003. The impacts of forest harvest and wildfire on soils and hydrology in temperate forests: A baseline to develop hypotheses for the Boreal Plain. Journal of Environmental Engineering and Science 2:S51-S62.

Cifuentes, L.A., and G.G. Salata. 2001. Significance of carbon isotope discrimination between bulk carbon and extracted phospholipid fatty acids in selected terrestrial and marine environments. Organic Geochemistry 32:613-621.

Crow, S.E., and K. Lajtha. 2004. Nitrogen additions as a result of long-term root removal affects soil organic matter dynamics American Geophysical Union Annual Meeting, San Francisco, CA.

Dalenberg, J.W., and G. Jager. 1989. Priming effect of some organic additions to C-14-labeled soil. Soil Biology & Biochemistry 21:443-448.

De Nobili, M., M. Contin, C. Mondini, and P.C. Brookes. 2001. Soil microbial biomass in triggered into activity by trace amounts of substrate. Soil Biology & Biochemistry 33:1163-1170.

DeForest, J.L., D.R. Zak, K.S. Pregitzer, and A.J. Burton. 2004. Atmospheric nitrate deposition and the microbial degradation of cellobiose and vanillin in a northern hardwood forest. Soil Biology & Biochemistry 36:965-971.

Dix, N.J., and J. Webster. 1995. Fungal Ecology Chapman and Hall, London.

Dixon, J.J. 2003. Applying GIS to soil-geomorphic landscape mapping in the Lookout Creek valley, Western Cascades, Oregon. M.S. Thesis, Oregon State University, Corvallis, OR.

Dufrene, M., and P. Legendre. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67:345-366.

Dutton, M.V., and C.S. Evans. 1996. Oxalate production by fungi: its role in pathogenicity and ecology in the soil environment. Canadian Journal of Microbiology 42:881-895.

Ekschmitt, K., and B.S. Griffiths. 1998. Soil biodiversity and its implications for ecosystem functioning in a heterogeneous and variable environment. Applied Soil Ecology 10:201-215.

Entry, J.A., P.K. Donnelly, and K. Cromack. 1991. Influence of Ectomycorrhizal Mat Soils on Lignin and Cellulose Degradation. Biology and Fertility of Soils 11:75-78.

Falchini, L., N. Naumova, P.J. Kuikman, J. Bloem, and P. Nannipieri. 2003. CO_2 evolution and denaturing gradient gel electrophoresis profiles of bacterial communities in soil following addition of low molecular weight substrates to simulate root exudation. Soil Biology & Biochemistry 35:775-782.

Fierer, N., and J.P. Schimel. 2002. Effects of drying-rewetting frequency of soil carbon and nitrogen transformations. Soil Biology & Biochemistry 34:777-787.

Fierer, N., J.P. Schimel, and P.A. Holden. 2003. Variation in microbial community composition through two soil depth profiles. Soil Biology & Biochemistry 35:167-176.

Fisk, M.C., and T.J. Fahey. 2001. Microbial biomass and nitrogen cycling responses to fertilization and litter removal in young northern hardwood forests. Biogeochemistry 53:201-223.

Fontaine, S., A. Mariotti, and L. Abbadie. 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology & Biochemistry 35:837-843.

Frey, S.D., J. Six, and E.T. Elliott. 2003. Reciprocal transfer of carbon and nitrogen by decomposer fungi at the soil-litter interface. Soil Biology & Biochemistry 35:1001-1004.

Frey, S.D., V.V.S.R. Gupta, E.T. Elliott, and K. Paustian. 2001. Protozoan grazing affects estimates of carbon utilization efficiency of the soil microbial community. Soil Biology & Biochemistry 33:1759-1768.

Frey, S.D., M. Knorr, J.L. Parrent, and R.T. Simpson. 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. Forest Ecology and Management 196:159-171.

Fritze, H., J. Pietikainen, and T. Pennanen. 2000. Distribution of microbial biomass and phospholipid fatty acids in Podzol profiles under coniferous forest. European Journal of Soil Science 51:565-573.

Griffiths, B.S., K. Ritz, N. Ebblewhite, and G. Dobson. 1999. Soil microbial community structure: Effects of substrate loading rates. Soil Biology & Biochemistry 31:145-153.

Griffiths, R.P., and A.K. Swanson. 2001. Forest soil characteristics in a chronosequence of harvested Douglas-fir forests. Canadian Journal of Forest Research 31:1871-1879.

Hamer, U., and B. Marschner. 2002. Priming effects of sugars, amino acids, organic acids and catechol on the mineralization of lignin and peat. Journal of Plant Nutrition and Soil Science 165:261-268.

Hamer, U., and B. Marschner. 2005. Priming effects in different soil types induced by fructose, alanine, oxalic acid and catechol additions. Soil Biology & Biochemistry 37:445-454.

Hanson, J., J. Macalady, D. Harris, and K. Scow. 1999. Linking toluene degradation with specific microbial populations in soil. Applied and Environmental Microbiology 65:5403-5408.

Hart, S.C., and P. Sollins. 1998. Soil carbon and nitrogen pools and processes in an old-growth conifer forest 13 years after trenching. Canadian Journal of Forest Research 28:1261-1265.

Hill, G.T., N.A. Mitkowski, L. Aldrich-Wolfe, L.R. Emele, D.D. Jurkonie, A. Ficke, S. Maldonado-Ramirez, S.T. Lynch, and E.B. Nelson. 2000. Methods for assessing the composition and diversity of soil microbial communities. Applied Soil Ecology 15:25-36.

HJA LTER. 2005. Primet soil temperature and moisture data set. Available on-line [http://www.fsl.orst.edu/lter/data.cfm?topnav=8] from the Forest Science Data Base, a partnership between the Department of Forest Science, Oregon State University, and the U.S. Forest Service Pacific Northwest Research Station. Corvallis, OR.

Högberg, P., and A. Ekblad. 1996. Substrate-induced respiration measured in situ in a C_3-plant ecosystem using additions of C_4-sucrose. Soil Biology & Biochemistry 28:1131-1138.

Högberg, P., A. Nordgren, N. Buchmann, A.F.S. Taylor, A. Ekblad, M.N. Högberg, G. Nyberg, M. Ottosson-Löfvenius, and D.J. Read. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411:789-792.

Holland, E.A., B.H. Braswell, J.M. Sulzman, and J.-F. Lamarque. 2004. Nitrogen deposition onto the United States and Western Europe. Data set. Available on-line [http://www.daac.ornl.gov] from Oak Ridge National Laboratory Distributed Active Archive Center. Oak Ridge, TN.

IPCC. 2001. Summary for policymakers, *In* R. Watson, ed. Climate change 2001: the scientific basis. Cambridge University Press, Cambridge, UK.

Johnsen, A.R., A. Winding, U. Karlson, and P. Roslev. 2002. Linking of microorganisms to phenanthrene metabolism in soil by analysis of C-13-labeled cell lipids. Applied and Environmental Microbiology 68:6106-6113.

Keirstead, H. 2004. Quantifying C and N contents and isotopic signatures of SOM pools in the H.J. Andrews DIRT plots. M.S. Thesis, Oregon State University, Corvallis, OR.

Kieft, T.L., E. Wilch, K. Oconnor, D.B. Ringelberg, and D.C. White. 1997. Survival and phospholipid fatty acid profiles of surface and subsurface bacteria in natural sediment microcosms. Applied and Environmental Microbiology 63:1531-1542.

Kirschbaum, M.U.F. 2000. Will changes in soil organic carbon act as a positive or negative feedback on global warming? Biogeochemistry 48:21-51.

Kourtev, P.S., J.G. Ehrenfeld, and M. Haggblom. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology 83:3152-3166.

Koutny, M., J. Ruzicka, and J. Chlachula. 2003. Screening for phenol-degrading bacteria in the pristine soils of south Siberia. Applied Soil Ecology 23:79-83.

Kruskal, J.B. 1964. Nonmetric multidimensional scaling: a numerical method. Psychometrika 29:115-129.

Kruskal, J.B. 1964. Nonmetric multidimentional scaling: a numerical method. Psychometrika 29:115-129.

Kuzyakov, Y., and G. Domanski. 2000. Carbon input by plants into the soil. Review. Journal of Plant Nutrition and Soil Science 163:421-431.

Kuzyakov, Y., and W. Cheng. 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology & Biochemistry 33:1915-1925.

Kuzyakov, Y., J.K. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. Soil Biology & Biochemistry 32:1485-1498.

Kuzyakov, Y., H. Ehrensberger, and K. Stahr. 2001. Carbon partitioning and below-ground translocation by Lolium perenne. Soil Biology & Biochemistry 33:61-74.

Lajtha, K., S. Crow, Y. Yano, S.S. Kaushal, E.W. Sulzman, and J.D.H. Spears. 2005. Detrital controls on soil solution N and dissolved organic matter in soils: a field experiment. Biogeochemistry (In Press).

Langley, J.A., and B.A. Hungate. 2003. Mycorrhizal controls on belowground litter quality. Ecology 84:2302-2312.

Lavigne, M.B., R.J. Foster, and G. Goodine. 2004. Seasonal and annual changes in soil respiration in relation to soil temperature, water potential and trenching. Tree Physiology 24:415-424.

Lavoie, M., and R.L. Bradley. 2003. Short-term increases in relative nitrification rates due to trenching in forest floor and mineral soil horizons of different forest types. Plant and Soil 252:367-384.

Li, Y.Q., M. Xu, O.J. Sun, and W.C. Cui. 2004. Effects of root and litter exclusion on soil CO_2 efflux and microbial biomass in wet tropical forests. Soil Biology & Biochemistry 36:2111-2114.

Litton, C.M., M.G. Ryan, D.H. Knight, and P.D. Stahl. 2003. Soil-surface carbon dioxide efflux and microbial biomass in relation to tree density 13 years after

a stand replacing fire in a lodgepole pine ecosystem. Global Change Biology 9:680-696.

Lundquist, E.J., K.M. Scow, L.E. Jackson, S.L. Uesugi, and C.R. Johnson. 1999. Rapid response of soil microbial communities from conventional low input, and organic farming systems to a wet/dry cycle. Soil Biology & Biochemistry 31:1661-1675.

Macdonald, L.M., E. Paterson, L.A. Dawson, and A.J.S. McDonald. 2004. Short-term effects of defoliation on the soil microbial community associated with two contrasting Lolium perenne cultivars. Soil Biology & Biochemistry 36:489-498.

Manefield, M., A.S. Whiteley, R.I. Griffiths, and M.J. Bailey. 2002. RNA stable isotope probing, a novel means of linking microbial community function to Phylogeny. Applied and Environmental Microbiology 68:5367-5373.

Mather, P.M. 1976. Computation methods of multivariate analysis in physical geography J. Wiley and Sons, London.

McCarthy, A.J., and S.T. Williams. 1992. Actinomycetes as agents of biodegradation in the environment - a review. Gene 115:189-192.

McCune, B., and M.J. Mefford. 1999. Multivariate analysis on the PC-ORD system. Version 4 MjM Software, Gleneden Beach, Oregon.

McCune, B., and J.B. Grace. 2002. Analysis of Ecological Communities MjM Software Design, Gleneden Beach, Oregon.

Merila, P., R. Strommer, and H. Fritze. 2002. Soil microbial activity and community structure along a primary succession transect on the land-uplift coast in western Finland. Soil Biology & Biochemistry 34:1647-1654.

Mielke Jr., P.W. 1984. Meteorological applications of permutation techniques based on distance functions, *In* P. R. Krishnaiah and P. K. Sen, eds. Handbook of Statistics, Vol. 4. Elsevier Science Publishers.

Mielke Jr., P.W., and K.J. Berry. 2001. Permutation Methods: A Distance Function Approach.

Mooney, H.A., J. Canadell, F.S. Chapin III, J.R. Ehleringer, C. Korner, R.E. McMurtrie, W.J. Parton, L.F. Pitelka, and E.D. Schulze. 1999. Ecosystem physiology responses to global change, p. 141-189, *In* B. H. Walker, et al., eds. The Terrestrial Biosphere: Global Change Implications for Natural and Managed Ecosystems. Cambridge University Press, Cambridge, UK.

Myers, R.T., D.R. Zak, D.C. White, and A. Peacock. 2001. Landscape-level Patterns of microbial community composition and substrate use in upland forest ecosystems. Soil Science Society of America Journal 65:359-367.

Nadelhoffer, K.J., R.D. Boone, R.D. Bowden, J.D. Canary, J. Kaye, P. Micks, A. Ricca, J.A. Aitkenhead, K. Lajtha, and W.H. McDowell. 2004. The DIRT Experiment: Litter and Root Influences on Forest Soil Organic Matter Stocks and Function, *In* D. Foster and J. Aber, eds. Forest Landscape Dynamics in New England: Ecosystem Structure and Function as a Consequence of 5000 years of Change. Oxford University Press, New York.

Nguyen, C., and A. Guckert. 2001. Short-term utilisation of C-14-[U]glucose by soil microorganisms in relation to carbon availability. Soil Biology & Biochemistry 33:53-60.

Olsson, P.A., and A. Johansen. 2000. Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. Mycological Research 104:429-434.

Padmanabhan, P., S. Padmanabhan, C. DeRito, A. Gray, D. Gannon, J. Snape, C. Tsai, W. Park, C. Jeon, and E. Madsen. 2003. Respiration of ^{13}C-labeled substrates added to soil in the field and subsequent 16S rRNA gene analysis of ^{13}C-labeled soil DNA. Applied and Environmental Microbiology 69:1614-1622.

Park, J.H., and E. Matzner. 2003. Controls on the release of dissolved organic carbon and nitrogen from a deciduous forest floor investigated by manipulations of aboveground litter inputs and water flux. Biogeochemistry 66:265-286.

Parker, J.L., I.J. Fernandex, L.E. Rustad, and S.A. Norton. 2002. Soil organic matter fractions in experimental forested watersheds. Water Air and Soil Pollution 138:101-121.

Pendall, E., S. Bridgham, P.J. Hanson, B.A. Hungate, D.W. Kicklighter, D.W. Johnson, B.E. Law, Y.Q. Luo, J.P. Megonigal, M. Olsrun, M.G. Ryan, and S.Q. Wan. 2004. Below-ground process responses to elevated CO_2 and temperature: a discussion of observations, measurement methods, and models. New Phytologist 162:311-322.

Phillips, R.L., D.R. Zak, W.E. Holmes, and D.C. White. 2002. Microbial community composition and function beneath temperate trees exposed to elevated atmospheric carbon dioxide and ozone. Oecologia 131:236-244.

Post, W.M., T.-H. Peng, W.R. Emanual, A.W. King, V.H. Dale, and D.L. DeAngelis. 1990. The global carbon cycle. American Scientist 78:310-326.

Rey, A., E. Pegoraro, V. Tedeschi, I. De Parri, P.G. Jarvis, and R. Valentini. 2002. Annual variation in soil respiration and its components in a coppice oak forest in Central Italy. Global Change Biology 8:851-866.

Ross, D.J., N.A. Scott, K.R. Tate, N.J. Rodda, and J.A. Townsend. 2001. Root effects on soil carbon and nitrogen cycling in a Pinus radiata D. Don plantation on a coastal sand. Australian Journal of Soil Research 39:1027-1039.

Sahin, N. 2003. Oxalotrophic bacteria. Research In Microbiology 154:399-407.

Schimel, D.S. 1995. Terrestrial ecosystems and the carbon cycle. Global Change Biology 1:77-91.

Schimel, D.S., J.M. Melillo, J. Tian, A.D. McGuire, D.W. Kicklighter, T.G.F. Kittel, N. Rosenbloom, S. Running, P. Thornton, D. Ojima, W. Parton, R. Kelly, M. Sykes, R.P. Neilson, and B. Rizzo. 2000. Contribution of increasing CO_2 and climate to carbon storage by ecosystems in the United States. Science 287:2004-2006.

Schimel, J.P. 1995. Ecosystem consequences of microbial diversity and community structure, *In* F. S. Chapin III and C. Korner, eds. Arctic and Alpine

Biodiversity: Patterns, Causes and Ecosystem consequences, Vol. 113. Springer-Verlag, New York.

Schimel, J.P., and J. Gulledge. 1998. Microbial community structure and global trace gases. Global Change Biology 4:745-758.

Schimel, J.P., J.M. Gulledge, J.S. Clein-Curley, J.E. Lindstrom, and J.F. Baddock. 1999. Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. Soil Biology & Biochemistry 31:831-838.

Schlesinger, W.H. 1997. Biogeochemistry: An Analysis of Global Change Academic Press, San Diego, Ca.

Setala, H., and M.A. McLean. 2004. Decompostion rate of organic substrates in relation to the species diversity of soil saprophytic fungi. Oecologia 139:98-107.

Shen, J., and R. Bartha. 1996. Priming effect of substrate addition in soil-based biodegradation tests. Applied and Environmental Microbiology 62:1428-1430.

Siira-Pietikainen, A., J. Haimi, and H. Fritze. 2003. Organisms, decomposition, and growth of pine seedlings in boreal forest soil affected by sod cutting and trenching. Biology and Fertility of Soils 37:163-174.

Siira-Pietikainen, A., J. Haimi, A. Kanninen, J. Pietikainen, and H. Fritze. 2001. Responses of decomposer community to root-isolation and addition of slash. Soil Biology & Biochemistry 33:1993-2004.

Silver, W.L., and R.K. Miya. 2001. Global patterns in root decomposition: comparisons of climate and litter quality effects. Oecologia 129:407-419.

Simard, S.W., D.A. Perry, J.E. Smith, and R. Molina. 1997. Effects of soil trenching on occurrence of ectomycorrhizas on Pseudotsuga menziesii seedlings grown in mature forests of Betula papyrifera and Pseudotsuga menziesii. New Phytologist 136:327-340.

Smith, J.L., and E.A. Paul. 1990. The significance of soil microbial biomass estimations, p. 357-393, *In* J. Bollag and G. Stotsky, eds. Soil Biochemistry, Vol. 8. Mercel Dekker, New York, New York.

Spears, J.D.H., S.M. Holub, M.E. Harmon, and K. Lajtha. 2003. The influence of decomposing logs on soil biology and nutrient cycling in an old-growth mixed coniferous forest in Oregon, USA. Canadian Journal of Forest Research 33:2193-2201.

Subke, J.A., V. Hahn, G. Battipaglia, S. Linder, N. Buchmann, and M.F. Cotrufo. 2004. Feedback interactions between needle litter decomposition and rhizosphere activity. Oecologia 139:551-559.

Sulzman, E.W., J.B. Brant, R.D. Bowden, and K. Lajtha. 2005. Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO_2 efflux in an old growth coniferous forest. Biogeochemistry (In Press).

Tingey, D.T., D.L. Phillips, M.G. Johnson, P.T. Rygiewicz, P.A. Beedlow, and W.E. Hogsett. 2005. Estimates of Douglas-fir fine root production and mortality from minirhizotrons. Forest Ecology and Management 204:359-370.

Vance, E.D., P.C. Brookes, and D.S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry 19:703-707.

Vanderbilt, K.L., K. Lajtha, and F.J. Swanson. 2003. Biogeochemistry of unpolluted forest watersheds in the Oregon Cascades: temporal patterns of precipitation and stream nitrogen fluxes. Biogeochemistry 62:87-117.

Wagner, G.H., and D.C. Wolf. 1999. Carbon transformations and soil organic matter formation, *In* D. M. Sylvia, et al., eds. Principles and Applications of Soil Microbiology. Prentice Hall, Upper Saddle River, NJ.

Waldrop, M.P., and M.K. Firestone. 2004. Altered utilization patterns of young and old soil C by microorganisms caused by temperature shifts and N additions. Biogeochemistry 67:235-248.

Waldrop, M.P., and M.K. Firestone. 2004. Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities. Oecologia 138:275-284.

Waldrop, M.P., T.C. Balser, and M.K. Firestone. 2000. Linking microbial community composition to function in a tropical soil. Soil Biology & Biochemistry 32:1837-1846.

Waldrop, M.P., D.R. Zak, and R.L. Sinsabaugh. 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biology & Biochemistry 36:1443-1451.

Wallander, H., L.-O. Nilsson, D. Hagerberg, and E. Bååth. 2001. Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. New Phytologist 151:753-760.

Waring, R.H., and J.F. Franklin. 1979. Evergreen coniferous forests of the Pacific Northwest. Science 204:1380-1386.

White, D.C., and D.B. Ringelberg. 1998. Signature lipid biomarker analysis, p. 255-272, *In* R. S. Burlage, et al., eds. Techniques in Microbial Ecology. Oxford University Press, New York, NY.

Wiemken, V., E. Laczko, K. Ineichen, and T. Boller. 2001. Effects of elevated carbon dioxide and nitrogen fertilization on mycorrhizal fine roots and the soil microbial community in beech-spruce ecosystems on siliceous and calcareous soil. Microbial Ecology 42:126-135.

Wilkinson, S.C., J.M. Anderson, S.P. Scardelis, M. Tisiafouli, A. Taylor, and V. Wolter. 2002. PLFA profiles of microbial communities in decomposing conifer litters subject to moisture stress. Soil Biology & Biochemistry 34:189-200.

Wu, J.G., P.C. Brookes, and D.S. Jenkinson. 1993. Formation and destruction of microbial biomass during the decomposition of glucose and ryegrass in soil. Soil Biology & Biochemistry 25:1435-1441.

Yano, Y., K. Lajtha, P. Sollins, and B.A. Caldwell. 2005. Chemistry and dynamics of dissolved organic matter in a temperate coniferous forest on andic soils: effects of litter quality. Ecosystems (In Press).

Zak, D.R., K.S. Pregitzer, P.S. Curtis, and W.E. Holmes. 2000. Atmospheric CO_2 and the composition and function of soil microbial communities. Ecological Applications 10:47-59.

Zelles, L. 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review. Biology & Fertility of Soils 29:111-129.

Appendix

Justin B. Brant

Phospholipid Fatty Acid Extraction Protocol (modified for forest soil)

Reagents

- All reagents should be HPLC grade or better
- N_2 gas should be grade 4.0 or better
- All glassware should be acid-washed
- All steps should be performed in a fume hood

Phase 1

- Methanol
- Chloroform
- Phosphate buffer (1:1 solution of 0.1 M KH_2PO_4 and 0.1 M $K_2HPO_4 \cdot 3H_2O$)
  - Final pH of 1:1 solution of two buffer component 7.1 ± 0.1
    - If pH of final solution different adjust concentration of components
- Deionized H_2O

Phase 2

- Methanol
- Chloroform
- 3 M NaCl
- Sodium sulfate (Na_2SO_4)

Phase 3

- Chloroform
- Methanol
- Acetone

Phase 4

- Methanol (needs to be of known $\delta^{13}C$ if doing ^{13}C-PLFA analysis)
- 0.2 M methanolic KOH (5.6 g KOH in 500 mL methanol)
- toluene
- 1 M acetic acid (5.75 mL 17.4 M acetic acid in 100 mL dH_2O)
- Hexanes
- Chloroform

Procedure

Phase 1

1. Weight 2 g (dry wt.) soil into 250 mL serum vials
2. Add Bligh Dyer solution in approximately 4:1 ratio with dry soil (i.e. 2 g soil needs ~8 mL solution)
    a. Bligh Dyer solution consists of 2:1:0.8 ratio solution of methanol: chloroform: 1:1 phosphate buffer (i.e. 5 mL methanol: 2.5 mL chloroform: 2 mL phosphate buffer)
    b. 1:1 buffer is mixed at 2 x necessary concentration. Dilute to correct strength using 2 mL ddH_2O. Include H_2O in soil to added water. (i.e. if field moist soil contains 0.5 mL H_2O add an additional 1.5 mL H_2O)
3. Cover serum vials with tin foil. Secure tin foil with rubber band.
4. Shake on orbital shaker at 250 rpm for 2 hours
5. Let stand overnight at room temperature.

Phase 2

1. Wash glass funnels with chloroform and methanol. Insert Whatman #1 filter paper. Filter into Erlenmeyer flask or beaker
2. Centrifuge soil at 1000 rpm for 5 m.
3. Add the liquid form each sample to filter paper. Be careful to pour both methanol and chloroform phases into filter paper. Try to get as little soils as possible on filter paper, as it slows filtering, causing reagents to evaporate
4. Add another 5 mL of methanol and 2.5 mL of chloroform to each serum vial and swirl to remix.
5. Centrifuge as above, and filter through same filter paper into same flask.
6. Repeat steps 4 and 5.
7. Flush filter paper with 5 mL chloroform
8. Add 10 mL 3 M NaCl and ~0.5 g Na_2SO_4 to each flask.
9. Allow phases to separate and for chloroform phase to become clear (~ 1 h).
10. Remove chloroform phase (bottom phase) with a pipette and put in 60 mL serum vial
11. Rinse each flask with 5 mL chloroform and remove to same serum vial.
12. Dry each sample under N_2 in warm water bath (< 32°C)
    a. Do not bubble N_2 through sample
13. Seal serum bottle with septum and crimp-top
14. Evacuate bottles for 1 m.
15. Flush with N_2 for 1 m (put venting needle in after 10 s of flushing)
16. Repeat steps 14 and 15.
17. Remove venting needle from septum before shutting off gas to create positive pressure
18. Wrap top of vial with Teflon tape and Parafilm.
19. Store at -80°C.

Phase 3

1. Rinse SPE manifold in chloroform, acetone, and methanol
2. Put 3 mL Supelclean SPE columns on manifold (Supelco part #505048)
3. Prepare column by adding 1 mL chloroform to each column
4. Collect desired phase in acid-washed 15 x 85 mm borosilicate glass culture tubes (i.e. VWR part # 47729-574)
    a. chloroform phase = neutral lipids
    b. acetone phase = glycolipids
    c. methanol phase = phospholipids
5. Add 2 mL to dried sample. Swirl and add to column (cover tubes with aluminum foil after sample addition to prevent cross-contamination)
6. Wash serum vial twice with 1 mL chloroform and add to same column
7. Add 2 mL chloroform (2x) to each column when most of sample has gone through
8. Add 2 mL acetone 3x to each sample
9. Add 2 mL methanol 3x to each sample
10. Dry each collected sample under N_2 in warm water bath (<32°C)
11. If not proceeding directly to Phase 4 store dried samples under N_2 at -80°C in mason jar (try to perform Phase 3 and 4 within 2 d of each other).

Phase 4

1. Add 1 mL 1:1 methanol: toluene (prepare in beaker)
2. Add 1 mL 0.2 M methanolic KOH (prepare with methanol of known $\delta^{13}C$ if doing ^{13}C PLFA)
3. Heat at 32-37°C for 15 m
4. Add 2 mL 4:1 hexanes:chlorofrom
5. 200 µL of 1 M acetic acid
6. 2 mL ddH_2O (make sure acetic acid and methanolic KOH are pH balanced, remake solutions if 1 mL of KOH solution and 200 µL acetic acid don't return H_2O to original pH when added in sequence).
7. Transfer top layer (hexane/toluene/chloroform) to new acid-washed culture tube
8. Add another 2 mL hexanes: chloroform to sample, let sit for a few minutes and transfer to same tube.
9. Dry under N_2 in warm H_2O bath.
10. After drying, add 50 µL chloroform to tube
    - Remove without much agitation as most lipid at bottom of tube
11. Put in 200 µL insert in 2 mL Agilent vial
12. Repeat step 10 twice with the exception that tube should be flicked a few times to mix
13. Dry under N_2 using 1.5" 20G needles at very low pressure as to not blow any sample out of tube.
    - May have to increase pressure at very end to dry last bit of sample
14. Add 40 µL hexane to insert, cap and store at -20°C until analysis.