# LINKING LITTER QUALITY, SOIL MICROBIAL AND FAUNAL COMMUNITIES AND SOIL PROCESSES

by

### DUILIA YOLIMA CARRILLO

(Under the Direction of Carl Jordan)

### ABSTRACT

The decomposition and mineralization of plant litter and soil organic matter is known to be regulated by a set of hierarchically-organized interacting factors (climate, clay mineralogy, nutrient status of the soil, quality of decomposing resources and the activity of soil organisms). Although it is possible to predict decomposition and mineralization rates, the relative importance of the actual controlling factors and the mechanisms and effects of their interactions are not well understood. In particular, the interaction of the quality of resources and the soil organisms is not well understood. This dissertation presents results of field, laboratory experiments and simulation modeling that investigated some aspects of the interaction between the chemical quality of plant litter and the structure of the soil community. Chapter 2 investigates the short term effect that the chemical composition of one-time surface applied litter materials has on the soil microbial and mesofaunal communities in the mineral soil. Chapter 3 examines whether the changes brought about in soil by the quality of litter can influence the ability of the soil community to mineralize and decompose freshly added substrates and materials already present in the soil and whether the composition of the faunal community would affect this ability. In Chapter 4 we explore one potential way in which the soil fauna could affect nitrogen

mineralization: by mediating the control that the quality of litter exerts on the structure of the micro-food web. Chapter 5 uses simulation modeling of soil food webs to (a) assess the importance of the soil community changes brought about by the quality of litter on carbon and nitrogen mineralization from litter and soil, (b) evaluate the whether the role of the soil communities and their trophic interactions varies depending on the quality of the degrading substrate and (c) investigate whether all soil communities are equally suited to degrade substrates of all qualities. Chapter 6 used food web modeling to test the hypothesis that taking into account the soil biota structure and dynamics in addition to litter quality is important in explaining short-term nitrogen mineralization patterns from plant litter in an agricultural system.

INDEX WORDS: Litter quality, food webs, soil, fauna, nitrogen, carbon, mineralization, microbial communities

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### DUILIA YOLIMA CARRILLO

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## DUILIA YOLIMA CARRILLO

Major Professor:

Carl Jordan

Committee:

Mark Bradford Miguel Cabrera David Coleman Paul Hendrix

Electronic Version Approved:

Maureen Grasso Dean of the Graduate School The University of Georgia August 2007

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### **CHAPTER 1**

### INTRODUCTION AND LITERATURE REVIEW

Most of the primary production in terrestrial ecosystems is not processed directly by herbivores but by decomposers (Moore et al., 2004). The majority of primary production enters the decomposer sub-system as dead plant litter (Bardgett, 2005). The input of litter by plants constitutes a key linkage between the aboveground and belowground dimensions of terrestrial systems that can influence the functioning of the whole ecosystem (Wardle, 2002). Both the quantity and the quality of plant litter have important consequences for the decomposer system belowground. In turn, the functioning of the decomposer system influences the aboveground community by regulating nutrient availability and sequestration of carbon and nitrogen in the soil -among others.

The decomposition and mineralization –as a sub-process of decomposition-- of plant litter and soil organic matter is known to be regulated by a suite of hierarchically-organized interacting factors, including climate, clay mineralogy, nutrient status of the soil, quality of decomposing resources and the activity of soil organisms (Lavelle et al., 1993). Although it is possible to predict decomposition and mineralization rates from indices of chemical quality (Aerts, 1997; Swift et al., 1979), these indices act only as surrogates for the actual regulators of the decomposition process (Meentemeyer, 1978). The relative importance of the actual controlling factors and the mechanisms and effects of their interactions are still not fully understood (Bardgett, 2005). In particular the interactions of the quality of resources and the activity and structure of the soil community are not well known. This dissertation investigates some aspects of the interaction between the chemical quality of plant litter and the structure of the soil community. This introductory chapter presents a general summary of our understanding of the role of litter quality and soil organisms and the effects of their interactions on soil processes and identifies the research questions that are addressed in the following chapters.

### Role of soil organisms

The complete soil community, including microbial and non-microbial components and the direct and indirect interactions between them are involved in the process of decomposition and mineralization (Coleman et al., 2004). The decomposition of organic materials is a biological process in which microorganisms play the most direct role as primary consumers. The great taxonomic and metabolic diversity of the members of the soil microbial community as well as the differences in their abiotic requirements has long been known (Paul and Clark, 1996). It has also been shown that composition of the microbial communities in soil is responsive to agricultural management (e.g. Schutter and Dick, 2002), environmental change (e.g. Frey et al., 2004), the structure of the aboveground communities (e.g. Hackl et al., 2005) and the complexity of the available carbon substrates (e.g. Schutter and Dick, 2001) among other variables. However, the link between these responses and their effects on soil processes has been hard to draw due to intrinsic difficulty in studying the function of microbial communities. Recently though, several studies have established with some confidence that alterations in the composition of the microbial community can lead to modifications in soil and ecosystem functions (Balser and Firestone, 2005; Orwin et al., 2006; Waldrop and Firestone, 2004b).

It is well known that composition of the soil fauna can influence the decomposition and mineralization of litter and soil organic matter. Impacts of the members of the fauna can be attributed to multiple mechanisms including: (a) their direct impacts on soil physical and chemical characteristics that can modify the environment for decomposition such as the creation of burrows or other soil structures, (b) direct consumption of organic materials, (c) the modification, "conditioning" of organic materials that could alter their palatability or degradability, (d) the "creation" of new materials for decomposition such as the production of fecal pellets, (e) the translocation of organic material which can make them more accessible to the microbial community, (f) the direct modification of the microbial community composition via, for example, gut passage and (g) the establishment of trophic interactions with other members of the soil community which has the potential to lead to excretion of waste material, changes in their population sizes and in their activities and turnover and result in changes in function (Brussaard, 1998; Coleman et al., 2004; Edwards, 2000; Lavelle et al., 1993; Petersen and Luxton, 1982).

### Role of chemical quality of litter

Plant species differ greatly in the chemical composition of their tissues which translates into great variation in the quality of plant litter input to soil. The relative proportion of the carbon constituents and the concentration of nutrients and secondary compounds determine the susceptibility of a substrate to attack by decomposers and thus controls the rates of decomposition and nutrient release from litter materials (Heal et al., 1997). Indices of litter quality such as initial nitrogen concentration, carbon-to-nitrogen ratio, nitrogen-to-phophorus ratio, lignin-to-nitrogen ratio among others, are good predictors of decomposition and nutrient

release rates (Meentemeyer, 1978; Melillo et al., 1982; Parton et al., 2007; Taylor et al., 1991). It has been suggested however, that the effect of litter quality on soil processes is driven by its effects on the soil organisms responsible for the processes (Wardle, 2002).

### Effects of chemical quality of litter on soil community structure and on soil function

Although much attention has been given to the amount of resources regulating consumer populations in soil, the quality of resources may be their strongest driver and therefore that of the processes for which they are responsible (Scheu et al., 2003; Wardle, 2002).

Litter quality can determine the composition of the community that inhabits it. Of the microbial members of the soil food web the fungal community tends to be the most sensitive to resource quality (e.g. Robinson et al., 1994; Wardle et al., 1995). However, all members of the micro-food web have been seen to respond to litter quality. For example Beare et al. (1989), Bjornlund and Christensen (2005) and Parmelee (1989) found differences in the abundances of bacteria and fungi and their faunal grazers between plant residues that differed in their quality. Ilieva-Makulec (2006) also found clear differences in the abundances of nematodes, mites and sprigtails in three different litters. Studies looking at the mineral soil communities after incorporation of litter into soil have also found marked differences in microbial community composition (Bending et al., 2002; McMahon et al., 2005; Schutter and Dick, 2001).

In natural or managed systems in which plant litter does not get incorporated into the mineral soil (e.g. no-till systems) or in which incorporation occurs slowly, it is important to distinguish between the litter communities and the mineral soil communities. The input of litter can potentially affect mineral soil communities by generating changes in nutrient content and energy sources through litter redistribution by transport by water and soil fauna or through the

adsorption of organic matter to mineral particles where they come into close contact (Heal et al., 1997). Few studies have looked at the effects of unincorporated substrates on the community structure of the underlying mineral soils. Chapter 2 investigates the short term effect that the chemical composition of one-time surface applied amendments has on the soil microbial and micro and mesofauna community in the mineral soil.

How the mineral soil communities respond to the chemical quality of surface litter can have implications on the dynamics of nutrients and indigenous soil organic matter (Waldrop and Firestone, 2004a). The link between the change brought about in the soil communities by the chemical quality of plant material and the functioning of the soil community has not been addressed by many studies. Cookson et al. (1998) found that wheat litter decomposed faster in soils that had previously been exposed to wheat residue and suggested that conditioning the microbial community to specific plant residue types -therefore with different chemical composition- could to some degree override the influence of residue quality on decomposition. Dehlin et al. (2006) detected strong effects of the identity of organic substrates added to soil on microbial community structure and activity. Orwin et al. (2006) however, found that although the identity of carbon substrates added to soil significantly affected the structure of the microbial community, decomposition was mainly influenced by the effects on the chemistry of soil caused by the added substrates rather than by the changes in microbial community. Soil chemical variables can be affected by the chemical composition of organic matter inputs (Bulluck et al., 2002; Tirol-Padre et al., 2007). Changes in the chemical environment of soil may have direct impacts on soil processes but may also influence the structure and functioning of the soil community thus indirectly affecting processes. The results of a laboratory experiment presented in Chapter 3 examine whether the changes brought about in soil by the quality of litter could

influence the ability of the soil community to mineralize and decompose newly added substrates and materials already present in the mineral soil and whether the faunal composition would affect this ability.

### Interactions of faunal community and substrate quality

Interactive effects on mineralization and decomposition of the soil fauna (manipulated through exclusions or by creating artificial communities) and the quality of available substrates have been documented. Fauna can increase decomposition and mineralization rates of low or intermediate quality litter (Couteaux et al., 1991; Tian et al., 1992). Tian et al. (1992) hypothesized that low quality litter was easily decomposable by microbes so the role of fauna was less important. Tian et al. (1992) also suggested that fauna could enhance the decomposition of low quality substrates by reducing the inhibiting effects of polyphenols through the digestion of plant residues.

Some studies have shown that the composition of the faunal community can affect the level of control of litter quality on soil processes. In particular, a more complex fauna seems to enhance the degree of control of litter quality on decomposition (Schadler and Brandl, 2005; Smith and Bradford, 2003). It has been shown that microarthropods due to their high mobility can seek high quality substrates (Griffiths and Caul, 1993; Rantalainen et al., 2004) and make litter more accessible to microbes (Petersen and Luxton, 1982), which would in turn accentuate the effects of quality on microbial populations and as a consequence on the processes they drive. Chapter 4 explores one potential way in which soil fauna could affect nitrogen mineralization: by mediating the control that the quality of litter exerts on the structure of the micro-food web. In a field setting, we exposed soil to surface-applied litter of contrasting chemical compositions and

restricted the access of size-classes of fauna to mineral soil while monitoring the faunal and microbial community.

### Litter quality and trophic interactions in the soil food web

By affecting the soil community structure, litter quality can also affect the trophic interactions occurring in the litter and soil habitat. Trophic interactions between the microbes and their grazers in the soil food web have major effects on carbon and nutrient mineralization. Carbon mineralization can increase as a result of higher turnover rate activity and respiration of consumed populations due to grazing (Bardgett et al., 1993) while nitrogen mineralization occurs mainly due to excretion of excess nitrogen by the consumer (Woods et al., 1982). Furthermore, it has been suggested that the result of trophic transfers between the members of the soil food web can depend on the quality of resources (Bardgett, 2005; Herlitzius, 1983; Wardle, 2002); but there is very little experimental evidence of this interaction. Hanlon (1981) presented results that indicated that influence on fungal respiration exerted by a collembolan grazer depended on the nutrient concentration of the growth medium. Thus, the quality of litter has the potential to influence the dynamics of nutrients and carbon due to its inherent chemical degradability and due to its effects on the trophic interactions among soil populations. These two factors may in turn interact to affect mineralization.

The complexity of the decomposition process and biological interactions in soil makes modeling approaches necessary. Organism-oriented models, which explicitly incorporate soil organisms and their interactions with the biophysical environment, have a high explanatory value and permit the evaluation of the effects of intervention and management (Paustian, 1994; Smith et al., 1998). The organism-oriented modeling approach initiated by Hunt et al. (1987) has been

applied to several natural and agricultural systems (Berg et al., 2001; de Ruiter et al., 1994; Hassink et al., 1994; Schroter et al., 2003) and has helped in the understanding the link between community structure and ecosystem level processes such as OM decomposition and subsequent carbon and nitrogen mineralization (Moore et al., 1996). In Chapter 5 we use the soil food web model scheme of Hunt et al. (1987) to simulate carbon and nitrogen mineralization from surface applied litter of differing chemical qualities and from mineral soil based on observed population sizes and the trophic interactions among the members of the soil food web. We assessed the performance of the model and then ran simulations to investigate several aspects of the interaction of the soil community and the quality of litter including (a) the importance of the soil community changes brought about by the quality of litter on carbon mineralization and nitrogen mineralization from litter and soil (b) whether the role of the soil communities and their trophic interactions varies depending on the quality of the degrading substrate, and (c) whether some communities are better suited to degrade substrates of certain quality.

### Plant litter quality and soil biota in agroecosystems

Most agricultural practices aiming at enhancing economic and environmental sustainability involve, at some point, the use of organic soil amendments such as crop residues or green manures. Soil organic amendments can have an important role not only in building soil organic matter and in conservation of soil and water, but also in supplying nutrients to subsequent crops in rotations and to simultaneous crops (Gliessman, 1998). Whenever the use of inorganic fertilizers is being reduced in favor of organic materials, the availability of nutrients in soil depends increasingly on the natural processes of decomposition and mineralization. The soil biota plays an important role in regulating organic matter decomposition and nutrient

mineralization in agroecosystems (Andren et al., 1990). Hendrix et al. (1990) stressed the concept of soil biota as fundamental components of sustainable agroecosystems in particular as nutrient mineralization regulators.

Whereas the first two levels of the hierarchy of factors that regulate decomposition (climate, and clay mineralogy) are rather fixed conditions at intermediate spatial and temporal scales, the last two (quality of resources and the activity of organisms) are very susceptible to manipulation through management practices. In agroecosystems, for instance, practices such as soil amendment with crop residues or green manures can affect population size and dynamics of organisms in soil food webs (Forge et al., 2003; Wardle et al., 1999; Zwart et al., 1994) and as a consequence affect nutrient mineralization (de Ruiter et al., 1994). In turn, the choice of the amendments and the rate and timing of application determine the quality and quantity of resources that are made available to the soil community. Thus, in agroecosystems the interplay of plant input quality and the soil community is of special importance and can be a determining factor in the functioning and sustainability of an agricultural strategy.

An example of an agricultural strategy involving the use of green manures is alley cropping. Alley cropping is an intercropping system in which tree rows are planted creating alleyways for growing companion crops. The tree component is intended to provide mulch from leaf fall or prunings. Nutrient cycling processes interlinking the above and belowground components are greatly affected by trees and consequently, one of the most important potential benefits of agroforestry is the enhancement of nutrient availability for the crop plant. The main mechanism that accounts for this results from the production and decomposition of tree biomass –mainly leaf litter and prunings but also roots (Nair et al., 1997). Chapter 6 is an application of soil food web modeling to an agricultural system to test the hypothesis that taking into account

the soil biota structure and dynamics -in addition to the chemical quality of litter- is important

for explaining short-term nitrogen mineralization patterns from green manures and other plant

residues in alley cropping systems.

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## **CHAPTER 2**

# CHEMICAL QUALITY OF LITTER AS A DRIVER OF DETRITAL COMMUNITY ASSEMBLAGE IN MINERAL SOIL $^{\ast}$

<sup>\*</sup> Carrillo, Y., Ball, B. Molina, M.; to be submitted to Soil Biology and Biochemistry

### ABSTRACT

Although much attention has been given to the amount of resources regulating consumer populations in soil, the quality of resources may be their strongest driver. Studies that deal with the effect of litter quality on soil populations have focused on the litter communities; however, how the mineral soil communities respond to the chemical quality of surface litter can have implications in soil carbon and nutrient dynamics. In a field setting in North-East Georgia (USA) we studied the short term effect of surface application of five plant materials and a mixture on the microbial community, nematodes and microarthropods in the mineral soil. We used five different substrates and a mixture representing a gradient of different quality parameters (%C, %N, C/N, %P, % lignin, % cellulose and % hemicellulose, Lignin/N) and attempted to determine (a) the effect of substrate type on mineral soil communities and (b) what biochemical parameters most influenced the soil micro-food web groups' abundance during the first six months of decomposition. We identified some patterns of response to litter quality parameters by microbial groups. Fungi and Gram-negative bacteria responded positively to high nutrient content in litter and negatively to %C, C/N and %lignin. Gram-positive bacteria and actinomycetes were stimulated by high %C and % lignin. C/N was the main driver of the fungi-to-bacteria ratio. We also found that different quality variables were related to the responses of the soil community at different times. Early during decomposition those responses were mostly related with nutrient content while later %C, lignin and lignin-to-N had the greatest influence. Microbial feeding and omnivorous nematodes showed a response to substrate type that was correlated with the response of the bacteria. No pattern of response by microarthropods was observed. By affecting populations in the mineral soil at different trophic levels, litter quality thus has the potential to

influence processes in the mineral soil that are driven by trophic interactions such as nutrient mineralization and soil organic matter decomposition.

KEYWORDS: litter quality, microbial community, microarthropods, nematodes, soil

### INTRODUCTION

The structure of the soil food web is an important factor determining soil processes and ecosystem function (Hunt and Wall, 2002). One of the factors that influence the structure of the soil food web is the chemical quality of litter produced by plants. Although much attention has been given to the amount of resources regulating consumer populations in soil, the quality of resources may be their strongest driver and therefore that of the processes for which they are responsible (Scheu et al., 2003; Wardle, 2002). Changes in the quality of plant litter input to the soil may occur due to environmental change or simply due to management decisions as is the case in agricultural systems. Most studies looking at the effect of plant litter quality on communities have focused on the communities inhabiting the litter (e.g. Bjornlund and Christensen, 2005; Wardle et al., 2006) or on soil communities after substrate incorporation (e.g. Bending et al., 2002; Salamanca et al., 2006) but few have looked at the effects of unincorporated substrates. However, in natural or managed systems in which plant litter does not get incorporated into the mineral soil (e.g. no-till systems) or in which incorporation occurs slowly, it is crucial to distinguish between the litter communities and the mineral soil communities as (a) they utilize different organic matter pools as energy and nutrient resources and thus can play different roles in driving processes and (b) the populations that compose them can establish interactions that may influence carbon and nutrient cycling and organic matter accumulation (Fontaine et al., 2004). How the mineral soil communities respond to the chemical quality of surface litter can have implications on the dynamics of nutrients and indigenous soil organic matter (Waldrop and Firestone, 2004). Understanding the short term effects of plant litter quality on soil detrital communities is of special importance in integrated agricultural systems

where organic soil amendments such as green manure and crop residues are seasonally applied as a strategy to enhance soil fertility.

This study investigates the short term effect that the chemical composition of one-time surface-applied amendments has on the soil microbial and micro and mesofauna community in the mineral soil. We used five different substrates and a mixture representing a gradient of different quality parameters and attempted to determine (a) the effect of substrate type on litter and soil communities and (b) what biochemical parameters most influence the soil micro food web groups' abundance during the first six months of decomposition.

The input of litter can potentially affect mineral soil communities by generating changes in nutrient content and energy sources through litter redistribution by transport by water and soil fauna or through the adsorption of organic matter to mineral particles where they come into close contact (Heal et al, 1997). Changes in soil chemical properties such as pH have also been reported (Orwin et al., 2006). It would be expected that the chemical quality of litter will determine the extent of these influences.

The role of plant litter quality on the structure of microbial and faunal communities is usually studied as a contrast between only two or three plant materials (low to high quality, usually meaning high/low C/N ratio) and other individual quality parameters are not taken into account as specific drivers of the response. Some studies have looked at the response of microbial communities to carbon substrates after incorporation into the soil. Schutter and Dick (2001) and Orwin et al. (2006) found that carbon substrates of different complexity levels affected soil community structure by stimulating specific microbial groups over the course of decomposition. Plant materials are mixtures of different types of C-substrates and nutrients, which makes interactions very likely. However, it is reasonable to think that within such

mixtures, some quality parameters will be more important than others in driving the response of the microbial community at different times during decomposition.

It has been observed that some microbial groups in soil mainly consume fresh organic materials while some groups mainly derive their substrate from more recalcitrant materials in mineral soil (Kramer and Gleixner, 2006; Williams et al., 2007). It would be expected that those groups that are supported by soil organic materials will be less affected in the short term by the quality of added substrates.

### **METHODS**

In 2005 a field study was conducted in experimental plots in a previously abandoned conventional farm in the Piedmont region of Georgia USA (33°57'N lat. 83°19'W long). Soil is classified as Pacolet sandy clay loam (kaolinitic, thermic typic hapludult) and had 0.7% C and 0.1%N content. In June of 2005 standing vegetation was removed from a 100 m<sup>2</sup> plot. Removed vegetation consisted of grasses and forbs. The plot was kept vegetation-free throughout the duration of the study by periodically pulling out any plants. The plot was divided into 24 2 x 2 m sub-plots and sheets of aluminum flashing were buried down to 4-5 cm and placed as dividers. Soil from the first 5 cm of a randomly located area of 35 x 35 cm within each plot was collected and sieved to 4 mm. This soil was then frozen at -80°C for 72 h to kill fauna. After thawing, soil was placed back in the field in metallic trays (35 cm x 35 cm x 5 cm) made of 5-mm mesh. Soil was left bare and allowed to re-colonize and stabilize for 2 months. Six quality treatments, five substrates and a mixture, were randomly assigned to four of the plots and were surface applied at a rate of 327g/m<sup>2</sup> both to the inside and to the outside of the tray on July 22. Substrates applied were air dried green litter of cereal rye (*Secale cereale* L.), air dried green litter of crimson

clover (*Trifolium incarnatum* L.) air dried green litter of false indigo (*Amorpha fruticosa* L., hereafter "amorpha"),wheat hay (*Triticum aestivum* L.), pine needles (*Pinus taeda* L.), and an even mixture of these by weight.

### Sample collection and processing

Soil samples from each tray were collected 21, 91 and 165 days (August, October and February from herein) after substrate application. Soils and litter were immediately processed for fauna, kept refrigerated for up to a day then freeze-dried for PLFA extraction and mineral nitrogen. Approximately 4 grams of soil were extracted for NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> by shaking in 20 ml of 2 M KCl for one hour. Extracts were analyzed with an Alpkem Continuous Flow Analyzer. Soil moisture content was determined by drying at 100°C for 24h. pH was measured in water with a 1:1 water to soil ratio.

### **Biochemical quality of substrates**

Dry samples of the initial litter were ground using a Spex CertiPrep 8000-D Mixer Mill and used for chemical analyses and estimation of ash free dry mass for 3 hours at 500°C. For C:N analysis samples were analyzed on a Carlo Erba Elemental Analyzer and reported as carbon and nitrogen percent dry weights. Total phosphorus was measured from 0.5g of sample that was ashed, extracted in aqua regia acid, and analyzed on an automated Murphy-Riley Colorimetric Analyzer. Cellulose, hemicellulose, and lignin concentrations were measured from 0.5g of each sample that underwent sequential neutral detergent/acid detergent digestion on an Ankom A200 fiber analyzer.

### Microbial community

Phospholipid fatty acids were extracted from 2-mm sieved and freeze-dried soils. Five grams of soil were extracted with the Bligh-Dyer technique (Bligh and Dyer, 1959) by shaking for 2 h with a single phase mixture of methanol-chloroform-phosphate buffer (2:1:0.8 ratio in volume) in a teflon bottle. The mixture was centrifuged to decant soil. Extra volumes of chloroform and methanol were added and the sample was centrifuged again to separate the organic phase containing the lipids which was then fractionated into neutral lipids, glycolipids and phospholipids (Zelles and Bai, 1993) with an activated silica gel column (BondElut; Varian, Palo Alto, CA). Methylation of phospholipids was done with mild alkaline methanolysis (White et al., 1979) to produce fatty acid methyl esters (FAMEs). FAMEs were purified with NH<sub>2</sub> aminopropyl columns (Bond, Elut; Varian, Palo Alto, CA).

Samples were analyzed with a Hewlett-Packard (HP; Palo Alto, CA) 6890 series GC with a flame ionization detector and a 30 meter DB-5 (film thickness = 0.25 microns, internal diameter 0.32 mm; Agilent, Santa Clara, CA). Individual PLFAs were quantified in relation to an external standard (20:0 ethyl ester) that was run in duplicate with every batch of samples. Compounds were identified by comparison of their retention times with those of a prepared mixture standard containing 36 FAMES (Matreya, PA; Sigma-Aldrich, MO; Nu-Chek, MN) that was run with each batch. Analysis by GC-MS was used to identify FAMEs common in the samples that were not contained in the standard mixture. Fatty acid notation used follows that in Frostegard and Baath (1996).

### Soil fauna

Nematodes were extracted from approximately six grams of fresh soil with the Baermann funnel method for 72 hours. Nematodes collected were preserved in 5% formaldehyde. Total nematode counts to the level of trophic group were performed for each sample. Tylenchidae were included in the fungivorous trophic group. Microarthopods were extracted for four days on Tullgreen-type extractors. They were enumerated and sorted into Collembola, Prostigmata, Mesostigmata, and Oribatida.

### Statistical Analyses

Phospholipid fatty acid methyl ester concentrations were converted to mole percentage. PLFA community profiles were then subjected to principal components analysis (PCA). Significance of principal components was assessed with one-way ANOVAs. Correlations between PCs and litter quality parameters and soil variables were assessed with Pearson correlation analysis. The response of total PLFAs and the ratio of fungal to bacterial PLFA markers to substrates over time were analyzed with repeated measures ANOVA. Nematodes and microarthropods abundance data over time were analyzed by repeated measures ANOVA. Data were log transformed prior to analysis. Pearson's correlations coefficients were determined for relationships of litter quality and soil variables with soil community variables. All analyses were performed using JMP®.A probability level of 0.05 was used to determine significant effects.

### RESULTS

### Chemical quality of substrates

Initial chemical composition of applied substrates is shown in Table 2.1. Pine needles had the lowest concentration of nitrogen and phophorus, the highest C/N ratio and lignin percentage and the lowest percentage of cellulose. Hay had the second lowest values of N and P and followed pine needles in its C/N ratio; it had a low concentration of lignin but the highest of cellulose and hemicellulose. The composition of rye was similar to that of hay except for its considerably lower C/N ratio. Amorpha and clover showed the lowest C/N ratios and the highest concentration of P. Among these two, amorpha had the highest percentage of N and P and the lowest C/N ratio but the highest percentage of lignin after pine needles. The mixture of the five substrates had the average concentration for all parameters.

### Effects of substrate type on soil characteristics

Soil moisture did not differ significantly between substrate types on any of the sampling dates (p>0.05), data not shown). Soil pH was measured only for the October date. No significant difference between substrates was observed (p>0.05), data not shown). Substrate type had a significant effect on soil  $NO_3^-$  on all sampling dates and on  $NH_4^+$  concentrations only in August (Table 2.2).

### Total Phospholipid Fatty Acids

All identified and unidentified PLFAs were summed to obtain total phospholipid fatty acid content per gram of soil (as  $\mu$ g of PLFA/g of soil). There was a significant interaction of substrate by time but no overall effect of substrate on total PLFAs (Table 2.4). Total PLFAs decreased between August and October and increased between October and February, except for pine in which it decreased throughout the course of the study (Figure 2.1). A significant difference between substrate treatments was observed only for February (p=0.0014), when pine had the lowest concentration and amorpha, rye and the mixture the highest.

### Microbial community

Seventeen phospholipid fatty acids that were identified and common to all samples were used for PCA analysis. PLFA profiles were influenced more strongly by sampling date than by substrate (Figure 2.2). The relationships of PC1 and PC2 with time were statistically significant (p<0.001 and p=0.019 respectively). Samples collected in October separated along PC1 from samples collected in August and February. On the basis of eigenvector loadings, communities from October were enriched in i17:0 and 15:0, whereas communities from February contained greater amounts of  $18:1\omega7$ ,  $18:1\omega9$  and  $16:1\omega5$ . Communities from August were located in between but tended to be closer to those from February. PC2 distinguished communities from October and February, which had high loadings of a17:0, 10Me18:0 and cy19:0 from those of August, which were enriched in 14:0,  $18:2\omega9$  and  $20:3\omega6$ .

Community PLFA profiles were analyzed by PCA one date at a time to remove sampling date effects and focus on the effect of substrate. For this, identified PLFAs were grouped into 5 categories (Table 2.3) to maintain a high ratio of samples to variables. In August (Figure 2.3a) substrates pine and hay separated from all other substrates along PC1 (P=0.04) due to greater abundances of Gram-positive bacteria and actinomycetes and increased presence of Gram-negative bacteria and fungi under the other substrates. PC1 was highly and positively correlated to soil NO<sub>3</sub><sup>-</sup> (r=0.61, p=0.0024) and NH<sub>4</sub><sup>+</sup> (r=0.70, p=0.0003) and litter %N (r=0.49, p=0.02), %P

(r=0.51, p=0.02) and negatively related to %C (r=-0.59, p=0.004) and C/N (r=-0.62, p=0.002). PC2 separated the rye community from other substrates. However, the relationship between PC2 and substrate was not significant. Amorpha, clover and the mixture form a cluster along PC2 associated with Gram-negative bacteria. In October, neither PC1 nor PC2 showed a significant relationship with substrate. However, pine, hay and the mixture appeared to be separated from rye clover and amorpha along PC2 (Figure 2.3b). Eigenvector loadings indicated higher relative abundances of Gram-positive bacteria in pine, mix and hay and greater importance of Gramnegative bacteria and fungi in amorpha, rye and clover. PC2 was positively correlated to %C (r=0.49, p=0.02), % lignin (r=0.46, p=0.03), and lignin-to-nitrogen ratio (r=0.51, p=0.01). In February (Figure 2.3c), pine, Amorpha and rye showed higher abundances of protozoan and fungal PLFA markers, whereas hay, clover and the mix showed enrichment of Gram-positive bacteria (PC1, p=0.12). Pine, amorpha and hay communities were distinctive for their high loadings of actinomycetes along PC2 while the mixture, clover and rye soils were enriched in the PLFA fungal marker. PC2 (p=0.0002) was positively correlated to %C (r=0.75, p<0.0001), % lignin (r=0.60, p=0.002) and lignin-to-N ratio (r=0.61, p=0.002).

### PLFA fungal-to-bacterial ratios

No interaction of substrate by time was observed (Table 2.4). Highly significant effects of substrate were detected at all sampling dates (p=0.001, 0.0002, 0.009 for August, October and February respectively). Rye, clover and amorpha had the highest F/B ratios while pine, hay and the mixture had the lowest. F/B declined under all substrates between August and October after which no general trend was observed (Figure 2.4). Differences in F:B between and within dates were driven by differences in the fungal PLFA marker (data not shown).

### Nematodes

Significant time/substrate interactions were found for bacterivorous and omnivorous nematodes (Table 2.4). The highest abundances of nematodes groups were found in August, except for the predatory group, which increased as decomposition progressed (Figure 2.5). The greatest differences among substrates treatments were also observed in August and tended to decrease over time, except for the predatory nematodes. Nematode abundances within dates showed high variability, but trends in the ranges were evident and therefore these are presented descriptively, not statistically. In August bacterivorous nematodes reached higher numbers under clover, amorpha and the mixture. Abundances remained high under clover by October. In October and February abundances under amorpha had the lowest values among all substrates. In August and October omnivorous nematodes under rye, clover and amorpha showed the greatest abundances whereas pine and hay had the lowest. Predatory nematodes showed no clear response to substrate addition in Augsut and their greatest response occurred in February when they were most abundant under clover and the mixture. Overall predatory nematodes increased as decomposition progressed except when hay was the added substrate, where they consistently decreased with time. Fungivorous nematodes were rare and constituted only up to 2% of all nematodes and were absent under the amorpha treatment.

### **Microarthopods**

No differences in the responses to time or substrate of oribatid, prostigmatid and mesostigmatid mites were observed. Pooled numbers of all mites are presented. Mites and collembola significantly responded to time and no overall effect of substrate or substrate/time interactions were found (Table 2.3). The greatest abundances as well as differences among substrates were
observed in August for mites and Collembola (Figure 2.6). On this date, although no significant effect was found, the highest numbers of mites occurred under rye and amorpha and those of Collembola under hay.

### Litter quality parameters and soil variables as drivers of relative abundances

Relationships of the abundances of PLFA biomarkers with initial plant quality variables as well as soil moisture and soil content of nitrate and ammonium were examined with Pearson correlation analysis (Table 2.5). Percentage of hemicellulose and soil moisture at sampling dates did not show any apparent relationship with group abundances. No plant quality or soil variable was related to Protozoan PLFA markers at any date. Several significant relationships were observed in August and fewer are found thereafter. No microbial group showed any relationship to litter cellulose, lignin or lignin to N ratio in August, however Gram-negative bacteria were positively affected by %P and %N and negatively affected by %C and C/N. The opposite was observed for Gram-positive bacteria. Fungi and F/B were negatively related to %C. Fungal markers were also negatively correlated with C/N. In October the only microbial group that was related to quality parameters was the Gram-negative bacteria. This group was negatively related to % lignin and lignin/N. In February the actinomycetes were strongly and positively correlated to %C and Lignin/N and % Lignin.

Microbial groups also showed significant strong relationships with soil mineral nitrogen but only in August, when fungal, fungal to bacterial markers ratio and Gram-negative bacteria were strongly and positively related to soil  $NH_4^+$ . Gram-negative bacteria and fungi were also positively related to  $NO_3^-$ . Actinomycetes had a negative correlation with soil  $NH_4^+$ .

Relationships between the abundance of nematode trophic groups and microbial

biomarker groups were also assessed for every date. In August bacterivorous nematodes were positively correlated with Gram-negative bacteria (r=0.55, p=0.007) and general bacterial makers (r=0.54, p=0.012). Fungivorous nematodes were negatively related to protozoan markers (r=-0.46, p=0.04). In October and February only omnivorous nematodes showed relationships with microbial markers. They were negatively correlated to Gram-negative bacteria (r=-0.56, p=0.007) and bacteria (r=-0.54, p=0.009) in October and positively related to fungi in February (r=0.44, p=0.044), when they also showed a negative relationship with actinomycetes (r=-0.46, p=0.03).

No significant relationships were found between microbial groups and microarthropod groups.

# DISCUSSION

Some studies that have looked at the long term effect of mulches (surface applied plant residues or other materials) on mineral soil communities (Forge et al., 2003; Tiquia et al., 2002; Yang et al., 2003), have found that after long-term exposure (one to several years) soil microbial and micro and mesofauna communities differ only when the mulching materials are considerably contrasting (e.g. compost vs. wood chips). In our six-month study we found that although sampling date had a greater effect on the structure of microbial communities than substrate type, within each sampling date the structure of the microbial communities in mineral soil was different under different plant substrates. This was true after 3 weeks, 12 weeks and 29 weeks of substrate addition. This suggests that different factors might be involved in the short term and long term effects of litter on soil communities. In the longer term soil characteristics such as organic matter composition and quality and soil pH can be influenced by the quality of litter

inputs (Binkley and Giardina, 1998) and in turn modify the soil community (Hackl et al., 2005). In the shorter term the influence is probably due to temporary changes in carbon and nutrients availability brought about by the added substrate. It is in the early stages of litter decomposition when the soluble fraction of litter is released and can be leached into the mineral soil and become available for mineral soil populations. McMahon et al. (2005) demonstrated that the soluble fraction of ryegrass litter served as carbon source for the microbial biomass in bulk soil.

We found the separation of the communities within dates obtained with PCA to be driven by different soil, litter and community variables at different times. By week 3 (August), increased abundances of Gram-positive bacteria and actinomycetes under pine and hay were responsible for the distinction between these communities and the communities under clover, amorpha, the mixture and rye which were in turn enriched in Gram-negative bacteria and fungi. This ordination was very strongly correlated with the N and P concentrations in litter and with mineral N in soil, indicating that the Gram-negative bacteria and fungi were favored by high nutrient availability and Gram-positive bacteria and the actinomycetes by low nutrient availability (under pine and hay). By week 12 (October), Gram-negative bacteria and fungi were more abundant under amorpha, rye and clover. The PCA ordination obtained (PC 2) was correlated with litter %C, % lignin and lignin-to-nitrogen ratio indicating that Gram-positive bacteria were favored when there were high carbon and lignin concentrations in litter and a high lignin-to-nitrogen ratio, while Gram- negative bacteria and fungi responded in the opposite manner. After 22 weeks (February), the ordination that distinguished pine, amorpha and hay communities from the other substrates was correlated with the same litter variables as in October. In this case, indicating that the actinomycetes were more abundant in soil when the

litter had high carbon and lignin concentrations and lignin-to-nitrogen ratio while fungi were negatively affected by these variables.

While the distinction between communities in August was associated with litter and soil nutrient content, in October and February %C, lignin and lignin-to-nitrogen ratio had the greatest influence. This seems to parallel what is known about the control of decomposition by quality variables: that the relative importance of quality parameters in controlling decomposition changes as decomposition progresses (Berg and Staaf, 1987; Heal et al., 1997) and specifically that nutrients are of greater importance as a controlling factor in the early stages and lignin plays a greater role in later stages. The fact that most microbial groups showed correlations with soil mineral N after three weeks of decomposition suggests that their early response to substrate quality was mainly indirect through the modification of soil nutrient content due to the quality of the added litter. Our findings then suggest that the effect of litter quality on mineral soil biota is indeed mediated by its effect on decomposition; in other words, that litter quality affects biota by affecting decomposition and therefore what gets released into the soil or remains a component of the litter.

From PCA and correlation analysis results it was possible to identify some patterns in the response of individual microbial groups to litter quality parameters. Fungi and Gram-negative bacteria in general responded positively to high nutrient content in litter and mineral nitrogen in soil and negatively to %C, C/N and lignin content. Gram-positive bacteria and actinomycetes were stimulated by high %C, % lignin and the lignin-to-nitrogen ratio. A positive response of the soil microbial biomass to mineral nitrogen has been documented before (Hart and Stark, 1997). Our results suggest that this is due to the response of only some members of the microbial community.

The fact that Gram-negative bacteria showed stronger and longer-lasting relationships with more litter quality parameters than the Gram-positive groups is consistent with the findings of Waldrop and Firestone (2004) who showed that Gram-negative organisms are more responsive to the addition of substrates and with Kramer and Gleixner (2006) who found that Gram-negative bacteria prefer recent plant material over soil organic matter. The positive relationship of fungi with litter and soil nutrient content and the negative response to high litter C/N contradicts what Eiland et al. (2001) found for hay during composting and what has been found for litter in experimental and natural systems: that fungi populations are larger in high C/N substrates and high concentrations of recalcitrant compounds (Bardgett, 2005; Ingham et al., 1989). This might indicate that the response to quality in litter and in mineral soil populations occurs through different mechanisms. It is possible that the positive response of fungi to nitrogen and phophorus was indirect, i.e., not that they were consuming the nutrient rich substrates but that the nutrients leached into the soil allowed them to exploit recalcitrant substrates already present in the soil. Other studies have also found that fungi can be positively affected by an increase in nutrient concentrations. For example, Flanagan and van Cleve (1983) found that nitrogen and phophorus mineralization rates were positively correlated with fungal hyphae in soil in forest types with differing litter input quality. Also, in a study of successional changes in sawdust, Wardle et al. (1995) found that only fungi and not bacteria were responsive to the improvement of quality over time (i.e. an increase in C/N ratio). The consistently significant effect of substrate on the fungal-to-bacterial PLFA ratio was mostly driven by the changes in the PLFA used as a fungal marker which would mean that nutrient concentrations and C/N ratio (main drivers of fungi) were the main drivers of F/B in soil.

In the case of actinomycetes, we found that the positive relationship with %C, % lignin

and lignin-to-nitrogen ratio became very strong at the end of the incubation. One possibility is that six months of decomposition were enough time for some litter material to be translocated to the soil where it could have served as a carbon source for the actinomycetes that are capable of degrading lignified substrates.

Total PLFAs can be used as an indicator of soil microbial biomass. The low concentrations of PLFA in soil on the October sampling date are probably a result of soil moisture content, which was very low in the days prior to sampling. Substrate type only had an effect on total PLFAs at the end of the incubation. In a 92-day laboratory study, Orwin et al. (2006) found very little difference in total PLFA after incorporation of several carbon substrates. Due to their chemical compositions, at any given point in time different substrates will be at different stages of degradation and the relative proportions of their chemical constituents will vary during decomposition. Bardgett (2005) states that the extent of the effect of quality of plant input on soil biota will be at its maximum when the differences in quality and quantity of resources among species are greatest. The fact that Total PLFAs only showed significant differences after six months of decomposition and not earlier might indicate that at this point in time the properties of litter associated with the total size of the microbial community were showing the greatest differences. One possibility is that after several months of decomposition some natural incorporation of litter material had occurred. Although it is at the beginning of decomposition when most of the soluble components of litter are leached into the mineral soil (Heal et al., 1997), with longer exposure the role of active transport by fauna of organic materials into mineral soil can become more apparent (e.g. Chamberlain et al., 2006) and therefore affect C and nutrient availability and potentially the total size of the microbial community.

Ilieva-Makulec et al. (2006) found that C/N ratio of plant residues was closely related to nematodes densities in litter but not in mineral soil. In this study, the microbial-feeding and omnivore nematodes in soil showed a response to substrate type. The correlations between omnivore and bacterial-feeding nematodes with microbial groups that responded to substrate quality suggest that the influence of substrate quality reached the consumer level by affecting the abundance of microbial groups, particularly that of the bacteria. This influence was stronger but not restricted to the very early phase of decomposition. Although fungi did respond to substrate quality, no relationship was found between fungal-feeding nematodes and the fungal PLFA marker. It is possible that the relationship was not detected due to the very low fungivorous nematodes densities typical of the study site that made it hard to estimate their abundances.

The greatest differences (although still not significant) in mites and collembola abundances occurred three weeks after substrate addition, however, no consistent pattern of response of microarthropods to quality was observed. Although fungal PLFA responded favorably to high concentrations of nutrients in soil due to high nutrient concentrations in litter, collembola did not show a similar response. Mites did not seem to respond to changes in the microbial groups observed in soil either. The fact that some response to substrate type was observed soon after substrate addition, but that this response was not associated with the changes in the microbial populations in soil, might indicate that the differences observed are a result of changes in the microarthropod populations in the litter, but since these organisms are mobile they were detected in soil. If this were the case, though, we would have expected to see differences between substrates later in decomposition. It is possible also that there was no direct effect of litter quality on the microarthropods in the litter as has been found in other studies (Gonzalez and

Seastedt, 2001) or in the mineral soil (Ilieva-Makulec et al., 2006) and the high densities and pronounced differences found in August were just a result of environmental conditions.

In this study we found that in the short term the chemical quality of one-time-added plant materials had substantial effects on the microbial communities in the mineral soil. We identified some patterns of response to litter quality parameters by soil groups. Fungi and Gram-negative bacteria responded positively to high nutrient content in litter and negatively to %C, C/N and %lignin. Gram-positive bacteria and actinomycetes were stimulated by high %C and % lignin. We also found that different quality variables were related to the responses of the soil community at different times. Early during decomposition those responses were mostly related with changes in the nutrient content in soil brought about by the added litter. There was some indication that after several months of decomposition litter quality began to influence the soil community in a more direct manner by controlling carbon availability. By affecting populations of detritivores substrate quality also influenced the consumer level (nematodes at least). By affecting populations in the mineral soil at different trophic levels, litter quality then would have the ability to influence processes in the mineral soil that are driven by trophic interactions such as nutrient mineralization and soil organic matter decomposition. In the context of agricultural systems, this study suggests that the chemical quality of organic amendments that are surface applied to soil on a seasonal basis might have repercussions for the mineral soil food web which can potentially influence soil fertility in the short and long term.

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	%N	N %C C/N %P %Cellulose %H		% Hemicellulose	% Lignin		
Rye	0.70(0.06)	45.04(0.95)	70.34(5.72)	0.19(0.02)	38.04(1.16)	27.95(0.35)	3.99(0.51)
Clover	2.02(0.10)	43.28(0.33)	22.42(1.46)	0.50(0.04)	33.36(0.75)	12.07(0.23)	7.97(0.34)
Amorpha fruticosa leaves	2.35(0.04)	47.10(0.17)	20.19(0.36)	0.61(0.02)	26.89(0.60)	13.91(0.20)	10.01(0.17)
Pine	0.43(0.05)	50.95(0.28)	127.90(6.88)	0.05(0.02)	30.51(0.47)	11.89(0.47)	19.19(0.62)
Hay	0.49(0.04)	47.07(0.12)	102.49(5.35)	0.16(0.01)	40.37(0.47)	30.05(0.44)	4.63(0.19)
Mixture	1.07(0.06)	46.08(0.93)	46.18(4.25)	0.28(0.08)	35.70(0.63)	19.46(0.48)	10.25(0.44)

 Table 2.1. Chemical composition of substrates. Standard error in parentheses

Table 2.2. Soil nitrate and ammonium ( $\mu g/g$  soil). Standard error in parentheses.

		Aug		Oct	Feb		
	Nitrate-N	Ammonium-N	Nitrate-N	Ammonium-N	Nitrate-N	Ammonium- N	
Rye	5.1 (0.2)	31.9 (2.5)	12.7 (2.2)	21.6 (2.7)	1.6 (0.1)	23.0 (2.7)	
Clover	8.9 (1.3)	30.1 (2.6)	23.8 (5.7)	24.4 (4.0)	3.7 (1.6)	25.7 (1.4)	
Amorpha	9.0 (1.7)	19.7 (1.5)	17.0 (2.7)	18.4 (1.4)	2.8 (0.5)	24.4 (2.9)	
Pine	2.2 (0.3)	24.7 (3.1)	10.2 (1.6)	20.3 (0.9)	1.3 (01)	21.8 (0.6)	
Hay	2.2 (0.2)	12.2 (2.2)	7.7 (0.8)	13.6 (2.8)	1.3 (0.3)	22.8 (5.0)	
Mixture	4.9 (0.8)	31.2 (3.3)	10.5 (1.0)	25.3 (0.9)	0.6 (0.3)	23.0 (1.4)	
F ratio	9.03	9.71	3.62	2.79	2.78	0.34	
P value	0.0003	0.0002	0.0222	0.0535	0.0493	0.8800	

Microbial group	PLFA biomarker					
Gram-negative bacteria	cy17:0, cy19:0, 18:1ω7c, 18:1ω9c					
Gram-positive bacteria	14:0, i15:0, a15:0, 15:0, i16:0, i17:0, a17:0					
Bacteria	i15:0, a15:0, 15:0, i16:0, cy17:0, 17:0, 18:1 w7c, cy19:0					
Fungi	18:2ω6,9c					
Actinomycetes	10Me16:0, 10Me17:0, 10Me18:0					
Protozoa	20:2\u00f36,9c; 20:3\u00f36,9,12c, 20:4\u00f36,9,12,15c					

**Table 2.3**. Phospholipid fatty acids used as biomarkers for microbial groups

**Table 2.4**. ANOVA table of F- values on the effect of substrate (rye, clover, A. fruticosa, pine needles, wheat hay and mixture) and sampling time (August, October and February) on mineral soil microbial PLFAs, fungal-to-bacterial PLFA markers and abundance nematodes and microarthropods (fauna densities were log n+1 transformed data prior to analysis). \* P<0.05, \*\* P<0.01, \*\*\*P<0.001

	Total PLFAs	F/B	Bacterivorous Nematoda	Fungivorous Nematoda	Omnivorous Nematoda	Predatory Nematoda	Collembola	Mites
Substrate	1.41	31.8***	5.3***	1	6.36***	1.28	0.6	1
Time	73***	38.3***	13.7***	3.3*	19.24***	5.15*	11.4***	12.1** *
S x T	5.07***	1.29	2.3*	1.6	3.2**	1.7	0.62	1.83

**Table 2.5**. Pearson correlation coefficients and p-values for relationships between PLFA biomarkers (summed percentages) and plant litter quality and soil variables. Only combinations that showed significant relationships are shown. (-): not significant (p>0.05).

		%P	%N	%C	C/N	%Cell	%Lignin	Lign/N	Soil NH4	Soil NO3
3 weeks	Fungi			-0.52 (0.013)	<b>-0.51</b> (0.016)				<b>0.73</b> (0.0001)	<b>0.46</b> (0.032)
	Gram + bacteria	<b>-0.43</b> (0.046)	<b>-0.44</b> (0.041)		<b>0.47</b> (0.028)					-0.50 (0.018)
	Gram - bacteria	<b>0.55</b> (0.008)	<b>0.53</b> (0.011)	- <b>0.55</b> (0.008)	-0.67 (0.0006)				<b>0.52</b> (0.012)	<b>0.61</b> (0.003)
	Actinomycetes			<b>0.44</b> (0.041)					<b>-0.57</b> (0.006)	
	F/B			- <b>0.47</b> (0.0287)					<b>0.65</b> (0.0012)	
12 weeks	Gram - bacteria						<b>-0.43</b> (0.044)	<b>-0.46</b> (0.0313)		
29 weeks	Actinomycetes			<b>0.72</b> (0.0001)		-0.47 (0.025)	<b>0.68</b> (0.0004)	<b>0.69</b> (0.0003)		



Figure 2.1. Changes in concentrations of total phospholipid fatty acids ( $\mu$ g PLFA/g soil) in mineral soil over the course of decomposition of five surface applied plant residues and a mixture. Error bar indicates standard error.



Figure 2.2. Plot of first and second principal components (PCs) from Principal Component Analysis of mole percentages of selected phospholipid fatty acids from samples from all dates and all substrates. Data points represent means of 4 replicates +/- 1 standard error. Amount of variability explained by each component is shown in parentheses.



Figure 2.3. Plot of first and second principal components (PCs) from Principal Component Analysis of mole percentages of phospholipid fatty acids biomarkers grouped by microbial guild by date. Data points represent means of 4 replicates +/- 1 standard error. PC1 (*x* axis) explained 22%, 27% and 26% of the variation for August, October and September respectively. PC2 (*y* axis) explained %, % and % of the variation for August, October and September respectively.



Figure 2.4. Changes in the fungal-to-bacterial phospholipid fatty acid markers in mineral soil over the course of decomposition of five surface applied plant residues and a mixture. Error bar indicates 1 standard error.



Figure 2.5. Changes in nematode trophic groups in mineral soil (no./g soil) over the course of decomposition of five surface applied plant residues and a mixture. Error bars indicate 1 standard error.



Figure 2.6. Changes in microarthopods (no./g soil). (a) Collembola and (b) mites (Oribatid+Prostigmatid+Mesostigmatid) in mineral soil over the course of decomposition of five surface applied plant residues and a mixture. Error bar indicates 1 standard error.

# **CHAPTER 3**

# EFFECT OF PLANT LITTER QUALITY ON THE ABILITY OF SOIL TO PROCESS FRESH SUBSTRATES IN THE PRESENCE OF DIFFERENT SOIL FAUNA $^\dagger$

<sup>&</sup>lt;sup>†</sup> Carrillo, Y., Ball, B., Strickland, M., Bradford, M.A.; to be submitted to Soil Biology and Biochemistry

# ABSTRACT

The quality of plant litter can influence the composition of the soil community as well as the chemical environment in soil. Some studies have found that the soil fauna can accentuate the control by litter quality of soil processes and of the soil microbial community. Our objective was to test if the changes brought about in soil by the quality of litter could influence the ability of soil to mineralize and decompose newly added substrates and materials already present in the mineral soil and if the composition of fauna would affect this ability. We set up a laboratory experiment in which the soil biotic and chemical environment was manipulated by exposing it to two plant litters of contrasting qualities for 16 months. We then monitored nitrogen and carbon mineralization and litter mass loss after removing the old litter and (a) adding fresh litter of the same species with which the soil had been pretreated, (b) adding fresh litter of the second species and (c) leaving soil bare. We found that the preexisting conditions in soil affected the evolution of CO<sub>2</sub> from fresh litter and overrode the effect of litter quality on nitrogen mineralization in the first week of decomposition. Also by modifying the soil environment litter quality affected the mass loss pattern of one of the litter materials. We found indications that the composition of fauna affected the influence of litter quality on nitrogen mineralization, but the effects on mass loss and carbon mineralization were not clear. Our results suggest that the effects of plant litter quality on soil function are not limited to determining its own decomposition and mineralization rate and can generate biotic and abiotic conditions that affect the soil's ability to process freshly added substrates. Our study also contributed some evidence that the composition of soil fauna can change the extent of the influence of litter quality on soil properties that can affect its function.

Keywords: litter quality, soil fauna, carbon, nitrogen, mineralization, decomposition

# INTRODUCTION

One of the ways in which plants influence ecosystem processes is through the quality of their litter. Indices of the chemical quality of plant litter are good predictors of the rates of decomposition and nutrient mineralization in soil (Aerts, 1997; Melillo et al., 1982). These indices however, act only as surrogates for the actual regulators of the decomposition and mineralization processes (Meentemeyer, 1978). Lavelle et al. (1993) described decomposition – and mineralization as a sub-process of decomposition- as being determined by a set of hierarchically-organized interacting factors: climate, clay mineralogy, nutrient status of soil, quality of decomposing resources and effect of organisms. However, the study of the regulation of decomposition has concentrated on isolated factors and studies of the interactions of the quality of resources and soil organisms are especially uncommon. Wardle (2002) hypothesized that the effects of plant species on ecosystem processes are ultimately due to their effects on the soil organisms that drive the processes. Indeed, the chemical composition of plant litter has been shown to influence the structure of litter and soil microbial (Schutter and Dick, 2001) and faunal (Hansen and Coleman, 1998; Parmelee et al., 1989) communities.

The link between the change brought about in the soil biotic and abiotic environment by the chemical quality of plant material and the functioning of the soil community has not been addressed by many studies. Cookson et al. (1998) found that wheat litter decomposed faster in soils that had previously been exposed to wheat residue and suggested that conditioning the microbial community to specific plant residue types –therefore with different chemical composition- could to some degree override the influence of residue quality on decomposition. Dehlin et al. (2006) detected strong effects of the identity of organic substrates added to soil on microbial community structure and activity. In an attempt to test the hypothesis that plant species

encourage soil biota that specializes in the decomposition of their litter, Ayres (2006) found evidence that plant species can modify the soil community to influence decomposition. Orwin (2006) found that although the identity of carbon substrates added to soil significantly affected the structure of the microbial community, decomposition was mainly influenced by the effects on the chemistry of soil caused by the added substrates rather than by the changes in microbial community. Soil chemical variables can be affected by the chemical composition of organic matter inputs (Bulluck et al., 2002; Tirol-Padre et al., 2007). These changes in the chemical environment of soil may have direct impacts on soil processes but may also influence the structure and functioning of the soil community, thus indirectly affecting processes. It seems likely that the control of processes by the quality of litter is a result of the joint influence of its effects on the soil community and the chemical environment in soil.

The composition of the soil faunal community is another factor influencing the rates of decomposition and mineralization in soil (Bradford et al., 2002; Coleman et al., 2004). In general, nutrient mineralization and decomposition increase with increasing body size of the soil animals and with increasing complexity of the faunal community. However, there is evidence of strong interactive effects of the soil community composition and the quality of litter (Couteaux et al., 1991). Some studies have shown that the composition of the faunal community can affect the level of control of litter quality on soil processes. In particular, a more complex fauna seems to enhance the degree of control of litter quality on decomposition (Schadler and Brandl, 2005; Smith and Bradford, 2003) and N mineralization (Chapter 4). There is some indication that fauna might act by accentuating the response of the microbial community to the quality of litter (Chapter 4). A proposed mechanism for this effect involves fauna making the litter more accessible to microbial detritivores. It has been shown that microarthropods due to their high

mobility can seek high quality substrates (Griffiths and Caul, 1993; Rantalainen et al., 2004) and make litter more accessible to microbes (Petersen and Luxton, 1982), which would in turn accentuate the effects of quality on microbial populations and as a consequence on the processes they drive.

We set up a laboratory experiment in which the soil biotic and chemical environment was manipulated by exposing it to two plant litters of contrasting qualities for 16 months. We then monitored nitrogen and carbon mineralization and litter mass loss after removing the old litter and (a) adding fresh litter of the same species with which the soil had been pretreated, (b) adding fresh litter of the second species and (c) leaving soil bare. We sought to test if the changes brought about in soil by the quality of litter could influence the ability of the soil community to mineralize and decompose newly added substrates and materials already present in the mineral soil. We wanted to test this in communities of different faunal composition, so the experiment was carried out with soils that had been frozen to kill fauna and with intact soils. If the same substrate was processed differently in soils pretreated with different substrates, it would be evidence that it was through changing the soil biotic or chemical environment that quality controlled the processes. On the other hand, if we observed no differences in the processes rates this would indicate that it was only the intrinsic degradability of the fresh substrate that mattered in determining its mineralization and decomposition. If fauna complexity can enhance the control of litter quality on soil processes and on soil microbial community by making litter more accessible to microbes, then we expected to observe greater differences in mineralization and decomposition of the same litter in soils pretreated with different substrates when the soils had not been frozen -i.e. when the faunal community was more complex- than when it had been subject to freezing -i.e. a simplified community-. The time span of the experiment was limited to

a month, because it was assumed that beyond that time the soil biota would not be reflecting the effect of the litter pretreatment but would have adapted to the freshly added substrate.

# **METHODS**

# Soil pretreatment and preparation of microcosms

We chose two plant materials as quality treatments: leaflets of *Amorpha fruticosa* L., a leguminous shrub and needles of loblolly pine (*Pinus taeda* L.). Amorpha had a C/N of 12.6 and contained 3.2 % of phophorus, and 11% of lignin. The C/N of pine was 76, and it contained 0.86% phophorus and 30% lignin. Microcosms consisted of a layer of soil and a layer of litter in a glass container of 0.4 L in volume covered with stainless steel mesh of 40-µm pore size to prevent colonization by foreign fauna. Prior to setting up the microcosms, the mesh was autoclaved for 30 minutes and frozen at -80°C for two days to kill any existing propagules of soil animals.

Soil from the collection site in the field is classified as a Pacolet sandy clay loam (kaolinitic, thermic typic hapludult) and has an average of 0.7% carbon content and 0.1% nitrogen content. Field capacity was measured using a 0.3 µPa pressure plate for 12 days at 0.01 µPa bars and was estimated to be 19% water content. Soils were exposed to the quality treatments (pretreated) in three phases, one in the field, another one in the laboratory and a third one once they had been frozen and the microcosms had been set up. Soils used in the microcosms were sieved, homogenized and exposed to surface applied litter (of Amorpha and pine respectively) for 8 months in the field, then brought to the laboratory and after the addition of new air dried litter, incubated at 20°C for another 8 months in 20 L plastic pots. While in the lab, pots were watered periodically to maintain soil moisture above ~70% field capacity.

Soil to be used in the microcosms was removed from pots from the upper 5 cm under the litter layer. Approximately 80 g of soil (dry weight) were added to each of 52 glass containers. Containers were covered with mesh and then frozen at -80°C for 5 days, after which they were thawed and frozen again for 2 days. When the containers were taken out of the freezer, 52 more microcosms were prepared but not frozen. Three grams of air dried litter that had been previously autoclaved twice for 20 minutes with 24 hours in between were added to each container (26 frozen and 26 unfrozen received Amorpha and the same was done with pine). Microcosms were then incubated at ~20°C on a laboratory bench. Moisture was kept between 80% and 100% field capacity by periodically adding water to reach a set target weight.

After 60 days of incubation, litter was removed from each container and a soil sample from each of four containers from each treatment was collected to be analyzed for  $NH_4^+$  and  $NO_3^-$  (see below), pH and total carbon and nitrogen and microbial biomass carbon (see below). pH was measured in a 2:1 water-to-soil ratio and total carbon and nitrogen content was obtained using the Dumas combustion methods on a Carlo Erba Elemental Analyzer.

Following litter removal, treatments were assigned as shown in Table 3.1. In brief, of the microcosms that had been conditioned with pine, 20 received pine, 20 received Amorpha and 12 were left bare. Of those, half had been frozen and half had not. The same was done with soils conditioned with Amorpha. Approximately 4 g of litter were added to each jar. During manipulation of the microcosms, precaution was taken to avoid contamination of the frozen microcosms with external fauna.

To assess mass loss, a litter bag containing  $\sim 1$  g of autoclaved plant material was placed on top of the litter layer in each of the microcosms except the ones that had no litter (see mass loss methods). Jars were covered with mesh and allowed to incubate at 20°C and between 80

and 100% field capacity. After 8 days, half of the microcosms from each treatment were destructively sampled and processed for litter mass loss, litter nematodes, litter microarthropods, soil nematodes and microarthropods, soil  $NO_3^-$  and  $NH_4^+$ , and soil microbial biomass. The second and final sampling took place 28 days after litter addition.

# Sample processing

### Mass loss

Mass loss was measured using 1g of litter in 8-cm diameter nylon litterbags with 2 mm mesh. After collecting the litter bags, they were used for microarthopods extraction (see below). Samples were then dried and ashed (3 h, 500°C). Data are expressed as ash free dry remaining mass.

## Soil and litter microfauna

Nematodes were extracted from approximately six grams of fresh soil and 1 gram of litter with the Baermann funnel method for 72 hours. Nematodes collected were preserved in 5% formaldehyde. Total nematode counts to the level of trophic group were performed for each sample. Tylenchidae were included in the fungivorous trophic group. Microarthopods were extracted from ~25 g of of fresh soil and from litter bags for five days on Tullgren-type extractors. They were identified to order.

# Soil microbial biomass

Microbial carbon in soil was determined in samples from the first sampling date using the chloroform fumigation extraction method (Vance et al., 1987). Five grams of 2-mm sieved fresh

soil were fumigated for 72 hours and extracted in 20 ml of  $0.5M K_2SO_4$ . Extracts were filtered and analyzed for total organic carbon (Shimadzu TOC-5000A). Microbial biomass was calculated using Kc=0.32.

# Nitrogen mineralization

Four grams of fresh soil were freeze-dried immediately after collection and later extracted by shaking in 2 M KCl for one hour (20 ml for the soils and 60ml for the resin-filled bags). Extracts were analyzed for  $NO_3^-$  and  $NH_4^+$  using an Alpkem Continuous Flow Analyzer. Net mineralization rate was calculated for each of the two incubation periods (0-8 days and 8-28 days) as the difference between final and initial total nitrogen ( $NO_3^- + NH_4^+$ ) divided by the number of days in each incubation period.

#### Carbon mineralization

Carbon mineralization rates were repeatedly measured 4, 10, 18 and 27 days after litter addition on 5 replicate samples per treatment using a static incubation procedure. This procedure consisted of sealing each microcosm for a period of 3 h with a gastight lid modified to accept a sub-seal. We measured CO<sub>2</sub> concentrations immediately after capping each microcosm and at the end of the 3-h incubation period using an infra red gas analyzer (Li-Cor Biosciences, Model LI-7000). Carbon dioxide production rates were corrected for the contribution of the corresponding soil inoculum by subtracting the mineralization rates of the bare soil microcosms with the same pretreatment. In all cases soil CO<sub>2</sub> production accounted for less than 20% of total CO<sub>2</sub> production and in most cases accounted for less than 10% of total CO<sub>2</sub> production.

#### Statistical analysis

Mass loss, nitrogen mineralization, microbial biomass, soil mineral nitrogen concentrations and fauna abundance data were analyzed with three way ANOVA for each of the two sampling dates. Carbon mineralization was analyzed with repeated measures two factor ANOVA for each litter treatment with time being treated as a discrete variable. t tests were used for comparisons of pairs of means and Tukey's test was used for multiple comparisons.

# RESULTS

## Soil chemical variables

Soil chemical variables were assessed after pretreatment and freezing but before incubation started. Concentrations of  $NO_3^-$  and  $NH_4^+$  in soil were substantially greater in soils pretreated with Amorpha than in soils pretreated with pine (Table 3.2). Freezing had a significant effect on the concentration of  $NH_4^+$  in soils pretreated with Amorpha. C/N was significantly smaller in soils pretreated with Amorpha (p<0.05). Freezing had no effect on C/N. pH of soils was slightly but significantly greater in soils pretreated with pine. There was no effect of freezing on soil pH.

Throughout the incubation period concentrations of mineral nitrogen in soils pretreated with Amorpha remained considerably greater than in soils pretreated with pine (Table 3.3). Differences between pretreatments became smaller after 4 weeks due to the influence of the added litter, i.e., net immobilization when pine litter was added and net nitrogen release when Amorpha litter was added (significant effect of interaction between pretreatment and litter, p<0.05). After 4 weeks differences between pretreatments were greater in unfrozen soils. However, no significant effect of interaction of community and pretreatment was found (p=0.12).

#### Characterization of biota

Before incubation started, there was not an effect of pretreatment on microbial biomass carbon, and soils that had been frozen contained significantly more microbial carbon that unfrozen soils (Table 3.2). After 1 week of incubation soil microbial biomass was significantly larger in the unfrozen microcosms (Figure 3.1, Table 3.4). The effect of pretreatment was not consistent across litter treatments (Table 3.4). Microbial biomass was significantly greater in soils pretreated with pine but this was only true for soils in which the added litter was Amorpha.

Only bacterial and fungal feeding nematodes were present in the microcosms. Pretreatment had a large impact on nematode abundances in soil (Figure 3.2). After 1 week of incubation soils pretreated with Amorpha had significantly more nematodes than soils pretreated with pine. Freezing caused an increase in nematode densities in frozen soil pretreated with Amorpha, and in contrast led to almost complete absence of nematodes in soils pretreated with pine (significant interaction of pretreatment and community, F=9.98, p=0.003, three way ANOVA on total abundance). In Amorpha-pretreated soils, freezing increased the relative abundance of fungal feeders.

Frozen soils had between half to a fourth of the microarthropods of unfrozen soils after 1 week (Figure 3.3) and this difference was significant (F=19.64, p<0.0001, three way ANOVA on total abundances for Week 1). As time progressed (Week 4, Figure 3.3), differences in total abundances between frozen and unfrozen soils decreased due to the proliferation of prostigmatid mites in soils that had been frozen. The prostigmatids composed the great majority of all individuals in frozen soils. Soils that were pre-treated with Amorpha had significantly greater densities of microarthropods than soils pre-treated with pine; this difference was significant after 1 week (F=4.44, p=0.04, three way ANOVA on total abundances for week 1). They also had a

more even representation of the three mites groups and were the only ones that contained collembola. Soils pre-treated with pine mainly contained oribatids and prostigmatids.

There was no overall significant effect of freezing or pretreatment on microarthropods abundance in litter (p>0.05). One week after litter addition densities of microarthropods in litter were very low (Figure 3.4). On this sampling date unfrozen microcosms pre-treated with Amorpha and where the added litter was Amorpha had the highest microarthropod densities. Of the microcosms pretreated with pine the frozen microcosms that received Amorpha had the highest densities. After one month densities had increased substantially in all treatments and most individuals present in samples belonged to the prostigmatids. Prostigmatids proliferated equally under all treatments.

### Carbon mineralization

Carbon mineralization was about ten times greater in Amorpha than in pine litter (Figure 3.5). No significant effect of freezing on C mineralization from either litter or bare soil was found (Table 3.5). However, in bare soils CO<sub>2</sub> flux was greater in unfrozen microcosms up to the 7<sup>th</sup> day of incubation (Figure 3.5). A significant interaction of pretreatment by time was observed for Amorpha and pine litter (Table 3.5). In both cases carbon mineralization was greater in soils pretreated with Amorpha early after litter addition (Figure 3.5). The effect disappeared after day 4 in Amorpha litter but remained until day 7 in pine litter.

#### Mass loss

Substantial fungal growth could be observed in litter during the four weeks of incubation, which led to net mass gain in all treatments (Figure 3.6). Overall, less mass was gained in Amorpha

litter than in pine litter. After 1 week there was a significant effect of the interaction communitypretreatment-litter (Table 3.6). Analysis of data by litter indicated that for pine and Amorpha litter the effect of pretreatment was not consistent across frozen and unfrozen communities (two way interaction, p<0.05). For pine litter, when soils had been pretreated with pine, significantly more mass was gained in unfrozen microcosms while the inverse occurred in soils pretreated with Amorpha. For Amorpha litter pretreatment had a significant effect on mass gain only in frozen soils where there was more mass gained in soils pretreated with pine.

After 4 weeks of incubation, an effect of interaction of community, pretreatment and litter was detected (Table 3.6). Analysis by litter indicated that the effect of freezing was not consistent across pretreatments for any litter but no significant patterns were observed. After 4 weeks pretreatment had no significant effect on mass gain in any treatment.

## Nitrogen mineralization

Figure 3.7 shows average net mineralization or immobilization rates after 1 and 4 weeks of incubation. One week after litter addition only net immobilization of nitrogen was observed except for the soils that were frozen and pretreated with pine. In these cases however, net mineralization was very low. There was no effect of litter (Table 3.7). While immobilization rates were lower in bare soil, immobilization rates in Amorpha and pine litter were very similar. Soils pretreated with Amorpha showed significantly greater immobilization rates than soils pretreated with pine. Immobilization was consistently and significantly lower in soils that were frozen (Table 3.7). The effect of freezing was not consistent across pretreatments (interaction pretreatment-community, Table 3.7). When soils had been pretreated with pine the effect of freezing —of decreasing immobilization- was stronger than when they had been pretreated with

Amorpha (except under bare soil). After 4 weeks of incubation net mineralization was observed when Amorpha litter had been added and in bare soil that had been not been frozen and had been pretreated with Amorpha. A significant interaction of pretreatment and litter was detected, which resulted from pretreatment being important in bare soil and pine litter but not under Amorpha litter. An interaction of pretreatment and community was also observed, which reflected that the effect of freezing was important in soils pretreated with Amorpha but not in those pretreated with pine. In soils pretreated with Amorpha, unfrozen soils showed greater net mineralization or less net immobilization than frozen soils.

# DISCUSSION

## Effect of soil pretreatment and freezing

We were interested in testing if the changes in soil generated under substrates of contrasting quality could influence the ability of soil to mineralize and decompose newly added substrates and materials already present in the mineral soil and whether the faunal composition would affect this ability. Manipulation of soil was successful at generating distinct chemical and biotic conditions in soil due to quality pretreatment and at generating different soil faunal community compositions through freezing. Exposing mineral soils for 16 months to pine and Amorpha litter caused differences in their C/N ratio, mineral nitrogen concentrations, pH, soil nematode and microarthropod abundance and composition. From microbial community composition analysis using phospholipids fatty acids (Chapter 2) we also know that pine and Amorpha litter produce distinct microbial communities. Freezing soils caused a decrease in microarthropods abundance and changed their community composition to a prostigmatid dominated community. Freezing soils increased nematode abundance in soils pretreated with Amorpha and caused a decrease in

soils pretreated with pine. Frozen soils contained less total mineral nitrogen and more microbial biomass than unfrozen soils.

Having assessed the actual effect of the soil manipulations, we proceeded to assess their effects on processes.

## Carbon mineralization

In the first week of decomposition carbon mineralization from pine and Amorpha litter was greater when being decomposed on soils that were pretreated with Amorpha than when being decomposed on soil pretreated with pine, which indicates that the control by the chemical quality of the litter was influenced by the preexisting conditions in soil and supports the hypothesis that through changing the soil environment, the quality of the pretreatment litter controlled CO<sub>2</sub> evolution from freshly added litter. Was this effect due to chemical or biotic differences in soil? It is likely that it was a combination of both. Mineral nitrogen concentration, which was considerably higher when soils were pretreated with Amorpha, might help explain the higher respiration rates. An increase in nitrogen concentrations often can stimulate the decomposition of labile materials in litter (Knorr et al., 2005) for which is it is often found to have a positive effect early in the decomposition process (Berg and Matzner, 1997). However, the fact that the difference in soil nitrogen concentrations persisted until the end of the incubation while the differences in CO<sub>2</sub> flux due to pretreatment lasted only until day 7 suggests that the difference between pretreatments was not exclusively driven by nitrogen availability and other factors – which were important only at the beginning of the incubation- might have been involved. It is reasonable to think that differences in the microbial community or their consumers could have been of relevance. For example, soils pretreated with pine had greater microbial biomass when

Amorpha litter had been added and less nematodes in all cases. These or other differences in the size or structure of the microbial community would have tended to decrease as the decomposition of the newly added litter progressed and the new litter had more time to influence the soil community. This is consistent with the disappearance of pretreatment effect after day 7 and supports that a combination of factors was responsible for the difference between pretreatments.

Freezing had no effect on  $CO_2$  evolution from litter. The main effect of freezing in the microcosms was to make the microarthropod community in soil smaller and less diverse. Yet, we observed that the colonization of litter by microarthropods was mainly driven by its identity and the faunal composition in the mineral soil did not have much influence. Thus, it is not surprising that freezing was not an important factor in respiration from litter.

We found no evidence to support the hypothesis that the presence of a more complex soil fauna (in unfrozen soils) enhanced the effect of the pretreatment on  $CO_2$  evolution from newly added litter. This indicates that the differences in faunal community structure generated by freezing did not affect the variables that were driving respiration from litter and that led to the differences in  $CO_2$  production between pretreatments.

In contrast to litter, we did not observe a consistent effect of pretreatment in bare soil but we did see indications of an effect of freezing in the first week of incubation. The lack of effect of pretreatment would indicate that C0<sub>2</sub> evolution from soil organic matter was not affected by a higher nitrogen availability, lower C/N ratio, lower pH and higher microarthropods and nematodes abundance in soils pretreated with Amorpha. One possibility is that microbes in bare soil were carbon limited, which would not be surprising given that the carbon content of the soil was 0.7%. Another possibility is that the members of the microbial community responsible for
degrading soil organic carbon were not affected by the litter pretreatment, which would be consistent with studies that observed that some microbial groups in soil mainly consume fresh organic materials while some groups mainly derive their substrate from more recalcitrant materials in mineral soil (Kramer and Gleixner, 2006; Williams et al., 2007).

Although this effect was not significant, unfrozen bare soils tended to have greater respiration rates at the beginning of the incubation. This is consistent with the greater microbial biomass carbon found in unfrozen soils 1 week into incubation and it might have been related to the high abundances of the microarthropod fauna in unfrozen microcosms which could have increased turnover rate and activity of microbial populations through grazing (Bardgett et al., 1993). We did not find any evidence that the presence of a more complex and abundant faunal community increased the effect of pretreatment on soil organic matter respiration, which does not support our hypothesis about the role of fauna as an enhancer of the role of litter quality.

#### Nitrogen mineralization

Over the course of the first week of incubation only net immobilization was observed. The very high C/N of pine litter would be expected to generate high demand for soil nitrogen leading to higher immobilization rates. However, after a week of incubation there was no difference in immobilization rates between Amorpha and pine litter. Pretreatment was the factor driving the differences in immobilization rates. Pretreating with Amorpha led to greater immobilization rates in all cases. This indicates that the preexisting conditions in soil overrode the effect of litter quality on nitrogen immobilization and supports our original hypothesis. Greater immobilization rates in Amorpha pretreated soils are likely a result of the higher concentration of mineral nitrogen after being pretreated with Amorpha, which could then be available for uptake by the

microbial biomass in the litter. Also, soil microbial biomass tended to be higher in Amorpha pretreated soils, which might have contributed to a greater demand of mineral nitrogen and as a result could have increased immobilization rates. High levels of polyphenols derived from pine litter can inhibit nitrogen mineralization (Northup et al. 1995) which might have further contributed to the decrease in mineral nitrogen concentrations observed.

During the last three weeks of incubation, net mineralization was observed for Amorpha litter but net immobilization remained for pine litter. This suggests that their differences in quality had now taken precedence over the preexisting conditions in soil in determining nitrogen processing. There was still an effect of pretreatment in the last three weeks of decomposition. In the case of Amorpha litter, pretreatment with Amorpha caused more net mineralization of nitrogen. The abundance of microbial feeding nematodes and microarthropods was substantially higher in soils pretreated with Amorpha, which might help explain this observation. The presence of both of these groups has been generally associated with greater mobilization of nitrogen and greater nitrogen availability (Bardgett, 2005; Bardgett and Chan, 1999; Ingham et al., 1985; Woods et al., 1982). Thus, after four weeks of decomposition it was still possible to observe differences in the nitrogen release from litter due to conditions generated by the pretreatment litter.

Freezing led to less immobilization (or to net mineralization) during the first week of incubation. This could have been a result of lower total mineral nitrogen available in soil at the start point of the incubation, however this difference was not dramatic and tended to disappear as incubation progressed. The differences in the biota are more likely to be responsible for this effect. A smaller microbial biomass, as found in frozen soils, would have demanded less nitrogen and consequently decreased immobilization. It is possible too that larger faunal populations (in

unfrozen soils) might have activated the microbial biomass and as a result, increased N demand by the microbes (Bardgett, 2005), causing more net immobilization. In contrast, in the last three weeks of incubation, freezing had the opposite effect and led to either more immobilization or less net mineralization. This effect is very strong in the case of bare soil, where pretreatment with Amorpha lead to net immobilization in frozen soils and net mineralization in unfrozen soils. One possibility for explaining this is that higher microarthropod abundance in unfrozen soils might have led to less microbial grazing and consequently less excretion of nitrogen (Woods et al., 1982). It is not clear however, what caused the switch in the effect of freezing from week 1 to week 4.

We did not find that the effect of pretreatment (calculated as the absolute difference between pretreated with Amorpha rate and pretreated with pine rate as a percentage of the former) was consistently greater in soils that were unfrozen. An enhancing effect of fauna was found in the case of Amorpha after four weeks of decomposition (although the effect of pretreatment was not significant). Also, four weeks into incubation of bare soils, not freezing seemed to have accentuated the effect of the "high quality" of Amorpha and the "low quality" of pine by producing net mineralization in Amorpha pretreated soils and net immobilization in pine pretreated soils (while it was the opposite in frozen soils). The general trend observed did not support the hypothesis that fauna act by enhancing the role of litter quality in determining nitrogen mineralization of freshly added substrates. However, the fact that enhancing effects were never observed in the mineralization of pine litter might be a result of specific properties of this substrate. An experiment in which the effect of pretreatment are examined in a variety of substrates would be necessary to distinguish effects of the identity and specific attributes of the litter from general patterns. The lack of consistency in the response across time is consistent with the findings of Smith and Bradford (2003) in regards to decomposition. In their case it was also found that the enhancing effect of fauna could be observed after 30 days, but not after 60. It is possible then that in our case one week was insufficient time for an effect to become apparent in any litter and later, after 4 weeks, an enhancing effect of fauna occurred in Amorpha litter and bare soil.

#### Mass loss

Only net mass gain was observed during incubation, which we attribute to the profuse fungal colonization that could be observed. Less mass gain occurred in Amorpha likely suggesting more actual mass loss, which would be expected given its higher degradability due to better chemical quality. There was an important exception: when Amorpha litter was being degraded on pine-pretreated and frozen soil it gained as much mass as the pine litter. This might suggest that pretreatment with pine inhibited mass loss of Amorpha and this effect was enhanced when the faunal community was smaller and less complex. This finding supports the hypothesis that by influencing the soil environment, the quality of litter influenced the ability of soil to decompose Amorpha litter. The fact that the difference between pretreatments is only significant in frozen soils suggests that the presence of a larger and more diverse faunal community (in unfrozen soils) might have acted by compensating for the effects of pretreating with pine, and although it does not support the hypothesis that fauna enhance the effects of litter quality on mass loss, it suggests that fauna can change the degree to which litter quality effects on soil influence the decomposition of fresh substrates.

Ours results indicated that the differences generated in the mineral soil after exposure to litter of contrasting qualities affected its ability to process freshly added substrates and could

even override the control by litter quality early in the decomposition process –as was the case of nitrogen mineralization. This result is consistent with Cookson et al.'s (1998) findings on the decomposition wheat residue. As for whether the processing of soil organic matter was affected by pretreatment, we found that this was the case in nitrogen mineralization but not in respiration. Since there were several soil biotic and abiotic variables that were affected by pretreatment, we tend to favor the explanation that a lack of response in respiration was a consequence of the low availability of carbon in the mineral soil. A study involving a range of soil carbon content and availability would provide more conclusive evidence. Although the structure of the soil community and the chemical properties in soil were not isolated in this experiment, there were indications that it was a combination of both that determined the effect of the quality pretreatment, which is in agreement with Orwin et al. (2006).

With regards to whether a larger and more diverse faunal community would enhance the role of litter quality in influencing the soil biotic and abiotic environment, we expected to observe greater differences in mineralization and decomposition of the same litter in soils pretreated with different substrates when the soils had a more complex faunal community. This was the case only for nitrogen mineralization for two of three litter treatments only for the last three weeks of the study. This could indicate that fauna, probably by making litter more accessible to microbes (Petersen and Luxton, 1982), had the ability to influence the soil variables that regulate nitrogen mineralization and thus enhance the effects of litter pretreatment on the mineralization of newly added litter and of soil organic matter. The fauna hypothesis was not supported by the mass loss and respiration results, which contradicts the findings of Smith and Bradford (2003) and Schaedler and Brandl (2005). Various factors might be responsible for this discrepancy. The choice of substrates used in the experiments might be an important factor, as

the role of fauna might be different depending on the accessibility and palatability of the litter materials. For example, if a substrate has very low palatability or is not very hospitable it might not be consumed or comminuted by the animals and as a consequence they might not have any effect in making it more accessible to the microbes. Studies are needed to determine the role of fauna in exposing litter materials of different qualities to attack by microbes. The mass loss data suggested that even though fauna did not accentuate the effect of pretreatment litter quality, it was able to alter the degree to which litter quality effects on soil influenced the decomposition of fresh substrates by promoting mass loss when the conditions generated by pretreatment tended to cause a slow down. These two distinct effects point out that the mechanisms for the fauna's role in the control of processes by litter quality might be multiple and deserve further and intense study.

In summary, this study provided evidence that the effects of plant litter quality on soil function are not limited to determining its own decomposition and mineralization rate and can generate biotic and abiotic conditions that affect the soil's ability to process freshly added substrates. Our results suggested that these conditions can even override the control by litter quality early in the decomposition process. Our study also contributed some evidence that the composition of soil fauna can change the extent of the influence of litter quality on soil properties that can affect its function. Further studies are needed to better understand if this effect is always an enhancing effect or if neutral or negative effects are possible.

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Community (Frozen/Unfrozen)	Pretreatment	Litter	
		Pine	
Frozen	Pine	Amorpha	
	T IIIC	Bare soil	
		Pine	
	Amorpha	Amorpha	
		Bare soil	
Unfrozen		Pine	
	Pine	Amorpha	
		Bare soil	
		Pine	
	Amorpha	Amorpha	
	· ····orpriu	Bare soil	

Table 3.1. Treatment structure

Table 3.2. Soil chemical variables and microbial carbon after pretreatment and before incubation. Standard error in parentheses. Values within columns followed by the same letter are not significantly different (p<0.05). \* Separate t-tests were performed for Amorpha and pine pretreatments.

Pretreatment	Community	NO3-N ug/g	NH4-N ug/g	C/N	рН	Microbial C (mg/g)
Amorpha	Frozen	65.75 (6.8) a	38.79 (7.6) a	6.18 (0.2) a	4.89 (0.05) a	0.83 (0.19) a
Amorpha	Unfrozen	82.00 (13.7) a	70.96 (10.7) b	6.56 (0.4) a	4.77 (0.05) a	0.49 (0.08) b
Pine	Frozen	1.36 (0.73) b	1.47 (0.24) c	7.16 (0.5) b	5.00 (0.07) b	0.87 (0.06) a
Pine	Unfrozen	3.28 (1.83) b	1.16 (0.05) c	8.31 (0.7) b	4.97 (0.05) b	0.47 (0.06) b

		Litter treatment	Pine pretreated	Amorpha pretreated	
		Pine	2.65 (0.59)	85.51 (10.60)	
	F	Amorpha	3.46(0.39)	87.36 (4.28)	
ek 1		Bare soil	3.45 (0.43)	103.87 (10.11)	
Wee		Pine	1.98 (0.27)	73.57 (16.25)	
	UF	Amorpha	2.92 (0.88)	67.96 (10.47)	
		Bare	4.48 (0.97)	107.90 (21.18)	
		Pine	2.18 (0.61)	50.33 (6.97)	
	F	Amorpha	58.94 (5.96)	145.17 (14.51)	
ek 4		Bare	5.12 (0.32)	94.60 (14.03)	
Wee	UF	Pine	2.40 (0.90)	64.79 (7.42)	
		Amorpha	61.98 (14.61)	156.88(14.59)	
		Bare	3.12 (0.44)	127.43 (17.93)	

Table 3.3. Concentration of mineral nitrogen in soil (NO<sub>3</sub><sup>-</sup> + NH<sub>4</sub><sup>+</sup>,  $\mu$ g/g soil) 1 and 4 weeks after litter addition.

Table 3.4. F ratios and p values from three way ANOVA on the effect of litter pre-treatment, community (frozen/unfrozen soils), litter and their interactions on soil microbial biomass carbon (mg/g soil) 1 week after litter addition.

Source	F	р
Pretreatment	0.08	0.79
Community	5.77	0.02
Litter	3.82	0.03
Pretreatment * community	1.32	0.25
Pretreatment * litter	4.94	0.01
Community * litter	0.09	0.91
Pretreatment * community * litter	0.12	0.88

Table 3.5. F ratios and (P values) from repeated measures ANOVA on the effect of time, litter pre-treatment, and community (frozen/unfrozen soils) and their interactions on carbon mineralization ( $\mu$ g CO<sub>2</sub>-C/g /hour) from Amorpha and pine litter and from bare soil.

	Amorpha		Pine		Bare soil	
Source	F	Р	F	р	F	р
Pretreatment	0.14	0.71	20.34	< 0.001	1.90	0.24
Community	2.44	0.12	0.08	0.78	3.45	0.14
Time	399.37	< 0.0001	502.258	<0.0001	4.13	0.35
Pretreatment * community	0.61	0.44	1.71	0.20	0.16	0.71
Time * community	0.51	0.73	0.71	0.59	0.88	0.65
Pretreatment * time	3.28	0.02	2.61	0.05	0.20	0.91
Comm. * pretreatment * time	1.85	0.13	1.00	0.4144	0.09	0.97

Table 3.6. F ratios and p values from three way ANOVA on the effect of litter pre-treatment, community (frozen/unfrozen soils), litter and their interactions on litter mass (as % of initial ash free dry mass present) after 1 and 4 weeks of incubation.

	Wee	ek 1	Week 2		
Source	F	р	F	Р	
Pretreatment	5.1823	0.0301	0.01	0.9249	
Community	1.4411	0.2394	0.27	0.6057	
Litter	25.9577	<.0001	23.21	<.0001	
Pretreatment*community	0.1064	0.7465	0.33	0.5708	
Pretreatment*litter	5.277	0.0288	0.12	0.7369	
Community*litter	3.4512	0.073	2.62	0.116	
Community*pretreatment*litter	12.8589	0.0012	5.45	0.0265	

Table 3.7. F ratios and p values from three way ANOVA on the effect of litter pre-treatment, community (frozen/unfrozen soils), litter and their interactions on nitrogen mineralization ( $\mu$ g/g soil/day) 1 and 4 weeks after litter addition.

	We	ek 1	Week 4	
Source	F	р	F	Р
Pretreatment	51.09	<.0001	0.13	0.72
Community	19.54	< 0.0001	9.31	0.0044
Litter	1.66	0.21	99.06	<.0001
Pretreatment*community	16.40	0.0003	8.95	0.005
Pretreatment*litter	1.37	0.27	7.44	0.002
Community*litter	0.44	0.65	0.93	0.41
Pretreatment*community*litter	0.38	0.69	1.61	0.22



Figure 3.1. Microbial biomass carbon in soil (mg/g soil) one week after substrate addition in frozen and unfrozen microcosms. Asterisk indicates significant difference (P < 0.05).



Figure 3.2. Densities of soil nematodes (no./g soil) in soil 1 week after litter addition in frozen (F) and unfrozen (UF) soils. Error bars indicate standard error.



Figure 3.3. Densities of microarthopods (no./g soil) in soil 1 and 4 weeks after litter addition in frozen (F) and unfrozen (UF) soils. Error bars indicate standard error.



Figure 3.4. Densities of microarthopods (no./g litter) in litter 1 and 4 weeks after substrate addition in frozen (F) and unfrozen (UF) microcosms. Error bars indicate standard error. For the sake of clarity error bars only shown for groups that were abundant.



Figure 3.5. CO<sub>2</sub>-C flux in microcosms with Amorpha and pine litter (ug/g litter/h) and in bare soil (ug/g soil/h) pretreated with Amorpha and pine. Frozen (F); unfrozen (UF).



Figure 3.6. Percentage of initial mass present in litter bags (as % ash free dry mass) after 1 and 4 weeks of incubation.



Figure 3.7. Average net nitrogen mineralization or immobilization ( $\mu$ g/g soil/day) in microcosms 1 week and 4 weeks after litter addition. Error bars indicate standard error. Asterisks indicate significant difference between pretreatments (p<0.05).

## **CHAPTER 4**

# MEDIATION BY SOIL FAUNA OF THE EFFECT OF LITTER QUALITY ON NITROGEN MINERALIZATION $^{\ddagger}$

Carrillo, Y., Ball, B., Molina, M., Jordan, C. To be submitted to Soil Biology and Biochemistry

## ABSTRACT

In this paper we explore one potential way in which fauna could affect nitrogen mineralization: by mediating the control that the quality of litter exerts on the structure of the micro-food web. In a factorial arrangement, we exposed soil to surface-applied litter of contrasting chemical compositions and restricted the access of size-classes of fauna to mineral soil. After six months of decomposition, we assessed the effects of substrate type on the structure of microbial community in the mineral soil when fauna had been excluded and when it was present, and measured nitrogen mineralization and litter decomposition under both scenarios after 21, 91 and 165 days. We also estimated the abundances of microarthropods and nematode trophic groups in soil. We found some evidence that the composition of soil fauna affected the control of nitrogen mineralization by the chemical quality of plant litter. Specifically, we found that the influence of the chemical composition of litter was enhanced when the larger members of the soil fauna were not excluded from the system. Our results suggested that the presence of fauna affected nitrogen mineralization by affecting the abundance of the consumer level of the micro-food web, the nematodes in particular. Our study also indicated that that the presence of fauna also accentuated the response of the microbial biomass to the quality of litter and therefore supported the hypothesis that the effect of fauna on mineralization can at least in part be due to its effects on the structure of the micro-food web.

Key words: nitrogen mineralization, soil fauna, litter quality, microbial communities

### INTRODUCTION

One of the ways in which plants influence ecosystem processes is through the quality of their litter. Control by plant litter quality of processes has been demonstrated with strong relationships found between plant quality parameters and rates of decomposition and nutrient mineralization (see e.g. Aerts, 1997; Melillo et al., 1982). Quality indices however, act only as surrogates for the actual regulators of the decomposition process (Meentemeyer, 1978). Wardle (2002) suggests that the effects of plant species on processes result from their effects on the organisms that drive the processes. In fact, the microflora is responsible for most of the decomposition and nutrient mineralization of soil organic matter and incoming plant material (Hunt et al., 1987) and it has been shown that chemical composition of substrates added to soil affects the structure of the microbial community (Schutter and Dick, 2002). Recently Orwin (2006) provided some evidence of the direct link between changes in microbial community due to the chemical composition of added substrates and the ability of the soil community to decompose an added substrate.

It is well known that composition of the soil fauna can influence the decomposition and mineralization of litter and soil organic matter (Bradford et al., 2002; Brussaard, 1998; Coleman et al., 2004; Edwards, 2000; Lavelle et al., 1993). In general, nutrient mineralization and decomposition increase with increasing body size of the soil animals and with increasing complexity of the faunal community. However, interactive effects on mineralization and decomposition of the soil fauna (manipulated through exclusions or by creating artificial communities) and the quality of available substrates have been documented. Fauna can increase decomposition and mineralization rates of low quality litter (Couteaux et al., 1991) (Tian et al., 1992). But the opposite has been observed as well (Schadler and Brandl, 2005). Gonzalez and

Seastedt (2001) on the other hand found no fauna-litter interactions. The discrepancies between these results might be reflecting the fact that there are multiple potential mechanisms in which fauna can affect function which might in turn be dependent on environmental factors and on time (Couteaux et al., 1991).

Some studies have shown that the composition of the faunal community can affect the level of control of litter quality on soil processes. In particular, a more complex fauna seems to enhance the degree of control of litter quality on decomposition (Schadler and Brandl, 2005; Smith and Bradford, 2003). It has been shown that microarthropods due to their high mobility can seek high quality substrates (Griffiths and Caul, 1993; Rantalainen et al., 2004) and make litter more accessible to microbes (Petersen and Luxton, 1982), which would in turn accentuate the effects of quality on microbial populations and as a consequence on the processes they drive.

In this paper we explore one potential way in which fauna could affect nitrogen mineralization: by mediating the control that the quality of litter exerts on the structure of the micro-food web (the microbial groups and their direct predators). In a factorial arrangement, we exposed soil to surface-applied litter of contrasting chemical compositions and restricted the access of size-classes of fauna to mineral soil. After six months of decomposition, we assessed the effects of substrate type on the structure of microbial community in the mineral soil when fauna had been excluded and when it was present, and measured nitrogen mineralization and litter decomposition under both scenarios after 21, 91 and 165 days. We also estimated the abundances of microarthropods and nematode trophic groups in soil. We asked (a) does soil fauna mediate the effect of substrate type on the structure of the microbial community and the micro foodweb in the mineral soil, and (c) is the effect of fauna on mineralization associated

with its effect on the microbial community and their predators? We expected that if an effect of fauna on the strength of the control of nitrogen mineralization by litter quality was detected, it would be associated with corresponding changes in the microbial community and its direct consumers.

## **METHODS**

The field study was conducted in 2005 in experimental plots in a previously abandoned conventional farm in the Piedmont region of Georgia USA (33°57'N lat. 83°19'W long). Soil is classified as Pacolet sandy clay loam (kaolinitic, thermic typic hapludult) and had 0.7% C content and 0.1% N. In June of 2005 standing vegetation was pulled from a 100 m<sup>2</sup> plot. Removed vegetation consisted of grasses and forbs. The plot was kept vegetation-free throughout the duration of the study by periodically pulling all sprouting plants. The plot was divided into 24 sub-plots of about 4 m<sup>2</sup> and sheets of aluminum flashing were buried down to 4-5 cm and placed as dividers. Soil from the first 5 cm of four areas of 25 x 50 cm within each subplot was collected, mixed and sieved to 4 mm. Care was taken to allow at least 20 cm of distance between the four areas and between their edges and the edge of the sub-plot. Collected soil was then frozen at -80°C for 72 h to kill existing fauna. After thawing, soil was placed back in the field in metallic frames (25 cm x 50 cm x 5 cm) that fitted the previously dug holes. Soil under and around the sides of the trays was relocated as necessary to make sure that there was contact between soil inside and outside the tray. The bottom and the sides of the metallic frames were lined with mesh of different sizes to restrict access of fauna. Mesh sizes used were 5 cm, 2 mm, 100 µm and 40 µm. Soil was added to the mesh-lined frames (boxes from here on) to reach a depth of approximately 3.5 cm to allow room for the addition of plant residue. The boxes were

open on top, so that the mesh restricted access to mineral soil dwellers but litter organisms could move in and out by jumping the 1-2 cm side wall of the boxes. Soil was left bare and allowed to re-colonize and equilibrate for 2 months.

Substrate treatments consisting of five plant residues and a mixture were randomly assigned to four of the plots (4 replicates) and were surface applied at a rate of  $327 \text{ g/m}^2$  both to the inside and to the outside of the box on July 22. Substrates applied were air dried green litter of cereal rye (*Secale cereale* L), crimson clover (*Trifolium incarnatum* L.) and false indigo (*Amorpha fruticosa* L.); wheat hay (*Triticum aestivum* L.), pine needles (*Pinus taeda* L), and an even mixture of these by weight.

Sampling for mass loss and nitrogen mineralization was carried out 21, 91 and 165 days (August, October and February from hereon) after substrate application for mesh-size treatments 40 µm and 5 mm (the most contrasting ones) and for all size-class treatments in August. Nematodes and microarthropods abundances were assessed on all three dates in mesh sizes 40 µm and 5 mm and microbial community composition only in August and for mesh sizes 40 µm and 5 mm. After collection, soils and litter were immediately processed for microfauna and then freeze-dried for PLFA extraction and mineral nitrogen.

#### Chemical quality of substrates

Dry samples of the initial litter were ground using a Spex CertiPrep 8000-D Mixer Mill and used for chemical analyses and estimation of ash free dry mass. For C/N samples were analyzed on a Carlo Erba Elemental Analyzer and reported as carbon and nitrogen percent dry weights. Total phosphorus was measured from 0.5 g of sample that was ashed, extracted in aqua regia acid, and analyzed on an automated Alpkem Analyzer. Cellulose, hemicellulose, and lignin concentrations were measured from 0.5g of each sample that underwent sequential neutral detergent/acid detergent digestion on an Ankom A200 fiber analyzer. Percentage N and C were determined for remaining biomass at the end of the incubation.

#### Litter Mass Loss

Mass loss was measured using 3g of litter in 13 mm x 14.5 mm nylon litterbags with a combination of 2 mm mesh (to prevent loss of litter by fragmentation) and ten 4mm-diameter holes (to allow macroarthropods in the 5mm mesh size treatment). In the case of the mixed litter treatment, component species were equally represented in the total of 3g. Three bags, one for each sampling, were placed in box on top of the litter layer. After collecting the litter bags, half of the material was used for nematodes extraction (see below) and the remainder was used for microarthopods (see below). Ash free dry mass of remaining litter was calculated after ignition for 3 hours at 500°C.

#### Nitrogen Mineralization

Initial soil concentrations of NO<sub>3</sub><sup>-</sup> and NH4<sup>+</sup> were estimated from six soil samples collected from randomly selected boxes immediately before substrate application. Release of NO<sub>3</sub><sup>-</sup> and NH4<sup>+</sup> from substrates and soil was determined by incubation of nylon bags containing anion and cation exchange resins which were buried under the soil layer in each box to retain NO<sub>3</sub><sup>-</sup> and NH4<sup>+</sup> leached from litter and soil (Binkley and Matson, 1983). Each nylon bag measured approximately 4.5 cm in diameter and contained 24 g of an even mixture of Na-saturated cation and Cl-saturated anion exchange resins (Sybron Ionac C-250 and ASB-1P, Sybron Chemicals, Birmingham, NJ). One nylon bag was buried in each box immediately before substrate addition,

removed after three weeks of incubation and replaced by a new one for the next incubation period (9 weeks). This procedure was repeated for the final incubation period (10 weeks). At each sampling date a soil sample was collected from each tray. Resins and soils (4 g) were extracted by shaking in 2M KCl for one hour (20 ml for the soils and 60ml for the resin-filled bags). Extracts were analyzed for NO<sub>3</sub><sup>-</sup> and NH4<sup>+</sup> using an Alpkem Continuous Flow Analyzer. Nitrate and ammonium from soil extractions were converted to µg per gram of soil. To calculate the amount of nitrate and ammonium derived from soil and litter that had accumulated in the resins, it was assumed that the majority of the nitrate and ammonium collected had been derived from the soil directly above it (approximately 64  $cc^3$  of soil, since the diameter of the resin bag was approximately 4.5cm and the depth of the soil above resin bag was 4 cm). Using an average value of bulk density for the site, nitrate and ammonium accumulated in the resin over each incubation period was converted into µg per gram of soil. Concentration of mineral nitrogen was calculated using the combined amounts of nitrate and ammonium in both soils and resins and net mineralization per day for each incubation period was calculated as the difference between final and initial total nitrogen (NO<sub>3</sub><sup>+</sup> + NH4<sup>+</sup>) divided by the number of days in each incubation period (Kolberg et al., 1997). Initial soil inorganic N used for the last two incubation periods was the average N content in soil from the four replicates collected at the end of the previous incubation period.

## Microbial community

Phospholipid fatty acids (PLFA) were extracted from 5 g of 2 mm sieved and freeze-dried soils and 1 g of freeze-dried and ground litter. Samples were extracted with the Bligh-Dyer technique (Bligh and Dyer, 1959) by shaking for two hours with a single phase mixture of methanolchloroform-phosphate buffer (2:1:0.8 ratio in volume) in a teflon bottle. The mixture was centrifuged to decant soil. Extra volumes of chloroform and methanol were added and the sample was centrifuged again to separate the organic phase containing the lipids which was then fractionated into neutral lipids, glycolipids and phospholipids (Zelles and Bai, 1993) with an activated silica gel column (BondElut; Varian, Palo Alto, CA). Methylation of phospholipids was done with mild alkaline methanolysis (White et al., 1979) to produce fatty acid methyl esters (FAMEs). FAMEs were purified with NH<sub>2</sub> aminopropyl columns (BondElut; Varian, Palo Alto, CA).

Samples were analyzed with a Hewlett-Packard (HP; Palo Alto, CA) 6890 series GC with a flame ionization detector and a 30 meter DB-5 (film thickness = 0.25 microns, internal diameter 0.32 mm; Agilent, Santa Clara, CA). Individual PLFAs were quantified in relation to an external standard (20:0 ethyl ester) that was run in duplicate with every batch of samples. Compounds were identified by comparison of their retention times with those of a prepared mixture standard containing 36 FAMES (Matreya, PA; Sigma-Aldrich, MO; Nu-Chek, MN) that was run with each batch. Analysis by GC-MS was used to identify FAMEs common in the samples that were not contained in the standard mixture. Fatty acid notation used follows that in Frostegard and Baath (1996). PLFA results are reported as percentage of total concentration. Total PLFA were obtained by adding the concentrations (as ug/g soil) of all detected PLFA.

#### <u>Mesofauna</u>

Nematodes were extracted from approximately six grams of fresh soil with the Baermann funnel method for 72 hours. Nematodes collected were preserved in 5% formaldehyde. Total nematode counts to the level of trophic group were performed for each sample. Tylenchidae were included

in the fungivorous trophic group. Microarthopods were extracted from  $\sim 25$  g of of fresh soil for five days on Tullgren-type extractors and they were identified to order.

#### Statistical Analyses

Mass loss, decomposition and fauna data were analyzed by repeated measures two factor ANOVA with mesh-size and substrate type as factors and time as the repeated measure. Fauna densities were log (n+1) transformed prior to analysis. Two-way ANOVA was used for microbial community characteristics in the last sampling date. Tukey's test and t ratios were used for means comparisons when appropriate. Simple linear regression was used to assess the control on mineralization rate by quality parameters.

## RESULTS

It is possible for surface applied plant substrates to cause differences in the moisture content of soil which could in turn alter mineralization rates and organisms densities. Different mesh sizes can potentially affect drainage of the soil contained in the trays which could also confound results. Soil moisture inside the trays was measured at all sampling dates and no significant differences were found between substrates or between mesh treatments (data not presented), so we have no evidence that the moisture conditions were substantially affected due to the experimental set-up.

#### Litter quality

Chemical composition of applied substrates is shown in Table 4.1. Pine needles had the lowest concentration of nitrogen and phophorus, the highest C/N ratio and lignin percentage and the

lowest percentage of cellulose. Hay had the second lowest values of nitrogen and phophorus and followed pine needles in its C/N ratio; it had a low concentration of lignin but the highest of cellulose and hemicellulose. The composition of rye was similar to that of hay except for its considerably lower C/N ratio. *Amorpha* and clover show the lowest C/N ratios and the highest concentration of P. Among these two, *Amorpha* had the highest percentage of N and P and the lowest C/N ratio but the highest percentage of lignin after pine needles. The mixture of the five substrates had the average concentration for all parameters.

## Mass loss

No significant overall effect of mesh size nor an interaction of mesh size by substrate type was observed in the percentage of mass loss remaining in litter bags at any date or for any substrate (Table 4.2). We therefore only present data for the 5mm mesh size. The percentage of mass remaining by February was positively related to %C (r=0.71, p<0.0001), C/N (r=0.55, p=0.006), lignin-to-nitrogen ratio (r=0.5, p=0.01), % lignin (r=0.43, p=0.007) and negatively related to %N (r=-0.53, p=0.007) and %P (r=-0.54, p=0.007). For all substrates, the greatest mass loss occurred in the first three weeks (Figure 4.1). By the end of the incubation clover had lost about 80% of its mass followed by *Amorpha* (60%). Hay and rye mass loss pattern was very similar throughout the study. About 45% of their mass remained after 6 months. Pine and the mixture only lost ~30% of their mass by February. Using the final remaining masses and nitrogen concentrations, direct nitrogen release from litter over the course of the incubation was calculated (Figure 4.2). The amount of nitrogen released from litter in the 40-µm and 5-mm mesh.

#### Nitrogen mineralization

Average net mineralization rate was greatest for the July-August period and was low and similar for the August-October and October-February periods (Figure 4.3). In general, rates were higher for clover, *Amorpha* and the mixture and lowest for pine, rye and hay, but the specific ordinations among these varied with time. There was net nitrogen immobilization under rye, pine and hay in August. In general, increasing the mesh size led to higher nitrogen mineralization rates (or lower immobilization rates) -although this difference was not always significant. This trend was clear at all sampling dates with four mesh sizes in August (Figure 4.4) and with the two extreme sizes (Figure 4.3) on all sampling dates. There was no three-way interaction of time, mesh size and substrate, but there were significant interactions between mesh size and substrate and between time and mesh size (Table 4.2) indicating that the effect of mesh size on the nitrogen mineralization rate was different for different substrates and was not the same over time. In general differences between mesh sizes were greatest and most consistent across substrates in the October-February period, when the rate under the 5 mm mesh treatment was 3 to 7 times as high as under the 40 µm treatment. In July-August and August-October the magnitude of the difference varied substantially with substrate. Rye and the mixture showed the greatest differences between mesh sizes in July-August and while clover did in August-October.

To further examine how mesh size affected the strength of the relationship between substrate quality and mineralization rates we regressed individual quality parameters against nitrogen mineralization rates. No significant relationships between mineralization rate and cellulose, hemicellulose, lignin or lignin-to-nitrogen ratio were found, so only C/N, %N, %P and %C are considered. In the first period, all linear relationships were significant and the estimated slope coefficient was always higher in the 5 mm mesh size treatment than in the small mesh size

(Table 4.3). In the August-October period the slope coefficient only was significant in the 5mm mesh size and was always higher than in the small mesh size. In the last incubation period slopes were higher in 5mm, although the only significant relationship found was with C/N.

#### Effect of substrates on microbial communities

The microbial community from February was examined by analyzing the response of PLFA biomarkers for microbial groups to treatments. Table 4.4 shows the PLFA used as markers for each microbial group. Percentages of each PLFA were summed for each group. Figure 4.5 presents relative abundances by group and Table 4.5 summarizes results of analysis of variance. There were no significant effects of substrate, mesh size or interactions on the percentage of Gram-positive bacteria. The effect of substrate on Gram-negative bacteria was different for different mesh sizes. In the 40 µm mesh, Gram-negative bacteria were more abundant under clover and with 5mm under Amorpha. The average difference in abundance of Gram-negative bacteria between substrates was the same for both mesh sizes. The effect of substrate type on the proportion of fungal PLFA was significant and consistent across mesh sizes. Fungi were lowest under Amorpha, pine and hay. No effect of mesh size was observed. The relative proportion of actinomycetes was significantly higher under the pine substrate. The effect of substrate was the same for both mesh sizes. For protozoa, although the overall effect of mesh size was not significant, abundance tended to be lower under the 5mm mesh size with the exception of the pine treatment where the situation was reversed. The fungi-to-bacteria ratio was driven by the percentage of fungi. The effect of substrate on fungi-to-bacteria ratios is consistent across mesh sizes and there is no evident effect of mesh size.

Total PLFA values (as ug PLFA/g soil) (Figure 4.6) were in general greater for the 5 mm mesh treatment although not always significantly. The effect of substrates was not consistent across mesh sizes. Total PLFA concentration was highest under rye and *Amorpha* with 5mm mesh and under rye, hay and the mixture with 40  $\mu$ m. In the 5mm mesh the differences between substrates were more pronounced than in the 40 microns mesh.

#### Microfauna

Fungivorous nematodes were very rare in all treatments and represented only about 2 percent of all individuals on average. Only total abundances and abundances of bacterivorous nematodes are shown (Figure 4.7). The effect of substrate on nematodes abundances was dependent on time in both cases (p<0.05) (Figure 4.7) and no general pattern of response to substrate quality was observed. The effect of mesh size was also dependent on time (p<0.05). In general, a tendency for higher abundances of nematodes in the 5mm treatment was observed in August and February for both groups. For the bacterivorous nematodes there was a significant interaction of mesh size by substrate (p<0.05) indicating that the effect of mesh on nematodes abundances was not consistent across substrates. This was especially true in October. A similar pattern (although not significant) was seen for total nematode abundance.

Low abundances of microarthropods (up to 0.4 individuals per gram of soil) were found in soil. Densities were greatest in August followed by February and October both for collembola and mites. No apparent effects of mesh size, substrate or the interaction of both on collembola densities were detected (p>0.05). No differences in the responses of oribatid, prostigmatid and mesostigmatid mites were observed, so numbers were pooled for further analysis. The response

of mites to substrate type was not consistent for both mesh sizes but no pattern of response to substrate quality was detected and the response to mesh size depended on the substrate (p<0.05).

## DISCUSSION

The size of the mesh did not affect mass loss which would be expected if the litter community had been restricted. It is likely that the reason was that litter dwelling fauna had equal access in both mesh size treatments, which was expected as the boxes were open on top.

Increasing the size of the mesh increased the rate of nitrogen mineralization measured in soil. This on one hand is consistent with what is known of the role of soil animals in nitrogen mineralization (Verhoef and Brussard, 1990) and on the other hand suggests that the exclusion treatment was successful at keeping some members of the fauna out of the boxes. We detected low densities of microarthropods (mites and collembola) in the mineral soil in all treatments which led us to assume that it was the larger members of the mesofauna and the macrofauna that were effectively excluded and to whom effects should be attributed. It also suggests that restricting the mineral soil community did not have a drastic effect on the abundances of microarthropods. It is possible that the species composition and/or trophic structure of the microarthropod community responded to the mesh treatment, but the resolution of our observations did not allow us to detect such a response.

The increase in nitrogen mineralization with mesh size from litter and soil measured with ion resins was not associated with an increase in litter decomposition rate. The fact that the direct measurement of nitrogen loss from litter materials showed that except for one substrate the loss of nitrogen was the same for both mesh sizes indicates that nitrogen was released from litter in similar amounts in both mesh sizes. It is possible that the presence of larger fauna in the mineral soil 5-mm mesh size boxes led the released nitrogen to be more susceptible to being captured by the resin, which involves being in ionic form, while in the absence of larger fauna it was sequestered in the microbial biomass. This explanation is consistent with earlier findings that fauna can activate the microbial biomass and enhance nutrient mobilization (Couteaux et al., 1991; Huhta et al., 1988) which would subsequently allow more opportunities for leaching. However, the greater abundance of nematodes in the 5-mm mesh size can offer an alternative explanation. It is possible that the enhanced mobilization of nitrogen observed was a result of the grazing by nematodes as has been observed in other studies (Bonkowski et al., 2000; Ingham et al., 1985) and not a direct effect of larger fauna. It has been estimated that N mineralization due to the trophic interaction of microbial feeding nematodes and their prey can account for up to 10-15 percent of total mineralization by the soil food web (de Ruiter et al., 1994). The question that remains is why nematodes were more abundant in the 5-mm mesh size treatment. Total PLFA concentrations, which can be used as an indicator of active microbial biomass, were higher when the community was not restricted. This is consistent with the greater total nematode abundances, as a greater abundance of microbes could have supported greater populations of nematodes. However, a larger microbial biomass pool would also have the ability to immobilize more nitrogen, and thus decrease the amount of nitrogen that could be leached and captured by the resin bags. The fact that the opposite was observed suggests that the effect of predation by nematodes in increasing nitrogen mobilization was more important than the retention by microbes. Now, why did total PLFA increase in the unrestricted community? The presence of fauna could have increased the translocation of organic matter from the litter to the mineral soil (Frouz et al., 2006), increasing substrate availability in the mineral soil. However, we did not find greater litter mass losses in the unrestricted community. Another possibility is that the larger
members of the fauna activated the bacterial biomass through gut passage (Drake et al., 2006) or modified the accessibility or palatability of the substrates that were present in soil or that were leached from litter through the production of fecal pellets (Coleman et al., 2004) which could have in turn favored the microbial populations. Yet another option is that the microbial biomass in the restricted community was kept down by a direct or indirect top-down influence led by a microbial feeder that was not present in the unrestricted community. Top-down control on members of the microbial biomass has been documented in several studies (Lenoir et al., 2007; Wardle, 2002).

Comparison of differences in nitrogen collected in resins under different substrates within mesh-size treatments showed that the presence of fauna modified the pattern of response to substrate type by accentuating the differences in nitrogen availability under different substrates, especially in the two final incubation periods. This suggests that substrate type was more important as a driver of nitrogen availability in soil when the faunal community had not been restricted. This observation was confirmed when individual quality parameters were considered and the slopes of their linear relationships with mineralization rates were found to be greater or only became significant in the unrestricted community. However, when substrate identity (substrate type) was considered this effect was stronger at the end of the incubation (Figure 4.3) whereas when the control by quality parameters was quantified the effect was stronger at the beginning of the incubation (Table 4.3). This discrepancy might be reflecting the fact that plant litter is composed of different proportions of structural and non-structural compounds which can have different and even opposite effects on nutrient release and whose influence can come into play at different stages during decomposition (Bardgett, 2005). When the driving variable considered is substrate type multiple, combined and possibly interacting quality variables are

acting at once. Both variables, substrate type and quality parameters, nonetheless suggested greater control by substrate chemical composition when the soil community was not restricted.

How could the presence of fauna have enhanced the control by litter quality of nitrogen availability in soil? There are some reports of this effect being evident on the decomposition of litter. Smith and Bradford (2003) found that greater complexity of fauna was associated with a more pronounced quality-litter decomposition relationship after 30 days of decomposition although this was reversed afterwards. They attributed this effect to the ability of fauna to seek high quality litter and to make litter more accessible to microbes. Schaedler and Brandl (2005) also found that in the presence of larger invertebrates the role of nitrogen concentrations in litter on decomposition was greater. Here, we attempted to test if the effect of fauna on the control by quality of nitrogen mineralization was associated with its effect on the microbial community and the mesofauna that preys on it. If the effect of fauna on the control of mineralization by quality was a result of its effect on the structure of the microbial community, we expected to see that the presence of fauna had resulted in greater differences in the abundances of microbial groups among substrates, i.e. that fauna accentuated the response of microbial groups to the quality of the added substrates. We did not find much evidence of this effect by looking at individual microbial groups. We did find that to be the case for Total PLFA, where there were greater differences among substrates when the community was not restricted. We hypothesize that the presence of fauna facilitated the redistribution of litter-derived materials so that they were in closer contact with the mineral soil. These materials might have included inhibitory substances such as phenolic compounds or nutrients and carbon substrates, which could elicit a positive response, so that the overall response to quality was enhanced. Greater redistribution of litter

materials could occur through the combined effects of comminution, and direct transfer of litter material (Lavelle and Spain, 2001).

This study suggested that presence of fauna affected nitrogen mineralization by affecting the abundance of the consumer level of the micro-food web, the nematodes in particular. We found some evidence that the composition of soil fauna can mediate the control of nitrogen mineralization by the chemical quality of plant litter. Specifically, we found that the influence of the chemical composition of litter was enhanced when the larger members of the soil fauna were not excluded from the system. Our study suggested that the presence of fauna also accentuated the response of the microbial biomass to the quality of litter and therefore supported the hypothesis that the effect of fauna on mineralization can at least in part be due to its effects on the structure of the micro-food web. A demonstration of direct connection between such changes in soil community structure and its function would be the next necessary step to attribute the changes in mineralization to the response of the microbial community.

Understanding the short term interactions of substrate quality and the structure of the soil community is particularly relevant in integrated or organic agricultural systems, where organic amendments in the form of plant residues are seasonally applied as mulch to enhance soil fertility and improve soil physical qualities. This study suggests that the effects of the composition of plant residues on nutrient availability might be different in soils that naturally lack a complex soil community or whose community has been impoverished by management than in soils that house a diverse and complex community. By influencing the role of plant quality in nutrient availability, the soil fauna can potentially influence other ecosystem functions such as primary productivity and therefore the productivity and sustainability of agroecosystems.

Such interaction highlights the importance of considering soil biota when making management decisions.

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	%N	%C	C/N	% P	% Cellulose	% Hemicellulose	% Lignin
Rye	0.70(0.06)	45.04(0.95)	70.34(5.72)	0.19(0.02)	38.04(1.16)	27.95(0.35)	3.99(0.51)
Clover	2.02(0.10)	43.28(0.33)	22.42(1.46)	0.50(0.04)	33.36(0.75)	12.07(0.23)	7.97(0.34)
Amorpha fruticosa leaves	2.35(0.04)	47.10(0.17)	20.19(0.36)	0.61(0.02)	26.89(0.60)	13.91(0.20)	10.01(0.17)
Pine	0.43(0.05)	50.95(0.28)	127.90(6.88)	0.05(0.02)	30.51(0.47)	11.89(0.47)	19.19(0.62)
Hay	0.49(0.04)	47.07(0.12)	102.49(5.35)	0.16(0.01)	40.37(0.47)	30.05(0.44)	4.63(0.19)
Mixture	1.07(0.06)	46.08(0.93)	46.18(4.25)	0.28(0.08)	35.70(0.63)	19.46(0.48)	10.25(0.44)

Table 4.1. Initial chemical composition of plant materials

Table 4.2. F ratios and (P values) from repeated measures ANOVA on the effect of time, substrate type and mesh size and their interactions on nitrogen mineralization ( $\mu$ g mineral N/g soil/day) and litter mass loss (percentage of ash free dry mass remaining)

	Mineralization rate	% AFDM remaining
Substrate	14.01 (<0.0001)	60.09 (<0.0001)
Mesh size	32.42 (<0.0001)	0.08 (0.78)
S x MS	2.19 (0.08)	0.3 (0.91)
Time	28.9 (<0.001)	26 (<0.0001)
T x S	5.43 (<0.0001)	2.55 (0.01)
T x C	13.35 (<0.0001)	0.7 (0.5)
TxCxS	1.4 (0.20)	1.12 (0.32)

Table 4.3. Results of linear regression of average net nitrogen mineralization rates ( $\mu g NO_3^- + NH_4^+$ )/g soil/day) against selected initial litter quality parameters in mesh sizes 40  $\mu m$  and 5 mm. p-values of significant relationships shown in bold.

		40 µm				5 mm			
		r <sup>2</sup>	Slope	Std. error	р	r <sup>2</sup>	Slope	Std. error	р
JULY – AUGUST	C/N	0.700	-0.0266	0.0027	<0.0001	0.580	-0.0281	0.0052	<0.0001
	%N	0.726	0.9069	0.1313	<0.0001	0.395	1.2360	0.3341	0.0013
	%P	0.717	3.4484	0.5104	0.001	0.417	4.8095	1.2406	0.0009
	%С	0.414	-0.2257	0.0632	0.0022	0.308	-0.3417	0.1117	0.0059
AUGUST – OCTOBER	C/N	0.012	-0.0002	0.0004	0.60	0.353	-0.0020	0.0006	0.0028
	%N	0.022	0.0136	0.0195	0.49	0.455	0.1226	0.0293	0.0004
	%P	0.022	0.0525	0.0745	0.49	0.421	0.4508	0.1152	0.0008
	%С	0.001	-0.0007	0.0063	0.91	0.251	-0.0274	0.0103	0.01
OCTOBER- FEBRUARY	C/N	0.030	-0.0001	0.0001	0.41	0.165	-0.0014	0.0007	0.05
	%N	0.016	0.0046	0.0079	0.56	0.118	0.0627	0.0366	0.10
	%P	0.014	0.0168	0.0302	0.58	0.100	0.2199	0.1409	0.13
	%С	0.059	0.0029	0.0025	0.25	0.116	-0.0198	0.0117	0.10

Microbial group	PLFA marker
Gram-negative bacteria	cy17:0, cy19:0, 18:1ω7c, 18:1ω9c
Gram-positive bacteria	14:0, i15:0, a15:0, 15:0, i16:0, i17:0, a17:0
Bacteria	i15:0, a15:0, 15:0, i16:0, cy17:0, 17:0, 18:1 ω7c, cy19:0
Fungi	18:2\06,9c
Actinomycetes	10Me16:0, 10Me17:0, 10Me18:0
Protozoa	20:2\u00f36,9c; 20:3\u00f36,9,12c, 20:4\u00f36,9,12,15c

Table 4.4. Phospholipid fatty acids used as biomarkers for microbial groups

Table 4.5. F ratios and (P values) from two-way ANOVA on the effect of substrate type (S) and mesh size (MS) on relative abundance (as percentage of total) of PLFA markers for five microbial groups, the ratio of fungi to bacteria and Total PLFA (µg PLFA/g soil)

	S	MS	C x MS
Fungi	5.32 (0.0017)	0.04 (0.66)	0.91 (0.49)
Gram-negative	5.78 (0.001)	0.19 (0.66)	3.96 (0.0083)
Gram-positive	2.09 (0.40)	0.0001 (0.99)	1.16 (0.36)
Actinomycetes	17.51 (<0.0001)	0.66 (0.42)	1.54 (0.21)
Protozoa	0.88 (0.50)	0.51 (0.47)	1.92 (0.12)
F/B	4.62 (0.0038)	0.17 (0.68)	0.89 (0.499)
Total PLFA	3.74 (0.0097)	17.22 (0.0003)	2.13 (0.0898)



Figure 4.1. Percentage of ash free dry mass remaining in litter bags for substrates in boxes of 5 mm mesh size



Figure 4.2. Total nitrogen released (mg/g of litter) from substrates over six months of incubation. Asterisk indicates significant difference (p<0.05)



Figure 4.3. Average nitrogen mineralization rate ( $\mu g NO_3^- + NH_4^+$ )/g soil/day) for three incubation periods and mesh sizes 40 $\mu m$  and 5mm mm. Asterisks indicates significant differences (p<0.05).



Figure 4.4. Average nitrogen mineralization rate ( $\mu g NO_3^+ + NH_4^+$ )/g soil/day) for three incubation periods and mesh sizes 40 $\mu$ m, 100 $\mu$ m, 2mm and 5mm. Different letters indicate significant differences (p<0.05).





Figure 4.5. Relative abundance of microbial groups in soil after six months of decomposition under six substrates and in mesh sizes 40 microns and 5mm as percentage of total phospholipid fatty acids. Fungi-to-bacteria ratio (\*) is a dimensionless ratio.



Figure 4.6. Total phospholipid fatty acids (as ug PLFA/g soil) in soil after six months of decomposition under six substrates and in mesh sizes 40 microns and 5mm.

BACTERIVOROUS NEMATODES

ALL NEMATODES



Figure 4.7. Bacterivorous and total nematode densities (no./g soil) under six substrates and with  $40\mu m$  and 5mm mesh sizes

## CHAPTER 5

# MODELING THE EFFECT OF THE INTERACTION OF SOIL COMMUNITY STRUCTURE AND PLANT LITTER QUALITY ON C AND N MINERALIZATION<sup>4</sup>

<sup>&</sup>lt;sup>4</sup> Carrillo, Y., Jordan, C. Ball, B. To be submitted to Soil Biology and Biochemistry

#### ABSTRACT

By affecting the soil community structure, litter quality can affect the trophic interactions occurring in the litter and soil habitat. Trophic interactions in the soil food web have major effects on carbon and nutrient mineralization. It has been suggested that the result of trophic transfers between the members of the soil food web can depend on the quality of resources. In this paper we used a soil food web modeling approach to simulate carbon and nitrogen mineralization from surface applied litter of differing chemical qualities and from mineral soil based on observed population sizes and the feeding interactions among the members of the soil food web. The model included simple features to describe differential chemical composition of the litter, and differential abilities of fungi and bacteria to degrade organic matter fractions. To calibrate and validate the model we assessed the abundances of soil bacteria, fungi, protozoa, nematodes and microarthropods and measured nitrogen mineralization over six months of decomposition. The model behaved well in predicting net mineralization rates and was able to describe the effects of population size and predation pressure on the nitrogen release from litter and soil. We then used the model to investigate interactions between litter quality and soil community structure. Our simulations suggested that (a) soil food webs generated by plant litter of different chemical qualities have differential abilities to mineralize litter and soil organic matter, (b) soil communities are better able to mineralize substrates of quality that is similar to the one of the litter in which they had developed, and (c) the structure of the soil community is of less importance in determining mineralization rates when the degrading substrate is of intermediate quality.

**Keywords:** soil food web model, litter quality, soil community, nitrogen mineralization, respiration

#### INTRODUCTION

Decomposition and mineralization of plant litter and soil organic matter is known to be regulated by a suite of hierarchically-organized interacting factors, including climate, clay mineralogy, nutrient status of the soil, quality of decomposing resources and the activity of soil organisms (Lavelle et al., 1993). Although it is possible to predict decomposition and mineralization rates from indices of substrate quality (Aerts, 1997; Swift et al., 1979), these indices act only as surrogates for the actual regulators of the decomposition process (Meentemeyer, 1978). The relative importance of the actual controlling factors and the mechanisms and effects of their interactions are still not fully understood (Bardgett, 2005).

In particular, the effects of the interactions of the quality of resources and the activity and structure of the soil community are not well understood. It has been suggested that the effect of litter quality on soil processes is driven by its effects on the soil organisms responsible for the processes (Wardle, 2002). Plant litter quality determines the structure of the community that colonizes it (Beare et al., 1989) and can also affect the composition of the mineral soil community (Heal et al., 1997; Schutter and Dick, 2001). By affecting the soil community structure, litter quality can affect the trophic interactions occurring in the litter and soil habitat. Trophic interactions in the soil food web have major effects on carbon and nutrient mineralization. Carbon mineralization can increase as a result of higher turnover rate activity and respiration of consumed populations due to grazing (Bardgett et al., 1993) while nitrogen mineralization occurs mainly due of excretion of excess nitrogen by the consumer (Woods et al., 1982). Furthermore, it has been suggested that the result of trophic transfers between the members of the soil food web can depend on the quality of resources (Bardgett, 2005; Herlitzius, 1983; Wardle, 2002). Experimental evidence, however, is limited. In one of the few studies to

address this question, Hanlon et al. (1981) found that the influence on fungal respiration exerted by a collembolan grazer depended on the nutrient concentration of the growth medium.

Thus, the quality of litter has the potential to influence the dynamics of nutrients and carbon due to its inherent chemical degradability and due to its effects on the trophic interactions among soil populations. These two factors may in turn interact to affect mineralization. The inherently complex nature of these interactions makes them difficult to assess via experimental means as this would require isolating the effect of the soil populations and that of litter quality.

The complexity of the decomposition process and biological interactions in soil makes modeling approaches not just desirable but necessary. Organism-oriented models, which explicitly incorporate soil organisms and their interactions with the biophysical environment, have a high explanatory value and permit the evaluation of the effects of intervention and management (Paustian, 1994; Smith et al., 1998). The organism-oriented modeling approach initiated by Hunt et al. (1987) has been applied to several natural and agricultural systems (Berg et al., 2001; de Ruiter et al., 1994a; Hassink et al., 1994; Schroter et al., 2003). In this approach food webs are constructed by aggregating species into functional groups and the structure and functioning of the food webs are analyzed in relation to nutrient cycling. Soil food web models have proven useful in predicting C and N mineralization rates (de Ruiter et al., 1994b), in explaining rates in terms of the relative contribution of groups of organisms and particular trophic interactions (Berg et al., 2001).

In this paper we use the soil food web model scheme of Hunt et al. (1987) to simulate carbon and nitrogen mineralization from surface applied litter of differing chemical qualities and from mineral soil based on observed population sizes and the trophic interactions among the members of the soil food web. This model describes mineralization as being regulated not only

by trophic interactions or chemical quality limitations, but by the interaction of both. We add simple features to describe differential chemical composition of the litter and differential abilities of fungi and bacteria to degrade organic matter fractions. This approach allows us to isolate the effects of the changes in soil food web structure prompted by the added litter from the effects of the quality of litter on trophic transfers.

In a field experiment we assessed the abundances of soil and litter microbial detritivores, protozoa, nematodes and microarthropods and measured nitrogen mineralization over the course of six months after the surface application of six plant materials of contrasting chemical compositions. We calibrated the model using the measured soil populations and nitrogen mineralization after the application of one substrate and then assessed its performance with the other plant materials. We then used the model to investigate (a) the importance of the soil community changes brought about by the quality of litter on carbon mineralization and nitrogen mineralization from litter and soil; (b) whether nitrogen mineralization is better predicted by the interaction of soil community and litter quality than by each factor alone; (c) whether the role of the soil communities and their trophic interactions varies depending on the quality of the degrading substrate; and (d) whether some communities are better suited to degrade substrates of certain quality.

#### **METHODS**

A field study to assess populations' biomasses and measure nitrogen mineralization from plant litter and soil was conducted in experimental plots in a previously abandoned conventional farm in the Piedmont region of Georgia USA (33°57'N lat. 83°19'W long). Soil is classified as Pacolet sandy clay loam (kaolinitic, thermic typic hapludult) and has 0.7% C content and 0.1% N

content. Details of the experimental set up are included in Chapter 3. In brief, sieved and frozen mineral soil from the top 5 cm was placed back in the field in buried metallic frames of 35 x 35 x 5 cm made of 5 mm mesh. Here, soils were allowed to equilibrate and be re-colonized for two months, after which quality treatments (five substrates and a mixture) were randomly assigned to four of 24 plots. Plant materials were surface applied at a rate of  $327g/m^2$ . Substrates applied were air dried green litter of cereal rye (*Secale cereale* L.), air dried green litter of crimson clover (*Trifolium incarnatum* L.), air dried green litter of false indigo (*Amorpha fruticosa* L.), wheat hay (*Titricum aestivum* L.), pine needles (*Pinus taeda* L.), and an even mixture of these by weight. Initial carbon, nitrogen, phophorus, lignin, cellulose and hemicellulose contents were assessed (procedures in Chapter 3). Substrates were allowed to decompose for six months.

#### Nitrogen mineralization

Sampling for nitrogen mineralization was carried out 21, 91 and 165 days after substrate application. Nitrogen mineralization per gram of soil per day was determined via incubation of nylon bags containing anion and cation exchange resins which were buried under the soil layer in each tray to retain NO<sub>3</sub><sup>-</sup> and NH4<sup>+</sup> leached from litter and soil (Binkley and Matson, 1983). Average net mineralization rate was estimated for three consecutive incubation periods. Details of the processing and calculations to estimate average net mineralization rate for each period are presented in Chapter 3. Values were converted from  $\mu$ g NO<sub>3</sub><sup>-</sup>+NH<sub>4</sub><sup>+</sup>/g soil /day to mg NO<sub>3</sub><sup>-</sup> +NH<sub>4</sub><sup>+</sup>/m<sup>2</sup>/day for the upper 5cm of soil using measured soil bulk density of 1.1g/cc<sup>3</sup>. For model calibration and validation purposes an overall average mineralization rate for the whole period was calculated from the averages for each period.

#### Estimation of soil and litter populations' biomass

Soil microbial community composition (using phospholipid fatty acids, PLFA), nematodes and microarthropods abundances were assessed on all three dates. Details of the extraction and processing of soil samples are included in Chapter 2.

Litter populations were only assessed at the end of the incubation. Phospholipid fatty acids were extracted from 1gram of dry and ground litter and extracted as detailed in Chapter 2. Nematodes and microarthropods were extracted from litter and processed as detailed in Chapter 4. Nematodes were identified to feeding group and microarthropods to order.

The concentration of total and some individual phospholipid fatty acids (as  $\mu$ moles per gram of soil or litter) were used to obtain estimates of biomass of microbial groups. Total microbial biomass was estimated using the conversion factor in Bailey et al. (2002) and a Kc factor of 0.32. The fatty acid 18:2 $\omega$ 6 was used to estimate fungal biomass in soil using the conversion factor provided by Klamer and Baath (2004) for soil fungi. The biomass of bacteria was estimated as the difference between total microbial biomass and fungal biomass. Fungal biomass in litter was estimated using the relative differences observed in the concentration of the fatty acid 18:2 $\omega$ 6 in relation to its overall average concentration in the six litter materials studied. The relative deviation from the average (as percentage of the average) was applied to an assumed average ratio of fungi to bacteria in plant residues of 2 (Beare et al., 1990). Litter fungal and bacterial biomasses were estimated from total microbial biomass using the calculated ratio.

Existing methods for estimation of protozoa abundance are very time and labor intensive and can make population assessments in experimental contexts infeasible or impractical. A laboratory assay was conducted to obtain a conversion factor to estimate biomass of protozoa from the concentration of the phospholipid fatty acid biomarkers in soil and litter. Individual

PLFA can be used as markers for protists as their cell membranes contain specific polyunsaturated fatty acids (Desvilettes et al., 1997; White et al., 1996). The fatty acids  $20:2\omega6,9c$ ;  $20:3\omega6,9,12c$ ;  $20:4\omega6,9,12,15c$  have been used as relative indicators of protozoa abundance in soils (Cavigelli et al., 1995; Mauclaire et al., 2003). This is the first attempt to estimate a conversion factor from PLFA to protozoan biomass of which we are aware.

Protozoa from fresh soil samples from the field site were cultured for 2 days at 30C in phosphate agar as substrate and *Escherichia coli* as a food source. The cultures were then suspended in phosphate agar and the suspensions were pooled into one large flask. Immediately after, the abundance of ciliates, amoebae, and flagellates in this suspension (as numbers per milliliter) was estimated in five replicates using the dilution method (Most Probable Number) based on Singh (1946). Immediately after the enumeration, known volumes of the original suspension (10, 20 and 40 ml in triplicate) were placed in Teflon bottles and centrifuged at 24000 RCF for 30 minutes, after which the supernatant was poured and the samples were frozen at -80°C. PLFAs were extracted from thawed samples using the same extraction procedure as for soil and litter samples (Chapter 2).

From the concentrations of cells in the initial suspension obtained with MPN, biomass carbon of each protozoan group was estimated using the conversion factors in Beare et al. (1992) and total protozoan biomass per milliliter of suspension was calculated and extrapolated for the volumes used for PLFA extraction. A strong linear relationship ( $r^2=0.70$ ) was found between the concentration (µmoles/ml of suspension) of the fatty acid 20:4 $\omega$ 6,9,12,15c and the calculated protozoan biomass in the three analyzed volumes of culture suspension (as µg C). The following relationship was obtained:

ug C protozoa = 54860 ( $\mu$ moles of 20:4 $\omega$ 6,9,12,15c) + 3.5525.

This relationship was used to estimate protozoan biomass in soil and litter samples. Estimates of protozoan biomass ranged between 0.5% and 1.9% of total microbial biomass estimated with PLFA.

Numbers of nematodes and microarthropods per gram of soil or litter were converted to mg of biomass carbon using the conversion factors in Beare et al. (1992). Obtained values of biomass carbon for all microbial faunal groups were converted to mg per square meter of soil down to 5 cm using a bulk density value of 1.1g/cc<sup>3</sup>. Litter biomasses per

gram for litter were converted to biomass per square meter based on the initial rate of litter application and considering the amount of litter remaining in litter bags at the time of sampling (Chapter 3).

#### Food web modeling

The model was written using modeling software package STELLA 8.1. The food web model was based on the approach by Hunt (1987) to obtain mineralization rates from feeding interactions between soil functional groups of known biomass. We used this approach to simulate mineralization of carbon and nitrogen per day from litter and soil organic matter. Only the average biomasses—not the dynamics— of the major soil groups observed in mineral soil over the course of the field study, and at the end of the study for litter, were used as inputs to the model and played the role of driving the consumption of organic materials and their mineralization. Consequently, the simulated daily rate was considered the average rate for the study period. Carbon flows between trophic groups are derived from feeding rates which are in turn split into an excretion rate, a biomass production rate and a mineralization rate. Feeding rates are calculated assuming that the biomass production rate of a group balances the rate at

which material is being lost through natural death and predation. Feeding rate of a group on a prey or on a substrate is calculated as in de Ruiter et al. (1993):

#### F=((Dnat \* B)+P)/eass. eprod

where *F*: feeding rate; *Dnat*: natural death rate; *B*: biomass of functional group; *P*: predation rate; *eass*: assimilation efficiency and *eprod*: production efficiency. Nitrogen flows occur in parallel and in proportion to C flows through the use of the C/N ratios of organisms and organic fractions. Carbon and nitrogen mineralization rates are calculated as in de Ruiter et al. (1993):

#### Cmin = eass - (1 - eprod) \* F

#### *Nmin*= *eass*((1/*CNprey*)-(*eprod*/*CNpred*))\**F*

where C or *Nmin*: mineral carbon or nitrogen released per trophic transfer; *eass*: assimilation efficiency of the consumer; *CNprey*: carbon-to-nitrogen ratio of the prey or substrate; *eprod*: production efficiency of the consumer; *C/Npred*: carbon-to-nitrogen ratio of the consumer; *F*: feeding rate of consumer on prey. Specific death rates were made temperature dependent using a  $Q_{10}=3$  (Andren et al., 1990). Temperature was measured in the field every two hours throughout the sampling period. The overall average was used for simulations. Physiological parameters (CN, ea, ep, and death rates) for the functional groups in the litter and soil food webs were taken from the literature and are shown in Table 5.1.

Two separate food webs were modeled. Organisms found in litter were assumed to only consume litter material and prey inhabiting the litter; organisms found in soil were assumed to only consume material and prey in the mineral soil. The soil community was modeled as a simplified food web composed of five functional groups: bacteria, fungi, protozoa, nematodes and microarthropods. The specific trophic interactions modeled are shown in Figure 5.1. The differences in trophic transfers considered in litter and soil corresponded to observed differences

in the composition of the major functional groups assessed. For instance, in the case of litter, the great majority of nematodes were either bacterial or fungal. The specific proportion found for each group was included in the model as a factor modifying the total consumption demand by nematodes. Litter microarthropods were either oribatids or collembola which are both considered to be mainly fungivores (Coleman et al., 2004). Thus, in the litter food web model microarthropods only consumed fungi. In the case of soil, most nematodes found were omnivores or bacterial feeders. In the model, soil nematodes consumed bacteria, protozoa and fungi and their consumption of one particular prey was proportional to the relative abundance of that prey. Soil microarthropods were mostly prostigmatids followed by oribatids. As oribatids are considered fungivores and small soil prostigmatids are known to be important in regulating populations of nematodes (Coleman et al., 2004), the microarthropods in the model consumed fungi and nematodes. The specific proportion of oribatids and prostigmatids was used to split the consumption by microarthopods in soil.

Litter to be mineralized was divided into cellulosic/hemicellulosic material, lignin and labile material (estimated by default). Soil organic matter was split into labile and resistant materials 1 and 2 (1 easier to degrade than 2). The proportions of labile and resistant fractions 1 and 2 in soil were assumed to be 25%, 5% and 70% respectively. The three fractions are processed by bacteria and fungi. It was assumed that for each unit of mass demanded and consumed by bacteria 94% was labile, 5.5% was cellulose (or Resistant 1) and 0.5% lignin (or Resistant 2). These percentages were calculated to approximately reflect the relative proportions among the decay constants of these carbon pools used to model decomposition and mineralization in the original version of the CERES-N sub-model (Schomberg and Cabrera, 2001). The percentages were then modified to reflect known differences in substrate utilization

of bacteria and fungi, specifically the greater ability of fungi to degrade resistant fractions. Thus, in the case of fungi, 89% of consumed material was labile, 9.9% was cellulosic and 1.1% was lignin. Each functional group contributes to the residue pools through death and waste. Material return to the three organic matter pools was due to death and excretion using the fractions in Hunt et al. (1987) (Table 5.1).

C/N of the litter material was defined as the weighted average of the C/N of its three fractions. It was assumed that the C/N of the lignin fraction is 2 times that of the carbohydrate fraction and the C/N of the cellulose fraction is 3 times that of the carbohydrate fraction, so that:

CN litter: (2\*CN lignin\*lignin fraction)+(3\*CN cellulose\*cellulose fraction)+(CN labile fraction\*carbohydrate fraction)

The CN of the labile pool was calculated using the known CN of the litter and used to calculate the CN ratio of the other fractions. CN ratio of the organic fractions in soil was calculated in the same manner. The C/N ratio of soil organic materials that are available to decomposers was assumed to be 30. Initial and final (after six months of decomposition) C/N ratios of the litter materials were measured with the micro-Dumas combustion method and the average value of the two was used as the C/N of litter.

Nitrogen mineralized from litter is assumed to be leached into the soil and join the mineral nitrogen pool in soil, of which nitrogen mineralized in soil is also part. When the C/N of the prey or substrate is higher than that of the predator, then immobilization occurs and N is taken from the soil mineral nitrogen pool. The amount of nitrogen immobilized corresponds to all the nitrogen that would be required to meet the consumption demand by the predator according to its C/N.

The model was calibrated using the observed population biomasses, C/N of litter and average net mineralization rate for rye litter, which had an intermediate C/N value. To evaluate the performance of the model, we compared observed average net mineralization rates for the study period with modeled rates obtained with the observed population biomasses and average C/N ratios of the six litter materials studied.

#### **RESULTS AND DISCUSSION**

#### Chemical quality of substrates

Initial chemical composition of applied substrates is shown in Table 5.2. Pine needles had the lowest concentration of nitrogen and phophorus, the highest C/N ratio and lignin percentage and the lowest percentage of cellulose. Hay had the second lowest values of nitrogen and phophorus and followed pine needles in its C/N ratio; it had a low concentration of lignin but the highest of cellulose and hemicellulose. The composition of rye is similar to that of hay except for its considerably lower C/N ratio. *Amorpha fruticosa* (amorpha) and clover had the lowest C/N ratios and the highest concentration of phophorus. Of these two, amorpha had the highest percentage of nitrogen and phophorus and the lowest C/N ratio but the highest percentage of lignin after pine needles. The mixture of the five substrates had the average concentration for all parameters. At the end of the field incubation C/N ratios of all substrates had decreased. Amorpha had the lowest C/N followed by clover and the mixture. Rye and hay had intermediate values and rye continued to have a lower C/N than hay. Pine C/N was the highest.

#### Characterization of soil and litter populations' biomasses

Table 5.3 shows biomasses of the major groups in soil and litter after application of six plant litter materials. Since litter bacteria and fungi and soil fungi biomasses were estimated from total microbial biomass and since averages in soil were derived from several dates (not true replicates), only descriptive comparisons are presented. Although the concentration of biomass in litter per unit of mass is much greater than in mineral soil, when considering the amount of soil down to 5 cm in depth, the majority of the living mass per unit of area (between 80 and 90%) was found in soil. Soil bacteria represented between 60% - 70% of all biomass (soil and litter included). Soil fungi comprised between 17% - 28% of all biomass. The biomass of litter populations varied with litter type. Total microbial biomass was greater in clover and amorpha and was lowest in pine, hay and rye. Fungi were more abundant than bacteria; the fungi-tobacteria ratio was highest in rye, hay and the mixture and lowest in clover, amorpha and pine. Protozoan biomass was largest in clover and hay and lowest in pine, rye and amorpha. The percentage of nematodes in litter was never above 0.01%. Nematodes belonged either to the bacterial or fungal feeding groups. The biomass of nematodes in pine litter was substantially lower than in other materials and the bulk of the mass corresponded to fungal feeders. The proportion of bacterial feeding nematodes was lowest in (pine, rye and hay). Microarthopods' biomass in litter reached 1% of total biomass in the case of clover and rye and had the lowest biomass under pine, amorpha and the mixture.

Soil populations varied with litter type but differences were less pronounced than in litter. Soil total microbial biomass was highest in soils under amorpha, rye and the mixture and lowest under pine. Bacteria were more abundant than fungi in soil. Fungi-to-bacteria ratios also varied with litter type, but again, the differences were smaller. The lowest fungi-to-bacteria ratio occurred under pine and hay. Protozoan biomass in soil was not very responsive to litter type. The biomasses of nematodes and microarthropods in soil were comparable and represented up to

0.1% of total soil biomass. Soil nematodes were mostly omnivorous and bacterivorous. Microarthropods were mostly prostigmatids and oribatids.

#### Model performance

Previous sensitivity analyses of this soil food web modeling approach have determined that the model is highly sensitive to the variations in physiological parameters (production efficiency, assimilation efficiency, death rates and C/N of groups) (Berg et al., 2001; de Ruiter et al., 1994b; Hunt et al., 1987). The specific parameters for each group are hard to estimate empirically for every situation so values obtained and reported in other studies for other systems have to be assumed, as was the case for this study. This might be problematic especially when the model is being used to compare mineralization dynamics in systems with different abiotic conditions. In these cases it can be expected that organisms will show differences in physiological behavior, which would then make interpretations of simulations results difficult. Our application of the model was restricted to one site, so it was reasonable to assume that although the parameter values were not reflecting the real situation, they were not variable within the study site and therefore the same set of physiological parameters was used for every simulation. Our study involved comparisons in terms of the quality of the substrate being degraded and the structure of the soil communities. Since we had experimentally assessed both, we consider that the simulations results are good indications of the relative outcomes when different soil communities are degrading substrates of different qualities.

Two simple features were added to the model. One of them was the differential preference of fungi and bacteria for degrading the fractions of organic material. We defined that fungi consumed a greater proportion of the cellulose and lignin in litter and of the resistant

fractions in soil than bacteria. To test for the impact of this assumption we ran simulations in which the opposite was defined. We found that assuming that fungi had a greater preference for hard-to-degrade materials led to greater mineralization rates. This can be explained if the differences in the C/N of fungi and bacteria are considered. Bacteria have a lower C/N ratio than fungi, which implies that their demand for nitrogen is higher. Thus when bacteria consume a greater proportion of resistant material, which has higher C/N, the net release of nitrogen will be lower. This assumption and its effect seem reasonable but it would be helpful to find some empirical support. In our model the effects on mineralization rates of the differences in bacterial and fungal proportions that were observed in the communities will be affected by this assumption.

The second feature added was that the C/N ratio of the fractions was determined by the C/N ratio of the litter and the proportions of labile, cellulose and lignin. While these proportions were known for the litter, that was not the case for the mineral soil. The model however, was not very sensitive to changes in the proportions and it was the overall C/N ratio that mostly affected the predictions, as the greater the C/N of the overall material the greater the C/N of all fractions. An increase or decrease in the percentage of labile material of 20% determined a change of 11% in the mineralization rate while a 20% increase of decrease in C/N lead to a change in 50% of the predicted rate. The chosen mechanism for assigning C/N to the fractions was an arbitrary one because it is very difficult to determine what the true composition of every fraction is or even if this is a determining factor in the consumption and mineralization of litter and soil organic matter. Nonetheless, it allowed us to represent the differences in carbon and nitrogen compositions of the substrates being degraded.

To assess the model performance, simulations were run using the observed population biomasses, measured C/N and carbon composition of the litter fractions as inputs. The simulated net mineralization rates were then compared with the average net values measured in the field (Figure 5.1). The model was able to predict the general trend in observed net average mineralization rates with regards to C/N of substrates, that being that mineralization rates were higher with lower C/N. Simulated values were well within the observed range of values. No consistent pattern of overestimation or underestimation was detected. The model overestimated the mineralization rates of amorpha and clover but correctly predicted the relative order of the two rates (clover above amorpha), despite the fact that the C/N value of amorpha was lower than that of clover (although amorpha's C/N was higher than clover's initially, it became smaller by the end of the incubation so the average over the course of the study was lower). The greater mineralization simulated under clover reflected the effect of the greater population of litter bacteria in clover than in amorpha, which would have led to greater consumption of substrate and as a result, greater release of mineral nitrogen. Clover litter also had a considerably higher population of protozoa which by predating on the bacteria further contributed to more net release. The model also overestimated the mineralization rate of pine and in contrast underestimated the rate of hay, even though the C/N of pine is substantially higher that that of hay. Again here, these differences reflect the effect of the composition of the communities. In both of these substrates immobilization by the microbial groups was the dominant process given their high C/N ratios. However, except for the litter bacteria, populations in pine litter and soil were lower than in hay, which produced a lower demand for N which in turn resulted in less total immobilization or more net mineralization. The prediction of the mineralization rate of the mixture was very close to the observed rate. The prediction of mineralization rate under rye was

also very close to the observed but this was expected as this was the substrate used for model calibration. In summary, the model behaved reasonably well in predicting net mineralization rates and it was able to describe the effects of populations' sizes and predation pressures on the nitrogen release from litter and soil. This suggests that the mechanisms that the model included were an acceptable representation of the actual processes.

# Importance of differences in the structure of the soil and litter communities for carbon and nitrogen mineralization

To study the effect of the structure of the food webs generated by different litter qualities, we ran simulations in which the C/N and carbon compositions of litter and soil organic matter were held fixed, so that mineralization was a function solely of the different community structures in litter and soil associated with each litter type. The C/N value was fixed at 30. Simulated mineralization and respiration rates in soil and litter are shown in Figure 5.3.

The pattern of response of respiration rates and N mineralization rates to litter type was different for litter and soil. This reflects the fact that litter quality did not affect the litter and soil communities in the same manner. While litter communities are directly affected by the attributes of litter, as it constitutes their source of energy and nutrients as well as their habitat, the impact on the soil community is less direct and is mediated by processes of redistribution of the resource by fragmentation, leaching or translocation into the mineral soil (Heal et al., 1997). Therefore, we expect quality variables to generate different responses on soil and litter organisms.

The differences in the abundance of functional groups in mineral soil translated into differences in total respiration rates. The respiration rate in soils under amorpha was the highest, and 36% greater than respiration in soils under pine, which had the lowest rate. Rates were

proportional to the biomasses of groups and as a result total respiration was proportional to total biomass. The fraction of respiration due to fauna showed little variability, with most of the respiration due to protozoa.

Differences in respiration rates from litter types were more pronounced within litter than in soil, as were the contributions of different functional groups. This was consistent with the differences that were observed in the groups' biomasses. The respiration rate of clover (the litter type with the highest respiration) was 114% greater than rye (the litter type with the lowest respiration). Differences in predation pressure were evident in litter respiration and influenced the respiration rate of the detritivore groups. This can be observed in the respiration rates of bacteria which reflect (in direction and magnitude) the substantial differences in biomass of protozoa in different litter types.

As in the case of respiration, nitrogen mineralization showed stronger responses to different treatments in litter than in soil. The maximum difference between litter treatments in soil was 30%, while in litter it was 108%. This is consistent with what was observed in the biomasses of populations. As with respiration, nitrogen mineralization in litter was more influenced by the differences in fauna populations' biomasses than in soil. This was a result of a greater response of faunal populations to quality in litter combined with the influence of predation pressure on the bacteria and fungi. The latter can be evidenced in the total mineralization rates in litter which were more closely related to the abundance of protozoa than to biomass of bacteria, or to total soil food web biomass.

The effect of predation pressure on mineralization rates is a result of the steady-state assumption of the model, which is that groups consume as much as is needed to compensate for losses due to death and predation. This assumption determines that a larger biomass of the

predator will result in greater demand by the prey and thus prompt more mineralization. A consequence of this is that the influence of protozoa, nematodes and microarthropods (the predators in our food web) on total mineralization rate is greater than would be expected from their biomasses alone and therefore differences in the predators' populations as a response to quality cause considerable differences in the total mineralization by the whole food web. Although this assumption may not hold true in all cases in nature, there is evidence of compensatory responses to predation in soil food webs (Hedlund and Augustsson, 1995), which together with the predictions of the model indicates that at least in some cases, the differential responses of predatory fauna to the quality of substrates can lead to substantial differences in the mineralization of carbon and nutrients from litter and soil organic matter.

We hypothesize that because the effect (positive or negative) of the quality of a substrate can be consistent across groups (e.g. if a resource offers accessible carbon and nutrients both bacteria and fungi as well as their consumers will respond positively, as was observed in the clover treatment), then the effects of quality on respiration and mineralization will tend to be magnified with every trophic interaction (as opposed to cancelled out). This would constitute a potential mechanism of interaction of the soil food web structure and the quality of litter to determine mineralization.

Although the effects of litter quality on the mineral soil populations were more subtle than in litter, such effects still accounted for observable differences in simulated rates of mineralization of carbon and nitrogen from mineral soil. This result might suggest that the extent of the changes in soil organic matter dynamics observed after the addition of fresh organic carbon sources such as litter known as the priming effect (Bingeman et al., 1953; Kuzyakov et al., 2000) could be dependent on the quality of the added substrate and specifically on the
changes in the soil community and trophic interactions that it generates. Indeed, the predation interactions between soil microorganisms and soil fauna are regarded as key elements for understanding the priming effect (Kuzyakov et al., 2000). We hypothesize that because those interactions are affected by litter quality, a relationship between the extent of the priming effect and the quality of litter should exist. In fact, the quality of the litter produced by plant communities has been linked to the ability of soils to store carbon. Soils under communities that produce low quality litter tend to store less carbon (reviewed in Cebrian, 1999). It is possible that such relationship is mediated by the soil community's response to substrate quality. Experimental efforts that can link the effect of substrate quality on soil biota with the extent of the priming effect and the soil carbon and nutrient dynamics are warranted.

#### Dependence on quality of the role of the soil food web

To test whether the importance of the soil populations and their trophic interactions varied depending on the quality of the substrate being degraded, we ran simulations in which the observed field populations (in soil and litter) were set to degrade substrates of different C/N ratios ranging from 10 to 100. Table 5.4 shows the nitrogen mineralization rates obtained. Several patterns could be identified. The first is that the higher the C/N, the lower the net mineralization; this is expected because predators excrete excess nitrogen obtained from the tissue of the resource or prey. Second, when comparing the predictions in the different quality scenarios with the predictions obtained when the simulations used the real C/N ratio (Table 5.4), it was apparent that the predictions obtained combining the real populations and the actual C/N ratios were closer to the observed values than with any individual C/N in the range. Such a pattern indicates that nitrogen mineralization is better explained by the interaction of the

soil/litter community (and its trophic interactions) and the resource quality than by either factor alone. Wardle and Lavelle (1997) suggested that the effects of litter quality on soil biota regulate the extent to which the biota in turn facilitates the decomposition of plant litter. The extent and strength of this interaction however, are not well known. The model's predictions support Wardle and Lavelle's hypothesis and suggest that the specific community and trophic interactions generated as a result of the chemical quality of litter are a key factor in explaining nitrogen mineralization.

The examination of mineralization rates produced by communities under different C/N ratios indicated that not all communities were equally suited to mineralize all substrates. The clover community (produced by substrate with the "best" initial quality), produced the highest mineralization rates when degrading high quality substrates and the lowest rates when degrading low quality substrates. On the other hand the pine community (produced by substrate with the "worst" chemical quality) produced the lowest mineralization rates when degrading the high quality substrates (C/N=10, 20, 40) and average rates when mineralizing the low quality substrates (80, 100). The rye community (produced by intermediate-low quality substrate) generated the highest rates when degrading the low quality substrate and the mixture community produced mineralization values that are very close to the mean rate across the C/N range. Thus, food webs tended to produce the highest mineralization rates when degrading a substrate of quality similar to that of the substrate in which they were developed. This is a very interesting finding and it suggests that through their litter plants generate a food web that is to some extent specialized in the degradation of litter of similar quality and therefore of its own litter. The idea of plants being able to select for decomposer food webs with specific attributes has been recently put forward and has not yet been widely tested (Wardle, 2005). It has been proposed that by

promoting a soil community that is specialized in the decomposition of its own litter a plant species might gain a competitive advantage in terms of nutrient availability or capture (Bardgett, 2005). There is very limited experimental evidence of this feedback mechanism. Cookson et al. (1998) found that wheat residue decomposed faster in soil previously exposed to its litter and suggested that the initial exposure had "conditioned" the microbial community for the decomposition of this same litter. In contrast, in a laboratory experiment Ayres et al. (2006) found that litter from three different species did not decompose faster in the presence of "their" own soil biota. Our findings support the hypothesis that soil detrital food webs can show specialization in the degradation of litter materials and in a broader sense support the idea that litter quality might be one of the plant traits that contribute to the selection of decomposer communities.

How much the structure of the community mattered in determining nitrogen mineralization depended on the C/N. Intermediate C/N led to the smallest range in predictions, whereas extreme C/N led to the greatest differences (see standard deviation and maximum difference in Table 5.4). This could suggest that the attributes of the soil food webs that determine their differential ability to process materials are associated with specific abilities to decompose very low quality or very high quality substrates, so that this differential ability is better expressed when degrading these substrates and their performance is more similar when degrading substrates of intermediate quality. We hypothesize that the structure of the soil food web is more important in determining nitrogen mineralization when resource quality is very high or very low.

In summary, our simulation results using observed soil food webs suggest that (a) soil food webs generated by plant litter of different chemical qualities have differential abilities to

process litter and soil organic matter, (b) soil communities were better able to mineralize substrates of quality that was similar to the one of the litter in which they had developed, and (c) the structure of the soil community appears to be of less importance in determining mineralization rates when the degrading substrate is of intermediate quality. Thus, our modeling exercise proved to be a good tool to explore complex interactions between the quality of litter and soil communities and to put forward hypotheses for further exploration of how litter quality mediates the linkages between the structure of the soil food web and ecosystem functioning.

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Parameters (day <sup>-1</sup> )	Bacteria	Fungi	Protozoa	Nematodes	Microarthropods
Death rate <sup>a</sup>	0.0033	0.0033	0.02	0.006	0.005
C/N	5°	10 <sup>c</sup>	7 <sup>a</sup>	10 <sup>a</sup>	8 <sup>a</sup>
Production efficiency <sup>b</sup>	0.3	0.3	0.4	0.37	0.4
Assimilation efficiency <sup>b</sup>	1	1	0.95	0.43	0.5
Fraction to labile pool <sup>a</sup>	0.8	0.8	0.8	0.8	0.8
Fraction to cellulose pool <sup>a</sup>	0.2	0.2	0.2	0.2	0.2

Table 5.1. Model parameters. <sup>a</sup> From Hunt *et al.* (1987); <sup>b</sup> from de Ruiter (1993); <sup>c</sup> obtained through calibration

Table 5.2. Chemical composition of substrates. Standard error in parentheses

	%N	%C	Initial C/N	Final C/N	% P	% Cellulose	% Hemicellulose	% Lignin
Rye	0.70	45.04	70.34	51.79	0.19	38.04	27.95	3.99
	(0.06)	(0.95)	(5.72)	(2.99)	(0.02)	(1.16)	(0.35)	(0.51)
Clover	2.02	43.28	22.42	18.62	0.50	33.36	12.07	7.97
	(0.10)	(0.33)	(1.46)	(0.27)	(0.04)	(0.75)	(0.23)	(0.34)
Amorpha fruticosa leaves	2.35 (0.04)	47.10 (0.17)	20.19 (0.36)	17.21 (0.57)	0.61 (0.02)	26.89 (0.60)	13.91 (0.20)	10.01 (0.17)
Pine	0.43	50.95	127.90	100.36	0.05	30.51	11.89	19.19
	(0.05)	(0.28)	(6.88)	(7.38)	(0.02)	(0.47)	(0.47)	(0.62)
Hay	0.49 (0.04)	47.07 (0.12)	102.49 (5.35)	59.40 (1.39)	0.16 (0.01)	40.37 (0.47)	30.05 (0.44)	4.63 (0.19)
Mixture	1.07	46.08	46.18	33.51	0.28	35.70	19.46	10.25
	(0.06)	(0.93)	(4.25)	(2.96)	(0.08)	(0.63)	(0.48)	(0.44)

Table 5.3. Estimated biomass of functional groups in litter and soil (mg biomass C/m<sup>2</sup>). Standard errors in parentheses. Standard errors in litter correspond to four samples taken at the end of the sampling period. Standard errors in soil correspond to three sampling dates averages obtained from four replicates for each date. Values without a standard error were calculated from other estimates. \* Percentages of bacterial feeding nematodes in litter and fungal feeding microarthropods in soil were calculated from the average abundances of feeding groups and mites orders respectively (see methods foodweb modeling)

	Rye	Clover	Amorpha	Pine	Hay	Mixture
Litter bacteria	352	2227	1489	1170	606	931
Litter fungi	1850	2061	2273	1532	1843	2356
Litter total microbial biomass	2202 (476)	4288 (402)	3771 (262)	2702 (56)	2449 (371)	3282 (468)
Litter protozoa	98 (23)	238 (29)	53 (10)	91 (5)	284 (53)	162 (28)
Litter nematodes	0.181 (0.03)	0.077 (0.009)	0.172 (0.097)	0.028 (0.004)	0.337 (0.195)	0.170 (0.071)
% bacterial feeding nematodes in litter*	75	93	95	16	57	94
Litter microarthropods	27.5 (2.6)	46.3 (6.9)	10.3 (1.6)	3.1 (0.7)	20.1 (4.1)	13.4 (1.6)
Soil bacteria	17026	16897	22420	16701	18080	18288
Soil fungi	7812 (1015)	5474 (1865)	6822 (1620)	3973 (1423)	4240 (1667)	5491 (1832)
Soil total microbial biomass	24838 (28)	22371 (1739)	29242 (5406)	20674 (2212)	22320 (1893)	23779 (2314)
Soil protozoa	277 (7)	250 (14)	282 (22)	242 (2)	245 (9)	259 (19)
Soil nematodes	29 (12)	19 (6)	23 (16)	16 (5)	19 (4)	22 (8)
Soil microarthropods	22 (9)	14 (2)	20 (6)	15 (2)	14 (7)	16 (3)
% fungal feeding microarthropods in soil*	30	30	30	30	30	30

	Simulated							
C/N	10	20	40	80	100	Actual CN	Observea	
Amorpha	36.98	26.97	20.8	10.84	6.59	25.85	18.99	
Clover	48.15	29.3	18.41	-4.72	-9.44	27.27	23.45	
Mixture	39.43	26.43	18.65	4.26	-0.17	18.65	18.93	
Rye	35.06	26.52	21.22	12.89	9.13	13.36	15.19	
Hay	42.56	26.67	17.54	-2.45	-6.08	-2	2.23	
Pine	29.42	20.27	14.87	4.33	1.52	1.52	-0.01	
range	29-48	20-29	15-21	-4.7-13	-9.44 - 9.13	-2 - 27	-0.01 - 23.45	
mean	38.6	26.0	18.6	4.2	0.3	14.1	13.1	
stdev	6.4	3.0	2.3	7.0	7.1	12.2	9.7	
Maximum absolute difference	18.7	9.0	5.9	15.6	18.6	27.9	23.5	

Table 5.4. Nitrogen mineralization rates (mg/m<sup>2</sup>) simulated using observed populations' biomasses and different C/N ratios. Simulation results using the actual CN ratio of the litter and observed rate are also included.



Figure 5.1. Functional groups and trophic interactions considered in the soil food web model. In the interest of clarity, the arrows representing carbon and nitrogen mineralization are drawn for the whole soil and litter food webs although they are modeled for each individual feeding interaction. Nitrogen immobilization is also represented as a flow to the microbial biomass but it is modeled separately for bacteria and fungi. Nitrogen mineralized by the litter community joins the mineral nitrogen pool in soil from where both litter and soil microbial groups can uptake it according to their demand.



Figure 5.2. Observed and simulated net average nitrogen mineralization rates (mg inorganic  $N/m^2/day$ ) over the course of six months of decomposition. Bars indicate standard error. \* Observed value of pine too small to appear in figure. Pine mineralization rate was -0.01 mg/m<sup>2</sup>/day.



Figure 5.3. Simulated contribution to total respiration (mg CO2-C/m2) and nitrogen mineralization (mg N//m<sup>2</sup>) of bacteria, fungi and fauna in mineral soil and litter.

# **CHAPTER 6**

# MODELING GREEN MANURE ADDITIONS IN ALLEY CROPPING SYSTEMS: LINKING SOIL COMMUNITY DYNAMICS AND NITROGEN MINERALIZATION\*\*

<sup>\*\*</sup> Carrillo, Y. and Jordan, C. (in press) In. Towards Agroforestry Design: An Ecological Approach. Springer-Verlag . Reprinted here with permission of publisher.

# ABSTRACT

Understanding the short-term mineralization of nitrogen from biomass additions in alley cropping systems can help improve management strategies. Although initial chemical quality of materials can be a good predictor of mineralization rates, sometimes it is not sufficient to explain short-term mineralization patterns. Soil biota plays a crucial role in regulating nutrient mineralization. Trophic interactions among members of the soil community strongly influence nitrogen flow and storage. This study aimed at understanding how the addition of green manure influences the soil community and how this change in turn influences N mineralization patterns. We conducted field experiments to assess the impact of adding prunings of Albizia julibrissin Durazz on soil bacteria, fungi, protozoa, nematodes and microarthropods, and monitored nitrogen release over a growing season. We used a soil food web model to simulate nitrogen mineralization and test the hypothesis that changes in the soil biota brought about by plant material additions influence nitrogen mineralization dynamics. The main changes induced by prunings additions were larger total community biomass, greater relative importance of fungi in the microbial community and a larger representation of protozoa, nematodes and microarthropods. Incorporating these changes in the soil food web model increased the model's ability to predict nitrogen mineralization rates and soil nitrogen concentrations. This indicated that taking into account the soil biota structure and dynamics -in addition to biochemical qualityis useful for explaining short-term mineralization patterns from green manures and other plant residues in alley cropping systems.

Keywords: Albizia julibrissin, green manure, soil food web model, soil biota

# INTRODUCTION

The use of biomass in the form of litter, prunings or root as sources of nutrients for crops in alley cropping systems has proven helpful in enhancing nitrogen availability for crops (Nair et al., 1997). Important considerations in managing alley cropping systems are the amount and rate of N released that can benefit the crop in the alleys between the hedges. Understanding the short-term mineralization patterns of organic materials is a useful tool not only to assess but also to improve the suitability of hedgerow species (Isaac et al., 2000). Numerous studies in controlled and field conditions have successfully addressed the short-term decomposition and mineralization patterns of different species in relation to the initial chemical quality of substrates alone is sometimes not sufficient to explain short-term mineralization patterns. This is not surprising given the multiplicity of factors influencing decomposition and mineralization, including biochemical characteristics and soil biota composition and dynamics (Heal et al., 1997).

Soil biota plays an important role in regulating nutrient mineralization. Interactions among soil community members regulate the availability of the nutrients necessary for plant growth (Wardle, 2002). Direct trophic interactions are responsible for a great fraction of nutrient release. For example, (de Ruiter et al., 1994) estimated that protozoa feeding on bacteria were responsible for up to 95% of the total N released into the soil in two arable farming systems. Agricultural practices such as residue addition can affect population size and dynamics of organisms in soil food webs (Wardle et al., 1999) in turn affecting nutrient cycling processes. Understanding how the addition of green manure influences the soil community and how this

change in turn influences N mineralization patterns might help in managing soil biota and plant material selection and application to optimize nutrient utilization.

A potentially useful tool to study these interactions is the use organism oriented models. Organism- oriented mathematical models that track the flows of nutrients through groups of organisms have high explanatory value and can integrate the effects of management in the description of mineralization (Paustian, 1994). A relatively successful approach is the soil food web model by Hunt et al. (1987). Carbon and nitrogen mineralization rates can be satisfactorily derived from trophic interactions simulated through these mechanisms (de Ruiter et al., 1994). Soil food web models have proven useful for evaluating the relative contribution of functionally defined groups and particular trophic interactions to carbon and nitrogen cycling (Berg et al., 2001).

The purposes of this study are: to assess the impact of green manure addition on the soil community in an alley cropping system; to assess the performance of a soil food web model (Hunt et al., 1987) in simulating nitrogen mineralization rates from plant substrates, and to test the hypothesis that taking into account the soil biota structure and dynamics –in addition to biochemical quality- is important for explaining short-term mineralization patterns from green manures and other plant residues in alley cropping systems. For this, nitrogen release from three plant materials was measured over a growing season. Also the dynamics of the soil community were monitored in bare soil and after the addition of prunings of the leguminous tree *Albizia julibrissin* Durazz. These results were used to initialize and test the model. Simulations were then conducted to study how the influence of residue addition on the soil biota affects nitrogen mineralization.

# **METHODS**

# Study Site

This study was conducted in the Piedmont region of Georgia, USA (33°57'N lat. 83°19'W long). The climate is humid subtropical with an annual average temperature of 18°C. Precipitation is uniform throughout the year and averages 1200mm. The soil is an Ultisol (kaolinitic, thermic, typic, hapludult) with a sandy loam texture. Soils have a pH in water of 5.8. The site is located on a previously abandoned conventional monoculture farmland. In 1990 hedgerows of Albizia *(Albizia julibrissin Durazz)* were planted. Since then, cultivation of sorghum, wheat and corn in the alleys between the hedgerows has been alternated with fallow periods.

# Nitrogen mineralization

Nitrogen release was measured in the field under *A. julibrissin* prunings (from here on referred as albizia) addition and in bare soil. Prunings consisted of a weighted mixture of leaves and woody twigs. For model validation purposes, release from prunings of another hedge-row species (*Alnus serrulata* (Ait) Willd) and of the roots of albizia were also measured. Roots of albizia were chosen as a substrate to explore their mineralization rate because in some alley cropping systems roots are mechanically severed to prevent competition with crop species, and the severed roots subsequently decompose. Release of  $NO_3^-$  and  $NH_4^+$  from substrates was determined by incubation of undisturbed soil cores containing anion and cation exchange resins at their bottoms (Kolberg et al., 1997). Incubation cores were prepared by driving plastic sleeves (5-cm diameter x 8-cm long) into the soil and then withdrawing them with the soil inside. The bottom one cm of soil was removed and a nylon bag filled with resin was put in its place. The entire assembly was returned to the original hole. The resin used consisted of equal amounts (15-g) of a Na-saturated

cation and Cl-saturated anion exchange resins (Sybron Ionac C-250 and ASB-1P, Sybron Chemicals, Birmingham, NJ).

Substrates were oven dried (70°C- 48 hours) and 3.5-g of albizia (1.9-kg m<sup>-2</sup>), 2.5-g of albizia roots (1.4- kg m<sup>-2</sup>) and 2.5-g (1.4 kg m<sup>-2</sup>) of alder were placed on the surface of each core. The amounts were chosen to resemble yields from prunings and stocks in the soil. A plot parallel to an albizia hedgerow was chosen to be the experimental plot for in situ incubations. Naturally occurring litter and live plants were removed from the soil and no plants were allowed to grow within the plot. To obtain baseline levels of  $NO_3^-$  and  $NH_4^+$  ten randomly distributed composite samples were collected upon initiation of the study.

Cores were distributed in the plot and treatments were randomly assigned to the cores. Each core was considered to be a replicate. Resin bags were incubated for a maximum of 6 to 7 weeks after which they were replaced with new bags. Six soil cores per treatment were collected after 18, 48, 84, 115, 129 and 148 days. Complete assemblies were removed from the field, transported to the lab and refrigerated for up to a week until processed. Resin bags were washed with deionized water to remove soil and debris and then extracted for NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> by shaking intact bags in 60-ml of 2 M KCl for one hour. Each soil core was subsampled to measure gravimetric water content at 70°C for 48 hours, and NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> concentrations. An extraction was made from four grams of fresh soil by shaking in 20 ml of 2 M KCl for one hour. Resin and soil extracts were analyzed using an Alpkem Continous Flow Analyzer. Nitrate and NH<sub>4</sub><sup>+</sup> concentrations were corrected for soil moisture content and converted to mg per gram of dry soil. Concentrations of mineral N were calculated for each core using the combined amounts of NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> in both soils and resins. The concentration of total mineral N in all soil samples was converted to mg m<sup>-2</sup> using the averaged value of bulk density. Net mineralization for each incubation period was calculated as the difference between final and initial N concentrations.

Initial plant material was processed for total C and N using the micro-Dumas combustion assay and Neutral Detergent Fiber and Acid Detergent Fiber. Lignin, cellulose and hemicellulose percentages were calculated. At the end of the incubation period, final C/N ratio was determined.

ANOVAs were used for comparisons between dates and treatments. Simple linear regressions were performed between percentage of remaining C and N and quality parameters. Unless otherwise stated all significant differences are reported at the P< 0.05 level.

# Effect of addition of green manure on the soil community

The effect of the addition of Albizia prunings on the soil community was assessed in the field. Microbial biomass, Whole-Soil Fatty Acid profiles, biomass of protozoa, nematodes and microarthropods were monitored from May to September 2002 after the addition of Albizia prunings and in bare soil. Treatments were randomly assigned to four plots. Pruning of albizia trees was carried out in late May and a mulch bed was placed in two of the plots. Sampling for biomass measurements began upon addition on 20 May, and was repeated on 27 June, 22 August and 22 September. Eight 8-cm depth soil samples per treatment were collected on each date. Gravimetric moisture was determined for all samples ( $70^{\circ}C - 48$  h). Abundances were calculated on a per-dry-weight basis and then converted to mg C m<sup>-2</sup> using an averaged value of bulk density for the study area (1.13-gr cm<sup>-3</sup>).

Microbial carbon was determined using the Chloroform Fumigation Extraction method (CFE) (Vance et al., 1987). Four 20-g samples of fresh soils were fumigated with chloroform for 24-h. Samples were extracted by shaking for one hour with 80-ml of 0.5 M K<sub>2</sub>SO<sub>4</sub>. Extracts were

filtered and analyzed for Total Organic Carbon (TOC) (Shimadzu TOC-5000A). Microbial C was calculated using Kc=0.42.

Protozoa were processed with the most probable number method using 10-fold dilutions of 10-g subsamples and *Escherichia coli* as a food source. Samples were incubated at 25°C for three days after which individual wells were inspected and individuals enumerated. Biomass C was estimated from the cell numbers using an average volume of 300-µm<sup>3</sup> for ciliates and 50µm<sup>3</sup> for flagellates (Berg, 2001). Specific density was set as one, dry mass as 20% of fresh mass and C content as 50%. Protozoa counts were only carried out for June 26 and August 28 samples. Approximately six grams (fresh weight) of soil were extracted for nematodes with the Baermann Funnel method for 72 hours. Nematodes collected were preserved in 5% formaldehyde and later enumerated. Biomass C was estimated from the number of individuals using a mean individual biomass of 0.034625-µg dry weight (Sohlenius and Sandor, 1987) and the conversion factor of 50% for C content. Microarthropods were extracted (four days) on Tullgren-type extractors (Crossley and Blair, 1991). Number of individuals was converted to biomass C using average values for each major taxonomic group (Beare et al. 1992).

Whole-soil Fatty Acid Methyl Ester (FAME) analysis allows characterizing whole microbial communities in order to explain their relative differences and similarities. FAME profiles were obtained by the ester-linked method (Schutter and Dick, 2002). Duplicate analytical replicates were used for each of four samples-per-treatment. First, lipids were saponified by adding 15-mL of methanol-KOH (0.2 N) to 3-g air-dried soil samples and heating for 1 hour at 37°C with periodic vortexing. After neutralizing with 1N acetic acid, 10-ml of hexane were added and the mixture vortexed and centrifuged at 480 x g for 20 min. The top

phase was transferred by pipette to disposable test tubes that were placed in a 40°C water bath. Extracts were evaporated under a gentle stream of N<sub>2</sub>. FAMEs were resuspended in 0.5-ml of 1:1 hexane : methyl-tert-butyl ether and transferred to a GC vial for analysis by Hewlett-Packard 5890 gas chromatograph (Palo Alto, CA). FAMEs were identified using the standard Eukary chromatographic program and peak naming table as supplied by MIDI (Microbial, ID, Inc. Newark, DE). In order to examine the effect of date and treatment on community structure, FAMEs, as percentages of total FAMEs within a sample, were divided into chemical categories (Buyer et al., 2002) and analyzed using Principal Component Analysis (PCA). With PCA, multiple original variables (Fatty acid percentages) are reduced to a smaller number of uncorrelated variables called principal components that explain the overall variability. The first principal component accounts for as much of the variability as possible and succeeding principal components account for the remaining variability. Fatty acid biomarkers (as the areas of their peaks in the FAMEs profiles) were used as relative measures for bacteria and fungi as in Mummey et al. (2002). Biomarker fatty acids and biomass C of organisms were analyzed using repeated measures ANOVA to test for the effect of treatment and sampling date. Tukey-Kramer HSD tests were used when significant differences were found. Student t tests were performed for comparison between treatments by date. All biomass data were log transformed prior to statistical analysis. Significant differences are reported at the P<0.05 level.

#### Effect of the change in soil community on nitrogen mineralization

To study the influence of soil community changes after green manure addition, the soil community food web model developed by Hunt et al. (1987) was adapted to simulate the dynamics of nitrogen mineralization from green manure. The model simulates mineralization

from transfers of C among soil functional groups. In this study we use the model by applying it to the observed population sizes of the groups of organisms and C/N ratio of substrates. Physiological parameters such as assimilation rated and C/N ratios of organisms used for simulation were taken from Hunt et al. (1987). The model was calibrated using observed mineralization rates under bare soil. C flows are derived from feeding rates, which are in turn split into an excretion rate, a biomass production rate and a mineralization rate. Observed soil populations biomass changes through the growing season are incorporated into the model as inputs by adding the estimated rate of change in biomass to the rate of material loss due to natural death and predation (de Ruiter et al., 1993). Rate of change in biomass was estimating by dividing the difference between measurements by the number of days between measurements. Feeding rates are calculated assuming that the biomass production rate of a group balances the rate at which material is being lost through natural death and predation. Feeding rate of a group on a prey or on a substrate is calculated as follows:

#### $F=(DnatB+P+\Delta B)/eass. eprod$

where *F*: feeding rate; *Dnat*: natural death rate; *P*: predation rate;  $\Delta B$ : observed change in biomass; *eass*: assimilation efficiency and *eprod*: production efficiency. Nitrogen flows occur in parallel and in proportion to C flows through the use of the C/N ratios of organisms and organic matter. Nitrogen mineralization rate is calculated as:

#### *Nmin*= *eass*((1/*CNprey*)-(*eprod*/*CNpred*))*F*

where *Nmin*: mineral nitrogen released per trophic transfer; *eass*: assimilation efficiency of the consumer; *CNprey*: carbon to nitrogen ratio of the prey or substrate; *eprod*: production efficiency of the consumer; *C/Npred*: carbon to nitrogen ratio of the consumer; *F*: feeding rate of consumer on prey. Soil temperature was measured daily and death rates were related to temperature using a

 $Q_{10}=3$  (Andren et al., 1990). The model was applied to simulate daily N mineralization during one growing season (150 days). The soil community was modeled as a simplified food web composed of five functional groups: bacteria, fungi, protozoa, nematodes and microarthropods. The substrate to be mineralized was divided into labile material, cellulosic/hemicellulosic material and lignin. All forms are processed by bacteria and fungi. The specific groups and trophic interactions considered are shown in Figure 6.1. Total consumption demand by one group is split into the three substrates by adjusting the calibrated decomposition rate constants (k) of these substrates (Schomberg and Cabrera, 2001) to reflect bacteria and fungi differences in substrate utilization. The C/N ratio of substrates used was the weighed average of observed initial and final C/N. When no residue was added, a ratio of 25 was assumed for soil organic matter. To evaluate the performance of the model, it was applied to observed population sizes of the groups of organisms and the observed C/N ratios of albizia prunings, albizia roots and alder. Simulated and measured values were then compared. Two types of general population dynamics were used for simulations: one for when residue was applied and one for when no residue was added.

#### RESULTS

# Chemical composition of substrates and N dynamics

Initial chemical composition of substrates is summarized in Table 6.1. Albizia prunings had the lowest C/N ratio and Alder had the highest. Albizia prunings and albizia roots had similar N contents and C/N ratios but differed considerably in their lignin contents. Lignin content of roots was twice as high as prunings of albizia. Roots had the highest lignin concentrations (22%). Alder contains relatively low lignin and cellulosic material but its N content is lower than the

other substrates. Although alder had a higher C/N ratio that albizia at the beginning of the incubation, it was the lowest at the end followed by albizia roots Table 6.2.

Figure 6.2 presents field concentrations of mineral nitrogen (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) in soil. Initial mineral nitrogen concentration averaged 10000-mg m<sup>-2</sup>. At the end of the season, a net average increase of ca 11000-mg m<sup>-2</sup> was observed for both the albizia and albizia roots treatments. No net immobilization was observed in soils amended with albizia. Albizia roots, alder and bare soil showed alternating periods of net immobilization and net mineralization. The alder treatment consistently rendered the lowest N concentrations and no significant change in concentration was observed at the end of the season.

Albizia prunings showed the highest average mineralization rate (55-mg N m<sup>-2</sup> day<sup>-1</sup>, S.E.=7) closely followed by albizia roots (50-mg N m<sup>-2</sup> day<sup>-1</sup>, S.E.=27). Mineralization rate in bare soil was 23-mg N m<sup>-2</sup> day<sup>-1</sup>, S.E.=2 while the rate of alder was found to be close to zero and negative (-1.49-mg N m<sup>-2</sup> day<sup>-1</sup>, S.E.=10).

# Effects of addition of albizia prunings on the soil community

#### Microbial community

PCA of FAMEs demonstrated differences in the microbial communities associated with treatment and time. The first two principal components accounted for 65% of the total variance (Figure 6.3). The ordination shows separation by treatment along PC 1 (34% of variance) and no evident separation by date indicating that the presence of green manure rather than time was the strongest influence on the microbial community structure. Overall, fungal biomarkers peak areas were significantly greater in the albizia treatment (Figure 6.4). Bacterial biomarkers were greater in the albizia treatment as well, but this difference was not significant. The ratios of bacterial to

fungal biomarkers were consistently and significantly lower in the albizia treatment for all dates. Mean bacterial to fungal biomarkers ratios were 5.9 and 3.6 for the control and albizia treatments respectively.

# Biomass of soil groups

The amounts of biomass C in the various functional groups on the four sampling dates are presented in Figure 6.5. Table 6.1 shows the results of the repeated measures ANOVA for microbial biomass, nematodes and microarthropod biomass. Soil microbial biomass (Figure 6.5a) ranged from 7000 in May to 30000-mg C m<sup>-2</sup> in August. A significant effect of time was observed. No overall significant effect of albizia addition was observed, but microbial biomass was significantly higher under albizia in August. Protozoa biomass ranged from 15 to 85-mg C m<sup>-2</sup> (Figure 6.5b) and on average constituted 0.1% (bare soil) and 0.5% (albizia) of the total biomass. Under albizia addition, the population increased by a nine fold between June and August while it remained rather stable under bare soil.

On average microarthropods represented 0.1% (control) and 0.15% (albizia) of the total soil biomass. Values ranged from 10 to 40-mg C m <sup>-2</sup> (Figure 6.5c). A significant effect of time was observed. The highest biomass values for the albizia and control treatment occurred in September and August respectively. No significant overall effect of treatment was observed. However, microarthropod biomass was significantly higher under albizia in June and September.

On average nematodes constituted 0.05% (control) and 0.1% (albizia) of the total soil biomass. No overall effect of time was observed. A significant Time x Treatment interaction was observed. At the beginning of the season nematodes' biomass C was ca. 5-mg C m<sup>-2</sup> under both treatments (Figure 6.5d). It then significantly increased to 18-mg C m<sup>-2</sup> as the season

progressed for the albizia treatment but remained low and stable in bare soil. No overall significant effect of treatment was observed but late in the season, nematodes' biomass was significantly higher when residue was added than in the absence of residue.

#### Soil food web model performance

The model simulated fairly well albizia's average mineralization and average mineralization in bare soil (Figure 6.6). Average mineralization rate of albizia roots was over-estimated by a 50%. For alder, while the observed net mineralization is close to zero, the model produced an over estimated value similar to albizia roots'. Observed and measured concentrations of nitrogen in soil throughout the growing season are showed in Figure 6.7. Although the model was not able to simulate the periods of immobilization in the albizia roots treatment, the simulated general patterns and final concentrations are reasonably close to the observations for albizia prunings, albizia roots and bare soil. The concentrations of nitrogen in soil under alder are greatly over estimated by the model.

# Effects of change in the soil community on mineralization

The food web model was used to test the hypothesis that the change in soil community brought about by the addition of green manure influences N release patterns. For this, a simulation of albizia prunings mineralization incorporating the observed changes in soil community was run and then compared to a scenario where no change in the soil community was included (i.e. soil community observed in bare soil). Both simulations were compared to the observed nitrogen concentrations in soil (Figure 6.8). Nitrogen concentrations in soil obtained with simulations not taking into account the changes in the soil community underestimated concentrations by 27%.

Incorporating the changes in soil community caused by pruning addition considerably improved the model's ability to predict the concentrations of N in soil. The model still underestimated nitrogen content but only by a 14%.

# **DISCUSSION AND CONCLUSIONS**

#### Effects of addition of albizia prunings on the soil community

PCA of fatty acids indicated that the surface application of prunings has a stronger influence in changing the soil microbial community structure than seasonal environmental changes. Microbial groups' biomarkers dynamics supported this observation. Microbial biomass values indicated larger microbial populations at the end of the season when prunings were added, which is very likely attributable to a response to higher substrate availability by detritivores. The comparison of the dynamics of bacteria and fungi biomarkers under plus and minus pruning treatments indicated that the presence of prunings increased the relative proportion of fungi in soil. A greater response of fungi than bacteria is consistent with the conclusion of Wardle (2002) that fungi in soil appear to be regulated chiefly by resources. Seiter et al. (1999) also found larger soil fungal populations associated with green manure inputs in an alley cropping system. Fauna

Although no significant overall effect of treatment was observed for the biomass of faunal groups, biomass sizes of protozoa, microarthropods and nematodes were significantly higher under albizia than in bare soil at the end of the season. Larger microfaunal populations in the presence of added surface residue are commonly observed (Forge et al., 2003). Larger populations can be attributed to greater availability of organic materials and subsequently prey populations (bacteria and fungi) observed in this study. The difference between treatments became higher as temperature and moisture increased in the second half of the study period. These two factors were not monitored separately for each treatment, however, mulched soils present smaller variability and tend to be cooler and retain humidity better (Mathews et al., 2002). Greater relative increases in biomass amounts of all groups under albizia during the late season suggest an enhancing effect of the relatively more favorable microclimatic conditions on organisms' abundances.

As a percentage of total community biomass, faunal groups were between 50% and 100% higher in the albizia treatment, which indicates an influence of the prunings addition on the importance of fauna in the whole community. Zwart et al. (1994) found larger faunal percentages in conjunction with greater fungal populations in an integrated farming system involving addition of organic mulches in comparison to a conventional system.

# Model performance

The approach to soil food web modeling by Hunt et al. (1987) has been satisfactorily used to derive carbon and nitrogen mineralization dynamics from trophic interactions among members of the soil community (e.g. de Ruiter et al. 1994). More recently, Berg et al. (2001) used it to study N mineralization from organic matter in several stages of degradation. Here, we assessed the ability of the model to study mineralization from plant residues by running simulations for residues with different C/N ratios. The model was able to produce mineralization rates for albizia and bare soil that were close to the observed ones. Simulated concentrations of nitrogen for albizia roots were higher than observed. In the case of alder mineralization rates and soil nitrogen concentrations were greatly over-estimated. The discrepancies in the case of alder could be due at least in part to the fixed C/N ratio of organisms and substrates in the model, which

resulted in no net immobilization by either bacteria or fungi. In the field, a substantial alternation of net mineralization and immobilization periods was observed under alder, which could have been reflecting changes in substrate quality, changes in detritivores populations demand for nitrogen over the growing season or most likely, a combination of both. Changes in population's demand due to shifts in populations' growth or variation of their death rate are accounted for in the model, but change in substrate quality over the season was not described.

The over-estimation of albizia roots and alder mineralization rates might also be related to the fact that quality was represented in the model solely by the average observed C/N ratio of the substrates over the growing season. The examination of initial quality parameters suggested that lignin content of roots could be associated with their lower mineralization rate, which, based on its nitrogen content and ratio, could not be predicted. Since the model is driven only by one aspect of quality (C/N ratio), we obtained an over-estimation, because the C/N of roots is rather low. Another factor that could explain the discrepancy between simulated and observed values for alder and albizia roots is the fact that soil populations were only monitored after the addition of albizia and different residues could have prompted differences in community assemblages (Forge et al., 2003). The success of the model in predicting nitrogen release under albizia and in bare soil –where populations were measured- supports this claim.

In general, the model was successful for some residues but not for others, suggesting that a fixed C/N ratio as the only quality parameter might not be sufficient to explain differences in mineralization rates and patterns of different plant materials and indicating the need to include observed soil populations for all plant materials considered. Since the performance of the model was satisfactory for albizia, we proceeded to use the model to test the hypothesis that the change in soil community brought about by the addition of albizia prunings influences nitrogen mineralization.

#### Effect of changes in the soil biota on N mineralization

Including the changes in the soil community caused by the addition of prunings increased the mineralization rate and soil nitrogen concentrations and therefore the model's ability to predict nitrogen mineralization rates and soil nitrogen concentrations. The main changes induced by albizia prunings additions were larger total community biomass, greater relative importance of fungi in the microbial community and larger proportion of fauna (protozoa, nematodes and microarthropods) as a percentage of the whole community. Higher simulated mineralization rates and N concentrations are expected given the larger population sizes of soil organisms, which constituted the main effect of residue addition on the soil community. Larger biomass pools imply more consumption demand and therefore more N released in inorganic form with every mass transfer.

An important finding of this study is the enhancing effect of residue on fungal biomass. Fungi tend to have a better ability than bacteria to process resistant substrates and mobilize the N contained in them (Paul and Clark, 1996). Also, fungi generally have a higher C/N ratio (Wardle, 2002), which provides them with lower N demand levels resulting in greater amounts of released N. Greater N mineralization rates would then be expected if fungal biomass increases. This is consistent with both the observed and simulated patterns. Members of the mesofauna have been shown to enhance nutrient mineralization in soil (Coleman et al., 2004). An increase in fauna's representation in the soil community would then be expected to increase nitrogen mineralization rates. This was also consistent with observed and modeled results. Improving the prediction of nitrogen mineralization by incorporating the soil community supports our initial hypothesis and indicates that taking into account the soil biota structure and dynamics —in addition to biochemical quality- is important for explaining short-term mineralization patterns from green manures and other plant residues in alley cropping systems. This research highlights the importance of understanding how the role of soil organisms in the mineralization process can be affected by management. As the success of alley cropping systems relies to a large extent on their nitrogen cycling, this understanding can be key in enhancing their sustainability.

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Table 6.1. Repeated measures analysis of biomass of soil organisms over time under bare soil and soil amended with albizia prunnings in an alley cropping system in Georgia, USA. P values are showed for significant effects. N.S.: Not significant.

	Microbial biomass	Nematodes	Microarthropods
Time	P<0.0001	N.S.	P=0.0296
Treatment	N.S.	N.S.	N.S.
Time x Treatment	N.S.	P=0.0103	N.S.

Table 6.2. Initial quality parameters of *Albizia julibrissin* prunings, *A. julibrissin* roots and *Alnus serrulata* prunings and final C/N ratio after field incubations in an alley cropping system in Georgia, USA.

Parameters	Albizia prunings	Albizia roots	Alder prunings
Initial %C	45.01	41.74	46.3
Initial %N	2.49 2.09		1.92
Initial C/N	18.08	19.94	24.13
Final C/N	17.01	14.86	13.27
% Ligning	10.96	21.77	12.52
% Cellulose-Hemicellulose	43.34	43.62	33.34
Lignin/N	4.4	10.4	6.5



Figure 6.1. Trophic interactions described in the soil food web model used for an alley cropping system.


Figure 6.2. Soil concentrations of mineral nitrogen  $(NO_3^- + NH_4^+)$  (means+/- s.e) after addition of *Albizia julibrissin* prunings, *A. julibrissin* roots and *Alnus serrulata* prunings and in bare soil.



Figure 6.3. Principal Component Analysis of whole soil fatty acids extracted from soils amended with *Albizia julibrissin* prunings and from bare soils. Plot shows the separation of the treatments (A, Albizia and C, Control) using grouped FAMES. Bars indicate the standard error of four replicates. Community positions on PC1 and PC2 are averaged across treatments and dates.



\*Dimensionless ratio

Figure 6.4. Average peak areas  $(10^{-4})$  of fatty acid bacterial and fungal biomarkers and their ratios over the growing season in bare soil and in soils amended with *Albizia julibrissin* green manure (means +/- s.e). Different letters indicate significant differences between dates.



Figure 6.5. Biomass C (means +/- s.e) of soil organisms throughout the growing season in soils amended with *Albizia julibrissin* prunings and in bare soil. \* significant difference p<0.05.<sup>1</sup> no statistical analysis performed.



Figure 6.6. Observed and simulated nitrogen mineralization rates after addition of *Albizia julibrissin* prunings, *A. julibrissin* roots and *Alnus serrulata* prunings and in bare soil.



Figure 6.7. Observed (O) and simulated (S) soil nitrogen concentrations after addition of *Albizia julibrissin* prunings, *A. julibrissin* roots and *Alnus serrulata* prunings and in bare soil.



Figure 6.8. Observed soil nitrogen concentrations in an alley cropping system after addition of *Albizia julibrissin* prunings and simulated concentrations obtained with and without including soil community responses to the amendment.

# CHAPTER 7

#### CONCLUSIONS

#### Short-term effect of chemical quality of litter on the structure of communities

The chemical quality of surface applied plant litter had substantial effects on the structure of the microbial communities in the underlying mineral soil. Different microbial groups responded differently to quality parameters. Fungi and Gram-negative bacteria responded positively to high nutrient content in litter and negatively to % C, C/N and % lignin, while Gram-positive bacteria and actinomycetes were stimulated by high % C and % lignin. We found that the relative importance of quality parameters in driving the microbial community varied with time. Whereas early in decomposition responses were associated with nutrient content, there were indications that at later stages the carbon composition of substrates took precedence. The microbial community inhabiting the litter material was also affected by chemical quality and its response was more pronounced than in the mineral soil. The pattern of response of respiration rates and N mineralization rates to litter type was different for litter and soil. These differences suggested that there are distinct factors involved in determining the effect of litter quality on mineral soil and litter communities. Further studies should address this distinction.

In regards to the effect of quality on faunal populations we found that the effect of litter quality on mineral soil and litter populations reached the consumer level by affecting bacterial and omnivorous nematodes abundances. These were correlated with bacterial abundance which suggested that their response to litter quality was an indirect result of the bacterial response to chemical composition. A clear effect of litter quality on soil or litter Collembola and mites was not evidenced. A greater sampling intensity study would be necessary to ascertain that in the short term, the microarthorpod community is not responsive to litter quality variations. In general we found that the responses of nematodes and protozoa to litter quality were stronger in litter than in the mineral soil.

#### Functional effects of changes in the soil community brought about by the quality of litter

The food web modeling approach proved to be a good tool to explore the interactions between the quality of litter and the structure of soil food webs. Simulations indicated that differences in the abundance of functional groups and their trophic interactions in mineral soil and litter were sufficient to generate considerable differences in total respiration rates and nitrogen mineralization rates. Differences in respiration rates from litter types were more pronounced within litter than in soil. This was to a great extent due to the greater responses of the faunal groups in litter than in soil which dictated greater differences in the predation pressures on the bacteria and fungi. A consequence of this is that the influence of protozoa, nematodes and microarthropods on total mineralization rate was greater than would be expected from their biomasses alone and therefore differences in the predators' populations as a response to quality caused considerable differences in the total mineralization by the whole food web. We concluded that at least in some cases, the differential responses of predatory fauna to the quality of substrates can magnify the influence of litter quality on the mineralization of carbon and nutrients from litter and soil organic matter.

Our simulation results led us to hypothesize that because the effect (positive or negative) of the quality of a substrate can be consistent across groups, the effects of quality on respiration

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and mineralization will tend to be magnified with every trophic interaction (as opposed to cancelled out). This would constitute a potential mechanism of interaction of the soil food web structure and the quality of litter to determine mineralization.

We also hypothesized that a relationship between the extent of the priming effect and the quality of litter should exist and could be dependent on the quality of the added substrates and specifically on the changes in the soil community and trophic interactions that it generates. Experimental efforts that can link the effect of substrate quality on soil biota with the extent of the priming effect and the soil carbon and nutrient dynamics are warranted.

Our results also suggested that the importance of the structure of the soil food web in determining nitrogen mineralization is dependent on resource quality. Specifically, we found that community structure was more important when resource quality was very low or very high. Although it has been suggested that the results of trophic interactions in the soil food web can be dependent on resource quality, very little evidence exists. This study supports the existence of such relationship.

Our study indicated that soil food webs can be better suited to degrade substrates of quality similar to that of the substrate in which they were generated. This finding provides some support for the recently proposed hypothesis that plants can select for detrital communities that specialize in the decomposition of their litter and in particular suggests that litter quality could be one of the plant traits that contribute to the selection of detrital communities.

#### Influence of litter quality on the soils ability to process litter and soil organic matter

The results of the laboratory experiment presented in Chapter 3 indicated that exposing mineral soil to plant litter of contrasting qualities influenced its ability to process freshly added substrates

and soil organic matter in the short-term. Nitrogen mineralization, respiration and mass loss from fresh litter were affected by the quality of the litter with which soil had been pretreated. Only nitrogen mineralization from mineral soil was influenced by the quality of the pretreatment litter. We interpreted the absence of an effect on respiration from mineral soil as a potential result of the low organic carbon content (and presumably availability) in the soils used. We suggest that a study involving a range of soil carbon content and availability would provide more conclusive evidence.

Our experiment did not isolate the effects of litter quality on the soil community and on soil chemical variables. However, we detected changes in the faunal community and microbial biomass due to quality of pretreatment a well as changes in the chemical environment in soil. This, together with the behavior overtime of the variables measured suggested that a combination of biotic and abiotic effects was involved in determining the effect of the quality of pretreatment litter, although it was not possible to determine their relative importance.

In sum, our results indicated that the effects of plant litter quality on soil function are not limited to determining its own decomposition and can create biotic and abiotic conditions that can in turn affect the processing of other materials.

## Mediation by fauna of the role of litter quality

In regards to the relationship of soil fauna and litter quality, the study presented in Chapter 4 showed that the control by litter quality of nitrogen mineralization was affected by the composition of the faunal community. Specifically, the control by litter quality was stronger when the soil fauna had not been restricted i.e. when members of all sizes were allowed access. Observations of the response of the microbial biomass to litter quality in the presence of restricted and unrestricted communities suggested that fauna accentuated the control by litter quality by enhancing the response of the microbial biomass to litter quality. It has been proposed that fauna could have the opposite effect, that is, that by comminuting and digesting litter it can diminish the differences in chemical quality of litter from different plant species, and thereby decrease the effect of litter quality. However, our study supported the view that fauna could affect nitrogen mineralization through affecting the microbial community specifically by making litter more accessible.

In Chapter 3, we found that the composition of the fauna (Collembola and mites) can affect the extent of the influence of litter quality on soil properties that could in turn affect how substrates are decomposed. Here, though, we expected that fauna would enhance the effect of quality, but found mixed results (no effect on respiration, enhancing effect on nitrogen mineralization and negative effect on mass loss). A drawback of this experiment is that only two substrates were used, which didn't allow us to distinguish between effects due to specific attributes of the litter and general effects of litter quality. Studies involving a range of substrates (such as Chapter 4) and that directly address the two competing hypotheses of the mediation by fauna of the control by litter quality are necessary to understand in what circumstances fauna can have enhancing, negative or neutral effects on the role of litter quality in controlling soil processes.

### The relevance of the biota/litter quality relationship in agroecosystems

Organic soil amendments such as plant residues and green manures are key components of lowinput or integrated agricultural systems and can help in improving soil fertility and soil physical properties. Surface application of amendments as a management strategy is useful when plant

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resides are used for mulching purposes and is the only alternative in no-till sytems. In this study we showed that the chemical quality of surface-applied organic amendments can have important effects on the structure of the detrital food web and in the chemical environment in soil, which can in turn potentially affect how plant residues and soil indigenous organic matter are processed. Chapters 3 and 5 suggested that the chemical quality of an organic amendment does not only dictate its own decomposition rate but may also affect how materials subsequently added to soil will be processed. Chapters 3 and 4 indicated that the effects of the chemical quality of plant residues on nitrogen availability might be dependent on the composition of the soil fauna. We found evidence that a more complex faunal community could enhance the influence of litter quality in determining nitrogen mineralization. In Chapter 6 we found that taking into account the soil biota structure and dynamics in addition to the chemical quality of litter is important for explaining short-term nitrogen mineralization patterns from green manures. Overall, these findings demonstrate that the interplay of the quality of organic amendments and the structure of the soil community has the potential of influencing the functioning of an agroecosystem and should be taken into consideration when making management decisions.