ECOSYSTEM CARBON CYCLING: RELATIONSHIPS TO SOIL MICROBIAL COMMUNITY STRUCTURE

by

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(Under the Direction of Mark A. Bradford)

ABSTRACT

Many processes related to carbon-cycling in terrestrial ecosystems are carried out by soil microbes. Our understanding of the relationship between carbon cycling and microbial community structure is, at best, rudimentary. The role of microbial community structure in carbon-cycling processes has, for the most part, been treated as either functionally redundant or driven by binary distinctions (e.g. fungal:bacterial ratios). It was the purpose of this work to test these generalizations and advance our understanding how soil microbes regulate ecosystem carbon dynamics. This purpose was accomplished using field-based observation and controlled lab-based experimentation. In the first of the field-based studies, I determine the mineralization dynamics of glucose and its relationship to land-use, microbial community and edaphic characteristics across a southeastern U.S. landscape. I find that the size, activity, and fungal:bacterial dominance of the microbial community is unrelated to glucose mineralization but that land-use and the underlying edaphic variable, soil phosphorus, explains spatial variation in this process. In the second field-based study, I examine how an invasive grass affects belowground carbon-cycling in a southeastern forest. Again, I find no role of fungal:bacterial dominance but observe
that the presence of the invasive grass accelerates carbon-cycling and depletes soil carbon stocks, likely via ‘priming’ of the microbial community’s activity. Using lab-based experimentation, I explore the role of microbial community structure separate to the influence of confounding variables. I find that litter mineralization rates are dependent on the microbial community and that these communities exhibit ‘home-field advantage’. These results refute theories related to functional redundancy. In a follow-up experiment, I find that the perception of litter quality, of two chemically-distinct litters, is dependent on the microbial community. Specifically, communities sourced from herbaceous habitats perceive more chemically-simple litter to be of higher quality than more chemically-complex litter, whereas communities from forest habitats mineralize both litters similarly. Together, my findings show that although microbial community structure may appear unrelated to carbon dynamics in situ, controlled-experimentation reveals that this structure may play an important role in determining rates of ecosystem processes. Future work needs to resolve why there is an apparent disconnect between results from observational and controlled-experimental studies.

INDEX WORDS: carbon-cycling, microbial communities, bacteria, fungi, land-use, invasive species, glucose, stable isotopes, priming effect, preferential substrate utilization, leaf litter
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DEDICATION

I dedicate this dissertation to Delmer and Minnie Strickland, the best parents in the world. I could not have accomplished this without your love and support.
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CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

Fungal-to-bacterial dominance in soil – A review

Soil microbial communities are an integral component of many ecosystem level processes from N-fixation and methane oxidation to the mineralization of both simple and complex organic compounds (Bedard and Knowles 1989, Zak et al. 2006, Jackson et al. 2007). Because of this, the role that these communities play at the ecosystem level has become a major line of study in both soil and microbial ecology (Jackson et al. 2007). Although a seemingly straightforward proposition, actually gaining an understanding of these communities regarding their relationship to environmental factors and ecosystem function, as well as developing the methods to accurately assess them, has often proven difficult (Barns et al. 1999). One reason for this is likely the fact that these soil communities are some of the most diverse on Earth, with in excess of \(4 \times 10^4\) species found in a single soil sample (Torsvik et al. 2002, Fierer et al. 2007b). A second contributor to these difficulties is the opaque nature of the soil environment itself, which makes the direct observation of soil communities nearly, if not totally, impossible.

Given both of these difficulties and likely others, the predominant approach to understanding soil microbial communities has often been to simplify the community by dividing it into two categories of ecologically meaningful groups (Koch 2001). An example of this is the idea of copiotrophs versus oligotrophs where copiotrophs are

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organisms which thrive under high resource conditions and oligotrophs thrive under low resource conditions (Poindexter 1981, Koch 2001, Fierer et al. 2007a). Other examples of these sorts of distinctions include Winogradsky’s idea of autochthonous versus zymogenous microbial biomass (Winogradsky 1924) and $r$-versus $K$-selected organisms (Fierer et al. 2007a). Although these sorts of distinctions have aided greatly in our theoretical understanding of soil microbial communities, they all suffer from the fact that they are largely unmeasurable in the environment. That is, assessing whether or not a community is largely composed of copiotrophs or oligotrophs, for example, requires researchers to both know the abundance of specific taxonomic groups in a given soil as well as their life-history characteristics (Jackson et al. 2007, Green et al. 2008). Such an understanding of specific organisms is currently lacking, making measurements based on these community distinctions impossible (Jackson et al. 2007, Green et al. 2008).

One measure of the soil microbial community which is likely to be relevant to both taxonomy and life history characteristics is the dominance of fungi to bacteria (Wardle et al. 2004, van der Heijden et al. 2008). Although the methods used to determine fungal:bacterial dominance should not necessarily be considered easy, fungi and bacteria in general differ with regards to key traits (e.g. PLFA markers) which allow researchers to distinguish between them (Anderson and Domsch 1973, Frostegård and Bååth 1996). Furthermore, these two groups are expected to differ with regards to their life history characteristics which in turn are likely to influence both their response to environmental change as well as their impact on ecosystem processes (Hendrix et al. 1986, Holland and Coleman 1987, Yeates et al. 1997, Bardgett and McAlister 1999). That is the fungal:bacterial dominance of a soil community is both taxonomically and
likely ecologically relevant (van der Heijden et al. 2008). For this reason as well as the difficulties associated with making other categorical distinctions of the microbial community, assessing soil communities based on their fungal:bacterial dominance has been widely used (Bardgett et al. 1996, Yeates et al. 1997, Bardgett and McAlister 1999, Rousk et al. 2009, Rousk and Nadkarni 2009). This simple but elegant approach has provided insight into the opaque soil system and led to concepts which are widely used and discussed in today’s soil ecology literature (Figure 1).

The purpose of this review will be to examine many of these concepts. First we will briefly review and discuss the prominent methods employed to assess fungal:bacterial dominance (Section I). In Section II, we will examine some of the major environmental factors which are likely to lead to differences in the fungal:bacterial dominance of soil microbial communities. The third section will discuss the relationship between fungal:bacterial dominance and two major ecosystem processes: carbon (C) sequestration and litter decomposition. Finally, we will conclude by examining our current understanding of fungal:bacterial dominance and highlight areas where greater knowledge may lead to advances in this concept. Ultimately, we hope that this review will provide a general overview on concepts related to the fungal:bacterial dominance of soil microbial communities.

Section I – Techniques to measure fungal:bacterial dominance

Over the years a wide array of techniques have been developed which allow for the assessment of soil microbial communities based on their fungal:bacterial dominance (Joergensen and Wichern 2008). Although many of these measures utilize different
approaches, they are all driven by the use of key distinctions which can be made between fungi and bacteria. For example, Frostegård and Bååth (1996) used PLFA’s which were distinct to bacteria and fungi to estimate the biomass of each group in soil. Alternatively, Anderson and Domsch (1973) utilized selective inhibition with antibiotics which inhibit either fungi or bacteria to similarly estimate their dominance. Both of these techniques, as well as direct observation (i.e. microscopy) and the use of cell wall derived indicators of fungi and bacteria (e.g. chitin and muramic acid), were recently reviewed by Joergensen and Wichern (2008). This review found that across major land-use groups (i.e. cultivated, grassland, forest, and litter layers) these methods generally gave comparable results (Joergensen and Wichern 2008). The fact that each of these methods is ultimately an estimate of the biomass of the fungi and bacteria may have led to these similarities. Measures of whole microbial biomass using different methods are often found to correlate across large spatial scales but are not necessarily 1:1 at smaller spatial scales (Wardle and Ghani 1995). This discrepancy as resolution increases is largely attributed to the possibility that different biomass measures actually gauge slightly different components/characteristics of the microbial community (Wardle and Ghani 1995). Whether this holds true for estimates of fungal:bacterial dominance determined via different methods remains unknown. Furthermore, it is often difficult to ascertain the status/activity of the microbial biomass in soil (Rousk and Bååth 2007b). Meaning that methods such as these make the assumption that the biomass of a given group is a direct reflection of that groups contribution to a certain ecosystem process or its response to environmental change (Wright et al. 1995, Bååth 1998). Such assumptions have been frequently questioned (Wright et al. 1995, Bååth 1998, Rousk and Bååth 2007b).
A recent alternative measure for assessing fungal:bacterial dominance, is the use of DNA-based approaches. One of the more widely used of these approaches is quantitative polymerase chain reaction (qPCR). This approach uses the accumulation of a fluorescent reporter molecule during the PCR reaction coupled with primers specific to either bacteria or fungi (which typically target the 16s or 18s rRNA gene segments, respectively) to determine the relative abundance of these groups (Fierer et al. 2005). qPCR represents both a rapid and quantifiable approach to assess fungal:bacterial dominance in soils (Fierer et al. 2005). However, like with the above biomass measures there are caveats associated with this technique. Foremost, is that fungal:bacterial dominance as determined by qPCR may not be indicative of the true abundances of these groups in soil especially on a per biomass basis. Reasons for this include the fact that fungal cells may include many or no nuclei, leading to an over or underestimation of fungal abundance, DNA extraction efficiencies may differ between these two groups, and the amplification of genes may not be consistent across all fungal and bacterial taxa (Martin-Laurent et al. 2001). Finally, like with biomass measures, assessments of fungal:bacterial dominance based on DNA may not necessarily be related to a given ecosystem process or response to environmental change. This is because DNA is present in both active and inactive cells (Nocker and Camper 2009). Fungal:bacterial dominance determined via RNA based approaches may ultimately prove more informative when assessing relationships which are likely to be driven by active organisms (Poulsen et al. 1993, Aviv et al. 1996, Vestergard et al. 2008, Nocker and Camper 2009).

Another method which is distinct from biomass and DNA based approaches, is the determination of bacterial and fungal growth (Bååth 1992, Rousk and Bååth 2007b).
This method uses leucine/thymidine incorporation to measure bacterial growth and acetate-in-ergosterol incorporation to measure fungal growth (Bååth 1992, Rousk and Bååth 2007b). Both techniques estimate the biomass production of bacteria or fungi and may provide a direct estimate of the relationship between these groups and a given ecosystem process or their response to environmental change (Rousk and Bååth 2007b). In fact these techniques have been successfully used to determine relative changes in fungal and bacterial contribution to decomposition (Rousk and Bååth 2007a). However, a lack of adequate conversion factors have made quantification of bacterial and fungal growth in units C difficult, although developments suggest progress for both bacteria (Bååth 1994) and fungi (Rousk and Bååth, 2007b). Furthermore the accuracy of these assessments of fungal:bacterial dominance are contingent upon certain requirements being met such as balanced growth or that all bacterial or fungal taxa can take up their respective indicator molecule through the cell membrane (Winding et al. 2005).

In summary, all the techniques used to determine fungal:bacterial dominance in soil are by necessity indirect and are thus susceptible to various confounding factors that should be carefully considered when relating them to either ecosystem processes or a community’s response to environmental change. It is also likely that these relationships will often be dependent on the method used to assess fungal:bacterial dominance. The possibility that different methodologies lead to different results or at least stronger/weaker relationships should be addressed in the future. This will likely require collaborations between researchers whose expertise lay in assessing fungal:bacterial dominance via differing methods. Currently the wide array of methods used to assess fungal:bacterial dominance and the fact that researchers often relate this ratio to a wide
array of ecosystem processes and environmental factors makes a formal meta-analysis nearly impossible. However, generalizations related to the environmental factors likely to affect fungal:bacterial dominance and the impact fungal:bacterial dominance has on ecosystem processes can still be made.

**Section II – Environmental factors affecting fungal:bacterial dominance**

Much of the rationale for using fungal:bacterial dominance as an assessment of the soil microbial community rests on the proposition that these two groups of organisms respond dissimilarly to an array of environmental factors (van der Heijden et al. 2008). Such environmental factors include physical disturbance of the soil (e.g. tilling), nutrient availability, soil moisture, and soil pH. As these factors and others differ in direction and/or intensity then the general expectation is that fungi and bacteria will often exhibit contrasting responses (Hendrix et al. 1986, Holland and Coleman 1987, van der Heijden et al. 2008). The general consensus has been that highly disturbed and/or nutrient rich soils are dominated by bacteria whereas fungi dominate less disturbed and/or nutrient poor soils (Bardgett and McAlister 1999, van der Heijden et al. 2008). For the remainder of this section we will explore some of the key environmental factors likely to lead to differences in the fungal:bacterial dominance of soil communities.

*Physical disturbance and tilling effects*

Much of the interest in the fungal:bacterial dominance of soils has been related to agricultural practices (Hendrix et al. 1986, Holland and Coleman 1987, Drijber et al. 2000, Bailey et al. 2002, Helgason et al. 2009). Hendrix et al. (1986) proposed that no-till
agricultural practices would result in a fungal dominated system as opposed to the bacterial dominated one expected under conventional tillage practices. This expected outcome is largely based on key differences in the growth forms of fungi and bacteria (Hendrix et al. 1986). Most fungi exhibit a hyphal growth form while most soil bacteria are present as individual cells (Hendrix et al. 1986). The hyphal growth form of fungi likely conveys several advantages to these organisms. Chief of which is the translocation of nutrients and resources from microsites where these are abundant to sites where they are limiting via hyphal networks (Hendrix et al. 1986, Frey et al. 2003). This phenomenon has been commonly referred to as the hyphal bridge (Hendrix et al. 1986, Frey et al. 2003). This fungal advantage though may be unfavourable under conventional agricultural practices where the hyphae are continuously severed or disrupted due to tillage (Hendrix et al. 1986, Helgason et al. 2009).

In addition to any direct damage caused by tillage, any advantage fungi gain from hyphal bridges would likely be negated (Hendrix et al. 1986). Bacteria on the other hand would be relatively unaffected by tillage due to their presence as individual cells (Hendrix et al. 1986). Furthermore, tillage homogenizes the soil, evenly distributing nutrients and resources. Just as surely as severing hyphae, this too negates many of the advantages hyphal bridges convey fungi (Bardgett et al. 1996, Bardgett et al. 1999, Bardgett and McAlister 1999). Add to this the generally held idea that bacteria are more efficient colonizers of the organic resources (e.g. newly available SOM and dead microorganisms) made available post-till than are fungi and it seems safe to conclude that fungal:bacterial dominance will decrease under conventional tillage practices (Holland and Coleman 1987). Similar arguments have been made to describe the negative impacts
earthworms have on fungal dominance as well as other factors which physically disturb the soil (Butenschoen et al. 2007). Empirical evidence in support of such impacts on fungal:bacterial dominance are far from definitive however.

Of five studies which explicitly compared the fungal:bacterial dominance of communities exposed to conventional tillage practices to those exposed to no-till practices, none found consistent evidence of tillage effects (Frey et al. 1999, Bailey et al. 2002, Pankhurst et al. 2002, Spedding et al. 2004, Helgason et al. 2009). Of these studies, three found that changes to no-till or a reduction in tillage did lead to an overall increase in microbial biomass but this increase was proportional for both bacteria and fungi (Pankhurst et al. 2002, Spedding et al. 2004, Helgason et al. 2009). Bailey et al. (2002) found that fungal dominance determined via selective inhibition increased under no-till practices but the opposite was true when fungal dominance was determined using PLFA. Although the PLFA measurements in this study were not replicated, it does suggest that different methodologies may lead to dissimilar results (see Section I). Fungal:bacterial dominance was determined via PLFA by Frey et al. (1999) and they found that fungal dominance did increase under no-till practices. However, closer examination of these results showed that tillage covaried with soil moisture and once this was accounted for, tillage had no impact on fungal:bacterial dominance (Frey et al. 1999). Although studies such as these raise doubt concerning the impact that physical disturbance of soil will have on the fungal:bacterial dominance of the microbial community, they also demonstrate that other factors may well influence the relative dominance of these two groups.
Direct and indirect effects of nutrients

Another characteristic likely to influence the fungal:bacterial dominance of soil microbial communities is nutrient availability (Bardgett and McAlister 1999, Suzuki et al. 2009). This like physical disturbance has been largely related to agricultural based management decisions such as fertilizer inputs (Bardgett et al. 1996, Bardgett et al. 1999, Bardgett and McAlister 1999). In many ways the hypothesized impacts fertilizer inputs are likely to have on fungal:bacterial dominance are similar to those hypothesized for tillage. For example, fertilizer applications in effect homogenize soil nutrient availability and likely negate any advantages associated with the fungal growth form.

Stoichiometric differences between bacteria and fungi is another possible reason why fungal:bacterial dominance is expected to decrease as nutrient inputs into the soil increase (van der Heijden et al. 2008). Generally the carbon:nitrogen (C:N) ratio of bacterial biomass is expected to be ~ 3-6 while the C:N ratio of fungal biomass is expected to be ~ 5-15 (McGill et al. 1981). Because it can generally be assumed that fungal biomass has a greater C:N ratio than bacterial biomass then nutrient applications are likely to favour bacteria over fungi (Gusewell and Gessner 2009). This rationale is not solely limited to explaining fertilizer inputs. It has also been used to explain the effects of litter quality on fungal:bacterial dominance (see Section III for further details related to this). However, whether or not the biomass stoichiometry of bacteria is distinct enough from that of fungi to rationalize such an outcome is relatively unexplored.

Much of what is known about bacterial biomass C:N can be attributed to cultured organisms and more often than not these organisms were grown on relatively nutrient rich media (McGill et al. 1981). Bacteria under more natural settings are less
likely to encounter such nutrient rich environments and their actual C:N ratio may be much higher than previously assumed. In fact a compilation of studies examining communities of aquatic bacteria and cultures grown on nutrient poor media have found that bacteria range in C:N from ~ 3-12 (Fig. 2). Furthermore, assessments of the C:N ratio of fungi, based largely on sporocarps, show a range of ~3-51 for mycorrhizal fungi and ~ 4-60 for saprotrophic fungi (Fig. 2). This in the least suggests the possibility that a large proportion of fungi and bacteria, in general, overlap with regards to biomass C:N (Fig. 2). In soils, less is known concerning this possible overlap in the fungal and bacterial C:N ratio largely because of the difficulty in both measuring pure bacterial biomass *in situ* and culturing specific soil bacteria (Madsen 2005, Kreuzer-Martin 2007).

Cleveland and Liptzin (2007) have provided some circumstantial evidence that the C:N ratio of soil bacteria and fungi may not be as distinct a characteristic as previously thought. They found no difference in microbial biomass C:N ratios across a range of ecosystem types where fungal:bacterial dominance would be expected to differ (Cleveland and Liptzin 2007). If bacteria and fungi are distinct with regards to their biomass C:N ratio then the expectation would have been that total microbial biomass C:N in fungal-dominated systems would have been greater than in bacteria-dominated systems. This was not the case and in fact forests which are expected to have greater fungal dominance than grasslands actually had a lower microbial biomass C:N ratio. These results are suggestive of either a significant overlap in the C:N ratio of bacteria and fungi or that the most abundant organisms in either group are stoichiometrically similar.

Regardless of this lack of stoichiometric distinction, several studies have found that N fertilization impacts fungal:bacterial dominance. A prominent example is
Bardgett and McAlister (1999), who observed that pastures which received little or no inputs of fertilizer N typically had a greater fungal:bacterial dominance than did sites which received large inputs of fertilizer N. An experimental component of Bardgett and McAlister’s (1999) study, however, found that fertilizer cessation did not lead to a significant increase in fungal:bacterial dominance after 6 years. They suggested that this may have been due to high residual fertility.

Similar studies have also reported conflicting results for both pasture and forest sites (de Vries et al. 2006, Demoling et al. 2008). de Vries et al. (2006) found using a 2 year field-based experimental study that low fertilization rates led to an increase in fungal:bacterial dominance. On the other hand, a follow up field observation found that fungal:bacterial dominance was not related to changes in management intensity (de Vries et al. 2007). This was because both bacterial and fungal biomass responded similarly to changes in management intensity (de Vries et al. 2007). Demoling et al. (2008) found that across an N deposition gradient in coniferous forests that fungal:bacterial dominance (estimated using PLFA) increased as deposition decreased. Interestingly however, Demoling et al. (2008) did not find a concomitant change in fungal growth (estimated using acetate-in-ergosterol incorporation). The likely explanation for this was attributed to a shift toward more mycorrhizal fungi as N-deposition decreased since the fungal growth estimate (i.e. acetate-in-ergosterol incorporation) is likely only relevant to saprotrophs. This increase in the biomass of mycorrhizal fungi raises an interesting and less direct means that changes in nutrient levels are likely to affect fungal:bacterial dominance.
The nutrient acquisition strategies of plants often change concomitantly with changes in the concentration of soil nutrients (Lambers et al. 2008). In a review by Lambers et al. (2008), plants in young soils which are typically rich in P but poor in N were associated with relatively low levels of mycorrhizae and the same was true for ancient soils, poor in both nutrients. On the other hand, the association between plants and mycorrhizae are typically highest in soils where the N:P ratio is greatest (Lambers et al. 2008). Soils typical of this sort of nutrient status are by large found in temperate zones around the world where many studies investigating fungal:bacterial dominance have likely been conducted. Additionally, changes in the status of soil nutrients have been shown to dramatically impact mycorrhizal fungi with increased nutrient additions often leading to their decline (Oehl et al. 2004, Cruz et al. 2009, but see Birkhofer et al. 2008). These findings would suggest that plant nutrient status is likely to be a major factor impacting the fungal:bacterial dominance of the microbial community (van der Heijden et al. 2008). If researchers are interested in the effect that nutrient status has on fungal:bacterial dominance then close consideration of mycorrhizal fungi may not be necessary. On the other hand, if researchers are interested in relating ecosystem processes to fungal:bacterial dominance then the distinction between mycorrhizal and saprotrophic fungi is likely to prove important. Especially given the likely distinct ecological strategies exhibited by these two groups.

**Other effects**

Other factors which have been proposed to impact fungal:bacterial dominance include soil pH, temperature, and moisture (Holland and Coleman 1987, Rousk et al. 2009). Of
these soil pH, considered a sort of master variable, is likely to have significant impacts on the fungal:bacterial dominance of microbial communities (Fierer and Jackson 2006). Recent work has demonstrated that one of the major controls in soil on both the diversity and composition of bacterial communities is pH (Fierer and Jackson 2006, Lauber et al. In Review). This work has shown that bacterial diversity is related unimodally to soil pH with a peak in diversity found around pH 6-7 (Fierer and Jackson 2006, Lauber et al. In Review). Soil pH also appears to be related to fungal dominance but this possible relationship has been less intensively studied (Joergensen and Wichern 2008, Rousk et al. 2009). In general the expectation has been that fungi are likely more acid tolerant than are bacteria which in turn leads to increased fungal dominance in acidic soils (Hogberg et al. 2007, Joergensen and Wichern 2008, Rousk et al. 2009).

This though does not appear to be a conclusive pattern with alterations in pH sometimes resulting in increased, decreased, or unchanged levels of fungal dominance (Marstorp et al. 2000, Bååth and Anderson 2003, Hogberg et al. 2007, Rousk et al. 2009). One possible explanation for these seemingly inconsistent patterns may be that like soil bacteria specific fungal taxa are impacted to a greater or lesser degree by changes in pH. For example, Rousk et al. (2009) suggested that such observed variation may in part be driven by an increase in ectomycorrhizal fungi in acidic soils due ultimately to a shift in plant species composition. In this same work, they found that, like bacterial diversity, fungal dominance followed a similar unimodal pattern (Rousk et al. 2009). They also suggest that this pattern was largely driven by saprotrophic fungi because no ectomycorrhizae were present at their study site (Rousk et al. 2009). Another possibility is that fungal-dominance or the composition of the fungal community is simply not
impacted to the same degree by pH as the bacterial community is. Work conducted across a land-use gradient in the Southeastern U.S. found the relative abundance of fungal taxa were more strongly related to soil P and C:N ratios than any other edaphic characteristic (Lauber et al. 2008). If this pattern holds true across a range of systems then it might imply that the fungal component of the soil microbial community is likely to be impacted to a lesser degree than is the bacterial community by changes in soil pH.

Our understanding of the impact that moisture and temperature have on soil microbial communities has often been related to their role as mediators of ecosystem processes such as decomposition (Aerts 1997). However, given the likelihood that temperature and moisture regimes are apt to change both in degree and frequency across broad geographical regions in the face of global climate change, these factors and their effect on fungal:bacterial dominance have garnered increased attention (Allison and Treseder 2008). In general as with most expected effects of environmental perturbations, it has been proposed that fungi will exhibit less of a response to changing temperature and moisture than will bacteria (Holland and Coleman 1987). This is in many ways related to the fact that fungi have thick cells walls composed of chitin which are apt to be more resistant to desiccation caused by warm, dry conditions or to the stress caused by rapid changes in these conditions (Holland and Coleman 1987).

Frey et al. (1999) found, however, that fungal biomass was positively related to soil moisture in cultivated settings while bacterial biomass remained relatively constant. Yielding a fungal:bacterial dominance that was positively related to soil moisture (Frey et al. 1999). Similarly, Gordon et al. (2008) found that drying and subsequent rewetting events led to a decrease in fungal:bacterial dominance with no
change in bacterial biomass but a significant change in fungal biomass. Both studies show, in contrast to earlier expectations, that fungi are likely to be affected to a greater extent by changes in soil moisture than are bacteria. Frey et al. (1999) proposed that because bacteria are generally expected to inhabit soil pore spaces whereas fungal hyphae are generally found on the exterior of soil aggregates then bacteria are in effect physically protected from desiccation to some extent. Extending on this idea, bacteria would also be less affected by mild drying/rewetting events. This is not to say that bacteria are in some way physiologically more resistant/resilient to moisture effects but the habitat likely occupied by these organisms may well infer greater resistance/resilience (Six et al. 2006). If this is true then the protection from moisture stress inferred on the bacterial component of the microbial community is likely to be influenced greatly by soil texture (Six et al. 2006).

Temperature effects like moisture do not appear to follow the general consensus. In fact, the response of both bacteria and fungi to changing temperature appears to largely be in the same direction resulting in little to no change in fungal:bacterial dominance (Allison and Treseder 2008, Bárcenas-Moreno et al. 2009). For example, Allison and Treseder (2008) found that the relative abundance (determined via qPCR) of both bacteria and fungi declined by ~ 50% under experimental soil warming. This would have resulted in no change in fungal:bacterial dominance although specific fungal species were affected by warming (Allison and Treseder 2008). Another study, found that the actual growth rates of fungi and bacteria (determined via leucine/thymidine and acetate-in-ergosterol incorporation) responded similarly to changing temperatures (Bárcenas-Moreno et al. 2009). It would appear that, in the
absence of concomitant moisture effects related to temperature and barring any
differences in acclimatization between these groups, there is no clear evidence that
temperature itself influences fungal:bacterial dominance.

Section III – Functional implications of fungal:bacterial dominance

As detailed in Section II, above, there is the expectation in soil ecology that fungi and
bacteria differ with regards to several key traits (van der Heijden et al. 2008). These
differences on one hand are expected to relate to how each group is likely to respond to
environmental change and can largely be summed up, albeit highly simplified, as a
relationship where what is favorable to bacteria is unfavorable to fungi or vice versa.
Extending these ideas has led to the implication of fungal:bacterial dominance as a
determinant of ecosystem processes (Hendrix et al. 1986, Bardgett and McAlister 1999,
van der Heijden et al. 2008). In many ways this is a logical extension of the expected
differences between these two groups and is comparable, for example, to ideas relating
plant community composition to primary production (Tilman et al. 2001). This has led to
the suggestion that changes in fungal:bacterial dominance are likely to be related to such
ecosystem processes as decomposition, nutrient cycling, C-sequestration potential, and
ecosystem self-regulation (Hendrix et al. 1986, Bardgett and McAlister 1999, Bailey et

It is the purpose of this section to explore the rationale for these linkages
between fungal:bacterial dominance and ecosystem processes and to discuss the evidence
in favor and against such linkages. Clearly, the rationale for many of the linkages
between fungal:bacterial dominance and ecosystem processes are closely related to the
environmental response of each group, as described in Section II above, and therefore some redundancy between these sections is necessary. We have clearly cross referenced such occurrences. Furthermore, given the wide-array of ecosystem process which fungal:bacterial dominance may relate to, we have limited ourselves to the exploration of two processes: C-sequestration and decomposition. Both of these should serve as sufficient case studies of the rationale linking fungal:bacterial dominance to ecosystem processes and also provide a broad overview of the other processes expected to be affected.

*C-sequestration and fungal-to-bacterial dominance*

The C-sequestration potential of ecosystems has gained increased interest due to current concerns pertaining to climate change (Bailey et al. 2002). The fungal:bacterial dominance of a particular site has often been associated with that site’s C-sequestration potential with a greater potential associated with fungal dominance and a lesser one associated with bacterial dominance (Jastrow et al. 2007). Much of the evidence to date which relates fungal:bacterial dominance to C-sequestration has largely been gained from comparisons of intensively managed and less intensively managed systems. For example, Bailey et al. (2002) compared five land-use pairs which differed in management intensity and found that total soil C and fungal activity were both higher for four of the five pairs when management intensity was lower. Guggenberger et al. (1999) found the same for three of six sites but Busse et al. (2009) found almost the opposite with fungal-dominated sites having lower total soil C than bacterial dominated ones. The hypothesis that greater fungal dominance is synonymous with a greater C-sequestration potential has become a
widely held idea in soil ecology and like many ideas linking fungal:bacterial dominance to ecosystem processes is based on differences in specific traits between fungi and bacteria (Guggenberger et al. 1999, Bailey et al. 2002, Six et al. 2006, van der Heijden et al. 2008). Biomass stoichiometry, C-use efficiency (CUE), and the decomposability of their respective necromass are all examples of fungal and bacterial traits which are expected to differ and in turn impact C-sequestration.

It has been suggested that on average bacterial and fungal biomass will differ with regards to their stoichiometry (see Section I). Closely related to these expected differences in biomass stoichiometry and another trait expected to relate to greater C-sequestration in fungal-dominated systems is CUE also known as growth yield efficiency (e.g. Six et al 2006). It is expected that fungi on average produce more biomass-C per unit of C metabolized than do bacteria which again leads to a greater proportion of C stored in a fungal-dominated systems (high fungal:bacterial dominance) when compared to bacterial-dominated systems (low fungal:bacterial dominance).

Six et al. (2006) conducted an extensive review on the CUE of bacteria and fungi and found a significant degree of overlap between these two groups. More direct experimental tests conducted by Thiet et al. (2006) found no difference in CUE between sites differing in fungal:bacterial dominance and concluded that many of the differences in CUE associated with changes in fungal:bacterial dominance were purely due to methodological issues. In this experiment, though, Thiet et al. (2006) only determined the amount of C mineralized and the amount remaining in the soil. They did not determine the actual accumulation of C into the biomass of the respective groups. Furthermore, whether or not bacterial and fungal CUE is similar under different environmental stresses
in soils is currently unknown. It might be expected that fungi or bacteria are more or less resistant to certain types of stress such as drying/rewetting events (see Section II) and that these stress events will lead to subsequent impacts on CUE. Another factor to consider is how substrate C:N ratios influence CUE. It has been shown that as substrate C:N ratios increase, a decrease in decomposer CUE is noted (Manzoni et al. 2008). Whether fungi in comparison to bacteria reduce their CUE less as substrate C:N ratios increase is unknown and like many other factors related to fungal and bacterial CUE warrants further investigation.

The actual amount of time that C is stored in living fungal or bacterial biomass is another of the proposed links between fungal:bacterial dominance and C-sequestration (Six et al. 2006). Although there have been relatively few reports of biomass turnover for either group, Bååth (1994) estimated that bacterial biomass turnover ranged from days to weeks, averaging about one week. A similar estimate of turnover times for fungi showed that turnover was on the order of months (i.e. ~ 130-150 days [Rousk and Bååth 2007b]). This would suggest that C is stored longer in living fungal biomass than it is in living bacterial biomass and ultimately may lead to an increased residence time for C associated with the fungal component of the microbial community.

Like living biomass the decomposition of microbial necromass is another anticipated factor linking fungal:bacterial dominance to C-sequestration (Guggenberger et al. 1999). This is based on the expectation that fungal biomass is more chemically recalcitrant than is bacterial biomass. The rationale for the greater chemical recalcitrance of fungal biomass is due to cell-wall components which include chitin and membrane components such as the potentially recalcitrant ergosterol (Mille-Lindblom et al. 2004,
Zhao et al. 2005). Also, fungal biomass, as discussed above, is expected to have a wider C:N ratio than is bacterial biomass (McGill et al. 1981). Thus if a greater proportion of a systems dead microbial biomass is composed of fungi then the decomposition of that biomass may progress more slowly than if a greater proportion of that biomass were derived from bacteria (Guggenberger et al. 1999). Concurrently, more C would remain locked up over the same period of time in fungal rather than bacterial necromass leading to increased C-sequestration in fungal dominated systems (Guggenberger et al. 1999).

However, there have been relatively few studies which have explicitly looked at the decomposition rates of bacterial versus fungal biomass but in at least one instance the decomposition of fungal biomass was equivalent to that of bacterial biomass (Li and Brune 2005a). Although fungal and bacterial biomass in this study was only sourced from two cultured organisms and may not represent the full spectrum of possibilities, it none the less demonstrates that the necromass of certain species of bacteria and fungi may be equally decomposable (Li and Brune 2005a). On the other hand, Nakas and Klien (1979) found that the cell wall components of two bacteria species, in general, decomposed at faster rates than did three fungal species. One possible explanation for this discrepancy between studies may be that Li and Brune (2005) utilized a gram positive bacterium whereas Nakas and Klien (1979) utilized a gram negative. Gram positive bacteria have a thick cell wall composed of peptidoglycan whereas the cell wall of gram negative bacteria is composed of a much thinner layer of peptidoglycan and an outer lipopolysaccharide membrane. Since the cell walls of gram positive bacteria are composed primarily of peptidoglycan then the decomposition of this groups cell wall constituents may proceed at a slower rate than that of gram negative bacteria and may
even be slower than the chitinous cell walls of fungi. In fact a comparison, based on two studies, of the mineralization of chitin and peptidoglycan shows that chitin is mineralized at an equivalent or ~30% greater rate than peptidoglycan (Li and Brune 2005b, 2005a).

Although it can be argued that there is overlap in many of the characteristics of bacteria and fungi which are expected to impact C-sequestration, perhaps the most plausible mechanism leading to enhanced C-sequestration in a fungal-dominated system can be attributed to the fungal growth form. The majority of fungi exhibit a hyphal growth form which allows these organisms to grow into new habitats in order to access new substrates (Hendrix et al. 1986, Guggenberger et al. 1999). In comparison, bacteria, with the exception of the actinomycetes, are typically relegated to growth on the surface layers of substrates (Guggenberger et al. 1999). This difference between bacteria and fungi may lead to greater C-sequestration when a system is fungal-dominated for at least two reasons. First, if fungal hyphae grow into a habitat such as a soil aggregate and those hyphae die then they are largely protected from decomposition (Guggenberger et al. 1999, Six et al. 2006). Second, fungal activity may lead to increased aggregation via both mechanical processes (e.g. entanglement of soil particles by hyphae; Guggenberger et al. 1999, Six et al. 2006) and chemical processes (e.g. the exudation of glomalin [Guggenberger et al. 1999, Pikul et al. 2009]). An increase in soil aggregation in turn may lead to incorporation of SOC into the aggregate which is then biologically unavailable and sequestered (Guggenberger et al. 1999, Wilson et al. 2009). As fungal-dominance increases then the likelihood of either of these two occurrences may subsequently increase. Yet, many other physical and chemical properties of the soil are also likely to influence soil aggregation and fungal-dominance may only be one of many
at play (Wilson et al. 2009). The importance of fungal-dominance as it pertains to aggregate formation and possibly C-sequestration is an area where research is needed.

*Litter decomposition and fungal-to-bacterial dominance*

The decomposition of foliar leaf litter is perhaps one of the most widely studied processes in terrestrial ecosystems and is expected to be influenced by the microbial community. The role that fungi play in this process is expected to be distinct from the role played by bacteria such that the outcome of this process will be dependent on the fungal:bacterial dominance of the community (de Boer et al. 2005, van der Wal et al. 2006, Meidute et al. 2008). As litter recalcitrance increases then the role of fungi in decomposition is also expected to increase (van der Heijden et al. 2008). To some degree this is based on the C:N ratio of the leaf litter in question with a greater initial C:N expected to favor fungi and a smaller initial C:N expected to favor bacteria (Gusewell and Gessner 2009). Several mechanisms have been proposed to justify this expectation including stoichiometric differences between bacteria and fungi, the hyphal growth form of fungi which allows them to grow into leaf litter bypassing the more recalcitrant outer layers of the leaf, and that hyphal bridges allow fungi to alleviate nutrient limitations associated with litter decomposition by mining limiting nutrients from other sources (Hendrix et al. 1986, Holland and Coleman 1987). Also built into the expectation that recalcitrant litter will favor fungal growth is the lignin degrading ability of fungi (de Boer et al. 2005). There is a general consensus in the literature that fungi are capable of degrading lignin whereas bacteria are not (de Boer et al. 2005, van der Heijden et al. 2008). This allows fungi to exploit these structural components of litter leading to greater decomposition.
rates (de Boer et al. 2005, van der Heijden et al. 2008). In this section we will explore some of these mechanisms and, as above, assess the validity of relating fungal:bacterial dominance to leaf litter decomposition as well as the role both bacteria and fungi play in this process.

The argument that litter with a greater C:N ratio is likely to be primarily decomposed by fungi is in part based on the expectation that fungi have a greater biomass C:N ratio than bacteria (McGill et al. 1981). However, as mentioned above (see Section II) there may be significant overlap between these two groups and further examination with specific emphasis paid to those organisms inhabiting litter is necessary. In lieu of such investigations, Güsewell and Gessner (2009) have provided research which suggests that the role of fungi and bacteria in the decomposition of leaf litter may not be as clear cut as once expected. In their investigation they found that bacteria dominated leaf litter with a greater C:N ratio when phosphorous (P) was not limiting and this was attributed to heterotrophic N-fixation by the litter inhabiting bacteria. Under these conditions bacteria dominated when litter had a greater C:N ratio and ample P but fungi dominated when the C:N ratio was lower and P was limiting (Gusewell and Gessner 2009). Comparing treatments where there was a greater dominance of bacteria to those with a greater dominance of fungi showed similar overall decomposition rates and in at least one instance the bacterial-dominated treatment had a higher rate (Gusewell and Gessner 2009). Other studies have demonstrated that the role of bacteria and fungi in the decomposition of recalcitrant litter resources may be in part dependent on the specific organisms involved in the process (Hunt et al. 1988, Gholz et al. 2000, Ayres et al. 2009, Strickland et al. 2009a, Strickland et al. 2009b). In one of these studies the decomposition
of a recalcitrant litter resource was correlated both positively and negatively to both bacterial and fungal taxa (Strickland et al. 2009b). Although correlative, such a result does suggest the possibility that bacteria and fungi may overlap with regards to their degradative potentials but other explanations are also plausible such as antagonistic (Mille-Lindblom and Tranvik 2003; Mille-Lindblom et al. 2006) or synergistic (Bengtsson 1992) interactions between differing groups (Strickland et al. 2009b).

Both of these studies however were short-term (between 5 and 7 weeks) when compared to most other studies and may not have captured the successional dynamics associated with litter decomposition. For example, Poll et al. (2008) found in a microcosm study that initial colonization of litter was dominated by bacteria and latter stages where dominated by fungi. This was presumably driven by bacteria mineralizing the soluble, easily-available compounds leaving the more recalcitrant compounds behind (Poll et al. 2008). Although this may be true in some cases, it is not true for all litter types or for all microbial communities. Strickland et al. (2009a) found that in some cases bacterial dominance actually increased during decomposition and this was dependent on both the microbial community in question and the litter being degraded.

Another key argument made in favor of fungi being the primary decomposers of recalcitrant litter resources is based on their lignin degrading abilities (Tuomela et al. 2000, de Boer et al. 2005). It has been known for some time that fungi possess the necessary enzymes capable of degrading lignin while few bacteria have been documented which are capable of performing this same role (de Boer et al. 2005). Work has been conducted which demonstrates that in the presence of lignin degrading fungi, bacterial community composition changes. These bacteria are often ascribed to supporting roles in
lignin decomposition whereby they scavenge simple C-compounds released by fungal enzymes (Tornberg et al. 2003, Folman et al. 2008). However, recent work conducted by Vargas-García et al. (2007) in the field of biodegradation questions this. Vargas-García et al. (2007) isolated six strains of bacteria and seven strains of fungi from compost heaps and then measured their lignin degrading abilities. They found that four of the seven fungal strains led to a significant reduction in lignin. On the other hand, all six strains of bacteria led to a significant reduction in lignin. Furthermore, they found that one strain of bacteria (Bacillus licheniformis) demonstrated the greatest lignin degrading capability. Other results from this field have shown similar capabilities for bacteria but whether these outcomes will hold under field settings is as yet unknown (Perestelo et al. 1996).

Perhaps, like with C-sequestration, one of the most direct lines of evidence in support of greater decomposition of recalcitrant litter by fungi can be made by examining the fungal growth form. On one hand, fungi are expected to grow into litter material bypassing the recalcitrant outer-layers (Hendrix et al. 1986). This is likely true and is an ability that most bacteria lack but the actual importance of this mechanism to decomposition is largely unknown and unexplored. It also disregards the comminuting effects of other litter inhabiting organisms, such as collembola, mites, and earthworms, which may increase the surface area of litter available for bacterial colonization (Wardle 2002). Thus if soil fauna are in low abundance then the role of fungi in litter decomposition may be more important but if soil fauna are in greater abundance then the role of bacteria may be as important if not more so than that of fungi (Wardle 2002).

Another advantage that the growth form of fungi might incur is related to the idea of hyphal bridges/networks which allow for the translocation and integration of C and
nutrients across a wide spatial area (Briones and Ineson 1996, Frey et al. 2000, Frey et al. 2003). Because fungal hyphae can cover a great distance this allows fungi to subsidize the decomposition of a nutrient poor litter resource with nutrients translocated from a nutrient rich litter resource (Briones and Ineson 1996, Tiunov 2009). In fact it is this mechanism which has often been used to explain increased rates of decomposition associated with recalcitrant litters in litter mixtures (Tiunov 2009). This advantage of the fungal growth form though may also be negatively impacted by comminuting effects (Butenschoen et al. 2007). Tiunov (2009) found that in litter mixtures as particle size of the litters decrease then positive effects on decomposition rates were negated. The explanation proposed for this result was that inhibitory compounds, such as phenolics, could more easily diffuse throughout the litter layer thus negatively impacting microbial decomposers (Tiunov 2009). It is also likely that communciation impacts fungi in many of the same ways that tilling does (see Section II).

To conclude this section, we hope to have touched on how fungal:bacterial dominance is expected to relate to at least two ecosystem processes, C-sequestration and decomposition, and these two processes where meant to serve as case studies of this relationship. Although it should be noted that many of the arguments used in relating fungal:bacterial dominance to either C-sequestration or decomposition are also used to relate it to other ecosystem processes. We also hope that this section has pointed out some of the research needed to evaluate the linkage between fungal:bacterial dominance and ecosystem processes.
Section IV – Conclusions

Fungal:bacterial dominance has provided soil ecologists with a metric for assessing both the environmental impacts on and the functional implications of soil microbial communities. It has become both widely utilized and has provided many theoretical advances. It was the goal of this review to assess the implications of fungal:bacterial dominance as it relates to our current understanding of the soil environment in the hope of determining its efficacy. We believe that we have highlighted key areas where this assessment of the microbial community meets the general expectations, areas where it diverges from these expectations, and areas were more information is warranted.

For example, fungal biomass typically increases as management intensity decreases (i.e. tillage and fertilization). This result has been by and large the expectation for fungi under such changes (Hendrix et al. 1986, Bardgett and McAlister 1999). On the other hand, bacterial biomass also tended to increase as management intensity decreased typically leading to little if any change in fungal:bacterial dominance (Hendrix et al. 1986, Bardgett and McAlister 1999). This is generally underemphasized and suggests that management intensity related to both tillage and fertilizer inputs may result in parallel effects on both fungi and bacteria. Such effects on these two groups may also be expected due to temperature change. It might also be expected that bacteria and fungi significantly overlap with regards to the key traits expected to differentiate them such as biomass stoichiometry, CUE, and even their ability to decompose organic substrates. This is not a novel idea however. The preeminent microbiologist, Jackson W. Foster stated, “It appears incongruous to delineate metabolism of bacteria from that of fungi, since the two
overlap in so many respects and since each group displays, more than any other groups of microorganisms, a kaleidoscopic array of metabolic diversity.”

This may in many ways hold true especially given the large degree of taxonomic diversity found in both groups. In fact great diversity has often been a major pillar of arguments related to the functional redundancy of the whole microbial community and might also be applicable to the environmental response of fungi and bacteria (Andren and Balandreau 1999, Behan-Pelletier and Newton 1999, Wall and Virginia 1999). Although functional redundancy of whole soil communities has recently been questioned (Reed and Martiny 2007, Strickland et al. 2009a), this does not negate the possibility that fungi and bacteria on a global scale may have significant trait overlap. This might be one reason why changes in fungal:bacterial dominance are variable under similar conditions.

As more studies are conducted across regions which explore the impacts on fungal:bacterial dominance, then formal meta-analyses may be conducted which will ultimately provide broad generalizations related to the impact that environmental factors have on this measure of microbial community structure. It will also be necessary to formally assess many of the expected differences between fungi and bacteria under field conditions. For example, assessments of bacterial and fungal C:N ratios (especially bacteria) would prove enlightening and demonstrate whether or not there is significant overlap between these groups. In situ measures of fungal and bacterial biomass C:N ratios, until recently, have proven difficult if not impossible to attain. However, advances in the use of nano-scale secondary ion mass spectrometry (NanoSIMS) may allow researchers to explore these soil communities in ever greater detail (Herrmann et al. 2007). Using this method researcher will be able to determine the elemental composition
of individual cells and paired with stable isotopes, determine the biomass incorporation of specific elements (Herrmann et al. 2007).

The functional implications of fungal:bacterial dominance will likely also need further assessment. We did note that one of the key attributes of fungi likely to influence ecosystem processes is the fungal growth form. In many instances, when fungal biomass increased then soil C also tended to increase, although it is worth noting that this was not always associated with a decline in bacterial biomass. However, given this fact and evidence that fungi and bacteria are not clearly distinguishable via other characteristics (e.g. biomass C:N, CUE) then the hyphal growth form of fungi may be the trait most likely to influence such ecosystem processes. This highlights the need to fully assess the likely mechanism by which a change in fungal:bacterial dominance might influence a given ecosystem process and whether or not that mechanism is clearly distinct for bacteria and fungi. Then measurements clearly related to the assessment of the given mechanism should be employed to determine if fungal:bacterial dominance is truly at play. For example, if C-sequestration is the ecosystem process in question and it is decided that a change in the amount of fungal hyphae are likely to influence this process then measurements of fungal biomass or hyphae would likely be the most applicable.

Finally, we feel that it is important to realize that the implementation of fungal:bacterial dominance likely arose because it provided researchers with a taxonomically and ecologically tractable measure of the microbial community. Although this has provided researchers with a more complete view of the opaque soil environment, more recent methodological advances related to the soil community may provide even further insight. For example, via the use of DNA-based approaches researchers are now
capable of having an incredibly detailed taxonomic view of the soil microbial community. Such detail has allowed researchers to relate specific taxonomic groups of fungi and bacteria to environmental change as well as ecosystem processes (Lauber et al. 2008, Strickland et al. 2009b, Lauber et al. In Review). Also coupling these relationships to advances in culturing techniques and growth based measures may ultimately allow researchers to correlate specific fungal and bacterial species to a range of ecological classifications (Madsen 2005). In the end fungal:bacterial dominance has provided many insights related to an array of environmental factors and ecosystem processes and has, in the least, provided a stepping stone which allows for the assessment of soil microbial communities. It seems that the more manageable complexity of the microbial community achieved when dividing it into fungi and bacteria historically has enabled a quantitative and more mechanistic understanding of soil ecology. We predict that this approach has not yet been exhausted, and that insight may still be gained from it. Yet, future research will prove if this assessment of the microbial community will prove more reliable and/or informative in the face of new techniques also aimed at assessing these same soil communities.

**Introduction to the dissertation**

The above review was meant to highlight one of the predominant ways in which soil microbial communities are assessed, that is via its fungal:bacterial dominance. It was this assessment, at least initially, which was the principal driver of my dissertation research. In fact it was the relationship between the fungal:bacterial dominance of the microbial community and ecosystem C-cycling which formed the basis for the two field studies I
conducted. For these two studies, I wanted to examine the relationship between fungal:bacterial dominance first under differing land-use regimes and second under an invasive plant species. My expectation was that the fungal:bacterial dominance of the microbial community would differ in the face of these changes and would subsequently influence ecosystem processes related to C-cycling. Although the first of these expectations did hold some truth often it was other characteristics of the microbial community which likely influenced C-processes.

For instance in Chapter 2, I set out to examine how changing land-use/land-management regimes influence the soil microbial community as well as the mineralization of the low molecular weight organic carbon (LMWOC) compound, glucose. I found that fungal:bacterial dominance was not influenced by land-use/land-management regimes. Not surprisingly then was that fungal:bacterial dominance was unrelated to differences in the mineralization of glucose and other factors such as land-use and extractable soil P were more strongly related to glucose mineralization. Given that the microbial community acts as a direct control on the mineralization of glucose, this was one of the first signs that measures of fungal:bacterial dominance may not capture all of the intricacies of the soil microbial community which in turn influence ecosystem C-cycling.

Further evidence of this was found when I explored the impact that the invasive grass, Microstegium vimineum, had on microbial communities and the C-processes regulated by them (see Chapter 3). Again I expected that the microbial community would be dominated by bacteria in the presence of M. vimineum with the opposite occurring in the surrounding forest matrix. However, no difference in fungal:bacterial dominance was
detected but other characteristics related to the microbial community were noted with distinct effects on C-processes. Results from this study in addition to those described in Chapter 2 suggested that other factors related to the microbial community were likely at play and so I decided to investigate these possibilities using controlled lab-based experimentation.

In the first of these experimental studies (Chapter 4), I explored the functional redundancy of soil microbial communities. First, I found evidence which suggested that microbial communities were not functionally redundant with regards to the mineralization of leaf litter. Additionally, there was evidence which suggested that communities which shared a common history with a given litter resource often mineralized a greater proportion of that litter (i.e. ‘home-field advantage’ [Gholz et al. 2000]). I also noted that across the course of decomposition microbial communities were taxonomically distinct and this was related to both the bacterial and fungal components of the microbial community.

With regards to both home-field advantage and taxonomically distinct microbial communities, I further explored the role of these communities in a follow-up experiment examining the decomposition of novel litter resources (Chapter 5). In this work, I found that not all microbial communities perceived litter quality equally. I found that the perception of litter quality was in part related to the environment that the microbial community was sourced from as well as the actual composition of that community. Together, each of these studies has expanded on our understanding of the relationship between the structure of soil microbial communities and ecosystem C-cycling.
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Constraints on development of fungal biomass and decomposition processes

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Figure Legends

Figure 1.1 The prevalence of fungal:bacterial dominance in the literature today showing (A) the number of publications found using the search terms “fungal:bacterial” and “soil” and (B) the number of citations referring to those same publications from 1991 to 2008. Both works specifically related to fungal:bacterial dominance and the citations of these works have increased during this period of time. The number of publications and citations were identified using Web of Science. See Appendix A for the list of articles used to construct this figure.

Figure 1.2 The distribution of the C:N ratios of bacteria, saprotrophic fungi, and mycorrhizal fungi. Shown are box plots and distributions, notably fewer measurements of the C:N ratio of bacteria have been made. Results were compiled from the literature and included 1,099 observations from 18 studies. Most of the data on fungi was obtained in the field from sporocarps whereas most of the data for bacteria was obtained from cultures or aquatic samples. Closed circles = Mycorrhizal fungi; Open circles = Saprotrophic fungi; Closed squares = Bacteria. See Appendix B for the list of articles used to construct this figure.
Figure 1.1

(A) Publications

(B) Citations

Year

CHAPTER 2

RATES OF IN SITU CARBON MINERALIZATION IN RELATION TO LAND-USE, MICROBIAL COMMUNITY AND EDAPHIC CHARACTERISTICS

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Abstract

Plant-derived carbon compounds enter soils in a number of forms; two of the most abundant being leaf litter and rhizodeposition. Our knowledge concerning the predominant controls on the cycling of leaf litter far outweighs that for rhizodeposition even though the constituents of rhizodeposits includes a cocktail of low molecular weight organic compounds which represent a rapidly cycling source of carbon, readily available to soil microbes. We determined the mineralization dynamics of a major rhizodeposit, glucose, and its relationship to land-use, microbial community and edaphic characteristics across a landscape in the southeastern United States. The landscape consists of cultivated, pasture, pine plantation, and hardwood forest sites (n=3). Mineralization dynamics are resolved in both winter and summer using an in situ $^{13}$C-glucose pulse-chase approach. Mineralization rates of the labeled glucose declined exponentially across the 72 h measurement periods. This pattern and absolute mineralization rates are consistent across seasons. An information-theoretic approach revealed that land-use is a moderately strong predictor of cumulative glucose mineralization. Measures assessing the size, activity, and/or composition of the microbial community were poor predictors of glucose mineralization. The strongest predictor of glucose mineralization was soil-extractable phosphorus. It was positively related to glucose mineralization across seasons and explained 60% and 48% of variation in cumulative glucose mineralization in the summer and winter, respectively. We discuss potential mechanisms underlying the relationship between soil phosphorus and glucose mineralization. Our results suggest that specific soil characteristics often related to land-use and/or land-management decisions may be strong predictors of glucose mineralization rates across a landscape. We emphasize the need for
future research into the role of soil phosphorus availability and land-use history in determining soil organic carbon dynamics.

**Keywords:** Soil microbial communities, root exudates, low molecular weight compounds, fungal-to-bacterial ratios, land-use, rhizosphere, carbon cycling, decomposition

**Introduction**

Much of our understanding regarding the cycling of plant-derived carbon (C)-compounds has been developed from studies that examine the decomposition of leaf litter (Melillo et al. 1982, Couteaux et al. 1995, Aerts 1997, Gholz et al. 2000, Harmon et al. 2009). However, leaf litter is not the only plant-derived input of C to terrestrial systems. Of growing interest is the role that rhizodeposits play in ecosystem level C-processes (van Hees et al. 2005, Pollierer et al. 2007, Hogberg et al. 2008, Phillips et al. 2008). Rhizodeposits are in part composed of low molecular weight organic C (LMWOC) compounds, such as sugars (mono- and disaccharides), amino acids, and organic acids (Rovira 1969, Grayston et al. 1997, Dakora and Phillips 2002). Although the actual input of LMWOC compounds into a system have been estimated to account for less than 30% of total soil respiration per year (van Hees et al. 2005), the overall impact of these compounds on C-dynamics may be large (van Hees et al. 2005, Hogberg et al. 2008, Phillips et al. 2008). In part, this is because LMWOC compounds represent a highly labile, rapidly-cycling, form of C which is immediately available to the soil microbial community for growth, maintenance and respiration (van Hees et al. 2005, Hogberg et al. 2008). Indeed, the turnover of LMWOC compounds may explain significant variation in
soil respiration rates (Gu et al. 2004, Bengtson and Bengtsson 2007). Furthermore, the degree to which rhizodeposition interacts with the microbial community is likely to influence ecosystem C-sequestration, with increases or decreases in soil organic C pools expected under differing input rates of LMWOC compounds (Bradford et al. 2008a, Bradford et al. 2008b, Blagodatskaya et al. 2009). Less well understood is how variation in land-use, microbial community and edaphic characteristics will impact the mineralization rates of such rhizodeposits.

Overall there is the expectation that the size, activity, and composition of the soil microbial community will influence the mineralization rates of LMWOC compounds (van Hees et al. 2005, Fierer et al. 2007, Allison and Martiny 2008, Green et al. 2008, but see Kemmitt et al. 2008). An increase in the size and/or activity of the microbial community is expected to increase mineralization (van Hees et al. 2002, van Hees et al. 2005, Six et al. 2006). The influence of composition, on the other hand, is less clear. Compositional changes in the soil microbial community, associated with broad distinctions in life history strategies, are likely to have the greatest impact on mineralization dynamics. For example, using one of the traditional compositional distinctions, microbial communities largely composed of zymogenous (i.e. fresh organic matter decomposers) as opposed to autochthonous (i.e. soil organic matter decomposers) populations of microbes may have higher rates of LMWOC mineralization (Winogradsky 1924, Fontaine and Barot 2005). A phylogenetic distinction might posit that communities dominated by bacteria will have greater mineralization rates than those dominated by fungi (Bardgett and McAlister 1999, Six et al. 2006).
It is also feasible that microbial communities which have previously been exposed to a specific LMWOC compound will subsequently influence the future mineralization of that compound (Saggar et al. 2000). This expectation is similar to the hypothesis of ‘home-field advantage’, which was developed to explain differences in litter decomposition rates not accounted for by climate and/or litter quality (Hunt et al. 1988, Gholz et al. 2000, Ayres et al. 2009, Strickland et al. 2009a). The hypothesis suggests that communities pre-exposed to a given leaf litter are likely to mineralize that litter more rapidly than communities for which that litter is novel (Gholz et al. 2000, Ayres et al. 2009). Although the underlying mechanisms for home-field advantage may differ for mineralization rates of litter and root exudates, the scenario suggests that communities which derive the bulk of their C from rhizodeposits and/or LMWOC compounds may have greater mineralization rates than communities deriving their C from another source (Saggar et al. 2000, Stevenson et al. 2004). Based on this possibility, the mineralization rates of LMWOC compounds may be dependent on the plant community and factors such as land-use/land-cover, season, and nutrient availability (Grayston et al. 1997, Warembourg et al. 2003, van Hees et al. 2005). For example, where the dominant plant cover is grass (e.g. pastures), inputs of LMWOC compounds may be a more important C source than in forests, explaining higher mineralization rates of these compounds in herbaceous communities (Stevenson et al. 2004). Similarly, these inputs may vary dependent on both season and the nutrient demands of the plant itself (Nguyen 2003, Bais et al. 2006). Finally, factors typically related to soil C dynamics, such as temperature, moisture, pH, soil texture and nutrient availability, may all influence mineralization rates of LMWOC compounds (Saggar et al. 2000, Fierer and Jackson 2006, Yuste et al. 2007,
Bradford et al. 2008a, Bradford et al. 2008b). Specifically, each of these factors has been related to effects on the activity and/or composition of the soil microbial community but their effect on the mineralization rates of LMWOC compounds is lacking (van Hees et al. 2005).

One of the key constituents of LMWOC compounds is glucose (Dakora and Phillips 2002, Nguyen 2003, van Hees et al. 2005). Glucose is likely to be found in the rhizodeposits of nearly all plant species under an array of environmental conditions and is also found in the leachates of forest floor litter (Dakora and Phillips 2002, Nguyen 2003, van Hees et al. 2005). In light of the significance of glucose as a rhizodeposit and leachate-constituent, microbial mineralization of glucose has been widely studied. As early as the 1980s researchers have been adding glucose to soil and tracking the resulting mineralization dynamics. Notably, Voroney et al. (1989) found that the initial mineralization of glucose was rapid but that a large proportion (~ 25%) of it was later stabilized in the system, presumably as microbially-derived products. Using concentrations more typical of those found in situ, mineralization of glucose has been shown to follow Michaelis-Menten type kinetics (van Hees et al. 2005, van Hees et al. 2008). Soil microbial communities respond rapidly to glucose additions, even when these additions are in trace amounts, suggesting that at least certain components of the microbial community may be adapted to it and/or LMWOC compounds in general (De Nobili et al. 2001, Jones and Murphy 2007, Hanson et al. 2008, Hoyle et al. 2008). Lab-based studies of this phenomenon have demonstrated that this adaptation may be contingent on the source environment of the microbial community, with microbes from grasslands responding more rapidly than those from forests (Jones and Murphy 2007).
Such studies have added greatly to our understanding of the microbial response to glucose but few studies have assessed these dynamics in intact soil systems under field conditions. The exception is Boddy et al. (2007), who found that glucose mineralization was more rapid in the field than in the lab; this was attributed to an intact microbial community \textit{in situ} associated with the rhizosphere. Less well understood is whether the mineralization of glucose (or other LMWOC compounds) can be generalized across a landscape in much the same way that leaf litter decomposition has been (Barlow et al. 2007, Madritch and Cardinale 2007). That is, are there characteristics across space and time that likely explain variation in the mineralization rates of glucose \textit{in situ}?

The objective of our study was to determine the landscape-level controls on glucose mineralization rates. We amended soils with small amounts of $^{13}$C-labeled glucose and tracked its resultant mineralization, in both the summer and winter, across a representative rural landscape of the southeastern United States (see Richter et al. 1999, Richter and Markewitz 2001). This landscape consists of upland soils with four distinct land-use histories, currently with cultivated crops, pastures, old-field pine stands, and never cultivated hardwood forests. We expected that the size, activity, and composition of microbial communities, all of which could be considered potential determinants of glucose mineralization rates, would vary markedly. Our primary expectation was that either one or all three of these microbial community characteristics, and/or land-use, would be a major determinant of \textit{in situ} glucose mineralization rates. More specifically, we expected, due to likely differences in microbial community characteristics and rhizospheric inputs, that those sites currently under herbaceous plant cover (i.e. cultivated and pasture) would exhibit greater mineralization rates than would those under forest...
cover (i.e. pine and hardwood). To investigate these expectations we used an information-theoretic approach (Burnham and Anderson 1998) which permitted us to both determine the probable best model in a suite of candidate models, in addition to the most important model parameter, for explaining variation in glucose mineralization in situ. We assessed a secondary set of models that, in addition to microbial community characteristics and land-use, included a suite of soil characteristics which have been speculated to influence glucose mineralization (e.g. texture). To justify the use of an information theoretic approach we include potential explanatory variables for which there is an established mechanistic rationale for why they might explain variation in the response variable (see Burnham and Anderson 1998). These rationales are provided in the Methods. Our overall objective was to compare multiple, ecologically relevant models to provide a greater mechanistic understanding of mineralization dynamics of LMWOC compounds across a landscape.

Methods

Site descriptions

Sites used in this study were located in, or adjacent to, the Calhoun Experimental Forest (CEF), which is managed by the USDA Forest Service and located in the Southern Piedmont physiographic region (approximately 34.5°N, 82°W) of northwestern South Carolina, USA (Gaudinski et al. 2001, Callaham et al. 2006). The acidic Ultisols soils at these sites are classified as fine, kaolinitic, thermic Typic Kanhapludults of the Appling, Cecil, Hiwassee, and Madison series (Richter et al. 2006, Lauber et al. 2008, Grandy et al. 2009). All sites are on uplands and on interfluves from similar bedrock, thus
attempting to control native soil characteristics, geomorphology, and geology. Sites were
within 30 km of each other and each represented one of four land-use practices common
to the region (Lauber et al. 2008, Grandy et al. 2009): annual row-crop agriculture, cattle
pasture, old-field pine forest, and oak-hickory forest (n=3 in all cases).

Management regimes for both the cultivated and pasture sites have been in place
for at least the past 40 years. The cultivated sites are managed by the South Carolina
Department of Natural Resources as wildlife fields with annual crop rotation between
corn, millet, wheat, sorghum, sunflowers, and fallow using conventional tillage practices.
During the winter sampling (start date: 30th November 2006) two of the three cultivated
sites were fallow while the other (site name: Cultivated 3) was planted in winter wheat. In
the summer sampling (start date: 12 June 2007), cultivated sites were planted with corn
(Cultivated 1), sunflowers (Cultivated 2), and wheat/corn (Cultivated 3). Pasture sites are
dominated by rye and Bermuda grass and are, more or less, continuously cattle-grazed.
Both cultivated and pasture ecosystems are fertilized and limed. Two of the pine
plantations (i.e. Pine 1 and 3) are ~50 years old and consist of an overstory of loblolly
pine with an understory of oak and hickory species. The other pine plantation (i.e. Pine 2)
is a 10-year old loblolly pine monoculture. These pine forests are all growing on old
fields previously cultivated for cotton and have therefore been fertilized and limed but
not during the growth of pine. The hardwood sites are mature oak-hickory stands, ~75
years of age. One hardwood plot (i.e. Hardwood 2) is grazed by cattle and has little
understory vegetation. Average bulk density (for depth 0-7.5 cm) ± 1 S.E. was 1.23±0.15,
1.66±0.10, 1.32±0.09, and 1.16±0.17 g cm$^{-3}$ for cultivated, pasture, pine, and hardwood
land-uses, respectively.
This replicated land-use design allowed us to explore how variation in both the contemporary and historical management of these sites drove differences, which might be expected to influence glucose mineralization, in soil properties and microbial communities (Richter et al. 1999, Richter and Markewitz 2001). Differences in dominant plant cover per land-use could also be a significant determinant of glucose mineralization (e.g. Stevenson et al. 2004). It was our goal to select sites representative of the land-use practices and land-use legacies of this region, enabling a practical insight into the relationship between in situ glucose mineralization and soil chemical, physical and microbial community characteristics.

\textit{\textsuperscript{13}C-glucose pulse-chase}

To determine glucose mineralization rates across sites and seasons, we conducted a \textsuperscript{13}C-glucose pulse-chase experiment in both the winter and summer. The pulse-chase was conducted by making additions of 99 atom\% \textsuperscript{13}C-labeled glucose and tracking its mineralization as \textsuperscript{13}CO\textsubscript{2}. This was accomplished by placing two PVC collars (15.4 cm dia., inserted 5 cm into the soil) in each land-use plot. Water was then added to each core 24 h prior to sampling in order to alleviate any water stress between plots. Soil CO\textsubscript{2} efflux rates were determined using a closed-chamber approach (e.g. Bradford et al. 2001), where CO\textsubscript{2} concentrations were determined at the start and end of a 45 min capping period. We conducted a pilot study to determine the appropriate capping time and found that headspace CO\textsubscript{2} concentrations increase linearly from 0-45 minutes and flux rate estimates only begin to decrease after 60 minutes. Although this closed-chamber approach is likely to have caveats associated with it (i.e. underestimated CO\textsubscript{2} efflux
rates), it is nonetheless a widely used approach across an array of ecosystem types (Nay et al. 1994, Franzluebbers et al. 2002). Similar capping times and protocols as described here have been employed by other researchers (Iqbal et al. 2008, Mo et al. 2008).

Headspace samples were taken with 20 mL SGE gas syringes, transported to the laboratory in 12 mL Exetainers, and then CO₂ concentrations were determined using an infra-red gas analyzer (IRGA; Li-Cor Biosciences, Lincoln, NE, USA, Model LI-7000). A second sample was analyzed using continuous flow, isotope-ratio mass spectrometry (IRMS; Thermo, San Jose, CA, USA) to determine the δ₁³C value of the CO₂ in the sample. The initial headspace sampling provided the natural abundance values for the isotope mixing equations (see below). After this initial sampling, 1 L of 2.5 mM ¹³C-labeled glucose solution (99 atom%) was added to the collars and permitted to drain. The capping procedure was repeated post addition at 2, 5, 24, 48 and 72 h, permitting a negative exponential ‘decay’ of ¹³C label to be tracked in the soil CO₂ efflux, from which cumulative mineralization rates were estimated. The amount of C added to each collar was relatively small: <25 µg C g dry wt soil⁻¹, which is <0.0001% of the total soil carbon (from 0 to 7.5 cm depth in a 15.4-cm dia. PVC collar).

The contribution of ¹³C-labeled glucose to soil respiration was estimated using isotope mixing equations. The amount of CO₂ derived from glucose was calculated as follows (sensu Ineson et al. 1996): 

\[ C_{\text{glucose derived}} = C_{\text{total}} \times (\delta^{13}C_{\text{after}} - \delta^{13}C_{\text{before}})/(\delta^{13}C_{\text{glucose}} - \delta^{13}C_{\text{before}}) \]

where \( C_{\text{total}} \) is the total amount of C respired, \( \delta^{13}C_{\text{after}} \) is the \( \delta^{13}C \) value of the respired C after glucose was added, \( \delta^{13}C_{\text{before}} \) is the \( \delta^{13}C \) value of respired C before glucose was added (i.e. the natural abundance value), and \( \delta^{13}C_{\text{glucose}} \) is the value for the glucose itself. Values were calculated per PVC collar but for the statistical analyses the
mean of the pair of values for each site (per season) was used; in effect treating the pair of values as ‘experimental repeats’ and not replicates.

**Determination of microbial community structure and soil characteristics**

During both the summer and winter pulse-chase, we collected ten individual A horizon soil cores (8 cm dia., 0 – 7.5 cm depth) from each site using a stratified random approach. Soil cores were taken from areas adjacent (i.e. within 10 m) to the pulse-chase plots in each land-use. Soils were sieved (4 mm), homogenized, and stored at +5°C until analyzed. Analyses included those used to assess the soil microbial community and key soil characteristics expected to influence the mineralization of glucose. Mean values per land-use for both sets of characteristics are presented in Table 2.1 and the rationale for each is given below.

Although there is debate surrounding the applicability of different methods aimed at determining the size, activity, and composition of microbial communities, we decided to use three of the most common (Wardle and Ghani 1995). These included a modified chloroform fumigation-extraction (CFE) method to assess microbial biomass, substrate-induced respiration (SIR) as an estimate of activity, and determination of fungal-to-bacterial dominance via qPCR to estimate composition. All measures of microbial community size, activity, and composition were determined for both the winter and summer pulse-chase.

The modified CFE method as described in Fierer and Schimel (2002) and Fierer and Schimel (2003) allowed us to estimate microbial biomass C. Briefly this was estimated as the flush of DOC following fumigation with ethanol-free chloroform. Raw
values are reported for microbial biomass C; no correction factors were used. The SIR method we used follows Fierer & Schimel (2003) whereby soil slurries are incubated, after a 1 h pre-incubation with excess substrate (i.e. autolyzed yeast extract), for 4 h at 20°C. After the 4-h incubation, SIR is determined via infrared gas analysis of headspace CO₂ concentrations using a static incubation technique (Fierer et al. 2005a). Note that although both the CFE and SIR method both report measures of microbial biomass, these measures are not necessarily equivalent especially at finer spatial scales (i.e. <100 km) (Wardle and Ghani 1995). Where CFE is expected to estimate total biomass, SIR has been suggested to be a measure of active microbial biomass (Wardle and Ghani 1995).

The composition of the microbial community was assessed as the relative abundance of fungi-to-bacteria. The relative fungal-to-bacterial dominance of the microbial community is often taken as an indicator of belowground C processes with fungal-dominated systems being associated with greater C stores and/or slower C-cycling (Bardgett and McAlister 1999, Six et al. 2006). We determined these ratios using the quantitative PCR (qPCR) method described by Fierer et al. (2005b) and Lauber et al. (2008). Briefly, DNA was isolated from soil (kept at -80°C until use) using the MoBio Power Soil DNA Extraction kit (MoBio Laboratories, Carlsbad, CA) with modifications described in Lauber et al. (2008). Standard curves were constructed to estimate bacterial and fungal small-subunit rRNA gene abundances using E. coli 16S rRNA gene, or the Saccharomyces cerevisiae 18S rRNA gene, according to Lauber et al (2008). Ratios of fungal to bacterial gene copy numbers were generated by using a regression equation for each assay relating the cycle threshold (Ct) value to the known number of copies in the
standards. All qPCR reactions were run in quadruplicate. Additional details, including the specific qPCR reaction conditions, are provided in Lauber et al. (2008).

In addition to measures aimed at determining the size, activity, and composition of the soil microbial community we also identified and determined several soil characteristics which are likely to influence the mineralization of glucose both directly and indirectly. These included in the same surficial 7.5-cm deep samples, soil pH, soil temperature and moisture, soil texture, soil organic material C:N ratio, and extractable P. Soil pH, moisture, and temperature were determined for both the winter and summer pulse-chase. Soil texture, C:N, and extractable P were determined on 7 September 2006 as these measures are not expected to vary greatly across seasons (Richter et al. 2006).

Soil pH (1:1, soil:H₂O by volume) was measured with a bench-top pH meter. The overall rationale for including soil pH as a possible indicator of in situ glucose mineralization is partially related to its role as an indicator of microbial community composition and diversity (Fierer and Jackson 2006, Lauber et al. 2008, Jones et al. 2009). At broad spatial scales, as well at our study sites, soil pH is related to the relative abundance of specific microbial taxa, bacterial diversity, and fungal-to-bacterial dominance (Fierer and Jackson 2006, Lauber et al. 2008, Jones et al. 2009). Additionally, soil pH may directly impose stress on the microbial community affecting its activity and/or it may serve as an integrator of many soil characteristics likely to impact both the soil microbial community and as a result glucose mineralization (Fierer and Jackson 2006).

Soil temperature (determined at 7.5 cm depth) and moisture (determined across 0-10 cm depth) were determined at each pulse-chase measurement using hand-held
moisture and temperature probes. The average of both was estimated for the entire 72 h period of both the summer and winter pulse-chase. The rationale for determining temperature and moisture is based on the well known interaction between these factors and the decomposition of leaf litter (Couteaux et al. 1995, Yuste et al. 2007). This is likely due to the influence that these two factors have on the activity of the soil microbial community and it is likely that the interaction between these factors will also influence the mineralization of glucose.

Soil texture was estimated using a simplified version of the hydrometer method as described by Gee and Orr (2002). Texture has been shown to relate to the composition of microbial communities, including across our study landscape (Lauber et al. 2008). Generally, finer textured soils may protect microbial biomass from both predation and environmental stress, such as desiccation, and may influence the activity of the microbial community (Six et al. 2006). This in turn typically leads to a lower turnover of the microbial biomass, lower activity, possible shifts in community composition, and in turn likely differences in glucose mineralization (Six et al. 2006).

Soil C:N was determined using an NA1500 CHN Analyser (Carlo Erba Strumentazione, Milan, Italy). Extractable P was measured on an Alpkem auto-analyzer (OI Analytical, College Station, TX) using Murphy-Riley chemistry after extraction with Mehlich I double-acid (H₂SO₄ –HCl) using a 1:4 mass:volume ratio (Kuo, 1996). Both soil C:N and extractable P are suggestive of soil nutrient status and have been shown to influence the mineralization of an array of C-compounds (Bradford et al. 2008a, Bradford et al. 2008b, Gusewell and Gessner 2009, Strickland et al. 2009b). Both of these soil characteristics have also been found to relate to the composition of soil microbial
communities, specifically fungal communities, at the CEF as well as other ecosystems (Lauber et al. 2008, Clare et al. 2009, Gusewell and Gessner 2009, Hryniewicz et al. 2009). Due to the fact that both soil C:N and P are tied to nutrient availability as well as microbial community characteristics then they may also influence glucose mineralization.

*Statistical analyses*

Linear mixed-effects models (Pinheiro and Bates 2000) were used to examine the relationships between glucose mineralization and soil microbial community characteristics (i.e. size, activity, and composition), land-use, and select soil characteristics. For this approach microbial community characteristics, land-use, soil characteristics, and season (when applicable) were treated as fixed effects. Site identity was included as a random effect to account for repeated sampling (i.e. season).

Cumulative glucose mineralization was log$_e$-transformed to conform to assumptions of homoscedasticity (verified using model checking). Parameter estimates were calculated using restricted maximum likelihood. However, models compared using the information theoretic approach were constructed using maximum likelihood estimates as the fixed effects of these models varied (MacNeil et al. 2009). Further assessment of model fit was done using linear regression within a given season (i.e. the same linear model was fit to either data gathered in the winter or summer). Finally, analyses were also conducted which examined the effect of land-use on cumulative glucose mineralization and mineralization dynamics. We analyzed cumulative glucose mineralization using a linear mixed effects model where land-use and season were treated as fixed effects and site identity was treated as a random effect to account for repeated sampling across seasons;
land-use and season were allowed to interact in this model. Land-use was further examined within each season using ANOVA with land-use as a discrete variable and the Tukey method was used to assess differences between means. We analyzed the mineralization dynamics across the 72 h period using a linear mixed-effects model where land-use, season, and time since glucose addition were treated as fixed effects and site identity was treated as a random effect to account for repeated sampling across the 72 h. Time was treated as a continuous variable and all three variables were allowed to interact. Mineralization dynamics were also analyzed within a given season using a similar mixed-effects model except in this case there was no fixed effect of season. All analyses were conducted using the freeware statistical package R (http://cran.r-project.org/).

Model selection and comparison was conducted using an information-theoretic approach (Burnham and Anderson 1998). We initially compared models which related soil microbial community characteristics and land-use to cumulative glucose mineralization. This resulted in a set of 30 candidate models plus an additional intercept only model. This model set included a full factorial combination of soil microbial community characteristics with and without both land-use and season (see Appendix C for the full model list). Land-use was also included in this candidate set as a single parameter and in combination with season. Model parameters were only included as additive terms in order to both keep the number of candidate models manageable and because there was no a priori rationale for the inclusion of interactions. Akaike’s information criterion for small sample size (AICc) was used as the model selection criterion (Burnham and Anderson 1998). Model comparisons are based on the difference in AICc ($\Delta_i$) with models <2 AICc units apart considered nearly indistinguishable
Models $>10$ AICc units from the model with the minimum AICc value have no support (Burnham and Anderson 1998). We also calculated Akaike weights ($\omega_i$) which allowed us to quantify the support for one model relative to another, calculate the 95% confidence set of models, and determine the importance of a given model variable (Burnham and Anderson 1998). The last of these was calculated as the $\sum \omega_i$ for all models containing the variable in question (Burnham and Anderson 1998).

In addition to the initial 30 candidate models we also looked at the role of specific soil characteristics. The overall rationale for this was to determine, if any, which specific soil characteristics regardless of their relationship to land-use likely drove differences in cumulative glucose mineralization. In order to assess this possibility we incorporated five soil characteristics likely to impact the soil microbial community: pH, the interaction between soil temperature and moisture, silt + clay content, soil organic material C:N ratio, and extractable P. The rationale for inclusion of each is given above (see Determination of microbial community structure and soil characteristics). Each of these was included as an additive variable in models containing microbial community characteristics as well as a single factor and as an additive factor with season (see Appendix C for the full model list). This resulted in a total of 110 candidate models (plus an intercept only model) which included the original 30 candidate models. Model selection for this set of candidate models was conducted in the same manner as described above.
Results

Temporal dynamics and cumulative mineralization of glucose

Overall the addition of glucose caused relatively little if any change in total soil respiration within a land-use, regardless of season (Appendix D). The amount of glucose respired as $^{13}$CO$_2$ declined across the 72 h measurement period with the highest mineralization values recorded ~2 h after the addition of glucose and the lowest values occurring at ~48 or ~72 h (Fig. 2.1). This change in glucose mineralization rates across time was highly significant ($F_{1,96} = 278.93; P<0.0001$) and an interaction between these temporal dynamics and season was detected ($F_{1,96} = 5.27; P<0.05$). This interaction was likely explained by the similar mineralization rates regardless of season at the 2 and 5 h measurement periods but greater mineralization rates in the winter at the 24, 48, and 72 h measurement periods. It is worth noting that glucose mineralization rates tended to be similar across seasons in spite of the fact that total soil respiration was greater in the summer when compared to the winter (Fig. 2.1, Appendix D). Land-use was also a significant factor impacting glucose mineralization rates ($F_{3,8} = 9.89; P<0.01$) and this was consistent for both the winter ($F_{3,8} = 10.08; P<0.01$) and summer ($F_{3,8} = 9.57; P<0.01$) (Fig. 2.1a,b). In general we noted that pasture and cultivated sites typically had higher glucose mineralization rates across 72 h than did pine and hardwood sites, although this pattern was clearer in the summer than in the winter (Fig. 2.1).

Overall, land-use had a significant influence on cumulative glucose mineralization ($F_{3,8} = 10.34; P<0.01$). Neither season effects, ($F_{1,8} = 0.45; P = 0.52$), nor an interaction between season and land-use ($F_{3,8} = 1.36; P = 0.32$), were observed for the cumulative amount of glucose mineralized. Generally, cumulative mineralization was higher in
pasture sites while both the pine and hardwood sites tended to be lower (Fig. 2.1). The cultivated sites were not as easily generalized given that cumulative mineralization tended to be lower in the winter when compared to the summer. Additionally, during the summer there was a large degree of variation associated with this land-use, likely due to differences in crop cover between the three sites (see Methods and Discussion). The high variation in cultivated sites in the summer may explain why the land-use effect on cumulative glucose mineralization (Fig. 2.1c, d) was not consistent across seasons. That is, in the winter a clear land-use effect was noted ($F_{3,8} = 18.22; P < 0.001$) but this was not true for the summer ($F_{3,8} = 2.64; P = 0.12$), where larger error variances were observed (compare Fig. 2.1c, d). The large variation in cumulative glucose mineralized across cultivated sites may also explain why the temporal dynamic data (Fig. 2.1b) revealed a statistically significant land-use effect but the cumulative data (Fig. 2.1d) did not.

Relatively little of the added glucose was recovered as CO$_2$ and this was true regardless of season or land-use. The amount of C recovered as CO$_2$ ranged from a low of 0.57% (Hardwood 3 in the winter) to a high of 4.29% (Cultivated 3 in the summer) and across all land-uses and both seasons averaged 1.93%. This suggests that approximately 95-99% of the added glucose-C remained within the soil system. Of course, given that we did not begin tracking the mineralization of glucose until 2 h after its addition, and the fact that some of the respired glucose may not have been captured by our chamber method; the actual amount of C remaining may have been lower. However, low recovery rates of glucose-C as CO$_2$ are typical when glucose solutions are added to soils in low concentrations (Boddy et al. 2007).
Glucose mineralization and its relationship to microbial community structure and land-use

We initially set out to determine the importance of land-use and the size, activity, and composition of the microbial community as they relate to cumulative glucose mineralization in situ. In order to do this we utilized an information-theoretic approach to assess a series of 30 candidate models (Burnham and Anderson 1998). Given that land-use was significantly related to cumulative glucose mineralized (Fig. 2.1), it is not surprising that the best model (i.e. lowest AICc) included land-use as the sole explanatory variable (Table 2.2). The probability that this model was the actual best model in this set was 53% suggesting moderate support for this outcome. However, a model which only included an intercept term was within 2 AICc units of the top model suggesting that it was also a plausible top model candidate (Table 2.2). Furthermore, the evidence ratio (i.e. $\omega_1/\omega_2 = 1.89$) when comparing these two models is ~2 and we must then assume a high level of model selection uncertainty when considering these two models (Burnham and Anderson 1998). Additionally, all of the remaining models, including those that accounted for the size, activity, or composition of the microbial community, were > 4 AICc units from the top model indicating that there was overall considerably less support that any of these models was the ‘actual’ best model (Burnham and Anderson 1998). In fact when calculating the importance of each model parameter, we found that the size, activity, and composition of the microbial community were all relatively unimportant in the prediction of glucose mineralization ($\omega_i < 0.10$ for all 3; Fig. 2.2a) while land-use was moderately important ($\omega_i = 0.55$; Fig. 2.2a). Parameter estimates for models within the 95% confidence limit are reported in Appendix E.
Glucose mineralization and its relationship to microbial community structure, land-use, and soil characteristics

Given that only marginal support for any one model or model parameter was found in the initial candidate set, we decided to explore the relationship between several key soil characteristics at our sites and the cumulative mineralization of glucose. We found that the best model (i.e. lowest AICc) in this candidate set included soil P concentrations as the sole explanatory variable (Table 2.3). The probability that this model was the actual best model in this set was 73% suggesting moderately strong support for this outcome. In fact no other model was within 2 AICc units of this model and in comparison to the second ranked model in this set, the evidence ratio was 10.11. This suggests that given this set of candidate models that it is fairly certain that this model is in fact the top model. Further supporting this is the fact that no other models were <4 AICc units from this top model which indicates that there was overall considerably less support for any of the other 109 models (Burnham and Anderson 1998). Parameter estimates for all models within the 95% confidence limit are reported in Appendix E.

We also found that soil extractable P was the most important model parameter and was significantly related to land-use ($F_{3, 8} = 9.53; P<0.01$). This is not surprising given that soil P was both the sole parameter in the top model and was also found in all of the other top models (i.e. all models within 10 AICc units of the top model). Soil P also had an extremely high weight of evidence ($\omega_i = 0.99$; Fig. 2.2b), again demonstrating its importance as an indicator of cumulative glucose mineralization. No other parameter was found to be as important. In fact all other model parameters were > 10 times less likely to be as important as soil P (Fig. 2.2b). Soil P and cumulative glucose mineralized, after
accounting for variation among plots, were positively related ($\beta = 0.48 \pm 0.09$; $F_{1,10} = 30.04$; $P < 0.001$). Regression analysis found that this was generally consistent across seasons (Fig. 2.3) with soil P explaining 60 and 48% of the variation in cumulative glucose mineralized in the winter and summer, respectively (Fig. 2.3).

**Discussion**

In this study we amended four land-uses representative of a rural upland landscape with $^{13}$C–labeled glucose and tracked its mineralization across the course of 72 h in both the summer and winter in order to determine what factors, if any, were related to the mineralization of this common LMWOC compound in soils. We expected that cumulative glucose mineralization would be related to land-use and/or the size, activity, or composition of the soil microbial community. We also evaluated a second set of putative explanatory variables related to specific soil characteristics. We expected that these soil characteristics either alone or in combination with microbial community characteristics would affect cumulative glucose mineralization. Both of these sets of variables were examined using an information-theoretic approach that allowed us to determine the probable best model as well as the most important individual parameter for explaining glucose mineralization. The approach, when applied to evaluate and compare potential explanatory variables for which there is an established mechanistic rationale detailing why they might cause variation in a response variable, generates ecologically-relevant, regression models (see Burnham and Anderson 1998). Our overarching objective was to advance the understanding of factors that influence the *in situ* mineralization of LMWOC compounds across landscapes.
Across the course of 72 h we found that glucose mineralization rates decreased exponentially and this pattern was consistent across land-use and season (Fig. 2.1a, b). Such results are similar to the findings of an array of lab-based studies (Bremer and Vankessel 1990, Bremer and Kuikman 1994, Hoyle et al. 2008, van Hees et al. 2008), as well as the findings of field-based studies where relatively high concentrations of glucose have been applied (Voroney et al. 1989). The mineralization dynamics observed in this study where similar to those observed by Boddy et al. (2007), the only other in situ study which simulated glucose additions at amounts and concentrations comparable to those expected under root exudation. This may suggest that across a wide array of settings the mineralization dynamics of glucose and, perhaps other LMWOC compounds, follow a predictable pattern.

Of the glucose added, we found that as much as 4% was recovered as CO₂ meaning that ~96% of glucose-derived C remained in the soil. This is not an uncommon occurrence, with comparable studies such as Voroney et al. (1989) finding that ~25% of a large glucose addition remained after 7 years and Boddy et al. (2007) finding that ~70-80% of a small glucose addition remained after 48 h. In light of such findings, it has been suggested that relatively large amounts of added/exuded glucose are cycled through the microbial biomass and may to some degree be stabilized as microbial byproducts in the soil organic matter (Voroney et al. 1989). However, such processes may be slow and occur over decadal time scales (Richter et al. 1999). The stabilization (or not) of LMWOC in SOC sinks is an area requiring considerably more research attention, given the importance of the inputs of these compounds to soils.
We found that land-use affected the cumulative amount of glucose mineralized (Fig. 2.1c, d). Like temporal mineralization dynamics (Fig. 2.1a, b), we found that pasture sites typically had greater cumulative glucose mineralization than did pine or hardwood sites. These results concur with those of Stevenson et al. (2004), who showed, using lab-based catabolic response profiling, that grassland soils are typically associated with greater glucose mineralization than forest soils. Cumulative glucose mineralized in the cultivated sites was on average lower in the winter than in the summer and this was likely due to the presence of established crop cover in the summer. Specifically, cumulative mineralization in the cultivated sites in summer was often comparable to cumulative values for pastures. In the winter two of the cultivated sites were fallow and the third (Cultivated 3) had freshly sprouted winter wheat. Interestingly, variation in mineralization rates across cultivated sites in the summer was high (Fig. 2.1d) and this variation may be due to differences in crop species cover. However, the role of plant-cover species identity in regulating mineralization dynamics of LMWOC compounds is relatively unexplored (see Paterson et al. 2007).

Not surprising given that land-use was significantly related to cumulative glucose mineralized, the best explanatory model in the primary set of models analyzed using the information theoretic approach contained land-use as the sole parameter (Table 2.2). Land-use was also found to be the most important parameter for the prediction of cumulative glucose mineralization (Fig. 2.2a). Surprisingly, all models containing parameters related to the size, activity, or composition of the microbial community were likely poor predictors of cumulative glucose mineralization and overall they were not important parameters (Table 2.2, Fig. 2.2a). A possible explanation for the overall lack of
importance for all of these model variables may be the high functional redundancy in the microbial community with regards the mineralization of glucose (Jones and Murphy 2007, Hanson et al. 2008). However, we only used one method each to determine the size, activity, and composition of the microbial communities. Although these methods are commonly and widely employed, they are coarse and may not be indicative of the actual characteristics of the microbial community involved in the mineralization of glucose. Future research will need to assess the role of methodology as it pertains to measures of the microbial community that might explain glucose mineralization dynamics in situ.

Land-use and land-management regimes are likely to transform soil systems in many ways and this is apparent at the CEF (Richter and Markewitz 2001). Research at these sites has demonstrated that a suite of edaphic properties (e.g. soil P, texture) are affected by both the contemporary land-use as well as the legacy of land-management decisions (Richter and Markewitz 2001, Richter et al. 2006, Lauber et al. 2008, Grandy et al. 2009). For example, Richter et al. (2006) demonstrates across the same landscape that soil P is strongly influenced by management history such as fertilization, even for decades after it was last practiced. Notably, we found that soil P was a parameter of major importance in explaining cumulative glucose mineralization; it was over 10 times more important than any other parameter considered in the entire model set (Fig. 2.2b). Soil P was positively related to cumulative glucose mineralized and this was consistent across both the winter and summer (Fig. 2.3). These results may suggest that both past and present land-use/management decisions ultimately influence ecosystem C dynamics through influences on underlying variables including soil P. Saggar et al. (2000), using a lab-based study of pasture soils with different fertilizer input histories, observed that
glucose mineralization was positively related to soil P concentrations. They suggested that this occurs because the microbial biomass becomes less efficient at utilizing C-compounds as soil P becomes limiting. Similarly, Cleveland and Townsend (2006) found that when labile C (i.e. leached from litter) was plentiful then P-availability was the primary control on its mineralization under both field and lab conditions. There is also the likelihood that increasing P-availability increases overall plant biomass, including root biomass (Saggar et al. 1997, Saggar et al. 2000), leading to an increase in the size of the microbial community geared toward the mineralization of LMWOC compounds like glucose. A recent study conducted with soils from our CEF sites demonstrated that the decomposition of recalcitrant leaf litter material decreased as soil P increased (Strickland et al. 2009b). Together these studies, and our new results, suggest that soil P may be an indicator of shifts in the microbial community from (under higher P) organisms which specialize in utilizing LMWOC compounds to those (under lower P) which utilize more complex substrates such as leaf litter. This may be similar to distinctions relating certain components of the microbial community to r- and K-strategists (Fierer et al. 2007, Blagodatskaya et al. 2009). Alternatively, soil P may simply be co-correlated with characteristics of the soil microbial community that determine C-dynamics. For example, it has been shown that soil P is related to the relative abundance of different microbial taxa (Lauber et al. 2008). Increased resolution beyond the level of fungal-to-bacterial dominance may provide more relevant indices of community composition when attempting to predict glucose mineralization (e.g. Hanson et al. 2008).

Soil P may also be related to the ATP supply rate of the microbial community (Bradford et al. 2008b, Hoyle et al. 2008). Under this expectation soil P is associated with
an increased ATP store within the microbial community which may subsequently lead to greater glucose mineralization. On the other hand, a greater ATP supply rate may favor soil microbes that maintain a high intracellular concentration of ATP (De Nobili et al. 2001, Hoyle et al. 2008). These types of microorganisms have been described as exhibiting a ‘metabolically alert’ life history strategy (De Nobili et al. 2001). Such organisms might be expected to dominate in systems where LMWOC compounds are the predominant form of C inputs. If either of these possibilities hold true then assessments of ATP may be a better indicator of microbial activity with regards to glucose mineralization than the SIR method employed in this study (but see Landi et al. 2006).

In conclusion, it was the goal of this study to determine the in situ mineralization of the common rhizodeposit and leachate-constituent, glucose, and its relationship to land-use, soil microbial community and edaphic characteristics across a landscape. Surprisingly no measure of the microbial community was related to glucose mineralization. However, soil P and land-use were predictors of glucose mineralization. Given that land-use and soil P are intimately related at these sites, there is the indication that land management decisions which impact soil P may in turn lead to altered mineralization rates of LMWOC compounds (Richter and Markewitz 2001, Richter et al. 2006). This is not likely a trivial matter given that a large proportion of the Earth’s soils are similar to the low P Ultisols at our sites and that many of these same soils are apt to be or are already impacted by management decisions which increase soil P (Richter and Markewitz 2001). Such management decisions may lead to increased mineralization of LMWOC compounds, altering ecosystem C-cycling and C source-sink dynamics. Future studies will need to address these possibilities and decipher the underlying mechanisms
that explain the relationship between soil P and C dynamics. Such studies will further our understanding of the factors which control the cycling of belowground plant-C inputs and may ultimately lead to an understanding of LMWOC compound dynamics comparable to our current understanding of leaf litter dynamics.

Acknowledgements

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Table 2.1 Mean values for soil and microbial community characteristics per land-use in the winter and summer. Values in parentheses are standard errors across the three sites in each land-use. Silt + clay content, soil C:N, and soil P were determined in September 2006 only as these measures are not expected to vary greatly across seasons.

<table>
<thead>
<tr>
<th>Land-use</th>
<th>Microbial biomass (g C m(^{-2}))</th>
<th>SIR (g CO(_2)-C m(^{-2}) h(^{-1}))</th>
<th>F:B</th>
<th>pH</th>
<th>Soil Temperature (ºC)</th>
<th>Soil Moisture (%)</th>
<th>Silt + Clay (%)</th>
<th>Soil C:N</th>
<th>Ext P (g m(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cultivated</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>5.17</td>
<td>0.08</td>
<td>0.022</td>
<td>5.39</td>
<td>14.2</td>
<td>28.94</td>
<td>38.8</td>
<td>14.68</td>
<td>1.62</td>
</tr>
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<td></td>
<td>(2.33)</td>
<td>(0.008)</td>
<td>(0.013)</td>
<td>(0.14)</td>
<td>(0.23)</td>
<td>(2.72)</td>
<td>(2.87)</td>
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<tr>
<td></td>
<td>(1.95)</td>
<td>(0.011)</td>
<td>(0.000)</td>
<td>(0.22)</td>
<td>(0.52)</td>
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<td>21.0</td>
<td>14.11</td>
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<td>(1.40)</td>
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<td>(0.04)</td>
<td>(4.16)</td>
<td>(0.38)</td>
<td>(0.30)</td>
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<td>24.3</td>
<td>15.29</td>
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<td>(3.36)</td>
<td>(0.024)</td>
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<td>(0.08)</td>
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<td><strong>Pine</strong></td>
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<td>Winter</td>
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<td>(0.33)</td>
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<td>(0.21)</td>
<td>(3.13)</td>
<td>(4.95)</td>
<td>(0.39)</td>
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<td>(0.003)</td>
<td>(0.36)</td>
<td>(0.31)</td>
<td>(0.31)</td>
<td></td>
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<tr>
<td><strong>Hardwood</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Winter</td>
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<td>0.12</td>
<td>0.239</td>
<td>6.09</td>
<td>14.2</td>
<td>20.61</td>
<td>30.1</td>
<td>21.78</td>
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<td>(2.28)</td>
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<td>(0.159)</td>
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<td>(0.20)</td>
<td>(3.18)</td>
<td>(2.96)</td>
<td>(0.11)</td>
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<td>0.006</td>
<td>5.15</td>
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<td>17.04</td>
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<td>(6.36)</td>
<td>(0.028)</td>
<td>(0.002)</td>
<td>(0.30)</td>
<td>(0.42)</td>
<td>(0.42)</td>
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</tr>
</tbody>
</table>
Table 2.2 The 10 best models (i.e. ΔAICc<10 ) of the initial 30 candidate models considered which explain cumulative $^{13}$C-glucose mineralized across 72 h ($^{13}$CO$_2$).

<table>
<thead>
<tr>
<th>Model</th>
<th>K</th>
<th>log(L)</th>
<th>AICc</th>
<th>Δ$_i$ AICc</th>
<th>$\omega_i$</th>
<th>Evidence ratio</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{13}$CO$_2$ = Land*</td>
<td>6</td>
<td>-7.08</td>
<td>40.15</td>
<td>0.00</td>
<td>0.53</td>
<td>1.00</td>
<td>1</td>
</tr>
<tr>
<td>$^{13}$CO$_2$ = 1*</td>
<td>3</td>
<td>-16.38</td>
<td>41.42</td>
<td>1.27</td>
<td>0.28</td>
<td>1.89</td>
<td>2</td>
</tr>
<tr>
<td>$^{13}$CO$_2$ = MicC*</td>
<td>4</td>
<td>-15.73</td>
<td>44.47</td>
<td>4.32</td>
<td>0.06</td>
<td>8.65</td>
<td>3</td>
</tr>
<tr>
<td>$^{13}$CO$_2$ = F:B*</td>
<td>4</td>
<td>-16.25</td>
<td>45.51</td>
<td>5.36</td>
<td>0.04</td>
<td>14.57</td>
<td>4</td>
</tr>
<tr>
<td>$^{13}$CO$_2$ = SIR*</td>
<td>4</td>
<td>-16.36</td>
<td>45.72</td>
<td>5.57</td>
<td>0.03</td>
<td>16.18</td>
<td>5</td>
</tr>
<tr>
<td>$^{13}$CO$_2$ = MicC + SIR</td>
<td>5</td>
<td>-14.67</td>
<td>47.90</td>
<td>7.75</td>
<td>0.01</td>
<td>48.24</td>
<td>6</td>
</tr>
<tr>
<td>$^{13}$CO$_2$ = MicC + Seas.</td>
<td>5</td>
<td>-14.82</td>
<td>48.21</td>
<td>8.06</td>
<td>0.01</td>
<td>56.16</td>
<td>7</td>
</tr>
<tr>
<td>$^{13}$CO$_2$ = Land + MicC</td>
<td>7</td>
<td>-6.29</td>
<td>48.98</td>
<td>8.83</td>
<td>0.01</td>
<td>82.73</td>
<td>8</td>
</tr>
<tr>
<td>$^{13}$CO$_2$ = MicC + F:B</td>
<td>5</td>
<td>-15.39</td>
<td>49.36</td>
<td>9.21</td>
<td>0.01</td>
<td>99.92</td>
<td>9</td>
</tr>
<tr>
<td>$^{13}$CO$_2$ = Land + Seas.</td>
<td>7</td>
<td>-6.82</td>
<td>50.04</td>
<td>9.89</td>
<td>0.00</td>
<td>140.35</td>
<td>10</td>
</tr>
</tbody>
</table>

The table shows the number of parameters ($K$), maximized log-likelihood ($\log(L)$), AICc, AICc differences ($\Delta_i$ AICc), Akaike weights ($\omega_i$), evidence ratio ($\omega_1/\omega_n$), and the model rank. In bold are the best top models in this candidate set ($\Delta$AICc<2). Models within the 95% confidence interval are denoted with an * and parameter estimates for these models are given in Appendix E. All models had plot as a random effect in order to account for repeated sampling at the plot level.

SIR = substrate induced respiration, a measure of microbial activity; MicC = microbial biomass C as determined via CFE, a measure of the size of the microbial biomass; F:B = fungal-to-bacterial dominance as determined via qPCR, a measure of community composition; Seas. = Season, either winter or summer when the pulse-chase experiment was conducted; Land = land-use.
Table 2.3 The 6 best models (i.e. ΔAICc<10) of the initial 104 candidate models considered which explain cumulative $^{13}$C-glucose mineralized across 72 h ($^{13}$CO$_2$).

<table>
<thead>
<tr>
<th>Model</th>
<th>K</th>
<th>log(L)</th>
<th>AICc</th>
<th>Δ$_i$AICc</th>
<th>$\omega_i$</th>
<th>Evidence ratio</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{13}$CO$_2 = P*$</td>
<td>4</td>
<td>-8.10</td>
<td>29.20</td>
<td>0.00</td>
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<td>1</td>
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<td>$^{15}$CO$_2 = P +$ MicC$*$</td>
<td>5</td>
<td>-7.63</td>
<td>33.83</td>
<td>4.63</td>
<td>0.07</td>
<td>10.11</td>
<td>2</td>
</tr>
<tr>
<td>$^{13}$CO$_2 = P +$ SIR$*$</td>
<td>5</td>
<td>-7.84</td>
<td>34.26</td>
<td>5.06</td>
<td>0.06</td>
<td>12.53</td>
<td>3</td>
</tr>
<tr>
<td>$^{13}$CO$_2 = P +$ Seas.$*$</td>
<td>5</td>
<td>-7.87</td>
<td>34.30</td>
<td>5.10</td>
<td>0.06</td>
<td>12.82</td>
<td>4</td>
</tr>
<tr>
<td>$^{13}$CO$_2 = P +$ F:B</td>
<td>5</td>
<td>-8.06</td>
<td>34.68</td>
<td>5.48</td>
<td>0.05</td>
<td>15.50</td>
<td>5</td>
</tr>
<tr>
<td>$^{13}$CO$_2 = P +$ MicC + SIR</td>
<td>6</td>
<td>-5.52</td>
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<td>7.84</td>
<td>0.01</td>
<td>50.31</td>
<td>6</td>
</tr>
</tbody>
</table>

The table shows the number of parameters ($K$), maximized log-likelihood (log(L)), AICc, AICc differences ($\Delta_i$AICc), Akaike weights ($\omega_i$), evidence ratio ($\omega_1/\omega_n$), and the model rank. In bold are the best top models in this candidate set (ΔAICc<2). Models within the 95% confidence interval are denoted with an * and parameter estimates for these models are given in Appendix E. All models had plot as a random effect in order to account for repeated sampling at the plot level. SIR, MicC, F:B, Land, and Seas. are the same as in Table 2.2. P = extractable phosphorous.
**Figure 2.1** Results of the $^{13}$C-glucose pulse-chase conducted during the winter (December 2006) and summer (June 2007) associated with land-use. Panels a and b show glucose mineralization dynamics across 72 h for each land-use. Land-use differences were detected for these dynamics across seasons ($P<0.01$) and during both the winter ($P<0.01$) and summer ($P<0.01$). Panels c and d show the cumulative amount of glucose mineralized during the entire 72-h period. Although there was a significant land-use effect across seasons on cumulative glucose mineralization ($P<0.01$) this was not consistent within seasons. That is, a statistically significant land-use effect was observed in the winter ($P<0.001$) but not in the summer ($P=0.12$) for cumulative amounts. Values are means ± 1S.E. per land-use ($n=3$) and letters in panel c indicate significant differences between land-uses based on pair-wise comparisons. Note that mineralization dynamics and cumulative values were roughly equivalent for a given land-use regardless of season.

**Figure 2.2** The relative importance of variables from the primary (a) and secondary (b) set of candidate models which explain cumulative glucose mineralized. Relative importance was determined via the sum of Akaike weights ($\sum \omega_i$) for the models in which a given variable was present. The closer to 1 a variable is the more important it is in this given set. Numbers to the right of each bar indicate the number of models that a given variable was in (e.g. land-use was in 16 of the candidate models).

**Figure 2.3** The relationship between cumulative glucose mineralized and soil P for the winter (a) and summer (b). Notably this relationship was very similar in both the winter ($y = 0.51x + 2.73$) and summer ($y = 0.45x + 2.90$). Cumulative glucose mineralized was log$_e$-transformed in the regression analysis but untransformed values are shown here. Each symbol represents a specific plot with Cu1-3, Pa1-3, Pi1-3, and Ha1-3 representing
the individual sites ($n=3$) in the cultivated, pasture, pine, and hardwood land-uses, respectively.
Figure 2.1

(a) Winter (December)
Land-use effect: $P < 0.01$

(b) Summer (June)
Land-use effect: $P < 0.01$

(c) Winter (December)
Land-use effect: $P < 0.001$

(d) Summer (June)
Land-use effect: $P = 0.12$
Figure 2.2

(a) Initial candidate model set

(b) Secondary candidate model set

Model parameter

Land

P

MicC

SIR

F:B

M*T

C:N

Land

pH

S+C

16

16

16

16

16

16

16

16

16
Figure 2.3

Winter (December)

$\text{Cumulative glucose mineralized (mg }^{13}\text{CO}_2\text{-C m}^{-2})$

$\text{Extractable P(g m}^{-2})$

$r^2 = 0.60$

$P<0.01$

Summer (June)

$r^2 = 0.48$

$P<0.01$
CHAPTER 3

GRASS INVASION OF A HARDWOOD FOREST IS ASSOCIATED WITH DECLINES IN BELOWGROUND CARBON POOLS

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Abstract

Invasive plant species affect a wide range of ecosystem processes but their impact on belowground carbon pools is still relatively unexplored. This is particularly true for grass invasions of forested ecosystems. Such invasions may alter both the quantity and quality of inputs to forest floors. Dependent on both, two dominant theories, ‘priming’ and ‘preferential substrate utilization’, suggest these changes may decrease, increase, or leave unchanged native plant-derived soil carbon. A decrease is expected under ‘priming’ theory due to increased activity of the soil microbial community degrading soil carbon. Under the theory of ‘preferential substrate utilization’, either an increase or no change is expected because the invasive plant’s inputs are used by the microbial community instead of soil carbon. Here we examine how the widespread invasive *Microstegium vimineum* affects belowground carbon-cycling in a southeastern U.S. forest. Following predictions of priming theory, the presence of *M. vimineum* is associated with decreases in native-derived, soil carbon pools. For example, in September 2006 *M. vimineum* is associated with 24, 34, 36 and 72% declines in total organic, particulate organic matter, mineralizable, and microbial biomass carbon, respectively. Soil carbon formed from *M. vimineum* inputs does not fully compensate for these decreases, meaning that the sum of native- and invasive-derived carbon pools are smaller than native-derived carbon pools in uninvaded plots. Supporting our inferences that carbon-cycling is accelerated under invasion, the microbial community is more active per unit biomass: added $^{13}$C-glucose is respired more rapidly in invaded plots. Our work suggests that this invader may accelerate carbon-cycling in forest soils and deplete carbon stocks. The paucity of studies investigating impacts of grass invasion on soil carbon-cycling in forests highlights the
need to further study *M. vimineum* and other invasive grasses to assess their impacts on carbon sink strength and forest fertility.

**Key-words:** *Microstegium vimineum*, Japanese stiltgrass, Nepalese browntop, stable isotopes, carbon sequestration, carbon sink, priming effects, preferential substrate utilization, annual grass, exotic species

**Introduction**

Invasive plant species are capable of altering a range of ecosystem processes and properties from nutrient cycling to species interactions (Mack *et al.* 2000; Liao *et al.* 2008). In some cases, these impacts are the result of specific traits that are novel to the recipient community. For example, nitrogen (N)-fixing invasive species can dramatically impact decomposition rates and soil fertility in ecosystems lacking native N-fixers (Vitousek *et al.* 1987; Vitousek & Walker 1989; Allison & Vitousek 2004). Similarly, the introduction of invasive grass species can increase rates of fire disturbance and fire intensity, altering rates of carbon (C)-cycling (Mack *et al.* 2001; Mack & D'Antonio 2003; Bradley *et al.* 2006). We know far less about the more general effects of invaders, particularly the many species that do not exhibit particular traits such as N-fixation. Consequently, it is difficult to predict whether many invasive plant species will significantly alter ecosystem processes and/or the communities they invade.

Blumenthal (2006) noted that many invasive plant species are “high-resource” species. That is, these species show lower investment in defensive compounds and tend to be of higher chemical quality (e.g. lower C:N) than native plant species. Others have made similar observations (Pattison *et al.* 1998; Baruch & Goldstein 1999; D'Antonio &
Corbin 2003). Evidence also suggests that the presence of invasive plant species is, more often than not, associated with increased detrital inputs to native ecosystems (Ehrenfeld 2003; Liao et al. 2008). While such changes may not be true for all invasive plants, this highlights the likelihood that many invaders may alter ecosystem processes simply via changes in the quantity and/or quality of detrital inputs within a system (D'Antonio & Corbin 2003). This would be synonymous to those studies which demonstrate that changes in the composition of native detrital resources within an ecosystem affects that system’s decomposition rates (Ball et al. 2008; Harguindeguy et al. 2008). Specifically, the presence of an invasive plant species may alter the composition of detrital inputs entering a system via an increase in both the quality and quantity of inputs. The impacts of these altered inputs on belowground processes and properties, specifically with regard to soil C, is an area of debate in soil theory (Bradford et al. 2008c) and such impacts as they relate to invasive plant species are little studied (Hook et al. 2004; Valery et al. 2004; Batten et al. 2005; Litton et al. 2006, 2008).

Evidence on one hand suggests that increases in the degradability of C inputs, as perceived by the microbial community (Strickland et al. 2009), will have a negligible and in some cases a positive impact on belowground C pools (Dalenberg & Jager 1981; Wu et al. 1993; Fontaine & Barot 2005). This is referred to as the ‘preferential substrate utilization’ theory and is based on the premise that high quality C sources, such as leaf litter and rhizodeposits, will be utilized before lower quality soil C sources (Fontaine et al. 2004a; Fontaine & Barot 2005). Although the mechanism leading to preferential substrate utilization is debatable, it has been largely assumed that if novel inputs are of both a high enough quality and quantity in comparison to background inputs then a shift
in the microbial community toward organisms which specialize on less recalcitrant compounds will occur (Blagodatskaya & Kuzyakov 2008; Bradford et al. 2008c). Under such an expectation the component of the microbial community responsible for the degradation of more recalcitrant soil C sources may go unchanged leading to little change in soil C pools (Fontaine et al. 2003). Under more extreme cases, the increase in organisms specializing on less recalcitrant compounds may negatively impact (e.g. through competition) those specializing on more recalcitrant resources (e.g. soil C) leading to a decline in the latter’s population and an increase in the size of soil C pools (Fontaine et al. 2003). In contrast, ‘priming effects’ theory posits that high-quality inputs give rise to disproportionate impacts on soil C (Fontaine et al. 2003; Fontaine et al. 2004b; Dijkstra & Cheng 2007). Priming is theorized to occur because high quality inputs stimulate the activity of the microbial community, in particular those groups which specialize in decomposition of recalcitrant soil C pools. The result is enhanced degradation of belowground C pools, leading to declines in their size (Fontaine et al. 2004b). It seems reasonable to assume that the impact of invasive plant species on belowground C pools in native ecosystems will be dependent on the quality and quantity of invasive inputs relative to native ones, the proportion of labile invasive inputs to recalcitrant ones, and whether or not the outcomes of preferential substrate utilization or priming effects theory are realized. Regardless of which outcome occurs, and given that both theories suggest that a change in soil C-cycling will occur, altered C inputs with plant invasions will likely alter a site’s fertility, drought tolerance, C storage potential, and possibly the aboveground-belowground interactions between organisms (Lal 2004; Lal et al. 2007).
We studied an advancing invasion of *Microstegium vimineum* (Trin.) A. Camus (commonly named Japanese stiltgrass or Nepalese browntop) to measure associated changes in soil C-cycling and C pools. *Microstegium vimineum* is an annual C$_4$ grass, which produces relatively little root biomass, and invades the understory of a variety of forest types across a large portion of the southern and eastern United States (U.S.). The U.S. Department of Agriculture reports *M. vimineum* invasions in 25 states including all states east of the Mississippi River except Vermont, New Hampshire and Maine (http://plants.usda.gov/). Research in northeastern U.S. forests demonstrates that *M. vimineum* alters soil properties, including the activities of microbial exoenzymes that may affect soil C-cycling (Kourtev et al. 1998; Ehrenfeld et al. 2001; Kourtev et al. 2002). As yet no work has examined how *M. vimineum* invasion impacts belowground C pools. In fact, little is known generally about the impact of grass invasions on soil C in forest ecosystems (Kourtev et al. 2003; Mack & D'Antonio 2003; Litton et al. 2008). A recent meta-analysis of invasive plant impacts on C and N cycling shows only 23% of 94 studies measured some form of belowground C, and of those only two studies looked at the effect of grass invasion on belowground C pools in forests (Liao et al. 2008). The first of these studies examined how perennial grass replacement of forest, due to an increase in fire occurrence, impacted soil C (Mack & D'Antonio 2003). The second, a laboratory study, compared changes in soil C between invasive understory species (including *M. vimineum*) and a single native understory species (Kourtev et al. 2003). Mack & D'Antonio (2003) found no significant difference in mineralizable soil C between forest sites where an exotic grass had and had not been removed but did note a greater percentage of mineralizable soil C in the invaded sites during the dry season. Kourtev et
al. (2003) found that soils planted with *M. viminalis* when compared to soils planted with a native understory species had a greater percentage of soil organic matter but whether this difference held for an intact forest invaded by *M. viminalis* was not determined. A third and more recent study (Litton et al. 2008) assessed the impacts of invasive grass removal on soil C. The researchers found decreased rates of soil C flux for forests where the invasive grass was removed but did not find changes in soil C pools between removal and invaded plots. Despite the ubiquity of *M. viminalis* invasions of eastern U.S. forest understories and recent calls for research investigating understory invaders (Martin et al. 2009), as far as we are aware there has been no assessment of the impacts on soil C pools of intact, forest ecosystems where uninvaded and grass-invaded areas are compared.

What *M. viminalis*’s impact on belowground C pools in an intact forest landscape will be is unknown, but because the plant has invaded forests over a large portion of the eastern U.S. understanding its impact is important (Morrison et al. 2007). The objectives of this study were to compare the size of multiple soil C and N pools across an active *M. viminalis* invasion front, measure the contribution of native plant species and *M. viminalis* C to soil C pools, and evaluate differences in soil C pools across the invasion front with changes in soil microbial activity. In our study system C inputs from *M. viminalis* can be distinguished from native inputs using stable C isotope ratios because *M. viminalis* is the only species to use the C₄ photosynthetic pathway. Our overarching hypothesis was that *M. viminalis* invasion would alter belowground carbon cycling and we expected this alteration to be explained by one of two hypotheses (H₁ or H₂). We expected that in invaded plots either the predictions of (H₁) preferential substrate utilization theory would be observed through no change or an increase in native plant-
derived, soil C pools, or \((H_2)\) priming effect theory would be observed by decreases in the same soil C pools. A significant strength of our study is the investigation of an active invasion front in an intact forest ecosystem. At the same time this ‘observational’ approach means we need to consider alternative mechanisms that may contribute to those responses we document; we explore these through additional measurements and in the Discussion.

**Methods**

*Site description*

Twelve 2 m × 2 m study plots divided into 6 pairs (1 uninvaded and 1 invaded) were established across the edges of a rapidly progressing *M. vimineum* invasion front in a riparian forest within the Whitehall Experimental Forest, Athens, GA, USA (N33°53.27’ W 83°21.93’). The site is a former wood lot, managed as mixed hardwood for the past 70 years (Rhett Jackson pers. comm.). There is little evidence that these particular sites were actually farmed but much if not all of the surrounding land was (Rhett Jackson pers. comm.). Soils at this site are a sandy loam in the Madison series (Soil Survey Staff 2009). Bulk density was consistent across the site and was 1.08 g cm\(^{-3}\). Anecdotal reports indicate *M. vimineum* became apparent within the Whitehall Experimental Forest ~15 years ago, but we do not know the exact time of invasion for these study sites but given their size they are likely < 15 years old. It is likely that *M. vimineum* went largely unnoticed until it formed dense lawns and so pinpointing the exact invasion date is difficult (Martin *et al.* 2009). Notably, the invaded plot in each pair appeared to have developed from a discrete, invaded patch and remained discrete while our work was
conducted in 2006 and 2007. In the 2008 growing season these patches were joining-up to form a contiguous cover of *M. vimineum*. No confounding issues related to invasive earthworms were noted between invaded and uninvaded sites (see Results). The forest overstory was composed of *Acer rubrum*, *Quercus nigra*, *Platanus occidentalis*, and *Liquidambar styraciflua*. The uninvaded areas of the study site were generally depauperate in understory plants. The understory plant community was composed of <5% cover of *Vitis rotundifolia*, *Acer negundo*, *A. rubrum*, *Q. nigra*, *Parthenocissus quincifolia*, *Carex sp.*, *Ligustrum sinense*, *Lonicera japonica*, *Smilax bona-nox*, *Toxicodendron radicans*, *Euonymous sp.*, *Chasmanthium latifolium*, *Bignonia capriolata*, and *Aster sp.* (unpub. data). In the invaded areas, *M. vimineum* covered >90% of the understory on average with an average dry green biomass of ~63.04 g m\(^{-2}\) (unpub. data).

*M. vimineum* began to invade the uninvaded plots towards the end of our study in 2007 and in 2008 had invaded all of these control plots. This suggests that our uninvaded study plots were hospitable to *M. vimineum* and the plant had simply not invaded these areas yet. This is an important strength of our study. We deliberately worked across an advancing invasion to minimize the likelihood that invaded and uninvaded areas might exhibit differences in soil C cycling because of an historical factor other than the *M. vimineum* invasion. We do recognize, however, that our study design is not a controlled experiment and our inferences should be interpreted with this in mind. Further, although our sample plots are spatially independent and our results pronounced, they are limited to the description of a single forest site. Whether they can be generalized to other similar sites or to other grass invasions of forest understories is not yet known.
Standard sampling regime

Standard sampling consisted of taking three individual A horizon soil cores (8 cm dia., 0 - 10 cm depth) from each plot using a stratified random approach. Soils were sieved (4 mm), homogenized, and stored at +5ºC until analyzed. Standard samples were taken six times between September 2006 and July 2007 (12 September 2006, 13 November 2006, 19 January 2007, 14 February 2007, 8 May 2007, and 24 July 2007). *Microstegium vimineum* was actively growing during the sampling months of September, May, and July but had senesced by November and did not germinate prior to the January and February samplings.

Measurements taken during the seasonal sampling regime consisted of gravimetric soil moisture, pH, soil temperature (taken at 10 cm depth), soil CO$_2$ efflux, substrate induced respiration (SIR; a measure of microbial biomass), and mineralizable C (a measure of microbially-available C). Measurements of both moisture and pH were conducted on two analytical repeats per sample. Gravimetric soil moisture was determined by drying a soil sub-sample at 105ºC for 24 h and pH (1 : 1, soil : H$_2$O by volume) was determined using a bench-top pH meter. Soil CO$_2$ efflux, which is a measure of both heterotrophic and autotrophic respiration, was taken in the field using an infrared gas analyzer (IRGA; Li-Cor Biosciences, Lincoln, NE, USA, Model LI-8100). Specifically, given the small plot size, one PVC collar was used per plot (20 cm dia., inserted 5 cm into the soil), and one measurement per plot was taken during the late afternoon. Collars were placed on each sample day and measurements were taken at a minimum of 1 h after placement. The SIR method we used follows Fierer & Schimel
(2003) whereby soil slurries are incubated, after a 1 h pre-incubation with excess substrate, for 4 h at 20°C.

Mineralizable C was determined using 60-day C-mineralization assays and is often used to estimate the pool size of labile C in the soil. This is similar to measurements of dissolved organic carbon (DOC; see below) but the mineralizable C assays provide a more direct measure of microbially available C than do measurements of DOC (e.g. Bradford et al. 2008b). Mineralizable C was determined by maintaining soils at 20°C and 65% water-holding capacity (WHC) for 60 d with periodic determinations of respiration rates using a static incubation technique (Fierer et al. 2005a) and infra-red gas analysis of headspace CO\textsubscript{2} concentrations. Mineralizable C was estimated as the area under the curve derived by plotting CO\textsubscript{2} production against time.

**Intensive sampling regime**

Measurements for the intensive sampling regime were taken before (12 September 2006) and after (13 November 2006) *M. vimineum* had senesced. Due to the elongated growing season in the southeastern United States, *M. vimineum* biomass was still green in September. For the intensive sampling regime, we measured the mineral-associated and particulate organic matter (POM) C and N pools, the DOC pool, CFE microbial biomass C and N, and extractable inorganic and organic N. This fractionation approach, whereby we resolved C pools with different turnover times, provided a powerful approach for detecting changes in soil C that a single blanket measure of total soil C might have obscured (see Bradford et al. 2008b; Bradford et al. 2008c).
To determine the mineral-associated and POM C and N pools, the fractionation method described in Bradford *et al.* (2008c) was used. Briefly, duplicate soil samples (10 g of air-dry soil) from each site were dispersed with NaHMP (30 mL sample⁻¹) via shaking for 18 h and then passed through a 53 μm sieve. Material < 53 μm is considered mineral-associated and material > 53 μm is considered POM. Both mineral and POM material were dried (105°C), ball-milled to a fine powder, and percentage C and N determined using an NA1500 CHN Analyser (Carlo Erba Strumentazione, Milan, Italy). Of these two fractions, mineral-associated C pools are expected to have slower turnover times than are POM C pools (Schlesinger & Lichter 2001). Mineral-associated C pools are presumed to be primarily microbial-derived C whereas POM pools are presumed to be primarily plant-derived C (Grandy & Robertson 2007). Given the multi-annual turnover times of these pools we only measured them during the September sampling; i.e. further changes in pool size would not have been observed across the time course of our study.

During both the September and November sampling, DOC and organic and inorganic N pools were determined on two analytical repeats per sample. Samples were shaken with 0.5 M K₂SO₄ for 4 h, filtered, and DOC concentrations determined using a Total Organic Carbon Analyzer (Shimadzu, Columbia, USA). Dissolved organic N (DON) and inorganic N pools were quantified on a Lachat autoanalyzer (Milwaukee, WI, USA). The size of the DOC pool is often determined because it is one estimate of labile soil C (Bradford *et al.* 2008c). However, although its turnover time is more rapid than either POM or mineral-associated C pools, DOC is a mixture of both high and low
molecular-weight C compounds and so is likely less labile than the C pool resolved using our 60-day mineralization assays described above.

Microbial biomass C and N was estimated using a modified chloroform fumigation-extraction (CFE) method as described in Fierer & Schimel (2002) and Fierer & Schimel (2003). Microbial biomass C or N was estimated as the flush of DOC or DON, respectively, following fumigation. Raw values are reported for microbial biomass C and N; no correction factors are used. By determining microbial biomass C using the CFE method, we were able to determine the proportion of microbial biomass which was derived from *M. vimineum* (see below). Note that the relationship between SIR and the CFE method for microbial biomass is not necessarily one to one (Wardle & Ghani 1995). For example, where CFE is expected to estimate total biomass, SIR is suspected to measure active microbial biomass (Wardle & Ghani 1995). We used the SIR method for the standard seasonal sampling because it likely provides greater resolution of differences in soil microbial biomass within a site (Wardle & Ghani 1995). It could not though provide the information on native versus invasive-derived plant C in the microbial biomass that the CFE method could.

Finally, during intensive sampling we determined earthworm biomass, litter chemistry, and the fungal-to-bacterial dominance of the microbial community. *M. vimineum* invasion is linked to increased nonnative earthworm biomass in northeastern U. S. forests (Kourtev *et al.* 1998; Kourtev *et al.* 1999; Nuzzo *et al.* 2009) so we wanted to account for this potential confounding issue in our study. Earthworm biomass was determined by hand-extracting earthworms from 20 cm dia., 20 cm deep soil cores. Hand-extracting is commonly considered to be the most accurate sampling technique for
earthworm biomass (Lee 1985; Eisenhauer et al. 2008), which we report here as wet mass.

Total percentage C, nitrogen (N), cellulose, hemi-cellulose, and lignin were determined for native forest litter, *M. vimineum* leaf material, and *M. vimineum* stem material (Table 3.1). We also determined the ratio of leaf-to-stem material for *M. vimineum* which allowed us to determine whole plant litter chemistry. Total C and N were determined using an NA1500 CHN Analyzer (Carlo Erba Strumentazione, Milan, Italy). Fiber concentrations were determined using an Ankom A200 Fiber Analyzer (Ankom, Macedon, USA). Quantitative inputs of *M. vimineum* litter and native forest litter were assessed by taking 0.25 × 0.25 m quadrats from each plot post-senescence (i.e. November), drying the sample at 65°C, hand-sorting the material, and determining its mass (Table 3.1).

Fungal-to-bacterial dominance of the microbial community is often taken as an indicator of belowground C processes with fungal-dominated systems being associated with greater C stores. We determined these ratios using the quantitative PCR (qPCR) method described by Fierer et al. (2005b) and Lauber et al. (2008). Briefly, DNA was isolated from soil (kept at -80°C until use) using the MoBIO Power Soil DNA Extraction kit (MoBio Laboratories, Carlsbad, CA, USA) with modifications described in Lauber et al. (2008). Standard curves were constructed to estimate bacterial and fungal small-subunit rRNA gene abundances. After soil DNA concentrations were normalized, PCR reactions were carried out which allowed us to generate the ratios of fungal to bacterial gene copy numbers by using a regression equation for each assay relating the cycle threshold (Ct) value to the known number of copies in the standards. All qPCR reactions
were run in quadruplicate. Additional details, including the specific PCR conditions, are provided in Lauber et al. (2008).

**Determination of *M. vimineum* derived C**

To establish the amount of C derived from *M. vimineum*, we determined the $\delta^{13}$C value of the following C pools: mineralizable C, CFE microbial biomass, DOC, total soil C, POM C, mineral-associated C, and the litter layer. For mineralizable C, a gas sub-sample taken on the first day of each 60-day incubation was analyzed. The $\delta^{13}$C value of the CO$_2$ in the sample was determined using continuous-flow isotope-ratio mass spectrometry (IRMS; Thermo, San Jose, CA, USA). For the DOC and CFE biomass a TOC analyzer was coupled to the IRMS and the remaining solid samples were introduced to the IRMS via an elemental analyzer. Once $\delta^{13}$C values were determined, we were able to estimate the amount of *M. vimineum* derived C in each of the C pools by using the difference in the natural abundance, $\delta^{13}$C values of either the plant or soil C pools. The pre-invasion $\delta^{13}$C value for each C pool was determined from the plots where *M. vimineum* was absent and due to an absence of any native C$_4$-plant species these pools all exhibited a C$_3$-photosynthetic value which ranged from $-27.72 \pm 0.25$‰ (mean ± 1SE; $n = 6$) for native POM C to $-24.29 \pm 0.32$‰ (mean ± 1SE; $n = 6$) for the September DOC measurement. *M. vimineum* has a C$_4$-photosynthetic value ($n = 13$; mean ± 1SE = $-14.31 \pm 0.14$‰). The difference in the C isotope composition between *M. vimineum* and native C is sufficient to discriminate sources (Staddon 2004). The amount of C derived from *M. vimineum* was calculated as follows (sensu Ineson et al. 1996): 

$$C_{M.\ vimineum\ derived} = C_{pool} \times \frac{(\delta^{13}C_{invaded} - \delta^{13}C_{uninvaded})}{(\delta^{13}C_{M.\ vimineum} - \delta^{13}C_{uninvaded})},$$

where $C_{pool}$ is the measured size of the pool.
(total, POM, Mineral, DOC, Microbial C, mineralizable, or litter), $\delta^{13}C_{\text{invaded}}$ is the $\delta^{13}C$ value of the pool in plots where *M. vimineum* is present, $\delta^{13}C_{\text{uninvaded}}$ is the $\delta^{13}C$ value of the pool in plots where *M. vimineum* is absent, and $\delta^{13}C_{M. \text{vimineum}}$ is the value for *M. vimineum* itself.

**$^{13}C$-glucose pulse-chase**

To determine whether or not the presence of *M. vimineum* was associated with more rapidly cycling carbon pools, we conducted a $^{13}C$ pulse-chase in November 2006. The pulse-chase was conducted by making additions of $^{13}C$-labeled glucose and tracking its mineralization as $^{13}CO_2$. This was accomplished by placing two PVC collars (15.4 cm dia., inserted 5 cm into the soil) in three plots where *M. vimineum* was present and 3 plots where it was absent. Water was then added to each core 24 h prior to sampling in order to alleviate any water stress between plots. Soil $CO_2$ efflux rates were determined using a closed-chamber approach (e.g. Bradford *et al.* 2001), where $CO_2$ concentrations were determined at the start and end of a 45 min capping period. We conducted a pilot study to determine the appropriate capping time and found that headspace $CO_2$ concentrations increase linearly from 0 to 45 min; flux rate estimates only began to decrease after 60 min. Although this closed-chamber approach is likely to have caveats associated with it (i.e. underestimated $CO_2$ efflux rates), it is nonetheless a widely used approach across an array of ecosystem types (Nay *et al.* 1994; Franzluebbers *et al.* 2002). Similar capping times and protocols as described here have been employed by other researchers (Iqbal *et al.* 2008; Mo *et al.* 2008). Headspace samples were taken with 20 mL SGE gas syringes, transported to the laboratory in 12 mL Exetainers, and then $CO_2$ concentrations were
determined using an IRGA (Li-Cor Biosciences, Lincoln, NE, USA, Model LI-7000). A second sample was analyzed using continuous flow, IRMS to determine the stable C isotope composition (see above for details). The initial headspace sampling provided the natural abundance values for the isotope mixing equations. After this initial sampling, 1 L of 2.5 mM $^{13}$C-labeled glucose solution (99 atom %) was added to the collars and permitted to drain. The capping procedure was repeated post addition at 2, 5, 24, 48 and 72 h, permitting a negative exponential ‘decay’ of $^{13}$C label to be tracked in the soil CO$_2$ efflux, from which cumulative mineralization rates were estimated. The contribution of $^{13}$C-labeled glucose to soil respiration was estimated using isotope mixing equations similar to those described above.

**Statistical analysis**

Linear mixed-effects models were used to analyze the effect of *M. vimineum* presence/absence (Pinheiro & Bates 2000). For this approach the presence of *M. vimineum* and month (when applicable) were treated as fixed effects. Pair and plot were treated as random effects with plot nested within pair. The pair identity was included as a blocking variable to account for spatial heterogeneity among paired plots where *M. vimineum* was present and absent. If the removal of this blocking variable resulted in a more parsimonious model, as determined by AIC, then it was dropped from the analysis. Plot identity was included as a random effect to account for repeated sampling across months. When reported as such, data were log$_e$-transformed to conform to assumptions of homoscedasticity (verified using model checking). All analyses were conducted using the freeware statistical package R (http://cran.r-project.org/). In all cases we considered
results statistically significant at $P < 0.05$ and marginally significant at $P < 0.10$. Notably, it is acceptable practice to consider changes in soil C pools at $P < 0.10$ to be statistically significant because of the large spatial variation associated with them (e.g. Carney et al. 2007). However, we took a more conservative stance in this work.

**Results**

Of the standard measures taken at six time points spanning from September 2006 to July 2007, we observed an average decrease in mineralizable C of 35% in the presence of *M. vimineum* ($F_{1,5}=14.05; P<0.05; \text{Fig. 3.1a}$). Average declines in mineralizable C of 36, 18, 36, 39, 43, and 37% during the months of September, November, January, February, May, and July, respectively, were noted when the invader was present (Fig. 3.1a). A significant effect of sampling month was also detected ($F_{5,50}=2.59; P<0.05$) but the relative difference between sites where *M. vimineum* was present and where it was absent remained the same across all six sampling points (interaction: $F_{5,50}=1.29; P<0.28$).

Furthermore, of the initial mineralizable C (i.e. CO$_2$ evolved during the first of 60 incubation days), *M. vimineum* derived inputs accounted for approximately 10% on average across all sampling months (Fig. 3.1a). A high of approximately 20% was found for the May sampling and a low of approximately 4% was found for the November sampling (Fig. 3.1a). The presence of *M. vimineum* was not associated with a change in SIR biomass ($F_{1,10}=3.10; P=0.11; \text{Fig. 3.1b}$).

The presence of *M. vimineum* did not affect soil CO$_2$ efflux ($F_{1,10}=1.66; P=0.23; \text{Fig. 3.2}$), but did interact with soil moisture ($F_{5,50}=3.30; P<0.05$), temperature ($F_{5,50}=2.55; P<0.05$), and pH ($F_{5,50}=4.17; P<0.01$). Soil CO$_2$ efflux did vary across
sampling month (F_{5,50}=63.8; P<0.001; Fig. 3.2) with greater efflux during the growing season (i.e. September, May, and July) and lower efflux when plant species were not actively growing (i.e. November, January, and February). Soil moisture tended to be seasonally more stable in the presence of *M. vimineum* than in its absence. Soil temperature tended to be lower in the presence of *M. vimineum* during the months of November, January, and February but during the months of September, May, and July sites where it was present had similar or higher temperatures to sites where it was absent. In general, pH was typical higher in the presence of *M. vimineum*. This was dependent on sampling month with higher pH values for soils where *M. vimineum* was present during September, January, and May but similar pH values regardless of *M. vimineum* presence/absence for the months of November, February, and July.

Several soil C and N pools were intensively sampled in September and/or November 2006. Of the soil C pools, we noted a significant decrease of 64% and 41% (September and November, respectively) in CFE microbial biomass when *M. vimineum* was present (P<0.05; Table 3.2). Regardless of the invader’s status microbial biomass was marginally greater post-senescence (F_{1,10}=3.69; P=0.08). We also noted marginally significant declines of 22% and 29%, respectively in both total soil organic C (P=0.09) and POM-associated C (P=0.08; Table 3.2). The marginally significant decrease in total soil C in the presence of *M. vimineum* was likely driven by the decrease in POM C given that total C is the sum of POM and mineral-associated C. No significant change in mineral-associated soil C was noted, although the pool size was lower in invaded plots (P=0.13; Table 3.2). *Microstegium vimineum* presence also was not associated with a significant effect on DOC but again this pool was lower where the invader was present.
(P=0.19; Table 3.2). There were no obvious differences in any of the measured N pools (P>0.12 in all cases).

Inputs derived from *M. vimineum* contributed between 1 and 29% of the C found in the belowground pools of invaded plots (Table 3.2). These inputs did not, however, compensate fully for the observed declines in several native plant-derived C pools (Table 3.2). Specifically we noted significant declines in both native-derived CFE microbial biomass C (P<0.05) and POM-associated C (P<0.05), as well as marginally significant declines in native-derived total soil organic C (P =0.07) and DOC (P =0.10) in the presence of *M. vimineum* (Table 3.2). The presence of *M. vimineum* was associated with a 72% decline in native derived CFE microbial biomass C in September and a 43% decline in November (Table 3.2). Overall, native-derived CFE microbial biomass C tended to increase post-senescence in November (F_{1,10}=5.40; P<0.05). *Microstegium vimineum’s* presence was also associated with a 25% decline in native derived DOC (P=0.10; Table 3.2) and this was independent of sampling month (F_{1,10}=0.00; P=0.93). Native derived POM-associated soil C was 34% lower and total soil organic C was 24% lower in the presence of *M. vimineum* but there was no statistical support for changes in the mineral-associated soil C, albeit this pool was lower on average in invaded plots (Table 3.2).

Of the remaining measures taken in September and November 2006, no statistically significant differences in earthworm biomass m^{-2} were detected between invaded and uninvaded plots (F_{1,10}=0.04; P=0.84), nor in fungal-to-bacterial ratios (F_{1,10}=0.00; P=0.99). In contrast, the cumulative amount of 13C-glucose mineralized to 13CO2 was lower in sites where *M. vimineum* was absent and higher in sites where it was present (F_{1,2}=19.6; P<0.05; Fig. 3.3). Analysis of the time-series data showed that 13C-
glucose mineralized to $^{13}$CO$_2$ generally followed a pattern of exponential decay across the 72 h sampling period. The significant difference in cumulative values between sites was likely due to higher rates of $^{13}$C-glucose mineralized to $^{13}$CO$_2$ observed in the plots invaded by *M. vimineum* during the 2 h and 5 h sampling periods (Fig. 3.3).

**Discussion**

We evaluated whether the abundant invader, *M. vimineum*, impacted belowground C pools. We expected that if it impacted belowground C pools, the mechanism could likely be framed around the theories of preferential substrate utilization or priming. If preferential substrate utilization was the mechanism then our expectation ($H_1$) was that there would be either no change or an increase in belowground C pools, meaning that soil C turnover rates would likely remain the same or decrease (Fontaine et al. 2004a; Fontaine & Barot 2005; Bradford et al. 2008c). In contrast, if a priming effect ($H_2$) is the potential mechanism then our expectation was that *M. vimineum*’s presence would be associated with a decrease in the size of soil C pools and an increase in their turnover rates (Fontaine et al. 2004b). We observed that the presence of *M. vimineum* was associated with increased C turnover rates and declines in several belowground C pools. Our findings suggest that *M. vimineum* invasion is altering soil C cycling via a priming effect. We do, however, recognize that our study was observational and so causation could be attributed to other response variables. Given that both moisture and temperature were neither consistently higher nor lower in invaded sites and earthworm biomass was similar across invaded and uninvaded plots we discuss below why priming may be the
most plausible explanation and contrast our results with other studies that appear to have found preferential substrate utilization manifesting in grass-invaded forest soils.

We found a marginal decrease in both total organic and POM C associated with the invasion but mineral-associated C was not lower (Table 3.2). The decrease in total soil C appeared to be largely an effect of the decrease in POM C, which is part of the total C pool. The reason that a decrease in POM C was observed while no statistically measurable decrease in mineral-associated C was observed may be due to the greater stability of the mineral pool and its slower turnover rates (Schlesinger & Lichter 2001; Grandy & Robertson 2007; Bradford et al. 2008c). These declines in both POM and total soil C represent an average 29 and 22% decrease under M. vimineum. If expressed on an areal basis, this would be equivalent to a decline of ~375 g m⁻² of total C which represents a decrease of ~25 g m⁻² year⁻¹, conservatively assuming M. vimineum has been present at this site for 15 years. We recognize that we are basing some of our inferences on statistically marginal differences (see Table 3.1). However, we think this is legitimate given that a power analysis indicated the likelihood of detecting a significant effect associated with a 22% total or 29% POM C decline was extremely low (0.18 and 0.29, respectively) and even lower power would be associated with smaller percentage declines (e.g. a 10% decline is associated with a power of 0.08). In both instances to achieve the recommended power of 0.80 would have required a sample size of > 40 and > 20 replicates for total and POM C, respectively (Thomas 1997).

The lack of power to detect significant changes when measuring soil C pools, given their large spatial variation at fine scales, is a common issue (Throop & Archer 2008). Recent work by Saby et al. (2008) indicates that the ability to detect changes in
soil C pools is largely site specific and that annual changes in these pools are extremely
difficult to detect. This may have prompted the acceptance of studies conducted at fine
spatial and temporal scales to deem changes in soil C pools at $P<0.10$ significant (e.g.
Carney et al., 2007). We followed the more conventional approach of $P<0.05$ and
recognize this may be overly conservative. Given the widespread occurrence of *M.
vimineum* invasion and a call for increased emphasis on understory invaders of intact
forest systems (Martin et al. 2009), clearly there is a need for more studies such as ours
that assess the impacts of invasion on forest soil C pools. Use of fractionation and stable
isotope approaches will increase the ability to resolve changes in soil C stocks and
cycling. In conducting future studies, investigators need be aware that the power to detect
statistically significant changes in soil C pools is generally below recommended values
when logistically feasible replicate numbers are used. The failure to detect statistically
significant effects should not simply be interpreted as a null effect of grass invasion on
forest soil C pools. Undoubtedly, more studies and then meta-analysis of their collective
results will indicate the significance, direction, and size of the effect on soil C associated
with grass invasion of forest understories. The use of meta-analysis to explore the result
of multiple studies using relatively small sample sizes is successfully and widely-adopted
in medical fields (Gurevitch & Hedges 1999).

After accounting for the contribution of *M. vimineum*-derived C in invaded plots
there was relatively little change in the contribution of native-derived C to the mineral
pool (Table 3.2). *Microstegium vimineum*-derived C only accounted for ~1% of mineral-
associated C, which again is indicative of the greater stability of this pool and its slower
turnover time (Schlesinger & Lichter 2001; Grandy & Robertson 2007; Bradford et al.
2008c). It may also suggest that a lesser proportion of *M. vimineum*-derived C when compared to native-derived sources is microbially processed to the extent that it becomes stabilized in the mineral pool and more C derived from this invader may be lost as CO₂. This is simply speculation based in part on the knowledge that mineral-associated C may often be largely microbially-derived (Grandy & Robertson 2007). Alternatively, it may be that *M. vimineum* has not been present (i.e. <15 years) at this site long enough to affect the mineral-associated C pool to an extent that marked differences can be resolved; similar explanations have been suggested by others (e.g. Litton *et al.*, 2008).

In contrast to the mineral-associated C pool, C derived from native sources found in total organic and POM C were lower in the presence of *M. vimineum*. Although the POM C pool was smaller in the presence of *M. vimineum*, the invader accounted for ~6% of this pool (compared to 1% for the mineral-associated C pool). Thus, most of the decline in the POM C pool is from the loss of native-derived sources. Declines in native-derived POM C may indicate that native-derived C is cycling faster in plots where the invader is present due to invader inputs priming its decomposition. Furthermore, native-derived POM C decreased by ~34% and given that *M. vimineum*-derived C only accounts for ~6% of this pool then further declines in this pool may be expected. This outcome will be largely contingent on whether or not the declines in native-derived POM C have stabilized and/or inputs derived from *M. vimineum* remain the same. Future research will need to address these possibilities if we are to understand the long term impacts of this invader on soil C pools.

Total DOC (i.e. both native and *M. vimineum* derived) was not significantly lower in the presence of *M. vimineum*. However, DOC derived from native sources was
marginally lower in the presence of *M. vimineum*. Interestingly, DOC derived from *M. vimineum* was greater on average pre-senescence than post-senescence (Table 3.2). This may indicate that rhizodeposition from *M. vimineum* is an important source of C inputs to soils, despite their small root to shoot ratios (unpub. data). This possibility remains to be tested.

Like DOC, DON was not significantly impacted by the presence of *M. vimineum* and we found no significant difference in inorganic N pools. This last result contrasts with other studies conducted in the northeastern U.S. which showed that the presence of *M. vimineum* was associated with changes in available NO$_3^-$ and nitrification rates (Kourtev *et al.* 1999; Ehrenfeld 2003; Kourtev *et al.* 2003). We found that *M. vimineum* was not associated with a statistically significant change in available NO$_3^-$ although it is worth noting that pH, which typically increases when NO$_3^-$ is the dominant form of plant available N (Nye 1981; Ehrenfeld 2003), was significantly greater in the invader’s presence. One possible reason for the contrasting results between our study and studies conducted in northeastern forests may be the association between non-native earthworm biomass and *M. vimineum*. Non-native earthworm biomass is often associated with altered N availability and mineralization in soils (Scheu 1987; Kourtev *et al.* 1999; Eriksen-Hamel & Whalen 2008) and may have contributed to changes in N pools in northeastern US forests invaded by *M. vimineum* (Bohlen *et al.* 2004; Marhan & Scheu 2006; Eisenhauer *et al.* 2007). Given that there was no difference in earthworm biomass between our plots, we were able to explore the effect of *M. vimineum* in the presence of a uniform earthworm gradient.
Extractable microbial biomass C, both total and native-derived, was found to be significantly lower in plots invaded by *M. vimineum*. Microbial biomass C was determined using a modified CFE method and is expected to relate to the total active and inactive biomass (Wardle & Ghani 1995; Bradford *et al.* 2008c). Declines in microbial biomass C have often been associated with declines in soil C pools (Wardle & Ghani 1995; Bradford *et al.* 2008a; Bradford *et al.* 2008b). Since microbial biomass C is likely to be one of the more responsive belowground C pools then its decrease may be an indicator of an overall decline in soil C associated with *M. vimineum* invasion. We observed that a greater proportion of microbial biomass C was derived from *M. vimineum* pre-senescence than post-senescence (Table 3.2). Under actively growing *M. vimineum*, the microbial community derived ~30% of its C from the invader (Table 3.2). This is a remarkable percentage because it is much larger than the aboveground biomass of *M. vimineum* at the study site (relative to native plant biomass), and *M. vimineum* also has a rather superficial root system. That C derived from *M. vimineum* in the microbial biomass was much higher pre-senescence may suggest that the microbial community is attaining the bulk of *M. vimineum*-derived C via root exudates rather than foliar litter. Root exudates are in part composed of an array of low-molecular weight compounds, such as glucose, which are expected to be highly labile (van Hees *et al.* 2005). Such compounds have been shown to cause priming effects when applied to soils and may be the direct link between *M. vimineum* invasion and associated declines in soil C (Dalenberg & Jager 1981; Blagodatskaya *et al.* 2007; Carney *et al.* 2007). Further work is necessary to establish this linkage between *M. vimineum* root exudates, the microbial community, and declines in soil C pools.
Although the presence of *M. vimineum* was associated with a decrease in microbial biomass C, no change in the fungal-to-bacterial dominance of the microbial community was found. This result was somewhat unexpected because a shift toward bacterial dominance might be expected, due to this invasion, since *M. vimineum* represents inputs of higher chemical quality (Table 3.1) and its presence was also associated with a more neutral pH. Both factors are expected to favor bacteria over fungi (Bardgett & McAlister 1999; Six *et al.* 2006; van der Heijden *et al.* 2008). Changes in fungal-to-bacterial dominance may not have had long enough to occur (Bardgett *et al.* 1996; Bardgett & McAlister 1999) but some studies have noted changes across short time intervals (Carney *et al.* 2007). Another possibility is that fungal-to-bacterial dominance may be too coarse an estimate of microbial community structure in this case. A finer measure of the community may have shown differences associated with *M. vimineum* which have been reported elsewhere for this species (Ehrenfeld *et al.* 2001; Kourtev *et al.* 2002). Indeed, evidence is growing that specific taxa within fungal and bacterial communities are associated with specific ecological strategies, such as *r*- versus *K*-strategists (Fierer *et al.* 2007; Lauber *et al.* 2008). Shifts in the community at this level would not necessarily be detected simply by determining fungal-to-bacterial ratios. Such a shift (below the Kingdom level) might explain why we observed a decrease in the size of the microbial biomass pool but saw no concomitant decline in SIR biomass (Table 3.2; Fig. 3.1b). SIR biomass differs from CFE biomass because it may be more indicative of physiologically active microbial biomass rather than absolute biomass (Wardle & Ghani 1995; Bradford *et al.* 2008c). If true, then on a per unit biomass basis, the microbial community associated with *M. vimineum* was more active.
The mineralizable C pool, a measure of microbially available C (Bradford et al. 2008c), was significantly lower when associated with *M. vimineum* invasion (Fig. 3.1a). The decrease in the size of this pool under *M. vimineum* may indicate that it is cycling more rapidly. The soil CO$_2$ efflux data also support the idea that C is cycling more rapidly under *M. vimineum* invasion. Indeed, soil CO$_2$ efflux did not decrease under *M. vimineum* but yet declines in mineralizable C as well as other soil C pools were observed (Fig. 3.2; Table 3.2). This discrepancy may be explained by more rapid turnover of belowground C pools (assuming autotrophic respiration is unchanged). That is, if soil C pools were simply smaller and turnover rates were unchanged then soil CO$_2$ efflux would be smaller in invaded plots. Instead, soil CO$_2$ efflux rates were equivalent in the presence and absence of *M. vimineum*, indicating that labile C pools under *M. vimineum* may be turning over more rapidly (i.e. C atoms have reduced residence times in the soil in invaded plots). This scenario is synonymous with depletion of fast-cycling soil C pools under experimental soil warming, where soil CO$_2$ efflux rates in control and warmed plots are equivalent when plots reach equilibrium due to faster cycling of active soil C pools in the warmed plots (Kirschbaum 2004; Knorr et al. 2005; Bradford et al. 2008a).

The possibility that an increase in autotrophic respiration is associated with *M. vimineum* presence seems unlikely given that, if this were the case, soil CO$_2$ efflux in invaded plots should have been lower than in uninvaded plots once *M. vimineum* had senesced, but it was not. Although, if after *M. vimineum* senesced decomposition increased due to the invader’s litter inputs then the lack of a difference in CO$_2$ efflux between plots might be attributed to increased autotrophic respiration pre-senescence and increased heterotrophic respiration post-senescence. To help decipher the mechanism we conducted an *in situ*
pulse-chase with $^{13}$C-glucose. The more rapid mineralization of this added substrate supports our inference that soil C is turning over more rapidly in plots invaded by *M. vimineum*. Specifically, when *M. vimineum* was present more glucose was mineralized to CO$_2$ during the first 72 h after addition (Fig. 3.3). Notably the difference in cumulative values was largely driven by higher rates of glucose mineralization during the first five hours after glucose addition (Fig. 3.3).

The results of our study show that the presence of *M. vimineum* was associated with declines in several belowground C pools and these declines were likely associated with more rapid cycling. *Microstegium vimineum* represents a relatively minor quantitative detritus input (< 3% of litter layer biomass; Table 3.1) at our site but the higher chemical quality of *M. vimineum* litter (Table 3.1), in addition to its root exudates, appears to stimulate soil C decomposition through a possible priming effect. Other studies of invasive plant effects on belowground C pools, similar in context to our own (Stock *et al.* 1995; Mack *et al.* 2001; Mack & D'Antonio 2003; Bradley *et al.* 2006), have observed relatively little change in soil C pools (Hook *et al.* 2004; Valery *et al.* 2004; Koutika *et al.* 2007; Litton *et al.* 2008). We speculate that what appear to be inconsistencies between our study results and those of others may actually be consistent in the context of preferential substrate utilization versus priming effects. That is, the impact that an invasive plant species has on belowground C pools may be dependent on the quality and quantity of its C inputs relative to the quality and quantity of native inputs. If invasive plant species’ inputs are of high chemical quality (i.e. low C:N or low Lignin:N) but low quantity relative to native inputs then a priming effect may occur leading to a decrease in belowground carbon pools; however, if quantity is high then
preferential substrate utilization may instead occur. One key example of this is work conducted by Litton et al. (2008). They found that grass invasion in Hawaiian forests increased soil CO₂ efflux, represented a very large increase in litter inputs (i.e. 16 to 44 fold increase), but did not impact soil C pools. Such results are at least indicative of preferential substrate utilization. In our own study we found that the presence of *M. vimineum* did not change soil CO₂ efflux, its litter inputs only represented an ~3% increase in total litter inputs, and we noted declines in several soil C pools. These results are indicative of a priming effect and similar to other field based studies which have found and concluded the same (Carney et al. 2007). Furthermore, when considering both Litton et al.’s (2008) study and our own there is some support for the proposition that the strength of preferential substrate utilization versus priming may be dependent on the relative increase in inputs (Blagodatskaya & Kuzyakov 2008; Bradford et al. 2008c). This would be in agreement with Blagodatskaya & Kuzyakov (2008) who found that priming effects tended to decrease as relative inputs increase. It is also important to recognize that effects may not be consistent across all belowground C pools, with both C input rates and the nutrient content of these inputs affecting distinct soil C pools differentially (Bradford et al. 2008c). By incorporating priming and preferential substrate utilization theories with our current understanding of invasive plant species, we may be better able to understand and predict how invasive plants are likely to impact soil C cycling and hence long-term soil fertility.

In conclusion, we have demonstrated that at our study site *M. vimineum* invasion is associated with declines in the size of several belowground C pools and C inputs derived from *M. vimineum* do not offset the loss of native, plant-derived soil C. This loss
of C appears to be the result of a priming of the microbial community from higher quality
*M. vimineum* foliar litter inputs and root exudates, which leads to increased soil C
turnover rates where the invader is present. Whether our results can be generalized to *M.
vimineum* invasions at other similar sites is not yet known. Assuming they are
generalizable, then the results of this study indicate that invasions by *M. vimineum* may
have long term implications for forest soil fertility, carbon storage, and potentially the
flow of energy through detrital pathways to aboveground components of terrestrial
foodwebs.

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Experimental evidence for additive and non-additive effects. *Journal of Ecology*,
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Table 3.1 Litter chemistry and percentage of native and *Microstegium vimineum* derived inputs into *M. vimineum* invaded plots. *Microstegium vimineum* litter was divided into leaf material and stem material because of expected differences in their chemical recalcitrance. The proportion of *M. vimineum* stem and stem material was used to calculate whole litter chemistry for *M. vimineum*. Native litter was a mixture of several species found at this site. For litter chemistry the mean ± 1 S.E. is reported (n=3 analytical repeats). For the percentage of litter inputs the mean ± 1 S.E. is reported for all 6 invaded plots.

<table>
<thead>
<tr>
<th>Litter Source</th>
<th>Litter input (%)</th>
<th>C (%)</th>
<th>N (%)</th>
<th>Lignin (%)</th>
<th>Cellulose (%)</th>
<th>Hemi-cellulose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>97.25 ± 0.98</td>
<td>48.52 ± 0.72</td>
<td>0.87 ± 0.01</td>
<td>25.58 ± 1.81</td>
<td>18.66 ± 0.23</td>
<td>12.40 ± 0.52</td>
</tr>
<tr>
<td><em>M. vimineum</em></td>
<td>2.75 ± 0.98</td>
<td>42.50</td>
<td>1.10</td>
<td>17.97</td>
<td>25.17</td>
<td>21.03</td>
</tr>
<tr>
<td>Leaves</td>
<td>42.33 ± 1.81</td>
<td>1.34 ± 0.06</td>
<td>20.04 ± 2.38</td>
<td>20.54 ± 0.76</td>
<td>20.76 ± 0.54</td>
<td></td>
</tr>
<tr>
<td>Stems</td>
<td>42.83 ± 0.09</td>
<td>0.62 ± 0.03</td>
<td>13.99 ± 2.08</td>
<td>34.06 ± 1.09</td>
<td>21.54 ± 0.64</td>
<td></td>
</tr>
</tbody>
</table>

*Leaves represented 65.74 ± 0.02% of *M. vimineum* litter and stems represented 34.26 ± 0.02% (n=3).
Table 3.2 The mean ± 1S.E. of the *Total* (native + *M. vimineum*) and *Native* forest derived total soil organic C, POM associated C, mineral associated C, DOC, and microbial biomass C for plots where *M. vimineum* is absent and where it is present (*n* = 6). Where applicable, values are shown both before and after *M. vimineum* senesced (i.e. September and November samplings, respectively). Also, shown is the mean percentage ± 1S.E. that *M. vimineum* derived C contributed to the total C in each pool. Analyses compared both *Total* (native + *M. vimineum*) pool sizes between plots where *M. vimineum* was present/absent and *Native* (native only) C pool sizes. In uninvaded plots *Total* = *Native* and so a dash is shown in the table for these entries. Total C, POM C, and Mineral C pools were only measured pre-senescence. F-values, degrees of freedom, and *P*-values are reported for the main effect of *M. vimineum* presence/absence. Where measures were taken both pre- and post-senescence (e.g. DOC) these values are reported for the main effects across the two sampling points. Units for Total, POM, and mineral C pools are mg g dry wt soil$^{-1}$ and µg g dry wt soil$^{-1}$ for DOC and microbial biomass C. Where significant (*P*<0.05) *P*-values are reported in bold. Marginally significant (*P*<0.10) *P*-values are italicized.
Table 3.2 Cont.

<table>
<thead>
<tr>
<th></th>
<th>Pre-senescence</th>
<th>Post-senescence</th>
<th>F_{df}</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M. vimineum absent</td>
<td>M. vimineum present</td>
<td>% M. vimineum derived C</td>
<td>M. vimineum absent</td>
</tr>
<tr>
<td>Total C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22.21 ± 3.20</td>
<td>17.24 ± 3.56</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Native</td>
<td>–</td>
<td>16.80 ± 3.50</td>
<td>2.87 ± 0.64</td>
<td>NA</td>
</tr>
<tr>
<td>POM C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8.46 ± 1.24</td>
<td>5.99 ± 1.22</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Native</td>
<td>–</td>
<td>5.60 ± 1.16</td>
<td>6.01 ± 1.34</td>
<td>NA</td>
</tr>
<tr>
<td>Mineral C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>13.75 ± 2.41</td>
<td>11.25 ± 2.44</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Native</td>
<td>–</td>
<td>11.13 ± 2.43</td>
<td>1.29 ± 0.51</td>
<td>NA</td>
</tr>
<tr>
<td>DOC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>102.97 ± 12.96</td>
<td>87.10 ± 15.55</td>
<td>113.13 ± 18.32</td>
<td>87.46 ± 21.68</td>
</tr>
<tr>
<td>Native</td>
<td>–</td>
<td>77.16 ± 14.62</td>
<td>9.80 ± 2.12</td>
<td>–</td>
</tr>
<tr>
<td>Microbial biomass C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total^</td>
<td>68.56 ± 22.61</td>
<td>25.03 ± 10.48</td>
<td>74.50 ± 16.24</td>
<td>44.20 ± 6.86</td>
</tr>
<tr>
<td>Native^</td>
<td>–</td>
<td>19.11 ± 9.57</td>
<td>29.41 ± 7.50</td>
<td>–</td>
</tr>
</tbody>
</table>

*Data were log_{10} transformed.
Figure 3.1 Mineralizable C (a) and SIR microbial biomass (b) for plots where *Microstegium vimineum* was present and where it was absent (*n* = 6). Panel a shows the mean cumulative values ± 1S.E. for mineralizable C across each 60-day incubation. Mineralizable C is expected to represent microbially-available, labile C. Also shown in (a) is the percentage ± 1S.E. of CO$_2$-C derived from *M. vimineum* on the first day of each 60-day incubation for sites where *M. vimineum* was present (sub-set bar plot). Panel b shows the mean ± 1S.E. for SIR microbial biomass which may be an indicator of physiologically active microbial biomass. *Microstegium vimineum* was active during the sampling months of Sep., May, and Jul. (note it is an annual). * = *P* < 0.05; ** = *P* < 0.01; *** = *P* < 0.001; *ns* = not significant.

Figure 3.2 Soil CO$_2$ efflux (mean ± 1S.E.) measured in situ across all sampling dates for plots where *M. vimineum* was present and where it was absent (*n* = 6). This is a measure of both heterotrophic and autotrophic respiration. *Microstegium vimineum* was active during the sampling months of Sep., May, and Jul. (note it is an annual). Symbols are the same as in Fig. 3.1.

Figure 3.3 Results of the $^{13}$C-glucose pulse-chase experiment conducted during the November sampling. The panel insert shows the cumulative amount of $^{13}$C-glucose mineralized to $^{13}$CO$_2$-C (mean ± 1S.E.) across 72 h for plots where *M. vimineum* is present and where it is absent (*n* = 6). The main panel shows the time course of glucose mineralization. A marginally significant effect of *M. vimineum* presence was detected at 2 h (F$_{1,2} = 10.5$, *P* = 0.084) and 5 h (F$_{1,2} = 9.98$, *P* = 0.087) but no effect was detected at 24 h (F$_{1,2} = 0.13$, *P* = 0.76), 48 h (F$_{1,2} = 0.01$, *P* = 0.94), or 72 h (F$_{1,2} = 0.11$, *P* = 0.77). Symbols are the same as in Fig. 3.1.
Figure 3.1

**a**

- **M. vimineum absent**
- **M. vimineum present**

**M. vimineum presence ***

Sample month *

Interaction ns

**b**

**M. vimineum derived**

**M. vimineum presence ns**

Sample month ***

Interaction ns

Mineralizable C (mg CO$_2$-C g dry wt soil$^{-1}$)

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<th>Month</th>
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SIR (µg CO$_2$-C g dry wt soil$^{-1}$ h$^{-1}$)

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Figure 3.2

Soil CO₂ efflux (g CO₂-C m⁻² day⁻¹)

- **M. vimineum absent**
- **M. vimineum present**

Sample month ***
Interaction ns

M. vimineum presence ns
Figure 3.3

Glucose mineralization (mg $^{13}$CO$_2$-C m$^{-2}$ h$^{-1}$)

- $M.$ vinineum absent
- $M.$ vinineum present

- P = 0.08
- P = 0.09

Cumulative Mineralization (mg $^{13}$CO$_2$-C m$^{-2}$)

- Absent
- Present

M. vinineum presence*

ns

Time since glucose added (h)

0 20 40 60 80
CHAPTER 4

TESTING THE FUNCTIONAL SIGNIFICANCE OF MICROBIAL COMMUNITY COMPOSITION

Abstract

A critical assumption underlying terrestrial ecosystem models is that soil microbial communities, when placed in a common environment, will function in an identical manner regardless of the composition of that community. Given high species diversity in microbial communities and the ability of microbes to adapt rapidly to new conditions, this assumption of functional redundancy seems plausible. We test the assumption by comparing litter decomposition rates in experimental microcosms inoculated with distinct microbial communities. We find that rates of carbon dioxide production from litter decomposition were dependent upon the microbial inoculum, with differences in the microbial community alone accounting for substantial (~20%) variation in total carbon mineralized. Communities that shared a common history with a given foliar litter exhibited higher decomposition rates when compared to communities foreign to that habitat. Our results suggest that the implicit assumption in ecosystem models (i.e., microbial communities in the same environment are functionally equivalent) is incorrect. To predict accurately how biogeochemical processes will respond to global change may require consideration of the community composition and/or adaptation of microbial communities to past resource environments.

Keywords: bacteria, carbon mineralization, community composition, decomposition, functional diversity, functional equivalence, functional redundancy, fungi, leaf litter, microorganisms
Introduction

Many of the ecosystem processes critical to the functioning of ecosystems, such as decomposition, are carried out in whole or in part by microorganisms. Ecosystem models designed to explain and predict how the rates of these ecosystem processes change in response to changing environmental conditions, such as temperature and moisture, typically treat the microbial community as a single, homogenously functioning entity (Parton et al. 1983). Yet it is increasingly being recognized that microbial community composition plays a role in determining ecosystem process rates (Hendrix et al. 1986, Schimel and Gulledge 1998, Reed and Martiny 2007). However, even in those models (Hunt and Wall 2002) that explicitly consider microbial community composition (e.g., bacterial vs. fungal dominance), the assumption is still that the environment ultimately controls ecosystem process rates. That is, changes in microbial community composition exert proximate control on process rates but the contemporary environment ultimately structures the community. This hypothesis, which we refer to as “functional equivalence,” has recently been challenged (Reed and Martiny 2007) given the finding that historical contingencies, and not just the contemporary environment, structure microbial communities both at local and regional scales (Martiny et al. 2006, Ramette and Tiedje 2007). The counter-hypothesis, which we refer to as “functional dissimilarity,” is significant because it proposes that the responses of ecosystem processes to global changes are not ultimately fixed by environmental conditions (Balser and Firestone 2005). On the contrary, it proposes that microbial community composition (i.e., the whole community genotype) may, in combination with the environment, ultimately (not just proximally) determine ecosystem process rates.
The hypothesis of functional equivalence does appear intuitive. For example, given that there are many thousands of species of microorganisms in a given soil (Fierer et al. 2007) and that microbes can rapidly adapt to new environments (Goddard and Bradford 2003), one might assume that this equates with a high degree of functional redundancy (Andrén and Balandreau 1999, Behan-Pelletier and Newton 1999, Wall and Virginia 1999). Any spatial or temporal change in community composition would then produce a negligible change in ecosystem processes regulated by microbes (Bell et al. 2005, Franklin and Mills 2006, Wertz et al. 2006, Cardinale et al. 2007, Jiang 2007, Verity et al. 2007). There is an expectation that the greatest functional redundancy will be observed for biogeochemical processes performed by a broad array of soil microorganisms, such as the mineralization of carbon compounds during litter decomposition, rather than for processes such as nitrogen fixation (Schimel 1995, Bell et al. 2005) that are performed only by specific microbial groups. Work by Balser and Firestone (2005) tends to support this divide. They observed functional differences for microbial processes such as nitrogen mineralization but not for carbon mineralization. Indeed, equivalence in organic carbon degradation has been suggested by others among both local and regional heterotrophic microbial communities in both aquatic (Langenheder et al. 2005, 2006) and terrestrial habitats (Balser and Firestone 2005, Ayres et al. 2006, Wertz et al. 2006).

There is evidence from both plant and animal communities that changes in community composition impact ecosystem process rates (Tilman et al. 1996, 2001, Naeem and Wright 2003, Taylor et al. 2006, Cardinale et al. 2007). Similarly, evidence is now accumulating that microbial community composition influences ecosystem process
rates, albeit the identification of causation, as opposed to correlation, is not always conclusive (Schimel and Gulledge 1998, Behan-Pelletier and Newton 1999, Wall and Virginia 1999, Reed and Martiny 2007). To identify causation both common garden and reciprocal transplant approaches are required, where both ecosystem processes and community composition are monitored across time in different environments (Reed and Martiny 2007).

Here we used a long-term (300-day) experimental litter–soil system that combines both a common garden and a reciprocal transplant approach to test between the following competing hypotheses for litter mineralization: (1) different soil microbial communities are functionally equivalent vs. (2) different soil microbial communities are functionally dissimilar. The hypothesis of “functional equivalence” is based on the expectation that the high diversity and/or adaptive ability of soil microbes confer functional redundancy across different microbial communities (i.e., only the environment impacts function). The competing hypothesis of “functional dissimilarity” is based on the expectation that historical contingencies play a role in shaping the functioning of microbial communities. To test between the hypotheses, we collected soil inocula from three locations in the continental United States where we might expect the microbial community composition to differ either because of geographic distance (a surrogate for history) and/or contemporary habitat differences (Martiny et al. 2006). Litters from the dominant plant species at each of the three locations were also collected, sterilized, and then milled to generate three, distinct, common garden environments. We inoculated each litter in a factorial design with each of the three soils. If different inocula yielded distinct rates of litter decomposition then the hypothesis of functional dissimilarity was supported, and if
rates were similar then this supported the hypothesis of functional equivalence. We posed a sub-hypothesis under the hypothesis of functional dissimilarity that stated that soil inocula that shared a common history with a litter (i.e., soils collected from the same location as the litter) would mineralize that litter more rapidly than soil inocula without a shared history (the “home field advantage” hypothesis [Gholz et al. 2000]). Our findings support the hypotheses of functional dissimilarity and home field advantage, suggesting that differences in microbial community composition may contribute, as ultimate and not just proximal controls, to spatial and temporal variation in ecosystem carbon dynamics.

**Methods**

*Microcosm design*

Litter and soil were collected from three sites within the continental United States (see Plate 4.1). Grass litter (*Hordeum murinum*) was collected from Sedgwick Reserve, California (34°42′ N, 120°03′ W), pine (*Pinus taeda*) from Duke Forest, North Carolina (35°58′ N, 79°05′ W), and rhododendron (*Rhododendron maximum*) from the Coweeta Long Term Ecological Research site, North Carolina (35°00′ N, 83°30′ W). Pine and rhododendron were collected as recent litterfall and grass litter as standing-dead material. Litter was oven-dried (65°C) and then milled (2 mm). Litter was sterilized by autoclaving (121°C, 20 min) twice in succession and again 24 h later. Mineral soil (0–5 cm) was collected together with the litter. Soils were passed through a 2-mm sieve, homogenized, and then stored at 5°C until use as an inoculum. We refer to each soil inoculum as rhododendron, pine, or grass inoculum and each litter type as rhododendron, pine, or grass litter.
To 1 g of litter we added 0.5 g dry mass equivalent of soil inoculum and mixed them by vortexing in a 50-mL plastic centrifuge tube. The mixture was adjusted to and maintained at 50% water-holding capacity, which is favorable for microbial activity. Tubes were incubated at 20°C and 100% humidity during the 300-d experiment. Our design was a 3 × 3 combinatorial setup (i.e., all litters crossed with all soil inocula) plus additional “no-litter” soils. In all, 24 replicates per treatment were constructed, permitting destructive harvest of replicates on incubation days 25, 99, and 300 for chemical and microbial analyses (see Clone library construction and sequencing). The soil inoculum accounted for an average of <5% of the total CO₂ flux across all treatment combinations, and soil carbon (much of which is not likely to be readily bioavailable) accounted for <5% of total carbon in each microcosm (see Appendix F).

_Determination of carbon mineralization rates and litter chemistry_

Carbon mineralization rates were determined across 300 days using a static incubation procedure as described in Fierer et al. (2003). Carbon mineralization rates were determined on eight replicate samples per treatment combination at 22 time points during the course of the incubation, and carbon dioxide production rates were corrected for the contribution of the corresponding soil inoculum by subtracting the carbon mineralization rates of the “no-litter” soils. Since samples were harvested on days 25, 99, and 300 in order to assess the composition of the microbial community (see Clone library construction and sequencing below), we decided that those harvested microcosms should correspond to the same eight replicates per treatment measured up to a given harvest date. Specifically, those microcosms harvested on days 25, 99, or 300 were the same
microcosms on which we measured carbon mineralization rates during days 2–25, 26–99, or 100–300 of the incubation, respectively. This resulted in three incubation periods corresponding to days 2–25, 26–99, and 100–300. Since a treatment within each of these incubation periods was composed of different replicates, we measured all microcosms regardless of their specified incubation period on days 25 and 99 in order to insure that each set of replicates had similar carbon mineralization rates. It should be noted that microcosms from all three incubation periods (i.e., three sets of eight replicates) were measured on day 25, but only microcosms from the last two incubation periods (i.e., two sets of eight replicates) were measured on day 99, since the first set of microcosms had already been destructively harvested. No significant difference in mineralization rates was detected for samples from differing incubation periods measured on the same day ($F_{2, 357} = 0.12; P = 0.89$). Since no differences in mineralization rates were detected between samples measured on the same day but differing in incubation period we felt confident in using the entire data set to calculate cumulative carbon mineralized across the entire 300-d period of this experiment (See Statistical analyses below).

Total carbon, total nitrogen, and C:N ratios for each inocula and litter were determined using an NA1500 CHN analyzer (Carlo Erba Strumentazione, Milan, Italy).

Clone library construction and sequencing

For each of the nine unique litter–soil inoculum combinations, DNA was extracted from samples at three time points (days 25, 99, and 300 of the incubation periods) using the PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, California, USA), after grinding 1 g of each sample with a mortar and pestle in liquid nitrogen. We monitored
microbial community composition at each of these three time points in order to determine whether microbial community composition ever converged, an approach suggested by Reed and Martiny (2007). The DNA was extracted from three replicate samples per litter-soil inoculum combination at each time point, and these were pooled prior to polymerase chain reaction (PCR) amplification. This yielded a total of 27 unique DNA samples from which the clone libraries were constructed.

The DNA was also extracted and clone libraries were constructed from an additional 12 samples (two of the grass litter–grass soil inoculum treatments and two of the rhododendron litter–rhododendron soil inoculum treatments at each of the three time points). This was done to assess variability in microbial community composition within a given treatment at a given time point. In all cases, the replicate samples (the clone libraries constructed from the same litter–soil inoculum–time point combination) were essentially identical with respect to microbial community composition, and therefore we present only the results from the 27 clone libraries generated by the different treatments (nine litter type/soil inoculum combinations at three time points).

Clone libraries were constructed by amplifying the DNA samples with the universal primer pair 515f (5′-GTGCCAGCMGCAGCGGTAA-3′) and 1391r (5′-GACGGGCGGTGWGTRCA-3′). This primer pair amplifies small subunit rRNA genes from all three domains (Archaea, Bacteria, and Eukarya) (Baker et al. 2003, Cullen and MacFarlane 2005, Martiny et al. 2006) yielding a PCR product that is 850–1100 base pairs (bp) in length. Although this primer set may not necessarily amplify all taxonomic groups equally, any amplification bias should be consistent across all of the samples. Each 50-μL PCR reaction contained 1× PCR buffer, 2.5 mmol/L MgCl₂, 0.2 mmol/L of
each dNTP, 0.2 μmol/L of each primer, 0.5 units of Taq DNA polymerase (Invitrogen, Carlsbad, California, USA), and 2 μL of template DNA. Each DNA sample was amplified with three replicate PCR amplifications (30 cycles each, annealing temperature of 52°C) with the amplicons from each sample pooled prior to cloning. Amplicons were cleaned using the Promega WizardSV Gel Extraction and PCR Clean Up kit (Promega, Madison, Wisconsin, USA).

Amplicons were cloned using TOPO TA for Sequencing kit (Invitrogen) following the manufacturer's instructions. Plates were grown overnight at 37°C before plasmid amplification and sequencing. A total of 32 clones were sequenced per sample at Agencourt Bioscience (Beverly, Massachusetts, USA). Despite the small size of these libraries, they were sufficiently large to allow us to compare quantitatively community structure at a general level of phylogenetic resolution (see Sequence analyses).

Sequence analyses

Sequences were binned into major taxonomic groups (i.e., metazoan, bacteria, and fungi) using the BLAST algorithm (Altschul et al. 1997) against the complete European Ribosomal RNA database (http://www.psb.ugent.be/rRNA/index.html). Sequences with expect (E) values greater than $1 \times 10^{-100}$ to nearest neighbors were not included in the analyses. Of the clones sequenced, ~3% of the sequences were not included in the analyses as they were either of low quality, chimeric, or were neither bacterial nor fungal. The taxonomic classification of the fungal sequences was determined by BLAST search against the European Ribosomal RNA database. The bacterial sequences were aligned against the Greengenes database (DeSantis et al. 2006b) using the NAST aligner.
and classified into taxonomic groups with the Greengenes “classify” utility (http://greengenes.lbl.gov/cgi-bin/nph-classify.cgi). Only those sequences sharing >90% similarity to reference sequences over a 600-bp region were classified. Taxonomic characteristics of the microbial communities are provided in Table 4.1. Sequences were deposited in GenBank under the accession numbers EU729748–EU730583 and EU725929–EU726201 for bacteria and fungi, respectively.

The bacterial and fungal sequences were combined together and aligned against bacterial and fungal guide sequences downloaded from the Silva ssu RNA database (http://silva.mpi-bremen.de/). Sequences were aligned using MUSCLE (Edgar 2004) and a neighbor-joining tree containing all bacterial and fungal sequences from the constructed libraries. In addition, an outgroup (Haloflexx volcanii) was constructed in MEGA (Tamura et al. 2007).

We used the weighted UniFrac algorithm (Lozupone et al. 2006) to determine whether soil inocula affected the composition of the microbial communities on each litter type. UniFrac provides an overall estimate of the phylogenetic distance between each pair of communities by examining the fraction of the total branch length within a single phylogenetic tree that is unique to either of the two communities (as opposed to being shared by both; Fierer et al. 2007).

Statistical analyses
Statistical analyses of mineralization data were performed using S-Plus 7.0 (Insightful, Seattle, Washington, USA). To examine treatment effects on mineralization rates across time, a linear mixed-effects model was used in which time, inoculum source, and litter
type (all discrete variables) were treated as fixed effects and permitted to interact. Microcosm identity was included as a random effect to account for the repeated sampling across time of microcosms. We analyzed the cumulative carbon mineralization rates using ANOVA with litter type, inoculum source, and block as discrete variables; inoculum source and litter type were permitted to interact. Cumulative carbon mineralization rates were determined by integrating values under the curve of the 300-d sampling period. Since replicates were destructively harvested for molecular analyses, to generate cumulative values we blocked replicates within a treatment by mineralization rates and generated the cumulative values from samples within a block. This approach enabled us to determine whether litters crossed with their home (as opposed to foreign) soil inoculum resulted in the highest, cumulative carbon mineralization, permitting us to explore our “home field advantage” hypothesis. Pairwise comparisons of means were explored using the Tukey method to assess differences between inocula within the same litter type. For statistical significance we assumed an α level of 0.05 and, when reported as such, data were log$_{10}$-transformed to conform to assumptions of homoscedasticity (verified using model checking).

**Results**

*Carbon mineralization*

Across the course of the incubation we noted that in most cases, and regardless of the inoculum–litter combination, that carbon mineralization dynamics followed an approximately similar three-stage pattern. This consisted of an initial peak in mineralization rates during the first 25 d, a secondary peak lasting from about day 30 to
as much as day 150, and finally a decline phase characterized by a gradual decrease in mineralization rates over time (Fig. 4.1).

When examining each litter in turn, for rhododendron litter we found a significant two-way interaction between inoculum and time on carbon mineralization rates for each incubation period ($P < 0.0001$ in all cases; Table 4.2, Fig. 4.1A). The interactions, across all incubation periods (i.e., days 2–25, 26–99, and 100–300), showed that mineralization dynamics were different between at least two of the inocula and that these differences between the inocula were dependent upon time. For pine litter a significant two-way interaction between inoculum and time was found for the first two incubation periods ($P < 0.0001$ in both cases; Table 4.2, Fig. 4.1B). In the third incubation period (days 126–300), the interaction was not significant but the main effects of inoculum ($P < 0.0001$) and time ($P < 0.0001$) were (thus, relative differences between inocula were time independent). For grass litter we found a significant interaction between inoculum and time on carbon mineralization rates for incubation periods one, two, and three ($P < 0.05$, $P < 0.0001$, and $P < 0.001$, respectively; Table 4.2, Fig. 4.1C).

To examine further the dynamics we saw in the time course data, we looked at cumulative mineralization across the 300-d experiment (Fig. 4.2). This examination showed a significant two-way interaction term of inoculum and litter on cumulative carbon mineralization rates ($P < 0.001$; Table 4.3). We investigated this result by looking at the inoculum effect for each litter type individually to test our sub-hypothesis of “home field advantage.”

For each litter type we found a significant effect of inoculum on cumulative carbon mineralization ($P < 0.001$ in all cases). Post hoc analyses on rhododendron litter
showed that all three inocula differed, with the rhododendron inoculum yielding a higher cumulative value than either the pine or grass inoculum (Fig. 4.2A). For pine litter, all three inocula again differed except in this instance the pine inoculum yielded a higher cumulative value than either of the other two inocula (Fig. 4.2B). For grass litter, both grass and rhododendron inocula yielded a higher cumulative value than did the pine inoculum (Fig. 4.2C).

*Microbial community*

For the UniFrac analyses we examined each litter type separately (building a separate tree for each litter type), combining the sequences for each soil inoculum from the three time points. By doing this, we could examine the overall influence of the inoculum source on microbial community composition in each litter type across the entire incubation period. We found that, as expected, litter type influenced microbial community composition (data not shown). However, within each litter type, we also found a significant influence ($P < 0.01$) of soil inoculum on microbial community composition (Fig. 4.3), as determined by both the UniFrac significance test and the phylogenetic (P) test (Martin 2002). In most instances, the inoculum effects on the microbial communities were apparent at even the coarsest levels of taxonomic resolution (e.g., shifts in bacterial:fungal ratios or shifts in the dominance of particular bacterial phyla; Table 4.1). Notably, the resulting microbial community composition from the different inocula was most dissimilar in the grass litter microcosms and most similar in the rhododendron litter microcosms. That is, the more chemically complex the litter habitat, the more similar the resulting community compositions from the different inocula (Fig. 4.3, Table 4.1).
Furthermore, microbial communities on the same litter habitat never converged compositionally across the course of this experiment (Fig. 4.3, Table 4.1).

**Discussion**

The three-stage pattern observed in mineralization dynamics across time (Fig. 4.1) is consistent with a recently proposed mathematical model (Moorhead and Sinsabaugh 2006) that incorporates three guilds of microbial decomposers. However, we found that the magnitude and characteristics of each stage within a given litter habitat were strongly determined by the inoculum source (Fig. 4.1, Table 4.2). Indeed, the main effect of inoculum explained 20% of the variation in cumulative carbon mineralization across litter types, and its interaction with litter type explained an additional 25% of the variation (Fig. 4.2, Table 4.3). When examining the inoculum effect for each litter separately, it explained between 22% and 86% of the variation in cumulative carbon mineralization (Fig. 4.2, Table 4.3). The significant inoculum effects and inoculum interactions with time refute the hypothesis that microbial communities are functionally equivalent. These results are contrary to those studies (Balser and Firestone 2005, Langenheder et al. 2005, 2006, Ayres et al. 2006, Wertz et al. 2006) that did not observe differences in carbon mineralization rates between different microbial communities, and these results demonstrate that microbial communities may impact processes that are generally presumed to be carried out equivalently by a wide array of organisms (Schimel 1995). Indeed, the competing hypothesis of functional dissimilarity was supported across the course of the entire 300-day experiment. Given that the composition of the communities in a given litter habitat, from different inocula, were consistently distinct across time (Fig.
When the rhododendron and pine inocula were combined with their “home” litter resources, the combinations yielded significantly greater cumulative mineralization than instances in which the inocula were “foreign” to the litter (Fig. 4.2A, B, Table 4.3). The grass inoculum yielded cumulative mineralization values that were equivalent to the rhododendron inoculum and higher than the pine inoculum only with grass litter (Fig. 4.2C, Table 4.3). These results support the “home field advantage” hypothesis (Gholz et al. 2000, Ayres et al. 2006), suggesting that soil microbial communities display some adaptation (or pre-adaptation) to their past resource environment.

If adaptation to the past resource environment explains the “home field advantage” phenomenon that we observed (Fig. 4.2), this implies that decomposer communities may be selected for, at least in part, by the specific characteristics of past litter inputs as both Gholz et al. (2000) and Ayres et al. (2006) have suggested. More specifically, litter characteristics may lead to changes in the composition of a given microbial community, as observed in this study (Fig. 4.3, Table 4.1). Indeed, we chose three litter resources that presented a gradient in litter chemical complexity and recalcitrance, as indicated by both C:N ratios (Appendix F) and by in situ decomposition rates reported for identical or very similar litter types (Andren et al. 1992, Sanchez 2001, Ball et al. 2008), with rhododendron representing the most, and grass the least, complex and recalcitrant litter. Potentially then, these differences in resource quality could account for the strong home field advantage observed for both the rhododendron and pine inocula on their respective home litters and the weaker home field advantage observed for the
grass inoculum on the grass litter (Fig. 4.2, Table 4.3). Notably, Langenheder et al. (2005) observed that a microbial community inoculum from a lake with high-quality dissolved organic carbon (DOC) yielded respiration rates lower than inocula from low-quality DOC environments, when exposed to common garden environments with low-quality DOC. Also, soils pre-exposed to a pesticide or herbicide may degrade that compound more rapidly in subsequent applications (Taylor et al. 1996, Laha and Petrova 1997). Together, these results suggest that the ability of microbial communities to decompose carbon compounds may be contingent on past exposure to similar compounds (Taylor et al. 1996, Laha and Petrova 1997) and that the more chemically complex litter carbon is, the more likely community function will be contingent on history and not simply on chemical quality alone.

Our study cannot unambiguously disentangle whether the functional differences between the inocula were the result of differences in the abundance of different taxa or the degradative capacities of individuals from the same taxa from different inocula. If only the former mechanism applied then communities of more similar composition would function more similarly. We did not observe this relationship; instead, those communities that developed on rhododendron litter were most similar in composition (Fig. 4.3) and less similar with regards to their function (Figs. 4.1 and 2.2). This observation was investigated statistically and the similarity plots are shown in Appendix G. Intriguingly, these data suggest that the manner in which microbial communities respond compositionally to environmental variation may be a poor predictor of their impacts on ecosystem function and communities that are more similar in taxonomic composition may not necessarily be more similar with regards to their
functional capabilities. Or to rephrase that statement using the definitions from the biodiversity–ecosystem functioning literature, functional response groups may not correlate with functional effect groups (Naeem and Wright 2003). This may make prediction of the manner in which changing microbial community composition will impact ecosystem functioning highly uncertain. However, at least from the functional response group perspective, the microbes behaved like animal and plant communities. That is, the harsher environmental filter (i.e., rhododendron > pine > grass litter) generated communities of more similar phylogenetic composition (Fig. 4.3).

Ecosystem models are generally designed without incorporation of the potential effects of variation in microbial community composition or the functional capacities of individuals from the same taxonomic level on ecosystem processes (Zak et al. 2006). Yet, under similar environmental conditions, our results show that the effects of different microbial inocula can account for >85% of the variation in litter carbon mineralization (Table 4.3) and significantly alter the temporal dynamics of this process (Fig. 4.1). Before exploring the consequences of these findings and their possible implications for predicting ecosystem carbon dynamics, we propose two necessary areas of research. First we should resolve whether the taxonomic composition of microbial communities or the past exposure of communities to specific resource conditions predominately impacts ecosystem function through time. That is, does community composition impact future functioning and/or is functioning a product of the selection environment (Loehle and Pechmann 1988, Goddard and Bradford 2003)? Second, and building on ideas relating to adaptation, future work should resolve whether microbial communities from different selection environments functionally converge when placed in
a similar environment. Although our experiment ran for 300 days, to reliably test this possibility we would need an experimental design in which microbial communities sourced from differing environments were exposed to continuous inputs of the same resource. By using such an approach we may be able to determine how rapidly microbial communities adapt to new resource conditions and whether different communities functionally converge. Such studies might then inform us as to how rapid land use conversion may impact microbially mediated biogeochemical processes and would be vital to our understanding of the degree to which ecosystem functions are resistant and resilient to environmental change.

Our findings suggest that soil microbial communities of differing composition are functionally dissimilar. We propose future work to explore further the assumption of functional equivalence for soil microbial communities. If the weight of evidence supports the alternate hypothesis of functional dissimilarity, then the historical factors that shape microbial community functioning must be explored in ecosystem models to predict accurately how the carbon cycle will respond to global change.

Acknowledgements

We thank Tom Maddox in the Analytical Chemistry Laboratory of the Odum School of Ecology, University of Georgia, for element analyses. We also thank two anonymous reviewers who helped to significantly improve an earlier version of this manuscript. The authors acknowledge funding from the Andrew W. Mellon Foundation and from the National Science Foundation to the Coweeta LTER.
References


Table 4.1 Taxonomic description of the microbial communities of each litter type/soil inoculum combination at each of the three time points.

<table>
<thead>
<tr>
<th>Litter type</th>
<th>Soil Inoculum</th>
<th>Incubation Day</th>
<th>% Bacteria (all groups)</th>
<th>% Fungi (all groups)</th>
<th>% Proteobacteria</th>
<th>% Acidobacteria</th>
<th>% CFB</th>
<th>% Firmicutes</th>
<th>% Other bacteria</th>
<th>% Ascomycota</th>
<th>% Other Fungi</th>
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<tr>
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Table 4.1 Cont.

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<th>Litter type</th>
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<th>Incubation Day</th>
<th>% Bacteria (all groups)</th>
<th>% Fungi (all groups)</th>
<th>% Proteobacteria</th>
<th>% Acidobacteria</th>
<th>% Actinobacteria</th>
<th>% CFB</th>
<th>% Firmicutes</th>
<th>% Other bacteria</th>
<th>% Ascomycota</th>
<th>% Other Fungi</th>
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<td>8.9</td>
<td>4.0</td>
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Taxonomic description was determined by the 30-32 clones sequenced per library. Firmicutes refers to the ‘Bacillus-Clostridium’ group and CFB refers to the ‘Cytophaga-Flavobacterium-Bacteroides’ group. The “other bacteria” category consists primarily of sequences identified as either Planctomycetes or Verrucomicrobia. The “other fungi” category is evenly divided between sequences identified as Basidiomycota or Zygomyctota.
Table 4.2 Linear mixed-effects model results for the effect of inoculum and time on carbon mineralization rates.

<table>
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<th>Litter type</th>
<th>Incubation period (days)</th>
<th>Inoculum effect</th>
<th>Inoculum × Time effect</th>
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<td>2</td>
<td>21</td>
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<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>100-300</td>
<td>2</td>
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</tr>
<tr>
<td>Pine</td>
<td>2-25</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
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<td>26-99</td>
<td>2</td>
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<td></td>
<td>100-300</td>
<td>2</td>
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</tbody>
</table>

Notes: Results were analyzed based on litter type and incubation period. F and P values are reported for significant (P < 0.05) model terms; ns: not significant. All data were log$_{10}$-transformed to correct for heteroscedasticity. F-values and P-values are estimated in linear mixed-effects models using, in this case, restricted maximum likelihood (REML) estimates and are not calculated using least squares methods (Pinheiro and Bates 2000). Hence, means and sums of squares values are not calculated.
Table 4.3 ANOVA tables with percentage sums of squares explained (%SS) for the effects of inoculum across all litter types, and for the effect of inoculum within each litter type, on cumulative carbon mineralization.

<table>
<thead>
<tr>
<th>Litter Type</th>
<th>Source of Variation</th>
<th>d.f.</th>
<th>SS</th>
<th>% SS</th>
<th>F</th>
<th>P</th>
</tr>
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<td>1.8</td>
<td>6.2</td>
<td>16.3</td>
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<tr>
<td></td>
<td>Inoculum × Litter</td>
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All data were log_{10}-transformed.
Figure legends

**Figure 4.1** Time course of carbon mineralization for each litter type and soil inoculum combination. In all cases across all time periods there was a significant inoculum by time interaction on CO$_2$ production rates ($P<0.05$), except in the case of pine litter between incubation days 126 and 300 where there were significant main effects of inoculum source and time only ($P<0.0001$ and $P<0.0001$, respectively, see Table 2.2). In (A) mineralization dynamics for rhododendron litter are shown and in this instance rhododendron inoculum is native to this litter type. In (B) pine litter is represented and pine inoculum is native to this litter type. In (C) grass litter is represented and grass inoculum is native to this litter type. Means are shown for carbon mineralization rates ± 1 SE ($n=8$).

**Figure 4.2** Cumulative carbon mineralized for each litter type by inoculum across the 300 day experiment. The inoculum effects for each litter type were highly significant ($P<0.001$). Letters denote significant differences between treatments within each litter type using a Tukey post-hoc pair-wise comparison and those letters in bold denote the home litter by home inoculum combination, as do grey bars. In (A) cumulative carbon mineralization values are shown for rhododendron litter with rhododendron inoculum being native to this litter type. In (B) pine litter is represented and in (C) grass litter is represented. Means are shown ± 1 SE ($n=8$).

**Figure 4.3** Relative UniFrac distances between the decomposer communities developing from the three soil inocula in rhododendron litter (A), pine litter (B), and grass litter (C). The “home” soil inoculum is highlighted in bold type. The sequence data from the three sampling dates are combined for these analyses to illustrate overall differences in the
microbial communities. For each litter type, the three inocula always yielded distinct communities ($P<0.01$), as determined by the UniFrac significance test and the Phylogenetic (P) test (see text for details).
Figure 4.1

(A) Rhododendron litter

(B) Pine litter

(C) Grass litter

Carbon mineralization rate (µg C-CO$_2$ h$^{-1}$)

Time (incubation day)
Figure 4.3

A

Rhodo. soil
Grass soil
Pine soil

B

Rhodo. soil
Pine soil
Grass soil

C

Grass soil
Pine soil
Rhodo. soil

0.02
Plate legends

Plate 4.1. Sites (U.S.) where litter and soil inocula were collected: (top left) Sedgwick Reserve, California, (lower left) Duke Forest, North Carolina, and (right) Coweeta Long Term Ecological Research site, North Carolina (Photo credits: B. A. Ball).
Plate 4.1
CHAPTER 5

LITTER QUALITY IS IN THE EYE OF THE BEHOLDER: INITIAL DECOMPOSITION RATES AS A FUNCTION OF INOCULUM CHARACTERISTICS

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Abstract

1. The chemical composition of plant litter is commonly considered to indicate its quality as a resource for decomposer organisms. Litter quality, defined in this way, has been shown to be a major determinant of litter decomposition rates both within and across terrestrial ecosystems. Notably, the structure of the microbial community that is directly responsible for primary decomposition is rarely considered as an empirical predictor of litter decay rates.

2. Microbial communities are generally assumed to perceive litters of the same chemical composition to be of equivalent resource quality but evidence from field studies suggests that these same communities may adapt to the prevalent litter types at a given site. Here, we tested this assumption by assessing how microbial communities sourced from different forest- and herbaceous-dominated ecosystems perceive the quality of novel, foliar litters derived from a tree (*Rhododendron maximum*) and from a grass (*Panicum virgatum*) species. Based on chemical composition, we would expect *R. maximum* litter to be of lower quality than *P. virgatum* litter.

3. We used an experimental litter-soil system which employs a ‘common garden’ approach and measured rates of CO₂ production across 50 days; higher rates of production were assumed to indicate higher quality (i.e. more easily degradable) litter.

4. We found that communities sourced from habitats under differing plant cover perceived litter quality differently. Those communities sourced from herbaceous habitats perceived the grass litter to be of higher quality than the tree litter, whereas communities from forest habitats decomposed both litter types similarly. Within a litter type,
differences in both community composition and nutrient availability of the source habitat were related to decomposition rates.

5. Our results suggest that litter quality cannot necessarily be predicted solely from chemical characteristics; instead the perceived quality is dependent on the quality of past resource inputs a community has experienced and the structure of those microbial communities responsible for the initial stages of litter decomposition.

**Key-words:** Bacteria, Carbon mineralization, Fungi, Resource history, Soil properties

**Introduction**

The decomposition of plant litter by soil microbial and faunal communities is one of the most heavily researched areas in modern ecology and this process is one of the primary pathways in the terrestrial carbon cycle (Raich & Schlesinger 1992; Couteaux *et al.* 1995; Aerts 1997). Research on litter decomposition has shown that, at the global scale, decomposition rates of plant litter are strongly related to mean annual temperature (MAT) and precipitation (MAP) (Meentemeyer 1978; Vitousek *et al.* 1994; Aerts 1997). Within individual ecosystem or biome types, litter quality becomes a better determinant of decomposition rates than climate (Meentemeyer 1978; Aerts 1997). At the ecosystem scale, litter quality is most often related to the chemical characteristics of the litter, for example carbon-to-nitrogen ratios and/or lignin content (Aber *et al.* 1990; Aerts 1997). Support for this relationship is derived from a multitude of litter decomposition studies which often employ experimental set-ups in which a range of litter types are decomposed across a variety of different biomes (Vitousek *et al.* 1994; Gholz *et al.* 2000; Hobbie *et al.* 2006; Hobbie *et al.* 2007; Parton *et al.* 2007). Based on this evidence most models of
ecosystem carbon dynamics rely heavily on the effects of climate (i.e. MAT and MAP) as well as estimates of quality based on litter chemistry (Parton 1983; Heal 1997).

Models of decomposition based on climate and litter chemistry effectively ignore the potential influence of microbial community structure on rates of litter decomposition (Zak et al. 2006; Reed & Martiny 2007). This omission is partly due to the difficulty associated with manipulating microbial communities in situ and is also largely based on the assumption that given their abundance, diversity, and ubiquity soil microbial communities are, for the most part, redundant with regards to their functional attributes (Cardinale et al. 2007; Jiang 2007; Verity et al. 2007). This assumption has been questioned (Schimel 1995; Martiny et al. 2006; Zak et al. 2006; Ramette & Tiedje 2007; Reed & Martiny 2007) but there are surprisingly few studies directly testing how distinct microbial communities may perceive the decomposability of a given litter type. Indeed, we do know that microbial communities are not homogeneous, varying across space and time in composition due to environmental factors and/or historical contingencies (Andrén & Balandreau 1999; Behan-Pelletier & Newton 1999; Wall & Virginia 1999; Fierer & Jackson 2006; Martiny et al. 2006; Ramette & Tiedje 2007; Reed & Martiny 2007). Furthermore recent work has demonstrated both implicitly and explicitly that structural differences in the microbial community can influence ecosystem function (Balser & Firestone 2005; Reed & Martiny 2007; Strickland et al. 2009). Such results demonstrate that, like comparisons between individual species of microorganisms (Newell 1984; Dowson et al. 1989; Osono & Takeda 2002), whole microbial communities also differ with regards to their functional capacity and these differences may influence the process of litter decomposition.
The specific mechanisms which lead to functional dissimilarity (see Strickland et al. 2009) in litter decomposition amongst microbial communities remains poorly understood. One possible reason that functional dissimilarity may arise is due to a microbial community’s past exposure to litters of differing chemistry (Hunt et al. 1988; Gholz et al. 2000). Implicit evidence of this phenomenon has been shown for both the degradation of subsequent applications of pesticides and litter residues (Taylor et al. 1996; Laha & Petrova 1997; Cookson et al. 1998) as well as in reciprocal litter transplant experiments (Hunt et al. 1988; Gholz et al. 2000; Castanho & de Oliveira 2008; Vivanco & Austin 2008). However, it is unknown whether or not the past exposure of a microbial community to litter inputs of differing chemistry also relates to that community’s ability to decompose novel litters which themselves differ chemically. Such knowledge will improve our ability to understand, and possibly predict, the factors which influence litter decomposition rates.

To test whether or not past exposure of microbial communities to high or low quality litters influences the decomposition of novel litters (to those communities) which differ in litter chemistry, we used a ‘common garden’ experimental approach. We paired one of 12 soil inocula, six sourced from herbaceous habitats and six sourced from forest habitats, with one of two litters which differed in their litter chemistry. We hypothesized that soil microbial inocula from forested habitats would perceive the lower-nutrient, higher-lignin litters, taken from a tree species, to be of higher ‘quality’ than would inocula sourced from habitats dominated by herbaceous cover. Similarly, we reasoned that the higher-nutrient, lower-lignin litters, taken from a grass species, would be perceived of equivalent ‘quality’ by all inocula. This hypothesis was based on the
expectation that microbial communities which develop in forest habitats will be adapted (or pre-adapted) to decompose lower-nutrient, higher-lignin litters due to a history of exposure to them, which will have acted as a selection pressure and/or an environmental filter (sensu Lambers 1998). Hunt et al. (1988) present field data which may be explained by this hypothesis. To test the hypothesis our microcosm, experimental design permits effects associated with the microbial community to be separated from co-varying factors in the field. Henceforth, we use the term ‘perceived quality’. This is, essentially, a biotic definition of litter quality (see Fierer et al. 2005) because a litter’s decomposition rate in our experiment is a function of how it is “perceived” by the microbial community.

**Methods**

**Microcosm design**

The tree foliar litter was taken from *Rhododendron maximum* collected from the Coweeta Long Term Ecological Research (LTER) site, North Carolina, USA (35°00’N, 83°30’W) as recent, senesced litterfall. The herbaceous litter was taken from *Panicum virgatum* and was collected from the University of Georgia, Georgia, USA (33°53’N, 83°21’W) as recent, standing dead material. We chose these species because they have litters of low and high chemical quality, respectively (Table 5.1). That is, *R. maximum* litter had a C:N ratio which was nearly twice as great as, and a lignin concentration greater than twice that of, *P. virgatum* litter. Neither litter was present at the sites from where the inocula were sourced. This design ensured that effects of differing litter chemistries were not confounded by prior exposure to the litter species used (i.e. ‘home-field advantage’, sensu Gholz et al. 2000). Litters were air-dried, passed twice through a Wiley mill (2 mm
mesh), and then sterilised by autoclaving twice in succession and again 24 h later (121°C, 
20 min), before being dried to constant mass at 65°C. The milling was done to increase 
surface area for microbial colonization, to make the material between replicates more 
homogenous, and to remove the influence of physical litter structure on decomposition 
rates. For example, the slow decomposition rate of *R. maximum* foliar litters in the field 
(e.g. Ball *et al*. 2008) is thought to result from both its chemical composition and 
physically-tough leaves.

Soils for use as inocula were collected from, or adjacent to, the Calhoun 
Experimental Forest (CEF), which is managed by the US Department of Agriculture and 
located in the Piedmont region (approximately 34.5°N, 82°W) of northwestern South 
Carolina, USA (Gaudinski *et al*. 2001; Callaham *et al*. 2006). Twelve soil inocula were 
collected from four land-uses (cultivated, pasture, pine, and hardwood) within this region, 
each land-use being represented three times (4 land-uses × 3 locations = 12 inocula). This 
provided six inocula from forest habitats (pine and hardwood) and six from habitats 
dominated by herbaceous vegetation (cultivated and pasture). Each inoculum was a 
subsample from soil samples composed of 10 individual A horizon soil cores (8 cm dia., 
0 - 7.5 cm depth), which were collected from a 100 m² plot within each of the 12 sites 
using a stratified random sampling approach. Soils were sieved (4 mm), homogenized, 
and stored at +5°C until use.

Microcosms were constructed by adding 0.5 g dry mass equivalent of soil 
inoculum to 1 g of litter, which was then mixed by vortexing in a 50 mL plastic 
centrifuge tube. The mixture was adjusted to and maintained at 50% water-holding 
capacity, which is favourable for microbial activity. Tubes were incubated at 20°C and
100% humidity during the experiment. Our design was a $2 \times 12$ combinatorial set-up (i.e. all litters crossed with all soil inocula) plus additional “no-litter” soils. This resulted in two inocula treatments, herbaceous and forest. The herbaceous inocula were composed of three inocula sourced from cultivated sites and three sourced from pasture sites giving a total of 6 herbaceous replicates. The forest inocula were composed of three inocula sourced from hardwood stands and three inocula sourced from pine stands giving a total of 6 forest replicates. Six analytical repeats were constructed for each inoculum × litter combination giving 144 experimental units (i.e. $12$ inocula × $2$ litters × $6$ analytical repeats). To avoid pseudoreplication the mean was taken across the 6 analytical repeats for a given inoculum × litter combination resulting in 6 herbaceous and 6 forest replicates per litter type. The soil inocula accounted for an average of $\sim 5\%$ of the total carbon (C) dioxide ($CO_2$) flux across all treatment combinations and soil C (much of which is not likely to be bioavailable) accounted for $<20\%$ of total C in each microcosm (see Table 5.1, see Appendix H).

*Determination of decomposition rates and litter chemistry*

Litter decomposition rates were estimated by periodic measurement of CO$_2$ production rates from the microcosms. This was done across 50 days using a 24 h static incubation procedure described in Fierer *et al.* (2003). Before the start of the incubation period, soil-litter mixtures were incubated for 10 days to allow microbial colonization of the litters. Following this period CO$_2$ production rates were measured on days 1, 5, 10, 20, 30, 40, and 50 and were corrected for soil contributions by subtracting the production rates
measured from the corresponding ‘no-litter’ soils. Cumulative CO\textsubscript{2} production was calculated by integrating values under the curve for the incubation period.

Total percentage C, nitrogen (N), non-fibrous material, hemi-cellulose, cellulose, lignin, and pH, were determined for both litter types (Table 5.1). Total C and N were determined using an NA1500 CHN Analyzer (Carlo Erba Strumentazione, Milan, Italy). Non-fibrous material, hemi-cellulose, cellulose, and lignin concentrations were determined using an Ankom A200 Fiber Analyzer (Ankom, Macedon, USA). Litter pH was determined in water (2:1 ratio of water-to-litter) using a benchtop pH meter.

*Determinations of inoculum source edaphic characteristics*

For each site from which an inoculum was sourced, we determined a series of edaphic factors. Carbon and nutrient pools, microbial biomass, pH, soil texture, bulk density, and cation concentrations were calculated from three analytical replicates per soil sample from each inoculum source.

Total, POM (particulate organic material) associated, and mineral associated C and N were determined using an NA1500 CHN Analyser (Carlo Erba Strumentazione, Milan, Italy). Carbon and N associated with POM and mineral material were determined using the fractionation method described in Bradford *et al.* (2008). Microbial biomass C and N, and dissolved organic C (DOC), were determined using the method described in Fierer and Schimel (2003). Ammonium (NH\textsubscript{4}\textsuperscript{+}) and nitrate (NO\textsubscript{3}\textsuperscript{−}) were determined colorimetrically following Fierer and Schimel (2002). Extractable phosphorus (P) was measured on an Alpkem auto-analyzer (OI Analytical, College Station, TX) using Murphy-Riley chemistry after extraction with Mehlich I double-acid (H\textsubscript{2}SO\textsubscript{4}–HCl) using
a 1:4 mass:volume ratio (Kuo, 1996). Soil pH was determined in water (1:1 ratio of water-to-soil) using a benchtop pH meter. Silt and clay contents were measured using a simplified version of the hydrometer method as described by Gee and Orr (2002). Soil bulk density was calculated after correcting for the mass and volume of roots and stones (Culley 1993). Exchangeable cations (Ca\(^+\), Mg\(^+\), and K\(^+\)) were measured by atomic absorption in the presence of LaCl\(_3\) after extraction with 1 M NH\(_4\)OAc (pH 7) using a 1:10 mass:volume ratio (Sumner & Miller 1996). Edaphic data for each inoculum source are provided in Appendix H.

**Determination of the starting community composition of the soil inocula**

Soils for community analyses were collected and treated the same as those collected for edaphic characterization. A DNA-based rather than an RNA-based assessment of microbial communities was used because it probably better reflects the potential pool of microbes that might develop on our microcosm litter environments from the soil inocula; also DNA-based assessments are less variable and hence provide a more integrated assessment of differences in microbial community inocula. DNA was isolated from 3 replicate sub-samples of fresh soil per plot using the MoBio Power Soil DNA Extraction kit (MoBio Laboratories, Carlsbad, CA) with the modifications described in Lauber *et al.* (2008). Each DNA sample was amplified in triplicate using both bacterial and fungal specific primer sets described in Lauber *et al.* (2008) in order to assess bacterial and fungal community composition in each sample. For each plot, we constructed an individual clone library and sequenced 80-100 cloned amplicons per library following standard protocols. Additional details on the PCR conditions, clone library construction,
and sequencing are provided in Lauber et al. (2008). To classify the fungal sequences, we used the BLAST algorithm (Altschul et al. 1990) to compare the fungal sequences to an in-house data base of 100 fungal 18S sequences derived from the AFTOL data set (Lutzoni et al. 2004). Bacterial sequences were aligned against the Greengenes data base (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi) using the NAST alignment utility (DeSantis et al. 2006a) and fungal sequences were aligned using MUSCLE 3.6 (Edgar 2004a). The sequences were chimera-checked using utilities available on the Greengenes website (DeSantis et al. 2006b). Sequences which were of poor quality or suspected to be chimeric were eliminated from the analysis (less than 9% of the sequences). Sequences were aligned using MUSCLE 3.6 (Edgar 2004b) and a neighbor-joining phylogenetic tree containing either all of the bacterial or all of the fungal sequences was generated with PHYLIP 4.0. The phylogenetic distance between the bacterial and fungal communities within each soil inoculum was determined using the weighted-normalized UniFrac algorithm (Lozupone & Knight 2005). For full details and results concerning the composition of microbial communities, see Lauber et al. (2008). Briefly, Lauber et al. (2008) found that bacterial community composition was related to both soil pH and soil texture whereas fungal community composition was related to both extractable P and soil C:N ratios.

Statistical analyses

Statistical analyses of cumulative CO\textsubscript{2} production were performed in S-Plus 7.0 (Insightful Corp., Seattle, USA), using ANOVA with litter type and inoculum source as discrete variables that were permitted to interact. Using this approach, a significant
inoculum or inoculum by litter type interaction would support our hypothesis that resource history influences the perception of litter quality whereas only a litter type effect would refute this hypothesis. We further explored our results using correlation and regression approaches (see below). For statistical significance we assumed an $\alpha$-level of 0.05. When reported as such, data were log$_e$-transformed to conform to assumptions of homoscedasticity (verified using model checking).

Relationships between cumulative CO$_2$ production, edaphic characteristics of the source environment, and the starting community composition of the inocula were assessed using Plymouth Routines in Multivariate Ecological Research v5 software (Primer v5, Lutton, UK). Mantel tests were performed to determine if there were significant correlations between community function on either *R. maximum* or *P. virgatum* litter, edaphic characteristics of the source environment, and the starting whole fungal or whole bacterial community composition of the inoculum (individual taxa were examined using regression analyses, see below). Correlations between these factors were considered significant when $P<0.05$.

Linear regression analyses were performed to look at the relationships between cumulative CO$_2$ production on either *R. maximum* or *P. virgatum* and the starting edaphic characteristics of the inocula. This was only conducted for those edaphic characteristics which Mantel tests showed were significantly correlated to cumulative CO$_2$ production. Regression analyses were also used to look at relationships between cumulative CO$_2$ production and the dominant bacterial and fungal taxa in the starting inocula. We were able to look at the relationships between cumulative CO$_2$ production and the dominant bacterial and fungal taxa by performing phylogenetic independent analyses which
recorded the distribution of taxa for each site and normalized these values to represent the percentage of each taxon within a given inoculum. Regression analyses, like ANOVAs, were performed using S-Plus 7.0.

**Results**

*Litter decomposition*

During this 50 day incubation, between 2.4 and 3.1% of *R. maximum* litter C was lost as CO₂ and between 3.1 and 4.2% of *P. virgatum* litter C was lost as CO₂. When examining cumulative CO₂ production, we found a significant inoculum by litter type interaction (*P*<0.01; Fig. 5.1). This interaction suggested that the quality of past resources influences how the community inocula perceive the quality of the two litters. This difference is clearly represented in Fig. 5.1 - cumulative CO₂ production differs from one litter type to the other for the different inocula. Specifically, cumulative CO₂ production was nearly identical for the forest inoculum on either litter resource suggesting that there was no difference in perceived quality. In contrast, the herbaceous inocula perceived the *P. virgatum* litter to be of higher quality than the *R. maximum* litter. To further explore this interaction we examined each litter type separately. We did not detect a significant inoculum effect on cumulative CO₂ production for *P. virgatum* litter (*F*₁,₁₀ = 2.40; *P*=0.15) but did detect a significant inoculum effect on cumulative CO₂ production for *R. maximum* litter (*F*₁,₁₀ = 25.80; *P*<0.001). This suggested that the interaction between inoculum and litter type was driven by minimal differences in CO₂ production on *P. virgatum* litter while CO₂ production did differ between inocula on *R. maximum* litter. To further explain these results and to assess other cover-type related differences in the


perception of litter quality, we explored relationships between cumulative CO$_2$ production and the edaphic and microbial community characteristics of each inoculum (see next).

*Relationship of litter decomposition rates to inoculum characteristics*

When examining how cumulative CO$_2$ production from *R. maximum* litter was related to the microbial community composition of the inocula, we found significant relationships with both fungal and bacterial community composition ($P<0.05$ for fungi and $P<0.01$ for bacteria; Table 5.2). Regression analyses showed that the relative abundances of Sordariomycete, Leotiomycete, Chaetothyriomycete, and Agaricales (the dominant fungal taxa in these inocula; Lauber *et al.* (2008)) were significantly related to cumulative CO$_2$ production (Fig. 5.2a-d). The relative abundances of Leotiomycete and Sordariomycete were negatively related to cumulative CO$_2$ production (Fig. 5.2a-b). The relative abundances of Agaricales and Chaetothyriomycete were positively related (Fig. 5.2c-d). Of the bacterial taxa, the relative abundances of Acidobacteria in the starting inocula were positively related to cumulative CO$_2$ production on *R. maximum* (Fig. 5.2e).

We also found that site specific differences in the soil chemical and physical characteristics from which an inoculum was sourced were related to cumulative CO$_2$ production on *R. maximum* (Table 5.2). More specifically, we found that NO$_3^-$, NH$_4^+$, extractable P, K$^+$, and percentage clay were all significantly related to cumulative CO$_2$ production on *R. maximum* ($P<0.01$ for NO$_3^-$ and NH$_4^+$, $P<0.05$ for P, K$^+$, and %clay; Table 5.2). All except for NH$_4^+$ were negatively related to cumulative CO$_2$ production on *R. maximum* (Fig. 5.3).
When examining how cumulative CO$_2$ production from $P. \ virgatum$ litter was related to the microbial community composition of the inocula, we found that only the composition of the bacterial community was significantly related ($P<0.05$; Table 5.2), while the fungal community composition was not (Table 5.2). Regression analyses of bacterial taxa showed that only the relative abundance of Actinobacteria was positively related to cumulative CO$_2$ production (Fig. 5.2f). Of the soil chemical and physical characteristics, only K$^+$ concentration was positively related to cumulative CO$_2$ production ($P<0.05$; Table 5.2, Fig. 5.3f).

**Discussion**

We initially hypothesized that resource quality history would influence how microbial communities perceived litters which differed in chemical quality. Our expectation was that microbial communities which develop in forest habitats will be adapted (or pre-adapted) to decompose lower chemical quality litters due to their history of exposure to lower quality litters (Hunt et al. 1988; Gholz et al. 2000). We also expected that microbial communities, regardless of their previous history, would be equally well adapted to decompose high chemical quality litters (Hunt et al. 1988). As hypothesized, we found that those microbial communities sourced from forest habitats perceived the low chemical quality $R. \ maximum$ litter to be of higher quality than did the communities sourced from herbaceous habitats (Fig. 5.1). This result is similar to results from reciprocal litter transplant studies which have shown that litters often decompose more rapidly when placed in their native forests (Cookson et al. 1998; Castanho & de Oliveira 2008; Vivanco & Austin 2008). Also confirming our original hypothesis was the
observation that communities sourced from either habitat generally perceived the high chemical quality *P. virgatum* litter equally (Fig. 5.1), a result also observed in studies which have found no differences in decomposition rates across sites for the same litter type (Prescott *et al.* 2000; Ayres *et al.* 2006).

Initial litter decomposition rates with inocula sourced from herbaceous sites confirmed the expectation (Aber *et al.* 1990; Aerts 1997) that litter chemistry is a good predictor of decomposition rates. That is, the *R. maximum* litter with its greater C:N ratio and higher lignin content decomposed more slowly than the *P. virgatum* litter with its lower C:N ratio and lower lignin content (Fig. 5.1). In contrast to the inocula sourced from the herbaceous sites, the decomposition rates observed with the forest inocula were similar for the two litter types (Fig. 5.1). This result agrees with those studies which have found that litter chemistry is not a strong predictor of litter decomposition rates (Hunt *et al.* 1988; Gholz *et al.* 2000) and provides empirical support for the suggestion from these previous studies that the decomposer community characteristics affect litter decomposition rates. Overall, our results imply that an understanding of the microbial community and/or its past resource environment may increase our ability to predict decomposition dynamics. Indeed, the community or the resource history of that community may account for some of the unexplained variation in decomposition models where climate and litter quality are used as explanatory variables (e.g. Gholz *et al.* 2000, Parton *et al.* 2007). Our microcosm experiment demonstrates the potential for both decomposer communities and the resource history of the decomposer community to influence decomposition rates, but it remains to be determined if our results are relevant to decomposition dynamics observed in the field.
To further explore how differences in microbial community structure and resource quality history impact the perception of litter quality we assessed the relationship between cumulative CO$_2$ production of both litter types and the biological, chemical, and physical characteristics of the sites from which each inoculum was sourced. When examining the biological factors of the inocula sources which were related to cumulative CO$_2$ production from either *R. maximum* or *P. virgatum* litters, we found that both fungal and bacterial community composition was related to the cumulative CO$_2$ production from *R. maximum* litter but only the bacterial community composition was significantly related to the cumulative CO$_2$ production from *P. virgatum* litter (Table 5.2). The fact that the fungal community was related to cumulative CO$_2$ production on *R. maximum* but not on *P. virgatum* litter may be due to the ability of fungi to degrade litters of lower-nutrient and higher-lignin content, or at least out-compete bacteria in such environments (Six *et al.* 2006). When more closely examining the fungal community, we found that four fungal taxa (Sordariomycete, Leotiomycete, Chaetothyriomycete, and Agaricales) of the inocula sources were related to the cumulative CO$_2$ production on *R. maximum* litter. We found that those inocula sources which had comparably higher relative abundances of either Sordariomycetes or Leotiomycetes had lower cumulative CO$_2$ production on *R. maximum* litter than sites with lower abundances of these groups (Fig. 5.2a, 5.2b). Conversely, those inocula sourced from sites having a higher relative abundance of Chaetothyriomycete or Agaricales tended to have higher cumulative CO$_2$ production on *R. maximum* litter than did sites with lower abundances of either of these fungal taxa (Fig. 5.2c, 5.2d). This finding is similar to observations which have demonstrated differences in the litter degrading abilities of fungal phyla (Osono & Takeda 2002). Our
observations suggest that such differences may also occur within phyla given that within the phylum Ascomycota the abundance of one taxon (Chaetothyriomycete) was positively related, while two other taxa (Sordariomycetes and Leotiomycetes) were negatively related, to cumulative CO$_2$ production from *R. maximum* litter (Fig. 5.2a-d).

When considering the bacterial community, we found that those inocula sourced from sites with higher relative abundances of Acidobacteria and Actinobacteria were positively related to cumulative CO$_2$ production on *R. maximum* litter and *P. virgatum* litter, respectively (Fig. 5.2e, 5.2f). Acidobacteria have recently been associated with resource environments of lower quality (Fierer et al. 2007) and this may explain why a positive relationship was observed on *R. maximum* litter which has both a high C:N ratio and lignin content (Fig. 5.2e). When considering the Actinobacteria, relatively little is known concerning their ecological attributes. However, due to their filamentous growth form and a presumed ability to effectively scavenge nutrients (Goodfellow & Williams 1983; Steger et al. 2007), those inocula which started with a higher relative abundance of Actinobacteria may be better at decomposing *P. virgatum* litter across the course of our experiment.

Many more chemical and physical factors of the inocula sources were related to cumulative CO$_2$ production on *R. maximum* than on *P. virgatum* (Table 5.2). Of those factors that significantly correlated with CO$_2$ production on *R. maximum* litter we found that all but one was negatively related (Fig. 5.3a-e). Specifically, we observed that NO$_3^-$, extractable P, K$^+$, and percentage clay were negatively related to cumulative CO$_2$ production on *R. maximum* while NH$_4^+$ was positively related. Only K$^+$ was related to cumulative CO$_2$ production on *P. virgatum* and this relationship was positive (Table 5.2,
Soil physical and chemical factors are often products of management. In the region where our inocula were sourced NO$_3^-$, extractable P, and K$^+$ are generally higher in cultivated and pasture sites (than forests) due to annual fertilizer inputs (Richter et al. 2000). Percentage clay is generally higher in cultivated sites due to long-term intensive agriculture and weathering (Richter & Markewitz 2001; Callaham et al. 2006) and NH$_4^+$ is generally higher in forest sites due to lower levels of nitrification (Adams 1986). These relationships between chemical and physical variables and the source environments of the soil microbial inocula make it difficult to determine whether the chemistry of the litter inputs in the source environments, the nutrient status of the environments, or both were important factors in structuring the function of the microbial inocula in our litter microcosms. Notably, differences between the inocula in their chemical and physical characteristics, and microbial community composition, did not follow land use differences exactly (Table S1 and Lauber et al. 2008). This may suggest that litter chemistry (i.e. herbaceous vs. woody plant litters) was the over-riding cause of differences in the functioning of our inocula on the *R. maximum* and *P. virgatum* litters.

Our results provide a foundation upon which future studies can investigate how historical factors that may shape microbial communities may influence their function. In future work, investigation of how microbial community composition develops on different litter types and across time may facilitate a more detailed understanding of the relationships between the taxonomic composition of microbial communities and their function (Osono 2005; Trinder et al. 2008; Strickland et al. 2009). Such studies might address whether compositionally distinct communities, when faced with similar environments, develop in the same manner. They should consider which characteristics
(e.g. lignin content) of litter chemistry likely influence this development both at earlier and later stages of decomposition. They should also ask how long differences in the functioning of microbial communities might persist once different communities are exposed to the same environment. Given the rapid generation times of microbes it seems plausible that microbial community functioning might rapidly converge in the same environment (Strickland et al. 2009). Such information will add to our understanding of the process of decomposition and may also enable prediction of how a given microbial community will respond to environmental change.

Our study involved a short-term, laboratory, common garden approach to determine the significance of microbial community structure and its resource history for the initial decomposition rate of leaf litters of differing chemical composition. There are a number of criticisms that can be leveled at our approach. These include the fact that we do not know whether microfauna, which affect the activities of microbial decomposers, were present in our inocula or not. Certainly, the functional symbiosis of plants and mycorrhizal fungi, which may play a crucial role in decomposition (Talbot et al. 2008), was absent. In addition autoclaving, as any sterilization technique, impacts soil and potentially litter properties (Berns et al. 2008). However, in our study such non-target effects would have been consistent within a given litter type or inoculum and hence would not have been able to explain our most significant finding. That is litter chemistry impacting decomposition rates of litters inoculated with soils from herbaceous but not forested environments.

What our approach did provide was a tightly-controlled experiment which demonstrated that the past resource environment of a microbial community impacted its
ability to decompose litters differing in their chemistry. We cannot conclude to what extent this effect influences decomposition rates in the field, but it does offer one explanation for why different litter chemistries do not always lead to expected differences in decomposition rates in situ (Hunt et al. 1988; Gholz et al. 2000; Castanho & de Oliveira 2008; Vivanco & Austin 2008). Our work suggests that the differential perception of litter chemistry by different microbial inocula may be explained by the starting, community composition of microbial inocula and/or the resource status of the environment from which they were derived. To fully understand decomposition dynamics in the environment across space and time may require recognition and further exploration of the role of microbial community composition and that community’s resource history as a driving variable.

Acknowledgements

We gratefully acknowledge funding from the Andrew W. Mellon Foundation, and from the National Science Foundation to the Coweeta LTER. We thank Tom Maddox in the Analytical Chemistry Laboratory of the Odum School of Ecology, Univ. of Georgia, for element analyses; Dan Richter and Mac Callaham for establishing the permanent plots in the Calhoun Experimental Forest; and Mr. Jack Burnett for allowing us to collect samples on his property.
References


Table 5.1 Initial %C, %N, C:N ratios, %Lignin, and pH of the litters.

<table>
<thead>
<tr>
<th>Litter Type</th>
<th>%C</th>
<th>%N</th>
<th>C:N</th>
<th>%Non-fibrous</th>
<th>%Hemi-cellulose</th>
<th>%Cellulose</th>
<th>%Lignin</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. maximum</td>
<td>48.9 ±</td>
<td>0.42 ±</td>
<td>116 ±</td>
<td>60.91 ±</td>
<td>8.81 ±</td>
<td>17.73 ±</td>
<td>12.54 ±</td>
<td>4.5 ±</td>
</tr>
<tr>
<td></td>
<td>0.24</td>
<td>0.01</td>
<td>1.70</td>
<td>1.23</td>
<td>0.22</td>
<td>0.22</td>
<td>1.15</td>
<td>0.01</td>
</tr>
<tr>
<td>P. virgatum</td>
<td>41.8 ±</td>
<td>0.62 ±</td>
<td>68 ±</td>
<td>38.14 ±</td>
<td>27.53 ±</td>
<td>29.08 ±</td>
<td>5.24 ±</td>
<td>5.2 ±</td>
</tr>
<tr>
<td></td>
<td>0.07</td>
<td>0.02</td>
<td>2.32</td>
<td>0.25</td>
<td>0.52</td>
<td>0.18</td>
<td>0.38</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values shown are means ± 1 SE (n=3).
Table 5.2 Mantel test results showing correlations between either inoculum source edaphic factors or initial inoculum community composition and cumulative CO$_2$ production on either *R. maximum* or *P. virgatum* litter. Spearman’s correlation coefficients relate the calculated Euclidian distance between cumulative CO$_2$ production and edaphic factors; UniFrac distance is used for the community factors. Significant correlation coefficients are reported in bold (*P*<0.05).

<table>
<thead>
<tr>
<th>Edaphic factors</th>
<th>R. maximum litter</th>
<th>P. virgatum Litter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spearman's correlation coefficient</td>
<td>P-value</td>
</tr>
<tr>
<td>DOC (mg g dry weight soil$^{-1}$)</td>
<td>0.104</td>
<td>ns</td>
</tr>
<tr>
<td>NO$_3^-$ (μg g dry weight soil$^{-1}$)*</td>
<td><strong>0.750</strong></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NH$_4^+$ (μg g dry weight soil$^{-1}$)*</td>
<td><strong>0.480</strong></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total C (mg g dry weight soil$^{-1}$)</td>
<td>0.089</td>
<td>ns</td>
</tr>
<tr>
<td>POM C (mg g dry weight soil$^{-1}$)</td>
<td>0.034</td>
<td>ns</td>
</tr>
<tr>
<td>Mineral C (mg g dry weight soil$^{-1}$)</td>
<td>-0.072</td>
<td>ns</td>
</tr>
<tr>
<td>Total N (mg g dry weight soil$^{-1}$)</td>
<td>-0.013</td>
<td>ns</td>
</tr>
<tr>
<td>POM N (mg g dry weight soil$^{-1}$)</td>
<td>0.069</td>
<td>ns</td>
</tr>
<tr>
<td>Mineral N (mg g dry weight soil$^{-1}$)</td>
<td>0.062</td>
<td>ns</td>
</tr>
<tr>
<td>Extractable P (μg g dry weight soil$^{-1}$)*</td>
<td><strong>0.314</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ca$^+$ (mg g dry weight soil$^{-1}$)</td>
<td>0.026</td>
<td>ns</td>
</tr>
<tr>
<td>Mg$^+$ (mg g dry weight soil$^{-1}$)</td>
<td>-0.03</td>
<td>ns</td>
</tr>
<tr>
<td>K$^+$ (mg g dry weight soil$^{-1}$)*</td>
<td><strong>0.251</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>C:N</td>
<td>0.094</td>
<td>ns</td>
</tr>
<tr>
<td>C:P</td>
<td>0.152</td>
<td>ns</td>
</tr>
<tr>
<td>N:P</td>
<td>0.101</td>
<td>ns</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>0.134</td>
<td>ns</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>-0.145</td>
<td>ns</td>
</tr>
<tr>
<td>Clay (%)*</td>
<td><strong>0.244</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>pH</td>
<td>-0.061</td>
<td>ns</td>
</tr>
<tr>
<td>SIR (μg g dry weight soil$^{-1}$)</td>
<td>0.088</td>
<td>ns</td>
</tr>
<tr>
<td>Microbial C (μg g dry weight soil$^{-1}$)</td>
<td>0.060</td>
<td>ns</td>
</tr>
<tr>
<td>Microbial N (μg g dry weight soil$^{-1}$)</td>
<td>0.136</td>
<td>ns</td>
</tr>
</tbody>
</table>

Community factors

<table>
<thead>
<tr>
<th></th>
<th>R. maximum</th>
<th>P. virgatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td><strong>0.417</strong></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Fungi</td>
<td><strong>0.327</strong></td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*Data were log$_e$ transformed for both litters, except for K$^+$ which was only transformed for *R. maximum*. 
Figure legends

Figure 5.1 Cumulative CO₂ production from microcosms consisting of microbial inocula sourced from four land-uses representing two cover-types combined with either *R. maximum* or *P. virgatum* litters. Land-uses representing herbaceous cover-types are the cultivated and pasture inocula and land-uses representing forest cover-types are the pine and hardwood inocula. Values are means±1SE (*n* = 6). ANOVA results are based on cover-type. A significant interaction between inoculum and litter was detected for cumulative CO₂ production (*F*<sub>1,20</sub>=12.71; *P*<0.01). Main effects of litter type and inoculum were significant (*F*<sub>1,20</sub>=10.39; *P*<0.01) and not significant (*F*<sub>1,20</sub>=0.583; *P*=0.454), respectively.

Figure 5.2 Relationships between cumulative CO₂ production and the relative abundances of three fungal taxa (Sordariomycete, Leotiomycte, and Agaricales) and two bacterial taxa (Acidobacteria and Actinobacteria) on *R. maximum* (a-e) and *P. virgatum* litter (f). Circles, inverted triangles, diamonds, and squares represent cultivated, pasture, pine, and hardwood land-uses, respectively. Each individual plot within a given land-use is indicated by a different shade (e.g. Cultivated plot 1 (Cu1 in the legend) is represented by a white circle).

Figure 5.3 Relationships between cumulative CO₂ production and the edaphic characteristics of the site from which the inocula were sourced on *R. maximum* (a-e) and *P. virgatum* litter (f). Symbols are as in Fig. 5.2.
Figure 5.1

Inoculum ns
Inoculum x Litter **
Litter **
Cumulative CO₂-C (mg g litter⁻¹)

Cultivated inoculum
Pasture inoculum
Pine inoculum
Hardwood inoculum

Litter type
P. virgatum
R. maximum
Figure 5.2

Source habitat

---

![Diagram of source habitats: Cu1, Cu2, Cu3, Pa1, Pa2, Pa3, Pi1, Pi2, Pi3, Ha1, Ha2, Ha3.](image)

---

**Panel (a)**
- **R. maximum**
- $r^2 = 0.42$
- $P < 0.05$

**Panel (b)**
- **R. maximum**
- $r^2 = 0.46$
- $P < 0.01$

**Panel (c)**
- **R. maximum**
- $r^2 = 0.57$
- $P < 0.01$

**Panel (d)**
- **R. maximum**
- $r^2 = 0.36$
- $P < 0.05$

**Panel (e)**
- **R. maximum**
- $r^2 = 0.41$
- $P < 0.05$

**Panel (f)**
- **P. virgatum**
- $r^2 = 0.45$
- $P < 0.01$
Figure 5.3

(a) $R. \text{maximum}$

Cumulative CO$_2$-C (mg g litter$^{-1}$)

$r^2=0.54$

$P<0.01$

(b) $R. \text{maximum}$

Cumulative CO$_2$-C (mg g litter$^{-1}$)

$r^2=0.69$

$P<0.001$

(c) $R. \text{maximum}$

Cumulative CO$_2$-C (mg g litter$^{-1}$)

$r^2=0.63$

$P<0.01$

(d) $R. \text{maximum}$

Clay (%)

$r^2=0.63$

$P<0.01$

(e) $R. \text{maximum}$

K$^+$ (mg g dry weight soil$^{-1}$)

$r^2=0.33$

$P<0.05$

(f) $P. \text{virgatum}$

Extractable P (mg g dry weight soil$^{-1}$)

$r^2=0.30$

$P<0.05$
CHAPTER 6
CONCLUSIONS

The role that soil microbial community structure plays in regulating ecosystem processes has been thought redundant, or dependent on binary distinctions of microbes (e.g. fungal:bacterial dominance). In part the presumed functional redundancy of these communities began with the famous quote, “Everything is everywhere, the environment selects” (Beijerinck 1913, Baas-Becking 1934). Whether this was the intention of Beijerinck / Baas-Becking or not, this statement has been taken to suggest that either due to their incredible diversity or adaptive abilities that microbial community function is largely driven by the environment. Others have taken a more mechanistic approach to the role soil microbial communities’ play. Often this has relied on slotting components of these communities into one of two groups such as zymogenous vs. autochthonous, copiotrophs vs. oligotrophs, \( r \)-selected vs. \( K \)-selected, and bacteria vs. fungi (Winogradsky 1924, Koch 2001, Fierer et al. 2007, van der Heijden et al. 2008).

Although such distinctions made an incredibly diverse community of organisms more manageable, these binary distinctions overlook a grand spectrum of organisms and their respective traits. Ultimately this has left us with the question, “How is soil microbial community structure related to ecosystem function?”

My doctoral work was intended to begin addressing this question by examining how microbial communities are related to ecosystem carbon (C)-processes. In the first of these studies (Chapter 2) I set out to describe the role that soil microbial communities
played in the mineralization of glucose. Glucose is a low molecular weight organic compound found in root exudates, as well as leachates derived from plant litter, and likely represents a broadly available form of C to microbes (van Hees et al. 2005). I expected that the mineralization of this compound would likely be related to some component of the microbial community such as its size, activity, or fungal:bacterial dominance. Surprisingly, glucose mineralization was related to none of these variables but was related to the land-use that these communities were found in and more specifically land-use associated variation in soil phosphorus. This may well be an indication of the land-use legacy that these communities have endured (Richter and Markewitz 2001). That is, the land-use regimes found at the Calhoun Experimental Forest are for the most part products of 100 years of extensive cotton agriculture but upon cessation of these practices have been managed differently (Callaham et al. 2006). Specifically, that these communities share a similar history prior to cotton agriculture and at the time were likely broadly similar with regards to their structure and function. However, post-cotton these communities were exposed to differing land-use/management regimes which ultimately influenced these characteristics. In light of this finding it appears that at least microbial community function was influenced by its environment and further collaborative work at these sites indicated that community composition below the level of fungal:bacterial dominance was also influenced (Lauber et al. 2008).

Results from my examination of Microstegium vimineum invasion of hardwood forests revealed that changes in the soil microbial community altered C-processes (Chapter 3). Like my work in the Calhoun, I found that there was little change in the fungal:bacterial dominance of the microbial community but in spite of this, these
communities were functionally distinct. This functional distinction resulted in more rapid C-cycling associated with those communities where *M. vimineum* was present. This study also suggested that the function of the soil microbial communities was primed by inputs from the invasive plant. This outcome was likely contingent on the chemical quality of *M. vimineum* in comparison to the chemical quality of native-derived plant C (Kuzyakov et al. 2000, Litton et al. 2008). This may indicate that the functional response of soil microbial communities is in part related to their contemporary environment. This invasion may also indicate the sort of events which lead to functionally distinct microbial communities. Like the research at the Calhoun, the forest microbial community in this study shared a similar historical legacy but the invasion of *M. vimineum* altered this legacy for a spatial subset of the community. Whether this will lead to permanent functional and/or compositional divergence in these communities is as yet unknown. It does though suggest one mechanism, the historic resource environment, which is likely to influence the function of soil microbial communities with regards to C-processes.

The influence of the historic resource environment was resolved in the first of my lab-based experimental studies (Chapter 4). In this work I found that microbial communities were not functionally redundant and that communities paired with their ‘home’ litter resource typically exhibited the highest litter mineralization rates for that litter. This suggested that microbial communities which are pre-exposed to a given litter substrate are subsequently better at decomposing it. This phenomenon has been termed ‘home-field advantage’ (Hunt et al. 1988, Gholz et al. 2000, Ayres et al. 2009). Interestingly, I also found that communities sourced from different environments in combination with the most chemically-complex litter resource were compositionally the
most similar but functionally the most dissimilar. This may imply that the contemporary environment is a structuring force with regards to the composition of microbial communities but that the historical environment is the dominant structuring force with regards to function.

In my second lab-based experiment (Chapter 5) I found further confirmation of the influence of the historic environment on function. I paired two novel litter resources with microbial communities sourced from either forest or herbaceous habitats. We found that the mineralization of each litter (i.e. the perception of litter quality) was dependent on the environment that the microbial community was sourced from. Specifically, communities sourced from forest environments perceived no differences in quality between the chemically-complex and -simple litters. In contrast, communities sourced from herbaceous habitats perceived the chemically-simple litter to be of greater quality than the chemically-complex one. This indicates that the function of soil microbial communities might be further generalized with regards to their past exposure to different C-compounds (Table 6.1).

Madsen (2005) argued that one of the major unknowns in microbial ecology was the link between microbial communities and C-processes. Although my dissertation work does not link specific soil microorganisms to specific C-processes, it does suggest that the functioning of soil microbial communities is shaped by a history of differing C-inputs (Fig. 6.1). This provides us with one possible mechanism which leads to functional dissimilarity in soil microbial communities (Fig. 6.1). Although we may never be able to determine if everything is everywhere, we can say that in part the environment does
select with regards to function but that this selection is contingent on both the contemporary and historical environment of the soil microbial community.

**Future Directions**

One of the unanswered questions from my work is whether or not soil microbial communities from differing historic environments are likely to converge functionally, given enough time, when placed in the same contemporary environment. This is an area of research which I am currently exploring. I plan to first determine if functional convergence occurs. Second, if it does how long it takes and, finally, whether convergence is associated with loss of function in alternate environments (i.e. a ‘functional trade-off’). The results from this work will likely inform us as to the impact that a changing environment is likely to have on the role that soil microbial communities play in regulating ecosystem processes.

Other avenues of future research involve determining the legacy of historical inputs on the function of soil microbial communities. Specifically, if communities share a common history but a dissimilar contemporary environment then is function still broadly similar amongst these communities? For example, in the Calhoun microbial communities likely shared a common history but are now found in distinct contemporary environments. So, we can ask if the function of these Calhoun communities is more similar to each other than to historically-distinct communities, or whether it is the contemporary environment which exerts the strongest influence on function.

Finally, I believe it is important to determine whether it is the input history of C-compounds alone which impacts soil microbial community function. The structure of soil
microbial communities is influenced by an array of factors besides input history, such as soil pH and nutrient status. However, whether or not these structuring forces also impact the function of microbial communities has for the most part not been explored. I believe that controlled-experimentation should be first used to explore these questions, but ultimately that there is a need to explore the significance of these findings for the regulation of ecosystem processes \textit{in situ}. 

\textbf{References}


The carbon we do not see - the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biology & Biochemistry 37:1-13.

Table 6.1: Generalized functional implications for soil microbial communities with distinct histories of high and low chemical quality inputs as they relate to C-cycling. The realization and strength of these generalized outcomes will likely be contingent on the resource history of one microbial community relative to another. For example, a microbial community from an unfertilized grassland may be considered to have a high quality resource history when compared to an old growth forest but is likely low quality when compared to a fertilized grassland.

<table>
<thead>
<tr>
<th>Resource History</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High quality</strong></td>
</tr>
<tr>
<td>1. Mineralization of low molecular weight organic compounds (LMWOC) such as glucose is high. Possibly because LMWOC compounds are major C inputs in these systems or because communities are not C-limited.</td>
</tr>
<tr>
<td>2. Perception of litter quality is related to the litter’s chemical recalcitrance.</td>
</tr>
<tr>
<td>3. Increasing labile C inputs may lead to an increase or no change in soil C pools due to preferential substrate utilization.</td>
</tr>
<tr>
<td>4. Relatively poor decomposers of an array of litter resources.</td>
</tr>
</tbody>
</table>
**Figure Legends**

**Figure 6.1** A schematic showing how alterations in the quality of C-compounds may impact the function of soil microbial communities across time. Community X represents the ancestral community. Community $X_{1,2}$... represent communities distinct with regards to their function. Each node (●) represents environmental change leading to an alteration in the quality of C inputs a given subset of the ancestral community is exposed to. In this example, subsets of Community X are impacted by an environmental change which alters the quality of C inputs they are exposed to leading to two functionally dissimilar communities, Community $X_1$ and $X_2$. Communities $X_1$ and $X_2$ are again impacted by an environmental change leading to the functionally distinct communities of $X_{1.1}$, $X_{1.2}$, $X_{2.1}$, and $X_{2.2}$. Finally, Communities $X_{1.1}$ and $X_{1.2}$ are impacted by the same change experienced by the ancestral community (i.e. $X_1$) leading to convergence of these two communities and a return to the functioning of the ancestral community. On the other hand communities $X_{2.1}$ and $X_{2.2}$ experience a similar perturbation but never functionally converge. Environmental change may be as dramatic as land-use change or fertilizer inputs or even as minor as a change from one tree species to another within a forest. The question marks represent a change in input quality to those found one community back and is related to whether or not microbial communities will converge functionally.
Figure 6.1

Time

Community X

Community X

Community X

Community X

Community X

Community X

Community X

Community X

Community X

Community X

Community X

Community X
APPENDIX A

REFERENCES USED TO CONSTRUCTUCT FIGURE 1.1

References


APPENDIX B
REFERENCES USED TO CONSTRUCT FIGURE 1.2

References


APPENDIX C
CANDIDATE MODEL SET

Table C.1 List of candidate models used to describe cumulative glucose mineralized ($^{13}$CO$_2$). The first 30 models and the intercept only model were the initial set of candidate models used. All models were compared as the secondary set of candidate models.

<table>
<thead>
<tr>
<th>#</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$^{13}$CO$_2$ = 1</td>
</tr>
<tr>
<td>2</td>
<td>$^{13}$CO$_2$ = MicC</td>
</tr>
<tr>
<td>3</td>
<td>$^{13}$CO$_2$ = SIR</td>
</tr>
<tr>
<td>4</td>
<td>$^{13}$CO$_2$ = F:B</td>
</tr>
<tr>
<td>5</td>
<td>$^{13}$CO$_2$ = MicC + SIR</td>
</tr>
<tr>
<td>6</td>
<td>$^{13}$CO$_2$ = SIR + F:B</td>
</tr>
<tr>
<td>7</td>
<td>$^{13}$CO$_2$ = MicC + SIR + F:B</td>
</tr>
<tr>
<td>8</td>
<td>$^{13}$CO$_2$ = Seas. + MicC</td>
</tr>
<tr>
<td>9</td>
<td>$^{13}$CO$_2$ = Seas. + SIR</td>
</tr>
<tr>
<td>10</td>
<td>$^{13}$CO$_2$ = Seas. + F:B</td>
</tr>
<tr>
<td>11</td>
<td>$^{13}$CO$_2$ = Seas. + MicC + SIR</td>
</tr>
<tr>
<td>12</td>
<td>$^{13}$CO$_2$ = Seas. + MicC + F:B</td>
</tr>
<tr>
<td>13</td>
<td>$^{13}$CO$_2$ = Seas. + SIR + F:B</td>
</tr>
<tr>
<td>14</td>
<td>$^{13}$CO$_2$ = Seas. + MicC + SIR + F:B</td>
</tr>
<tr>
<td>15</td>
<td>$^{13}$CO$_2$ = Land</td>
</tr>
<tr>
<td>16</td>
<td>$^{13}$CO$_2$ = Land + Seas.</td>
</tr>
<tr>
<td>17</td>
<td>$^{13}$CO$_2$ = Land + MicC</td>
</tr>
<tr>
<td>18</td>
<td>$^{13}$CO$_2$ = Land + SIR</td>
</tr>
<tr>
<td>19</td>
<td>$^{13}$CO$_2$ = Land + F:B</td>
</tr>
<tr>
<td>20</td>
<td>$^{13}$CO$_2$ = Land + MicC + SIR</td>
</tr>
<tr>
<td>21</td>
<td>$^{13}$CO$_2$ = Land + MicC + F:B</td>
</tr>
<tr>
<td>22</td>
<td>$^{13}$CO$_2$ = Land + SIR + F:B</td>
</tr>
<tr>
<td>23</td>
<td>$^{13}$CO$_2$ = Land + Seas. + MicC</td>
</tr>
<tr>
<td>24</td>
<td>$^{13}$CO$_2$ = Land + Seas. + SIR</td>
</tr>
<tr>
<td>25</td>
<td>$^{13}$CO$_2$ = Land + Seas. + F:B</td>
</tr>
<tr>
<td>26</td>
<td>$^{13}$CO$_2$ = Land + Seas. + MicC + SIR</td>
</tr>
<tr>
<td>27</td>
<td>$^{13}$CO$_2$ = Land + Seas. + MicC + F:B</td>
</tr>
<tr>
<td>28</td>
<td>$^{13}$CO$_2$ = Land + Seas. + SIR + F:B</td>
</tr>
<tr>
<td>29</td>
<td>$^{13}$CO$_2$ = Land + Seas. + MicC + SIR + F:B</td>
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<tr>
<td>31</td>
<td>$^{13}$CO$_2$ = Moist.*Temp. + MicC</td>
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<tr>
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<td>$^{13}$CO$_2$ = Moist.*Temp. + MicC + SIR</td>
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<td>$^{13}$CO$_2$ = Moist.*Temp. + MicC + F:B</td>
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<td>77</td>
<td>$^{13}$CO$_2$ = S+C + Seas. + SIR + F:B</td>
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<td>109</td>
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SIR = substrate induced respiration, a measure of microbial activity; MicC = microbial biomass C as determined via sCFE, a measure of the size of the microbial biomass; F:B = fungal-to-bacterial dominance as determined via qPCR, a measure of community composition; Seas. = Season, either winter or summer when the pulse-chase experiment was conducted. Land = land-use. Moist.*Temp. = the interaction between moisture and temperature. C:N = the soil C:N ratio. S+C = silt plus clay content of the soil. pH = Soil pH. P = extractable phosphorous.
Figure D.1 Mean soil respiration ±1 S.E. per land-use (n=3) during the summer (a) and winter (b) across the 72h pulse-chase. The x-axis denotes the time since glucose was added. Glucose was added at time zero as indicated by the arrow.
APPENDIX E
PARAMETER ESTIMATES

Tables showing the restricted maximum likelihood (REML) estimates for models from Tables 2.2 and 2.3 which are within the 95% confidence interval.

**Table E.1** Restricted maximum likelihood (REML) estimates for models from Table 2.2 which are within the 95% confidence interval.

<table>
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<tr>
<th>Model</th>
<th>Factor</th>
<th>Estimate</th>
<th>SE</th>
<th>d.f.</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
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<td></td>
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<td>0.15</td>
<td>12</td>
<td>23.85</td>
<td>&lt; 0.001</td>
</tr>
<tr>
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<td>Land-Ha</td>
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<td>0.21</td>
<td>8</td>
<td>-3.46</td>
<td>&lt; 0.01</td>
</tr>
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<td>Land-Pa</td>
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<td>0.21</td>
<td>8</td>
<td>1.85</td>
<td>0.10</td>
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<td>0.21</td>
<td>8</td>
<td>-1.69</td>
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<tr>
<td>fixed effects</td>
<td>Intercept</td>
<td>3.33</td>
<td>0.14</td>
<td>12</td>
<td>24.09</td>
<td>&lt; 0.001</td>
</tr>
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<tr>
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<td>Intercept</td>
<td>3.48</td>
<td>0.19</td>
<td>11</td>
<td>18.06</td>
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<td>3.35</td>
<td>0.14</td>
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<td>0.27</td>
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Table E.2 Restricted maximum likelihood (REML) estimates for models from Table 3 which are within the 95% confidence interval.

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<th>P</th>
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APPENDIX F

TABLE SHOWING THE INITIAL %C, %N, AND THE C:N RATIOS OF THE LITTERS AND THE SOIL INOCULA

Table F.1

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<td>Rhododendron Litter</td>
<td>48.9 ± 0.24</td>
<td>0.42 ± 0.01</td>
<td>116 ± 1.70</td>
</tr>
<tr>
<td>Pine Litter</td>
<td>47.3 ± 0.67</td>
<td>0.66 ± 0.01</td>
<td>96.7 ± 1.81</td>
</tr>
<tr>
<td>Grass Litter</td>
<td>43.4 ± 0.27</td>
<td>0.49 ± 0.02</td>
<td>66.3 ± 1.60</td>
</tr>
<tr>
<td>Rhododendron Inoc.</td>
<td>4.5 ± 0.16</td>
<td>0.18 ± 0.01</td>
<td>25.3 ± 1.42</td>
</tr>
<tr>
<td>Pine Inoc.</td>
<td>2.7 ± 0.24</td>
<td>0.13 ± 0.01</td>
<td>21.5 ± 0.57</td>
</tr>
<tr>
<td>Grass Inoc.</td>
<td>3.3 ± 0.54</td>
<td>0.32 ± 0.03</td>
<td>10.1 ± 0.73</td>
</tr>
</tbody>
</table>

Values shown are means ± 1 SE (n=3).
### APPENDIX G

**BRAY-CURTIS SIMILARITY IN MICROBIAL COMMUNITY FUNCTION**

**Table G.1** Bray-Curtis similarity (%) matrix between the cumulative mineralization for each inoculum for rhododendron litter during the entire incubation period (All periods) and for each incubation period (Days 2-25, 26-99, and 100-300). Similarities were calculated from the mean of eight replicates per treatment. The home inoculum (Rhododendron) is in bold.

<table>
<thead>
<tr>
<th>Incubation period</th>
<th>Inoculum Source</th>
<th>Rhododendron</th>
<th>Pine</th>
<th>Grass</th>
</tr>
</thead>
<tbody>
<tr>
<td>All periods</td>
<td>Rhododendron</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pine</td>
<td>65.13</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grass</td>
<td>33.23</td>
<td>58.41</td>
<td>100.00</td>
</tr>
<tr>
<td>Days 2-25</td>
<td>Rhododendron</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pine</td>
<td>93.81</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grass</td>
<td>99.88</td>
<td>93.69</td>
<td>100.00</td>
</tr>
<tr>
<td>Days 26-99</td>
<td>Rhododendron</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pine</td>
<td>54.23</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grass</td>
<td>39.56</td>
<td>79.73</td>
<td>100.00</td>
</tr>
<tr>
<td>Days 100-300</td>
<td>Rhododendron</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pine</td>
<td>67.15</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grass</td>
<td>22.79</td>
<td>40.56</td>
<td>100.00</td>
</tr>
</tbody>
</table>
Table G.2 Bray-Curtis similarity (%) matrix between the cumulative mineralization for each inoculum for pine litter during the entire incubation period (All periods) and for each incubation period (Days 2-25, 26-99, and 100-300). Similarities were calculated from the mean of eight replicates per treatment. The home inoculum (Pine) is in bold.

<table>
<thead>
<tr>
<th>Incubation period</th>
<th>Inoculum Source</th>
<th>Rhododendron</th>
<th>Pine</th>
<th>Grass</th>
</tr>
</thead>
<tbody>
<tr>
<td>All periods</td>
<td>Rhododendron</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pine</td>
<td>84.42</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grass</td>
<td>76.56</td>
<td>62.36</td>
<td>100.00</td>
</tr>
<tr>
<td>Days 2-25</td>
<td>Rhododendron</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pine</td>
<td>99.83</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grass</td>
<td>98.28</td>
<td>98.45</td>
<td>100.00</td>
</tr>
<tr>
<td>Days 26-99</td>
<td>Rhododendron</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pine</td>
<td>57.11</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grass</td>
<td>84.15</td>
<td>45.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Days 100-300</td>
<td>Rhododendron</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pine</td>
<td>95.38</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grass</td>
<td>65.89</td>
<td>70.04</td>
<td>100.00</td>
</tr>
</tbody>
</table>
Table G.3 Bray-Curtis similarity (%) matrix between the cumulative mineralization for each inoculum for grass litter during the entire incubation period (All periods) and for each incubation period (Days 2-25, 26-99, and 100-300). Similarities were calculated from the mean of eight replicates per treatment. The home inoculum (Grass) is in bold.

<table>
<thead>
<tr>
<th>Incubation period</th>
<th>Inoculum Source</th>
<th>Rhododendron</th>
<th>Pine</th>
<th>Grass</th>
</tr>
</thead>
<tbody>
<tr>
<td>All periods</td>
<td>Rhododendron</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pine</td>
<td>93.67</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grass</td>
<td>97.70</td>
<td>91.38</td>
<td>100.00</td>
</tr>
<tr>
<td>Days 2-25</td>
<td>Rhododendron</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pine</td>
<td>93.00</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grass</td>
<td>86.12</td>
<td>93.05</td>
<td>100.00</td>
</tr>
<tr>
<td>Days 26-99</td>
<td>Rhododendron</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pine</td>
<td>89.48</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grass</td>
<td>84.05</td>
<td>94.48</td>
<td>100.00</td>
</tr>
<tr>
<td>Days 100-300</td>
<td>Rhododendron</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pine</td>
<td>95.14</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grass</td>
<td>83.94</td>
<td>79.24</td>
<td>100.00</td>
</tr>
</tbody>
</table>
**Figure G.1** Bray-Curtis similarity (%) between the decomposer community function across all incubation periods for the three soil inocula in rhododendron litter (A), pine litter (B), and grass litter (C). The “home” soil inoculum is highlighted in bold type.
APPENDIX H

EDAPHIC CHARACTERISTICS FOR THE CALHOUN PLOTS

Table H.1 Edaphic characteristics measured at each plot. Cultivated and pasture sites were classified as herbaceous-cover and pine and hardwood plots were classified as forest-cover. Means of three analytical repeats are shown.

<table>
<thead>
<tr>
<th>Land-use</th>
<th>Plot</th>
<th>DOC (μg g dry wt soil-1)</th>
<th>NO₃ (μg g dry wt soil-1)</th>
<th>NH₄⁺ (μg g dry wt soil-1)</th>
<th>Total C (mg g dry weight soil-1)</th>
<th>POM C (mg g dry weight soil-1)</th>
<th>Mineral C (mg g dry weight soil-1)</th>
<th>Total N (mg g dry weight soil-1)</th>
<th>POM N (mg g dry weight soil-1)</th>
<th>Mineral N (mg g dry weight soil-1)</th>
<th>Total P (μg g dry wt soil-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivated</td>
<td>Cu1</td>
<td>166.91</td>
<td>11.54</td>
<td>0.64</td>
<td>15.64</td>
<td>6.03</td>
<td>9.61</td>
<td>0.95</td>
<td>0.24</td>
<td>0.71</td>
<td>17.13</td>
</tr>
<tr>
<td>Cultivated</td>
<td>Cu2</td>
<td>129.94</td>
<td>18.04</td>
<td>0.58</td>
<td>10.96</td>
<td>3.57</td>
<td>7.39</td>
<td>0.88</td>
<td>0.19</td>
<td>0.69</td>
<td>16.74</td>
</tr>
<tr>
<td>Cultivated</td>
<td>Cu3</td>
<td>183.88</td>
<td>46.45</td>
<td>0.75</td>
<td>10.42</td>
<td>4.17</td>
<td>6.25</td>
<td>0.69</td>
<td>0.16</td>
<td>0.53</td>
<td>18.59</td>
</tr>
<tr>
<td>Pasture</td>
<td>Pa1</td>
<td>72.95</td>
<td>10.09</td>
<td>0.84</td>
<td>9.14</td>
<td>5.21</td>
<td>3.93</td>
<td>0.68</td>
<td>0.30</td>
<td>0.39</td>
<td>9.91</td>
</tr>
<tr>
<td>Pasture</td>
<td>Pa2</td>
<td>59.59</td>
<td>9.50</td>
<td>0.95</td>
<td>9.22</td>
<td>5.38</td>
<td>3.84</td>
<td>0.64</td>
<td>0.27</td>
<td>0.37</td>
<td>18.86</td>
</tr>
<tr>
<td>Pasture</td>
<td>Pa3</td>
<td>54.95</td>
<td>26.34</td>
<td>1.71</td>
<td>11.27</td>
<td>7.67</td>
<td>3.61</td>
<td>0.78</td>
<td>0.43</td>
<td>0.35</td>
<td>18.94</td>
</tr>
<tr>
<td>Pine</td>
<td>Pi1</td>
<td>38.64</td>
<td>0.04</td>
<td>5.47</td>
<td>8.21</td>
<td>4.52</td>
<td>3.69</td>
<td>0.33</td>
<td>0.12</td>
<td>0.21</td>
<td>1.02</td>
</tr>
<tr>
<td>Pine</td>
<td>Pi2</td>
<td>73.56</td>
<td>0.02</td>
<td>4.05</td>
<td>8.18</td>
<td>4.66</td>
<td>3.53</td>
<td>0.56</td>
<td>0.24</td>
<td>0.31</td>
<td>13.45</td>
</tr>
<tr>
<td>Pine</td>
<td>Pi3</td>
<td>114.33</td>
<td>0.00</td>
<td>0.77</td>
<td>9.52</td>
<td>5.20</td>
<td>4.32</td>
<td>0.30</td>
<td>0.12</td>
<td>0.18</td>
<td>2.30</td>
</tr>
<tr>
<td>Hardwood</td>
<td>Ha1</td>
<td>146.66</td>
<td>0.17</td>
<td>7.54</td>
<td>13.88</td>
<td>7.07</td>
<td>6.80</td>
<td>0.77</td>
<td>0.25</td>
<td>0.52</td>
<td>1.07</td>
</tr>
<tr>
<td>Hardwood</td>
<td>Ha2</td>
<td>242.11</td>
<td>0.00</td>
<td>3.23</td>
<td>24.94</td>
<td>14.73</td>
<td>10.21</td>
<td>0.90</td>
<td>0.45</td>
<td>0.45</td>
<td>3.55</td>
</tr>
<tr>
<td>Hardwood</td>
<td>Ha3</td>
<td>96.95</td>
<td>10.33</td>
<td>4.13</td>
<td>16.10</td>
<td>7.93</td>
<td>8.17</td>
<td>0.82</td>
<td>0.27</td>
<td>0.55</td>
<td>0.74</td>
</tr>
</tbody>
</table>