DECOMPOSITION AND NUTRIENT CYCLING IN A CHANGING ENVIRONMENT: IS GENETIC DIVERSITY REDUNDANT TO ECOSYSTEM FUNCTIONING?

by

MICHAEL D. MADRITCH

(Under the Direction of MARK D. HUNTER)

ABSTRACT

Given the drastic decline in biodiversity at all levels, it is imperative that we consider the potential affects of diversity within single species on ecosystem functioning. However, empirical data describing the relationship between intraspecific diversity and ecosystem functioning are lacking. We present field data demonstrating that the litter phenotype of individual trees affects carbon and nitrogen fluxes during decomposition and that single phenotype treatments differ in ecosystem processes from a phenotypic mix. Since nutrient dynamics are related to the chemistry of the litter, we then use the strength of the relationship between genetic distance and litter chemistry to infer the existence of genotypic effects on ecosystem functioning. In combination, our field results provide the first evidence that losses in intraspecific diversity can affect the ecosystem processes of carbon and nitrogen cycling.

We also present results from an experiment that simulated both a decline in biodiversity and an increase in nitrogen deposition. In soil microcosms, we tested effects of variation in intraspecific litter diversity and nitrogen deposition on soil respiration and
nitrogen leaching. Increases in intraspecific litter diversity increased soil respiration overall, with the greatest increases in respiration occurring under high nitrogen deposition. Our results demonstrate the potential for losses in genetic diversity to interact with other global environmental changes to influence terrestrial nutrient cycles.

We used a second microcosm experiment to determine whether the intraspecific diversity effects of turkey oak leaf litter on nutrient dynamics would be masked by the presence of naturally co-occurring longleaf pine litter. We varied the phenotypic diversity of oak litter in the presence and absence of pine litter and measured fluxes of carbon and nitrogen over a 42 week period. Soil C:N ratio peaked at intermediate levels of oak phenotypic diversity whereas the total amount of DOC leached from microcosms declined as oak phenotypic diversity increased. In no case did the presence or absence of pine litter influence the response of nutrient fluxes to changes in oak litter diversity. Our results indicate that phenotypic diversity can be important even in the presence of another species, and suggest that conservation efforts should consider both inter- and intraspecific diversity.

INDEX WORDS: biodiversity, decomposition, dissolved organic nitrogen, ecosystem functioning, intraspecific diversity, interspecific diversity, litter chemistry, nutrient cycling, phenotypic diversity, tannins
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CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

Ecosystem functioning describes the transfer of energy and cycling of materials through ecological systems. All organisms are in some way both dependent on, and responsible for, ecosystem functioning (Odum 1959). As such, changes in ecosystem functioning have the potential to affect all life. An issue of global concern is the drastic loss of biodiversity, and how this loss in biodiversity may affect ecosystem functioning (Ehrlich and Ehrlich 1981). In order to maintain ecosystem integrity in the future, it is essential to understand how diversity at all levels affects ecosystem functions such as nutrient cycling (Ehrlich and Wilson 1991). This project investigates how a loss of intraspecific genetic diversity affects the fluxes of carbon and nitrogen in an ecosystem through leaf litter decomposition.

Why conserve biodiversity? Communities with many constituents may have species which are functionally redundant (Frost et al. 1995). Species that presently perform similar functional roles may react differently to future environmental changes, possibly compromising their original functional ability. Falkowski (1998) suggests that high functional redundancy in plant communities helped to ensure photosynthetic establishment of the atmosphere during periods of severe climatic changes on prehistoric earth. The redundancy of photosynthetic organisms to compensate for changes in the past may again compensate for changes in the future if gene pools are sufficient. Biodiversity is most often examined at an interspecific level (i.e. species diversity).
However, in light of anthropogenic reduction of natural population sizes, it is necessary to devote more effort to studying the effects of reductions in intraspecific genetic diversity.

Many characteristics of forest litter that have large impacts on ecosystem processes are genetically-mediated. Soil carbon (C) and nitrogen (N) cycling are partially controlled by lignin content (Meentemeyer 1978), tannins (Gallardo and Merino 1992), and C:N ratios (Melillo et al. 1982) of litter. Lignin, a structural component of plant tissue, is the second most abundant natural polymer (Staley and Orians 1992) and it is well established that leaf litter lignin concentrations are negatively correlated to decomposition rates (Meentemeyer 1978, Melillo et al. 1982, Horner et al. 1988). Secondary metabolites (tannins and phenolics) also control aspects of carbon and nitrogen cycling in forest soils (Horner et al. 1988, Gallardo and Merino 1992, Jama and Nair 1996). Considerable variation in these traits can exist within a single species and are caused, in part, by genetic differences (Hunter and Hull 1993). Consequently, a loss in genetic variation in litter traits important to decomposition could affect nutrient and energy cycling.

This dissertation investigates the relative effects of intraspecific diversity of Quercus laevis leaf litter and environmental factors on decomposition and nutrient dynamics. The main objective was to determine if a loss in genetic diversity of turkey oak litter affected carbon and nitrogen cycling. These data are some of the first to explore potential effects of losses in intraspecific genetic diversity on ecosystem functioning. Two bodies of literature were important in developing this research. The first, a subset of the biodiversity-ecosystem function literature, deals with the relationship between
biodiversity and belowground processes. The second is the vast amount of soil ecology research that explains the activity of polyphenolics during decomposition and nutrient cycling.

**BIODIVERSITY AND ECOSYSTEM FUNCTIONING: BELOWGROUND RESPONSES**

The debate over biodiversity’s influence on ecosystem functioning has sparked several lively disputes and entrenched some ecologists opposite each other (see Tilman et al. 1996 and Huston 1997). Strong non-science and non-market values (e.g. aesthetic and existence values) can make it difficult to hold objective and fair debates over the importance of biodiversity. To further complicate the debate, biodiversity and ecosystem function are such general terms that their meaning has taken many forms throughout the volumes of existing literature. Unfortunately there is no simple answer to, “does biodiversity matter?” Most likely, it depends upon the specific context, the level of biodiversity, and the habitat in question. For instance, the level of taxonomic diversity can be important. Biodiversity can be used to describe simple species diversity (interspecific diversity), genetic diversity within a single species (intraspecific diversity), or diversity among different functional groups of organisms such as nitrogen-fixers, forbs, and grasses (functional diversity). In the field of ecology, we are now in the process of elucidating how, when, and under what circumstances biodiversity matters (Bengtsson 1998).

A common bias in biodiversity experiments is that ecosystem function is predominantly represented by aboveground biomass production. Since 90% of primary
production eventually enters the detrital pathway (Coleman and Crossley 1996), it is only reasonable that debates on biodiversity and ecosystem function also consider belowground processes (Wardle et al. 1997). Below I provide a brief review of biodiversity and belowground process research. Previous reviews exist for aboveground primary productivity (Johnson et al. 1996) and a smorgasbord of ecosystem properties (Chapin et al. 2000, Schwartz et al. 2000, Loreau et al. 2001), but no published reviews have focused primarily on belowground processes.

Mechanisms of Biodiversity Impacts on Ecosystem Processes

There are two main mechanistic theories of how biodiversity affects ecosystem functioning, and four theories of how ecosystem functions will actually respond to reductions in biodiversity. These are briefly described in turn before summarizing previous studies.

Mechanistic theories

*Niche partitioning* (Abrams 1983) a.k.a. *Complementary Niche Mechanism* (Tilman et al. 1996). Niche partitioning theory states that species coexist because they exploit different resources. Therefore, a very diverse system of plants would scavenge all available nitrogen from the soil since a greater diversity of plants would use a greater diversity of nitrogen forms or nutrient ratios (Tilman et al. 1996). Likewise, a very diverse microbial community would decompose litter faster since all forms of nutrients and carbon in the litter would be available to some type of microbe and/or microbial enzyme.
Sampling effect (Huston 1997). Huston (1997) initially described the sampling effect as a statistical error in Tilman et al.’s 1996 study. Simply put, the more species chosen at random from a large pool, the higher the probability of choosing a species with a significant functional trait. The presence of a functionally significant individual in high diversity treatments can lead to false conclusions that diversity matters when, in actuality, species identity matters. This same argument has been embraced by Tilman et al. (1997b) as a mechanism by which species diversity influences ecosystem function. Since the sampling effect is based on random selection, if natural diversity is reduced non-randomly, the selection effect may be more of a statistical error than a biological mechanism. The sampling effect and niche partitioning theories are not mutually exclusive, and biodiversity may influence ecosystem functioning via both mechanisms simultaneously.

Ecosystem Responses

Diversity stability hypothesis (MacArthur 1955), Figure 1.1A. The loss of any species will lead to a decline in ecosystem performance. This is based on the food web dynamics theory that more complex food webs are resistant to disturbance since many alternative pathways for energy flow exist (MacArthur 1955).

Ecosystem rivet hypothesis (Ehrlich and Ehrlich 1981), Figure 1.1B. The ecosystem rivet hypothesis states that most organisms are important to ecosystem functioning. A few species may be lost without much consequence, but a loss of several species will lead to a decline in ecosystem performance. Similar to the rivets holding an airplane together,
losing a few is of minor consequence, however the more that are lost, the weaker the plane becomes until it eventually falls apart.

*Functional redundancy* (Walker 1992, Lawton and Brown 1993), Figure 1.1C. Walker (1992) suggests that major ecosystem processes (e.g. primary production) are performed similarly by many species and thus ecosystems have inherent functional redundancy. Decreases in biodiversity will not affect ecosystem function unless biodiversity falls below the functionally redundant threshold, which is generally low.

*Idiosyncratic hypothesis* (Lawton 1994), Figure 1.1D. Biodiversity has significant, yet unpredictable effects on ecosystem function. Changes in biodiversity will likely affect ecosystem functioning; but the direction and degree of influence is random and unpredictable. Ecosystem function is more dependent on species specific traits such that species identity is far more important than is species diversity to ecosystem functioning.

**Aboveground biodiversity and belowground functions**

Two types of plant manipulations are common to biodiversity experiments that focus on belowground responses: plant species diversity and litter diversity. Litter studies generally focus on belowground processes in finer detail than do plant diversity manipulations which are primarily concerned with aboveground biomass.

Below, I review published reports of belowground responses to manipulation of aboveground resources. Studies were chosen based on the admittedly biased selection criteria of those found in electronic databases, which tend to favor more recent articles.
The databases *Web of Science* and *Current Contents* were used to search for manuscripts published between 1945 and 2002 that contained text relevant to biodiversity and ecosystem functioning. I limited search results to 25 manipulative experiments that tested for belowground effects of aboveground biodiversity. Articles are summarized in Tables 1.1 and 1.2.

**Plant diversity impact on soil processes**

Mass loss is the most basic and direct measurement of decomposition. However, it is also one of the most variable of belowground responses. Of the 7 studies that investigated litter mass loss during decomposition in Table 1.1, three reported no effect of plant diversity (Naeem et al. 1999, Wardle et al. 1999, Wardle et al. 2000), one found idiosyncratic effects such that litter diversity randomly, yet significantly affected mass loss (Wardle and Nicholson 1996), another reported immediate idiosyncratic effects, but no long-term effects (Naeem et al. 1994), and yet another reported that species diversity had no effect, but that functional diversity of plants increased mass loss rates (Hector et al. 2000). Diversity may also interact with other biotic factors to affect decomposition rates. For instance, Mulder et al. (1999) reported decreased decomposition in high diversity plots not sprayed with insecticides, and increased decomposition in high diversity plots sprayed with insecticides.

Soil nitrogen is an important aspect of ecosystem functioning as most plants are dependent on soil nitrogen pools for growth. In all of the following studies, decreasing soil nitrogen content was interpreted as increased plant uptake. Soil nitrogen relationships were as variable as mass loss data: three reported a negative relationship
between soil nitrogen and plant diversity (Tilman et al. 1996, Hooper and Vitousek 1997, Tilman et al. 1997a), while two others found no relationship (Symstad et al. 1998, Maly et al. 2000), and another reported idiosyncratic effects (Naeem et al. 1994).


**Litter diversity impact on soil processes**

At least thirteen studies have attempted to elucidate the specific effects of litter diversity on belowground function (Table 1.2). These differ from those mentioned above in that plant litter was specifically chosen as the controlled variable instead of live plant biomass. In general, leaf litter mass loss during decomposition is not affected by litter species diversity. Five of the ten studies showed no effect of species diversity, three reported idiosyncratic effects and one reported diversity effects only at high diversity treatment levels (Blair et al. 1990, Wardle et al. 1997, Bardgett and Shine 1999, Nilsson et al. 1999, Hector et al. 2000, and Fyles and Fyles 1993, McArthur et al. 1994, Wardle and Nicholson 1996, and Hansen and Coleman 1998, respectively). However, diversity of functional groups may be more important to mass loss as Wardle and Nicholson
(1996) reported an idiosyncratic response and Hector et al. (2000) reported a positive response (decomposition increased with litter diversity).

Only Chapman et al. (1988) specifically investigated soil nitrogen content, and found that litter species diversity had an idiosyncratic effect on soil nitrogen pools. Litter diversity usually significantly affected nitrogen release from litter, but not in any consistent manner; Blair et al. (1990) reported increased nitrogen release from high diversity litter, whereas McTiernann et al. (1997) reported decreased nitrogen release, and three others found idiosyncratic responses (Fyles and Fyles 1993, Briones and Ineson 1996, Wardle et al. 1997).

Based on existing research, there is no definitive effect of litter diversity on soil respiration since idiosyncratic (Chapman et al. 1988), positive (Briones and Ineson 1996, McTiernann et al. 1997, Salamanca et al. 1998), and no effect (Bardgett and Shine 1999) responses have all been reported. Similar varying results exist for microbial biomass (Wardle and Nicholson 1996, Wardle et al. 1997, Bardgett and Shine 1999, Nilsson et al. 1999). None of the studies listed in Tables 1.1 or 1.2 explored the effects of genetic diversity; all were limited to species and functional group diversity manipulations.

**Soil biodiversity effects on ecosystem processes**

The study of soil biodiversity and its impact on ecosystem processes is in its infancy due, in part, to the difficulty in assessing and manipulating soil biodiversity accurately. Four recent publications report three different effects of manipulated soil biodiversity on ecosystem function. Degens (1998) reduced soil microbial diversity and found that decomposition rates were depressed under optimal moisture regimes, but not
otherwise. Mikola and Setala (1998) reported that microbial respiration, and mineralization of nitrogen and carbon were all idiosyncratic in response to decreased soil microfauna diversity. Laakso and Setala (1999) found that primary production was relatively insensitive to reduced soil biodiversity, including trophic level diversity. Likewise, Griffiths et al. (2000) found no relationship between soil microbial diversity and soil processes.

**Summary**

Of the ecosystem parameters measured, it appears that fluxes of carbon occurring during decomposition are either resistant to, or respond in an idiosyncratic manner to losses in species diversity (Tables 1.1-1.2). Soil microbial biomass may increase with diversity, but this is not a universal trend (Tables 1.1-1.2). Nitrogen fluxes seem to be the most susceptible to changes in biodiversity, as 10 out of 11 studies reporting nitrogen results indicate some sort of biodiversity effect, whether it be at the species or functional group level (Tables 1.1-1.2). This agrees with a hypothesis put forth by Schimel (1995) that processes dealing with carbon fluxes may be more functionally redundant and, therefore, less responsive to losses in diversity compared with nitrogen transformations which, are performed by a less diverse group of organisms.

Tables 1.1 and 1.2 group reports based upon ecosystem type and level of diversity manipulated. There appears to be no pattern of ecosystem sensitivity to reduced biodiversity based on general classification such as grassland, herbaceous plants, or boreal forest. Studies conducted in temperate systems tend to elicit stronger responses to biodiversity reductions than others, but a lack of published reports in other systems
prevents any firm conclusions. There is also an apparent lack of any tropical studies investigating biodiversity and belowground ecosystem functioning.

More support exists for the idiosyncratic hypothesis put forth by Lawton (1994) than for any of the other three hypotheses mentioned above. This could, however, simply be due to a lack of empirical evidence. Runner up is the functional redundancy theory of Walker (1992), as in some cases extreme reductions in biodiversity did elicit ecosystem responses. Of the 25 studies analyzed, 14 report some sort of synergistic effect of biodiversity (Tables 1.1-1.2), meaning that high diversity treatments did not behave as an average of their single constituents. Five studies report no synergistic behavior while 6 did not properly test for such a relationship (Tables 1.1-1.2). Overall, belowground responses to reductions in biodiversity (above- or belowground) are incredibly variable, suggesting that theoretical mechanisms invoked for aboveground responses may not be as valid for belowground functions.

As previously noted, none of the above studies investigated the effects of intraspecific litter diversity on ecosystem functioning. Leaf litter chemistry plays an important role during decomposition, and variation in secondary metabolite production may be a mechanism through which intraspecific litter diversity affects ecosystem functioning.

THE INFLUENCE OF GENETICALLY-MEDIATED VARIATION IN POLYPHENOLICS ON NUTRIENT CYCLING AND DECOMPOSITION

There is a vast amount of literature focusing on the role of leaf secondary metabolites during litter decomposition. Entire books have been written on the
importance of litter quality to nutrient cycling (e.g. Cadisch and Giller 1997) and a comprehensive review is beyond the scope of this chapter. Rather, I will overview the production, regulation, and ecosystem effects of polyphenolics. Polyphenolics are particularly relevant to ecosystem functioning because they are the most widely distributed class of secondary metabolites and interact strongly with several aspects of nutrient cycling (Haettenschwiler and Vitousek 2000).

**Classes of phenolics**

Phenolics can be divided into three rough categories: 1) low molecular weight phenolics (simple phenolics), and 2) high molecular weight tannins, and 3) polymers of lignin. Simple phenolics are simple aromatic ring structures with at least one or more hydroxyl substitutions (phenols and polyphenols, respectively). They are similar to the simple monomers that make up the huge polymer structure of tannins. Tannins are high molecular weight (500 to >20,000 kD), non-structural carbon based polymers with free phenolic groups that characteristically form large complexes with proteins. Tannins are typically divided into condensed tannins (proanthocyanides) and hydrolysable tannins. Condensed tannins are polymers of flavanoid units connected by carbon bonds that are not susceptible to hydrolysis. Hydrolysable tannins have a sugar center with ester connections to phenolics such as gallic acid (gallotannins, e.g. the well-known “tannic acid” standard) and ellagic acid (ellagittannins). The frequent ester connections are relatively easily broken by hydrolysis and thus, hydrolysable tannins tend to be more water soluble than large condensed tannins.
Lignin is distinctly different from other polyphenolics since it actually plays structural roles within the cell walls of plants. Lignin is a complex polymer of phenylpropanoids that wraps in and out of the structural polysaccharides in cell walls. The seemingly random arrangement of subunits within lignin allows for extensive cross linking. These bonds that determine the overall structure are necessarily broken during extraction procedures, making a universal lignin polymer pattern elusive (Mann 1987).

**Biosynthesis of polyphenols**

Simple phenolics, tannins, and lignins all arise from similar biochemical pathways. Figure 1.2 summarizes the synthesis of simple phenolics, tannins, and lignin. The figure is only intended to represent generalities, the variation in phenolics is striking with over 20,000 described (Harborne 1997), all produced via slightly different pathways. For instance, several simple phenolics may undergo methylation, decarboxylation, reduction, and/or oxidation to create countless variations. Simple phenolics can also be created by hydrolysis of more complex polyphenols in plant tissues to create molecules ranging widely in complexity and mass (Brielmann 1999).

All phenols are carbon based and are derived ultimately from simple sugars produced during photosynthesis. The initial carbon substrate enters one of two pathways, either the Shikimic acid, or polyketide pathway. Simple phenolics are typically generated from products entering the polyketide pathway and standing stocks are typically low, since most become incorporated into tannin structures (Mann 1987). Condensed tannins are composed of flavonoid units made from products of both the Shikimic acid and polyketide pathways. Both lignin and hydrolysable tannins are
produced via the Shikimic acid pathway. Shikimic acid is typically converted into one of three phenols (coniferal, p-coumaryl, or sinapyl alcohol) which polymerize to form enormous lignin molecules. Shikimic acid can also be converted into gallic acid and bind with a central sugar via ester bonds to create gallotannin. Two gallic acid molecules may join to form ellagic acid which may also bind with a central sugar to form ellagitannins. While the biosynthesis of the monomeric starting units for polyphenolics is fairly well understood, the biochemical factors that regulate the construction of these giant polymers remain unclear (Haslam 1998).

**Regulation of polyphenol production: Extrinsic factors**

Environmental conditions that influence the production of polyphenolics can be divided into four categories: soil conditions, atmospheric conditions, seasonal climate changes, and plant damage (herbivory and other).

The carbon nitrogen balance hypothesis (CNBH) (Chapin 1980, Bryant et al. 1983) is based on fairly straightforward stoichiometry: when nitrogen is limiting, carbon based photosynthates are allocated to carbon based polyphenolics rather than to growth. Conversely, when nitrogen supplies are high, polyphenolic concentrations are reduced because plants allocate carbon to growth and not defense (Hunter and Schultz 1995, Mutikainin et al. 2000). A protein competition model of polyphenolic allocation (Jones and Hartley 1999) predicts the same pattern of high polyphenolic concentration in leaves of plants grown on low fertility soils. Since both proteins and phenolics compete for phenylalanine precursors, phenolic production receives the majority when nutrients are limiting. The primary difference between the two regulation theories is that the latter
specifies exactly where the tradeoff between growth and secondary metabolite production occurs. The resource availability hypothesis (Coley et al. 1985) explains that low soil nutrient availability can also act on an evolutionary time scale to affect polyphenolic concentration in plants. Plants that defend long-lived leaves with polyphenolics are more likely to succeed in low nutrient conditions, and in general, low nutrient soils tend to support plants that are slow growing and have long-lived leaves with high concentrations of polyphenolics (e.g., black water rivers in Amazonia, Janzen 1974). Soil characteristics other than nutrients are important to secondary metabolite production. For instance Louda et al. (1987) showed that secondary metabolite production can respond to soil moisture as well.

Two aspects of global climate change influence secondary metabolite production. Increased levels of CO\textsubscript{2} have been shown to increase polyphenolic concentrations (e.g. Penuelas et al. 1998, Mansfield et al. 1999), and the increase in UV-B as the result of ozone depletion has been shown to increase phenolic production and alter the Shikimic acid pathway (Caldwell et al. 1989, Rozema et al. 1997). High CO\textsubscript{2} levels probably increase polyphenolic production as a result of the carbon and nutrient balance mechanism (above) since increased CO\textsubscript{2} is usually associated with higher levels of photosynthetic activity (Swift et al. 1998). Increased levels of UV-B induce polyphenolic production as a means to protect protein and DNA from harmful radiation, as polyphenols probably played a major evolutionary role by allowing terrestrial vegetation to withstand UV bombardment on early earth (Rozema et al. 1997).

In general, polyphenolic concentrations in foliage are highest during the summer months (Feeny 1970). Summer coincides with both the onset of herbivory, and the
highest levels of photosynthetic activity. Herbivory-induced polyphenolic production is a well documented aspect of plant-insect interactions (Herms and Mattson 1992, Baldwin 1994). Some polyphenolics are constitutive, while others respond strongly to herbivory (Rossiter et al. 1998, Mutikainen et al. 2000). For instance in Betula pendula, condensed tannins appear to be an ever present herbivore deterrent, whereas hydrolysable tannins are produced in response to herbivory (Mutikainen et al. 2000). Herbivory can also have long lasting effects on phenolic production such that herbivory in previous years results in high polyphenolic production in the next (Schultz and Baldwin 1982). Hunter and Schultz (1995) found that soil nutrients can inhibit herbivore-induced production of polyphenolics, demonstrating that several environmental factors can influence polyphenolic production simultaneously. Physical damage such as ultrasound (Wu and Lin 2002) and mechanical wounding (Lagrimini et al. 1993) can also induce polyphenol production. Different types of physical damage can have similar overall effects. For example, Findlay et al. (1996) found that ozone and mite induced polyphenol production in leaves carried over into increased polyphenol content in cottonwood litter.

Regulation of phenol production: Genetic factors

In addition to various environmental factors mentioned above, polyphenolic production is also under genetic control both on large and small taxonomic scales. For instance, on a large scale, condensed tannins are very common in woody plants, but almost absent in herbaceous species (Haslam 1989). On a small scale, several polyphenolics are limited to a single species such as the ellagitannin Cercidinin A, isolated from the bark of Cercidiphyllum japonicum (Tanaka et al. 2001). The
concentration of polyphenolics produced by any plant is also under genetic control, and often there is considerable variation within the same species. For instance, Zangerl and Berenbaum (1990) found that genetic differences between populations of wild parsnips caused differences in the amount of constitutive and induced polyphenol production. Surveying all known species of the *Leucaena* genus grown in a common garden, Dalzell and Shelton (2002) found significant variation in the production of condensed tannins, both among species of the same genus (0-336 g tannin/ kg litter) and within single species (145 fold difference in total condensed tannin content for *L. trichandra*). Several studies also demonstrate genetically-mediated variation in oak species (Rossiter et al. 1988, Hunter and Shultz 1995, Hunter et al. 1996, Hunter 1997, Klaper et al. 2001) as well as other species such as the pacific cosmopolitan *Metrosideros polymorpha* (Haettenschwiler and Vitousek 2002).

Genetically-mediated variation in litter chemistries can lead to genotype by environment interactions. Lindroth et al. (2002) found that different genotypes of aspen (*Populus tremuloides*) responded differently to elevated carbon dioxide in the amount of condensed tannins that they produced. Likewise, Keinanen et al. (1999) found that condensed tannin production in silver birch (*Betula pendula*) varied by genotype, fertilization, and physical damage treatments, but was most affected by genotype. Although tree genotype by environment interactions are not widespread in the literature, the effect of genotype remains significant on phenolic production in several species.
**Polyphenolic Influences on Ecosystem Functions**

Although polyphenolics are best known for their herbivore and pathogen defense roles in living leaves, they also have several important consequences for terrestrial nutrient cycling during leaf litter decomposition (Haettenschwiler and Vitousek 2000). Large concentrations of polyphenolics probably also enter soils through senesced roots since roots can contain almost four times the concentration of polyphenolics as leaves (Gallet and Lebreton 1995). Polyphenolics influence nutrient cycles because 1) they often constitute a large percentage of the remaining litter (1-25%, Haettenschwiler and Vitousek 2000), and 2) they remain biologically active after tissue senescence (Horner et al. 1988).

Once leaves senesce and abscise, the relative concentration of simple phenolics decreases compared to condensed tannins (Gallet and Lebreton 1995). Simple phenolics can increase soil respiration by providing a simple carbon source for microorganisms (Horner et al. 1988, Schimel 1996). The remaining polyphenolics typically have four fates: 1) further biological breakdown, 2) incorporation into humic substances 3) adsorption to clay minerals, or 4) leached as dissolved organic carbon (Haettenschwiler and Vitousek 2000). Fungi do most of the catabolism work on complex polyphenolics through enzymatic degradation. However, they gain very little energy from polyphenols and need other available substrate for energy (Paul and Clark 1996). Earthworms and termites have some limited ability to metabolize polyphenolics (Haettenschwiler and Vitousek 2000), but most soil fauna aid in the decomposition through physical breakdown and mixing of litter and stimulation of the microbial community through grazing (Coleman and Crossley 1996).
Lignin is particularly recalcitrant and becomes incorporated into humic substances simply because very few organisms can catabolize it. Tannins are defined, in part, by their ability to bind to proteins (Bate-Smith 1975). If tannins bind covalently with proteins to form polyphenolic-protein complexes, they become very recalcitrant and only basidiomycetes with polyphenol oxidase and earthworms can take advantage of the complexed nitrogen sources (Hattenschwiler and Vitousek 2000). Tannins also have a limited ability to bind with carbohydrates and cellulose to form complexes that are equally recalcitrant (Horner 1988). The ability of polyphenolics to complex with proteins and other biochemicals is the primary method by which they influence soil respiration and soil nitrogen fluxes (discussed below).

As mentioned above, simple phenolics can increase microbial respiration, apparently by acting as a carbon source (Schimel 1996). More commonly reported is the negative effect of polyphenolics on soil respiration, attributed to the protein binding and outright toxicity of polyphenolics (Horner et al. 1988, Schimel et al. 1996, Harbone 1997). Polyphenolics are capable of generating oxygen radicals which are known to damage enzymes, cell membranes, and DNA molecules (Appel and Schultz 1999). Polyphenolic attributes that make them effective plant pathogen defenses also affect non-pathogenic fungi and microbes once litter enters the detrital food web; tannins do not discriminate between enzymes of plant pathogenic fungi or decomposing fungi, and have the same net effect on both.

Nitrogen cycling is also greatly influenced by plant polyphenolics for much of the same reasons. Ayres (1997) suggests that condensed tannins may be more important to nitrogen cycling than to herbivore defense, since condensed tannins frequently have no
anti-herbivory activity. By incapacitating microbial communities, tannins can reduce nitrogen mineralization rates, while at the same time binding proteins to immobilize nitrogen sources (Schimel 1995). Gallardo and Merino (1992) present a two-step process where nitrogen is imported into the litter via microbes, then precipitated by tannins and immobilized in the humic fraction. The net effect is a drastic reduction in mineral nitrogen availability. However, Northup et al. (1995) demonstrated that high concentrations of polyphenolics in pine litter increased the proportion of dissolved organic nitrogen leached relative to inorganic forms. They suggested that tying up nitrogen in organic forms may be a means by which plants conserve nitrogen and short circuit the microbial pathway. This mechanism may partially explain why polyphenolic production is so high in low nutrient conditions (in addition to the CNBH discussed above), however it is unclear whether or not high concentrations of polyphenolics actually increase ecosystem nitrogen retention and it is unknown whether plants can access nitrogen tied up in complex polyphenol-protein complexes (Hattenschwiler and Vitousek 2000). High levels of polyphenolic content may increase dissolved organic nitrogen concentration in leachate simply by inhibiting decomposition and mineralization rates.

The high reactivity and branching structure of reactive hydroxyl sites also allows polyphenolics to complex with clay particles in soil and thereby influence several micronutrient cycles also. For instance, polyphenols can reduce aluminum toxicity and increase phosphorus availability by competing for positive binding sites (Northup et al. 1998). Polyphenols can also retain inorganic cations and metals (Ca, Mg, K, Mn, Fe, and Cu) by providing sorption sites (Schnitzer et al. 1984).
CONCLUSION

Biodiversity and ecosystem functioning studies have tended to focus on aboveground ecosystem responses. Those that do investigate belowground responses to diversity manipulations do not form a consensus on the effects of biodiversity. Rather, belowground responses are extremely variable; positive, negative, idiosyncratic, and no effects of biodiversity have all been reported for most ecosystem functions measured. However, some general trends do emerge. For instance, litter diversity is likely to have an effect on belowground ecosystem functions, and especially on nitrogen fluxes in the soil and litter, although the direction of effect, and the shape of the ecosystem function response curve is far from being delineated. A primary mechanism through which litter diversity may affect decomposition processes is through polyphenolics and secondary chemistries.

Variation in plant polyphenolics is both genetically-mediated and important to ecosystem functioning. In addition to herbivore and plant pathogen defense mechanisms, polyphenolics also play major roles in nitrogen and carbon cycling during decomposition. In general, polyphenolics slow decomposition and nutrient cycles by complexing with proteins and inhibiting microbial activity. The variation in the specific chemical identity of polyphenolics is astounding, with equal variation in concentration of polyphenols produced. Polyphenolic concentrations vary widely both among and within species. Although polyphenol identity is also important to functioning, it is known that variability in the gross production of polyphenolics in some species such as *Metrosideros polymorpha* (Treseder and Vitousek 2001) and Cottonwood hybrids (Driebe and
Whitham 2000) can affect litter decomposition. It follows that intraspecific diversity has the potential to affect ecosystem processes via genetically-mediated variation in litter chemistries.

We conducted a series of field and lab experiments to test for effects of intraspecific diversity of *Q. laevis* litter on ecosystem functioning in an oak sandhills community. The results are presented in the following chapters. Chapter 2 is an analysis of the first 18 months of a 3-year *in situ* decomposition study of turkey oak litter at Savannah River Ecology Laboratory (SREL) in which we varied intraspecific diversity of turkey oak litter and monitored carbon and nitrogen fluxes during decomposition. Chapter 3 is a microcosm study where intraspecific diversity of leaf litter was varied along with nitrogen deposition simulations in order to test for interactions between two simultaneous aspects of global change: reductions in biodiversity and increases in nitrogen deposition. The 4th chapter is the complete 3-year *in situ* field study, which includes carbon and nitrogen cycle analyses, as well as microarthropod and microbial diversity analyses. The 5th chapter is a second microcosm study where we repeated intraspecific litter diversity manipulations of turkey oak litter with and without pine litter to determine if the presence of an additional species would mask any effects of intraspecific diversity. The 6th, and final, chapter summarizes our results.
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Mansfield, J. L., P. S. Curtis, D. R. Zak, and K.S. Pregitzer. (1999). Genotypic variation for condensed tannin production in trembling aspen (Populus tremuloides,


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From Johnson et al. (1996). The 4 major predicted ecosystem function and biodiversity relationships: a) diversity-stability b) ecosystem rivet c) functional redundancy d) idiosyncratic.
Figure 1.2. Summary of plant secondary metabolite biosynthesis.
CHAPTER 2

PHENOTYPIC DIVERSITY INFLUENCES ECOSYSTEM FUNCTIONING IN AN OAK SANDHILLS COMMUNITY

1Madritch, M.D. and M.D. Hunter. Ecology 83(8), pp. 2084-2090
ABSTRACT: Given the drastic decline in biodiversity at all levels, it is imperative that we consider the potential affects of diversity within single species on ecosystem functioning. However, empirical data describing the relationship between intraspecific diversity and ecosystem functioning are lacking. We present field data demonstrating that the litter phenotype of individual trees affects carbon and nitrogen fluxes during decomposition and that single phenotype treatments differ in ecosystem processes from a phenotypic mix. Since nutrient dynamics are related to the chemistry of the litter, we then use the strength of the relationship between genetic distance and litter chemistry to infer the existence of genotypic effects on ecosystem functioning. In combination, our results provide the first evidence that losses in intraspecific diversity can affect the ecosystem processes of carbon and nitrogen cycling.

Key words: biodiversity, ecosystem functioning, intraspecific diversity, decomposition, nutrient cycling.
INTRODUCTION

The current mass extinction period (Wilson 1992) has led ecologists to investigate the role that biodiversity plays in ecosystems. In particular, the role that biodiversity has on ecosystem processes such as carbon sequestration and nutrient retention has been of great and recent interest. Experiments linking biodiversity to ecosystem functioning have largely been limited to manipulations of species and functional group diversity. However, as natural areas decrease in size due to human activities, it is understood that both inter- and intraspecific genetic diversity will be lost (Purvis and Hector 2000). Due to a lack of empirical data, the consequences of losses in intraspecific genetic diversity for ecosystem functioning are presently unknown.

Ecosystem functioning is typically measured as some index of aboveground biomass production or, less frequently, some metric of soil nutrient retention. Studies by Tilman et al. (1994, 1996) and Naeem et al. (1994) suggested a positive effect of biodiversity on plant biomass production. Similar findings have been repeated throughout the literature but not without critical debate (See Chapin et al. 2000, McCann 2000, and Loreau et al. 2001 for recent reviews). Primary production is often measured because of methodological ease and agricultural influence. However, belowground processes are of equal importance, mediating what happens aboveground through nutrient availability and turnover. Recent work shows considerable variation in belowground responses to biodiversity treatments (Loreau et al. 2001). Since upwards of ninety percent of all aboveground biomass enters the detrital food web (Coleman and Crossley 1996), understanding the relationship between biodiversity, genetic or otherwise, and belowground processes is essential (Wardle et al. 1997, Hector et al. 2000).
Many of the genetically mediated characteristics of forest litter have large impacts on ecosystem level processes. Soil carbon (C) and nitrogen (N) cycling are partially controlled by litter lignin content (Meentemeyer 1978), tannin content (Gallardo and Merino 1992), and C:N ratios (Melillo et al. 1982). Several studies have found that secondary metabolites (tannins and phenolics) regulate aspects of carbon and nitrogen cycling in forest soils (Horner et al. 1988, Gallardo and Marino 1992, Jama and Nair 1996). Considerable variation in these traits can exist within a single tree species and are caused, in part, by genetic differences (Hunter and Hull 1993). Consequently, a loss in genetic variation in litter traits important to decomposition could affect nutrient and energy cycling.

In order to explore how intraspecific diversity affects ecosystem functioning, we chose nine genetically different Quercus laevis trees and established a litter decomposition experiment to observe the flows of carbon and nitrogen. We analyzed the decomposition of nine different single tree litter treatments (phenotypes) and one mixed treatment which consisted of litter from all nine trees. By comparing single litter treatments to the mixed treatment, we tested for an effect of intraspecific diversity on nutrient cycling during decomposition, hypothesizing that the underlying mechanism behind any effect of phenotype would be through variation in genetically mediated litter chemistries.

METHODS

Our field site is a 60+ hectare old growth stand of Quercus laevis, (85% canopy cover) and long leaf pine, Pinus palustris, (10% canopy cover) at the Department of
Energy’s Savannah River Site (SRS) in Aiken, SC. *Q. laevis* is endemic to the southeastern US coastal plain, often growing on poor, sandy soil. The study site lies on a gently sloping (5% grade) south-facing sandhill. Air photo coverage since 1943 shows no visible signs of recent disturbance. All *Q. laevis* individuals in a 3.2 ha plot within the stand were genotyped previously using 9 polymorphic allozyme loci (Berg and Hamrick 1994).

During leaf fall in 1998, we collected leaves from 9 individual *Q. laevis* trees (phenotypes) and established a decomposition study. All litter was collected by hand from known trees after senescence but before leaf-fall; turkey oaks generally hold their leaves for some weeks after senescence. Litter treatments were contained within open-bottomed, meter square boxes covered with vinyl coated hardware mesh to exclude non-treatment litter. Sets of 10 boxes, 9 with single phenotypes and 1 with an equal mix of all 9, were established at each of 9 sites within the 3.2ha plot (n=90 boxes total).

150 g of loose litter, corresponding to average litterfall per m$^2$, was added to each box at the beginning of the study and was added again after one year. Four litterbags, 1 mm$^2$ nylon mesh, containing 10 g of dry litter were also introduced into each box at the start of the experiment. The litter inside each bag was of the same phenotype (or a mix of all 9) as that in the box in which it was placed. Bags of litter were therefore imbedded within boxes of litter of the same phenotype. Bags were collected after 3, 6, 12, and 18 months and analyzed for carbon, nitrogen, litter chemistry, and microbial carbon content. Soil samples were taken at the same time intervals using a 2x10 cm soil corer at three different locations within each box. All three cores were then bulked, mixed and sieved of root matter before analyses for total carbon and nitrogen, microbial carbon, microbial
nitrogen, and pH. Soil moisture was also monitored every 3 months, but did not vary significantly across the site (data not shown).

Litter phenolic chemistry (total phenolics, condensed tannins, hydrolysable tannins) was estimated using previously established techniques (Rossiter et al. 1998, Hunter and Shultz 1995). Litter and soil total carbon and nitrogen values were determined using a Shimadzu total C and N analyzer. Lignin content was measured using an ANKOM automated fiber analyzer. Soil respiration was monitored monthly with a portable infrared gas analyzer (EGM-2 PP Systems). Microbial biomass carbon was determined by fumigation extraction (Ross and Sparling 1993) using published $k_{ec}$ values (Voroney et al. 1993). Microbial biomass nitrogen was determined by fumigation extraction (Vance et al. 1987) followed by persulfate digestion (Cabrera and Beare 1993) using published $k_{cn}$ values (Voroney et al. 1993). Ion exchange resin bags were used to estimate nitrate and ammonium availability in the soil (Binkley 1984). Two bags were buried at a depth of 8 cm within each box and left in the field for 3-month intervals, except during the 15-20 month interval when only one set was used. Resins were extracted in 1M KCl and analyzed for NH$_4^+$ and NO$_3^-$ using an Alpkem analyzer.

**Statistics**

All data were tested to fit the assumptions of normality using a Shapiro-Wilk W test and non-normal data were transformed as necessary. Phenotypic effects over time were estimated using the 9 sites as replicates while local environmental effects were estimated using the 9 phenotypes as replicates in repeated measures ANOVA procedures. By comparing the variance explained by phenotype and site, we were able to estimate the relative contributions of litter phenotype and local environment to the rate of
decomposition and nutrient availability. The mixed litter treatment served as a reference with which to compare single phenotype litters. To test for non-additive effects of mixing litter phenotypes, we compared the mixed litter treatment to the mean of the 9 single phenotype litters using repeated measures ANOVA procedures.

To assess the effects of litter chemistry on carbon and nutrient fluxes, it was necessary to account for changing litter chemistry over time. We therefore assumed that the rate of change in nutrient concentration between times t and t+1 would be related to the chemistry of litter at time t, similar to the analyses of population time series (Royama 1992, Berryman 1999). We calculated the change in the nutrient under consideration (X) as \( \ln \left( \frac{X_{t+1}}{X_t} \right) \), repeated this for each of the time steps (0, 3, 6, 12, and 18 months), combined data across all sites, and performed a stepwise regression between the nutrient flux of interest and our estimates of litter chemistry (Berryman 1999).

Given that variation in litter phenotype has both genetic and environmental components (Klaper and Hunter 1998), we used allozyme data (Berg and Hamrick 1994) and a Mantel test (Mantel 1967) to estimate the contribution of tree genotype to variation in litter phenotype. The genetic distance matrix consisted of 8 published allozyme markers (Berg and Hamrick 1994), while the litter chemistry matrix included day zero estimates of condensed tannin, hydrolysable tannin, phenolics, lignin, and C:N ratio.

**RESULTS**

We calculated changes in litter carbon and nitrogen concentrations for each litter phenotype at each sampling date as \( \frac{(current\ concentration - initial\ concentration)}{45} \)
(initial concentration). Positive values indicate nutrient immobilization (gain) whereas negative values indicate mobilization (loss). Data presented in all figures are untransformed.

After 18 months of decomposition, differences in nutrient dynamics had developed among litter phenotypes. Changes in litter carbon ($P<0.0001$) and litter nitrogen ($P<0.0001$) concentrations varied significantly among litter phenotypes (Table 2.1) with marked phenotypic differences in the tendency of a litter to mobilize or immobilize carbon and nitrogen relative to the mix of phenotypes (Figure 2.1). Carbon dynamics were also influenced somewhat by a site effect ($P=0.0008$, Table 2.1). Variation in the proportion of litter remaining on each sampling date had both phenotypic and site-related components (Table 2.1), whereas estimates of microbial biomass in litter were related only to site.

In comparison to the strong phenotypic effects upon nutrient dynamics in litter, nutrient dynamics in soil were dominated by site effects (Table 2.1). Soil respiration, percent carbon, percent nitrogen, microbial biomass carbon, microbial biomass nitrogen, and soil pH were all significantly affected by site and not by litter phenotype. However, one notable exception was the availability of ammonium in the soil. Ammonium availability in the soil directly underlying the litter treatments was significantly affected by the phenotype of litter above ($P=0.0227$, Table 2.1, Fig. 2.2). Neither site nor phenotype affected nitrate availability in the soil.

Despite a lack of phenotype treatment effects on soil properties, non-additive effects of mixed litter were found on soil carbon and nitrogen content as well as effects over time on soil microbial biomass carbon and soil pH (Table 2.1). By non-additive
effects, we mean that the responses of the mixed litter treatments differed significantly from the means of the 9 single phenotype treatments.

We hypothesized that our phenotypic effects upon nutrient dynamics were chemically based. Consequently, we assessed both the effects of phenotype upon litter chemistry and the effects of litter chemistry on nutrient dynamics. Litter phenotype affected C:N ratios and lignin, phenolic, condensed tannin, and hydrolysable tannin concentrations of litter (Table 2.1). There were also minor effects of site upon litter total phenolic and condensed tannin concentrations (Table 2.1).

Stepwise regression results showed a significant effect of at least one litter chemistry index on every ecosystem response measured except the nitrogen immobilized within soil microbial biomass (Table 2.2). Litter chemistry affected carbon and nitrogen fluxes in both litter and soil. In litter, the final regression models accounted for a significant proportion of the variation in rates of change of carbon (8.78%), nitrogen (31.70%), microbial carbon (22.90%) and litter mass (19.14%)(Table 2.2). In soil, litter chemistry accounted for a significant proportion of the variation in rates of change of respiration (28.00%), carbon (24.64%), nitrogen (9.56%), microbial carbon (2.59%), nitrate availability (12.27%), ammonium availability (21.41%), and soil pH (31.26%)(Table 2.2).

Having associated litter phenotype with litter chemistry, and litter chemistry with carbon and nitrogen dynamics, we sought to determine the contribution of genotype to variation in litter phenotype. In order to estimate the genetic component of the chemical phenotypes which affected carbon and nitrogen dynamics, we performed a regression between a genetic distance matrix and a litter chemistry matrix using a Mantel test.
We found a significant, positive relationship between genetic distance and variation in litter chemistry ($r^2=0.34$, $P<0.0001$). The same analysis run with a physical distance matrix and an identical litter chemistry matrix yielded no significant relationship ($P>0.18$). Thus genetic distance, but not physical distance, was correlated with variation in litter chemistry. We assume that the remaining variance includes contributions to litter phenotype from the environment in which trees were growing.

**DISCUSSION**

We have shown that litter phenotype influences nutrient dynamics under field conditions in an oak-sandhills community. The mechanism appears to be that tree genotype affects litter chemistry which has a strong impact on carbon and nitrogen dynamics. Using a Mantel test (Mantel 1967), we showed that 34% of the variation in litter chemistry is explained by genetic variation, which is roughly similar to an estimated 40% genetic contribution to variation in green-leaf chemistry of the same species (Klaper and Hunter 1998). The litter chemistry variation, in turn, had a large effect on almost all ecosystem responses measured (Table 2.2). Likewise, repeated measures ANOVA yielded significant effects of phenotype on litter nutrient dynamics as well as soil ammonium availability (Table 2.1).

There are potential implications for ecosystem functioning that might follow losses of genetic diversity. Figure 2.1 illustrates changes in litter nitrogen and carbon under single genotype litters. Depending upon the genotype, single litters may behave as net sources or sinks of nitrogen or carbon relative to diverse mixes. There is no coherence in the direction of difference from the mixed litter treatment, however significant
differences are prevalent. Simply put, losses in genetic diversity may influence ecosystem functioning in unpredictable ways. Single genotype litters elicit carbon and nitrogen responses that differ significantly, yet unpredictably, from the mixed litter treatment, in part, because no compensatory effects exist in low diversity treatments. Figure 2.2 illustrates that the identity of the litter on the soil surface also has a major affect upon the availability of ammonium in the soil beneath.

Several soil responses showed non-additive effects of mixing litter phenotypes. Soil carbon, nitrogen, microbial biomass, and pH under the mixed litter was different from the mean of the individual phenotypes. However, there was no significant effect of litter treatment itself on any of these soil characteristics (Table 2.1). We are unsure as to why these processes in particular showed a non-additive effect of intraspecific litter diversity, however others have found non-additive effects of biodiversity both above- (Tilman et al. 2001) and belowground (Wardle et al. 1997).

Some ecosystem responses were not susceptible to phenotype treatments, such as soil respiration and soil nitrate availability. Respiration is a process carried out by many different organisms and is not as likely to be affected by diversity treatments as is a process with fewer participants (Schimel 1995). Nitrate availability was not affected nearly as much as was the availability of ammonium, perhaps because ammonification is the first step in nitrogen transformation during decomposition.

The possibility exists that different genotypes preferentially establish in specific soil nutrient conditions. Soil nutrients could then be responsible for differences in litter chemistry, rather than the genotype itself directly affecting litter chemistry. However, there is no systematic variation in soil nitrogen concentration within the plot (Klaper and
nor is there any spatial autocorrelation in ammonium or nitrate availability (R. Klaper, S.L. Rathburn, M.D. Hunter, unpublished data). If genotypes were selective for certain soil nutrient types, some clonal clumping would be expected. However, Berg and Hamrick (1994, 1995) found no small-scale genetic structure or clumping in adult trees after surveying every tree in the same 160x160m forest plot. In addition, our trees were chosen at random across the plot. The lack of soil nutrient patterns along with the random small scale dispersal of adult genotypes across the site, combined with random tree selection effectively eliminates the possibility that litter chemistry was entirely driven by soil nutrient availability.

We conclude that biological diversity at all levels can contribute to variation in ecosystem functioning and that the challenge is to determine where and when such contributions matter. Various effects of species and/or functional diversity on ecosystem processes have been described previously (Naeem et al. 1994, Tilman and Downing 1994, Tilman et al. 1996, Chapin et al. 2000, Hector et al. 2000, McCann 2000) and here we present data that illustrate the potential for intraspecific diversity to affect carbon and nitrogen fluxes as well. While some have reported no effect of interspecific litter diversity on decomposition (Blair 1990, Hector et al. 2000), our results are similar to other’s who have shown significant, yet idiosyncratic, effects of interspecific litter diversity on belowground processes (Chapman et al. 1988, Wardle et al. 1997). The relative importance of different levels of diversity (genetic, species, functional group) may vary from system to system. Raw diversity itself may not be as important to ecosystem functioning as is the relative amount of diversity present compared to natural levels. The particular stand of *Q. laevis* used in this study exhibits relatively low species
diversity with only two dominant canopy species. Intraspecific diversity may be more important in such systems where species diversity is low (McGraw 1995) than in speciose ecosystems such as tropical forests.

ACKNOWLEDGEMENTS

This work was supported by grants from the Andrew W. Mellon Foundation and the National Science Foundation (Grants DEB-9906366 to M.D.H. and DEB-0104804 to M.D.M.). We also thank the Savannah River Ecology Laboratory for the use of their facilities and M. Cabrera, D. Coleman, J. Hamrick, P. Hendrix, R. Pulliam, R. Sharitz, R. Klaper, S. Connelly, B. Nuse, and J. Rogers for comments and/or laboratory assistance.

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Table 2.1. Repeated measures ANOVA of site and phenotype effects on litter and soil characteristics along with comparison of average single phenotype response with mixed litter response.

<table>
<thead>
<tr>
<th>Litter Responses</th>
<th>Phenotype Date *</th>
<th>Date* Site</th>
<th>Mean versus Mixed</th>
<th>Mean versus Mixed * date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter Carbon change</td>
<td>0.2840 (0.0001)</td>
<td>0.0330</td>
<td>(0.0008)</td>
<td></td>
</tr>
<tr>
<td>Litter Nitrogen change</td>
<td>0.0680 (0.0001)</td>
<td>0.0560</td>
<td>0.0940 (0.005)</td>
<td></td>
</tr>
<tr>
<td>Litter Microbial Carbon</td>
<td></td>
<td>0.0560</td>
<td>0.0940 (0.005)</td>
<td></td>
</tr>
<tr>
<td>Percent Litter Remaining</td>
<td>0.0370 (0.0021)</td>
<td>0.0450</td>
<td>0.0540 (0.0298)</td>
<td>0.0940 (0.0098)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Soil Responses</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration</td>
<td></td>
<td></td>
<td>0.0540 (0.001)</td>
<td></td>
</tr>
<tr>
<td>Soil Carbon</td>
<td>0.0670 (0.0433)</td>
<td>0.0868</td>
<td>(0.0068)</td>
<td></td>
</tr>
<tr>
<td>Soil Nitrogen</td>
<td>0.0460 (0.0271)</td>
<td>0.0810</td>
<td>0.0389 (0.0174)</td>
<td>(0.0411)</td>
</tr>
<tr>
<td>Soil Microbial Carbon</td>
<td>0.1200 (0.0002)</td>
<td>0.1680</td>
<td>0.1550 (0.0001)</td>
<td>(0.0080)</td>
</tr>
<tr>
<td>Soil Microbial Nitrogen</td>
<td>0.0990 (0.0023)</td>
<td>0.1440</td>
<td>(0.0006)</td>
<td></td>
</tr>
<tr>
<td>Soil Microbial C:N</td>
<td>0.0960 (0.0057)</td>
<td>0.2220</td>
<td>(0.0001)</td>
<td></td>
</tr>
<tr>
<td>Ammonium Availability</td>
<td></td>
<td></td>
<td>0.1870 (0.0227)</td>
<td></td>
</tr>
<tr>
<td>Soil pH</td>
<td>0.0790 (0.0102)</td>
<td>0.0660</td>
<td>0.0471 (0.0008)</td>
<td>(0.0470)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Litter Chemistry</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignin Content</td>
<td>0.0100 (0.0356)</td>
<td>0.0320</td>
<td>(0.0254)</td>
<td></td>
</tr>
<tr>
<td>Total Phenolics</td>
<td>0.0410 (0.0001)</td>
<td>0.0140</td>
<td>(0.0399)</td>
<td></td>
</tr>
<tr>
<td>Condensed Tannin</td>
<td>0.0780 (0.0001)</td>
<td>0.0850</td>
<td>0.0110 (0.0001)</td>
<td>(0.0331)</td>
</tr>
<tr>
<td>Litter C:N</td>
<td>0.0481 (0.0034)</td>
<td>0.0180</td>
<td>(0.0384)</td>
<td></td>
</tr>
<tr>
<td>Hydrolysable Tannin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Notes: Litter carbon and nitrogen change were calculated as (current concentration – initial concentration)/ (initial concentration). R-squared values are given for significant responses and $P$ values are reported directly below in parenthesis.
Table 2.2. Results of stepwise regression between litter chemistry values and nutrient dynamics in litter and soil.

<table>
<thead>
<tr>
<th>Litter response at time t+1</th>
<th>Litter chemistry at time t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignin Content</td>
<td>Total Phenolics</td>
</tr>
<tr>
<td>∆ Litter Carbon</td>
<td>0.0253</td>
</tr>
<tr>
<td></td>
<td>(0.0121)</td>
</tr>
<tr>
<td>∆ Litter Nitrogen</td>
<td>0.0193</td>
</tr>
<tr>
<td></td>
<td>(0.0045)</td>
</tr>
<tr>
<td>∆ Litter Microbial Carbon</td>
<td>0.0590</td>
</tr>
<tr>
<td></td>
<td>(0.0001)</td>
</tr>
<tr>
<td>∆ Percent Litter Remaining</td>
<td>0.1681</td>
</tr>
<tr>
<td></td>
<td>(0.0001)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Soils response at time t+1</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>∆ Respiration</td>
<td>0.0201</td>
</tr>
<tr>
<td></td>
<td>(0.0087)</td>
</tr>
<tr>
<td>∆ Soil Carbon</td>
<td>0.2056</td>
</tr>
<tr>
<td></td>
<td>(0.0001)</td>
</tr>
<tr>
<td>∆ Soil Nitrogen</td>
<td>0.0682</td>
</tr>
<tr>
<td></td>
<td>(0.0001)</td>
</tr>
<tr>
<td>∆ Soil Microbial Carbon</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>∆ Nitrate Availability</td>
<td>0.0450</td>
</tr>
<tr>
<td></td>
<td>(0.0001)</td>
</tr>
<tr>
<td>∆ Ammonium Availability</td>
<td>0.0125</td>
</tr>
<tr>
<td></td>
<td>(0.0037)</td>
</tr>
<tr>
<td>∆ Soil pH</td>
<td>0.0280</td>
</tr>
<tr>
<td></td>
<td>(0.0021)</td>
</tr>
</tbody>
</table>

Notes: $\Delta X$ was calculated as $\ln(X_{t+1}/X_t)$ where X is the variable of interest and $t=$time. Calculations were repeated for each replicate litter box across 4 time steps (0, 3, 6, 12, and 18 months) at 9 sites (n=360). The partial r-squared values are given for litter chemistry indices used in the final regression model. $P$ values are given in parenthesis directly below r-squared values.
Figure 2.1 Litter Carbon and Nitrogen Content. Changes in litter carbon and nitrogen were calculated as (current concentration - initial concentration)/(initial concentration).

The graph shows the response of each single phenotype litter and the mixed litter treatment, as well as the mean of the single phenotype treatments. Positive values indicate a net gain of carbon or nitrogen; negative values indicate a net loss. While there were significant differences among litter phenotypes ($P<0.0001$), they did not deviate in any predictable manner from the mixed litter treatment. The mean of 9 single litter treatments did not significantly differ from the mixed litter treatment. Bars are ±1 SE, n=9.
Figure 2.1

Change in Litter Nitrogen Concentration (%)

Change in Litter Carbon Concentration (%)

Litter Phenotype

- 3 month
- 6 month
- 12 month
- 18 month
**Figure 2.2 Soil Ammonium Availability.** Ammonium availability in the soil under single phenotype litters as measured with ion exchange resin bags. The graph shows the response of each single phenotype litter and the mixed litter treatment, as well as the mean of the single phenotype treatments. Ammonium in the soil varied under single phenotype treatments ($P<0.0001$), but did not deviate in any predictable manner from the mixed litter treatment. The mean of 9 single litter treatments did not significantly differ from the mixed litter treatment. Bars are ±1 SE, n=9.
Figure 2.2
CHAPTER 3
INTRASPECIFIC LITTER DIVERSITY AND NITROGEN DEPOSITION
AFFECT NUTRIENT DYNAMICS AND SOIL RESPIRATION¹

¹Madritch, M.D. and M.D. Hunter. Submitted to Oecologia 9/18/02
ABSTRACT: Anthropogenic forces are concurrently reducing biodiversity and altering terrestrial nutrient cycles. As natural populations decline, genetic diversity within single species also declines. The consequences of intraspecific genetic loss for ecosystem functions are poorly understood, and interactions among intraspecific diversity, nitrogen deposition, and nutrient cycling are unknown. We present results from an experiment that simulated both a decline in biodiversity and an increase in nitrogen deposition.

In soil microcosms, we tested effects of variation in intraspecific litter diversity and nitrogen deposition on soil respiration and nitrogen leaching. Increases in intraspecific litter diversity increased soil respiration overall, with the greatest increases in respiration occurring under high nitrogen deposition. Nitrogen deposition increased the amount of inorganic nitrogen leached, while the amount of dissolved organic nitrogen leached was correlated with initial litter chemistry (lignin concentration) and remained independent of litter diversity and nitrogen deposition treatments. Our results demonstrate the potential for losses in genetic diversity to interact with other global environmental changes to influence terrestrial nutrient cycles.

Key words: biodiversity, dissolved organic nitrogen, ecosystem functioning, litter quality, soil respiration
INTRODUCTION

Human activities drastically reduce biodiversity at various taxonomic levels (Purvis and Hector 2000). Although many reasons exist for the preservation of biodiversity, it is unknown whether biodiversity is essential for the maintenance of ecosystem functioning on a global scale (Loreau et al. 2001). Several studies have shown that some aspects of biodiversity, primarily species and functional group diversity, are indeed important for such ecosystem functions as primary production, nutrient cycling, as well as various measurements of stability (see Chapin et al. 2000, McCann 2000, and Loreau et al. 2001 for recent reviews). However, very little is known about how the reduction of diversity within single species can impact ecosystem functioning. Since reductions in intraspecific diversity usually precede species extinction as population sizes decline, and since human activities are known to reduce forest genetic diversity (Ledig 1992), it is imperative that we begin to look at intraspecific diversity and its relevance to ecosystem level processes.

In addition to reducing biodiversity, humans have also severely altered global nitrogen cycles (Norby 1998). Humans fix roughly 1.5 times as much atmospheric nitrogen as do natural systems (Kaiser 2001) and much of this inorganic nitrogen eventually returns to ecosystems through runoff and atmospheric deposition (Vitousek 1994). Detrimental effects of nitrogen deposition have been summarized by others (Vitousek 1994, Aber et al. 1998, Fenn et al. 1998) and include reduced biodiversity, restructured plant communities, decreased water quality, and acidified soils, among others. Since nitrogen deposition and reductions in biodiversity are happening in
concert, it is essential to understand how such global changes interact to affect ecosystem functioning (Bengtsson 1998).

In a previous field study, we explored the effects of reduced intraspecific diversity on ecosystem functioning in a turkey oak (*Quercus laevis*) sandhills community (Madritch and Hunter 2002). Reductions in the phenotypic and genetic diversity of turkey oak leaf litter significantly affected carbon and nitrogen cycles during decomposition. The mechanism appeared to be that genetically-mediated variation in litter chemistries, such as lignin and phenolics, affected litter decomposition. Similarly, Driebe and Whitham (2000) found that condensed tannin concentrations in cottonwood species and their hybrids differentially affect decomposition rates. Treseder and Vitousek (2001) also found that small genetic differentiation among populations of *Metrosideros polymorpha* can cause significant differences in nutrient cycling through litter quality. Since tannins are known to affect nitrogen cycling, and may shift soil nitrogen pathways from inorganic to organic (Northup *et al.* 1995), we hypothesized that genetically-mediated variation in litter chemistries may interact with nitrogen deposition to affect overall soil nitrogen retention. Using microcosms, we have expanded upon our previous field study to investigate how reductions in intraspecific diversity interact with nitrogen deposition to affect basic ecosystem processes, especially soil respiration and nitrogen retention.

**METHODS**

Soil and litter were collected from a 60-ha turkey oak (*Q. laevis*) stand located at the Savannah River Site, SC, USA. Soil was collected at 5 sites within the stand, then
bulked and passed through a 2-mm sieve. Litter from nine previously genotyped trees (Berg and Hamrick 1995) was hand-collected in November 2000 after senescence, but before abscission; turkey oak trees hold their leaves for some weeks after senescence. Although we have established previously that variation in litter chemistry among these nine *Q. laevis* trees has a strong genetic component (Madritch and Hunter 2002), replicate genotypes (clones) do not occur at our site. As such, our experiments test for effects of phenotypic diversity rather than genotypic diversity per se. Each microcosm consisted of 150 g of soil with a total of 2 grams of roughly ground litter and was incubated at 30° C and 90-100 % relative humidity for 60 days.

Random litter combinations of 0, 1, 3, and 6 genotypes were picked from the pool of 9 known genotypes as previously determined by 9 allozyme markers (Berg and Hamrick 1995). Regardless of the diversity treatment (1, 3, or 6), each microcosm contained a total of 2 grams of litter. Single phenotype treatments were replicated six times while the zero, three, and six genotype treatments were replicated 3 times. Each replication was a novel random selection of a genotype, or a mixture of genotypes. Identical genotype combinations within treatments were disallowed in an effort to test diversity itself, and not the identity of the litter treatments; the identity of the specific trees varied with each replication.

We increased the replicates of low diversity treatments in an effort to offset the sampling effect bias (Huston 1997). Briefly, the sampling effect is based on the fact that a functionally significant individual is more likely to be included in high diversity treatments than in low diversity treatments based on chance alone. The sampling effect has been cited as both a statistical error (Huston 1997) and as a valid mechanism by
which diversity affects ecosystem functioning (Tilman 1997). Although our design did not allow for statistical partitioning of selection effects as described by Loreau and Hector (2001), we attempted to minimize selection effect bias by increasing the number of low diversity treatments. For the same reason, we excluded a “9 phenotype” high diversity treatment, which would necessarily have included all individuals and could have been misinterpreted to show a false diversity effect.

Weekly additions of NH₄NO₃ solutions simulated 0, 15, and 30 kg N/ha/yr deposition rates. 30kg N/ha/yr is about the maximum level of nitrogen deposition in the southeastern U.S. according to review by Fenn et al. (1998). A factorial design crossed each diversity treatment with each nitrogen deposition treatment (1 phenotype*6 reps*3 N treatments + 3 phenotypes*3 reps*3 N treatments + 6 phenotypes*3 reps*3 N treatments + No litter*3 reps*3 N treatments= 45 total).

Respiration was measured at least every other day for 60 days using a PP System EGM-2 respiration meter with a modified soil chamber to fit microcosm openings. Soil cores were flushed weekly with 25 mL of DI water. Leachate from weeks 0, 2, 4, 6, and 9 were analyzed for mineral nitrogen using an Alpkem analyzer. Dissolved organic nitrogen (DON) was estimated using a persulfate digestion (Cabrera and Beare 1993), followed by subsequent analysis on an Alpkem analyzer. Leachate was also analyzed for dissolved organic carbon (DOC) using a Shimadzu TOC-500 analyzer. Denitrification rates were estimated at the end of the 2 month period. Headspace was sampled initially and 24 hr later for N₂O on a Varian 3000 gas chromatograph.

Litter phenolic chemistry (total phenolics, condensed tannins, hydrolysable tannins) was estimated using previously established techniques (Rossiter et al. 1998,
Hunter and Shultz 1995). Litter total carbon and nitrogen values were determined using a Carlo Erba NA 1500 CHN Analyzer (Carlo Erba Instrumentazione, Milan, Italy). Lignin content was measured using an A200 Fiber Analyzer (ANKOM, Fairport, New York, USA).

Data were non-normal, and were consequently analyzed with generalized linear models and Spearman correlations (Proc Genmod and Corr, SAS Inst. 1998), as repeated measures ANOVA’s and regressions are inappropriate for non-normal data (Sokal and Rohlf 2001). When testing for an effect of phenotype diversity, zero litter treatment controls were excluded from the generalized linear models. Final models were selected by back-stepping from full models to maximize log likelihood ratios (Agresti 1996).

RESULTS

Soil respiration increased with intraspecific litter diversity \( \chi^2 = 9.62, \text{d.f.} = 2, P = 0.0082 \), Figure 3.1). There was also a significant date*diversity*nitrogen deposition effect \( \chi^2 = 47.26, \text{d.f.} = 6, P < 0.0001 \), Figure 3.2), but no effect of nitrogen deposition alone \( \chi^2 = 2.38, \text{d.f.} = 2, P = 0.3049 \). Figure 3.2 shows that high diversity litter treatments respired most under the heavy, 30 kg N/ha/yr, nitrogen deposition treatment. Under the 0 kg N/ha/yr treatment, high diversity treatments respired similarly to low diversity treatments (Figure 3.2).

Not surprisingly, denitrification and the amount of dissolved inorganic nitrogen (DIN) leached both increased with nitrogen deposition \( \chi^2 = 6.97, \text{d.f.} = 2, P = 0.0307 \), and \( \chi^2 = 47.01, \text{d.f.} = 2, P < 0.0001 \), respectively). Nitrogen deposition also decreased the relative contribution of DON to the total amount of nitrogen lost \( \chi^2 = 10.99, \text{d.f.} = 2, P = 0.0001 \).
0.0041), but simply because DIN leachate increased. However, there were no other significant effects of diversity or nitrogen treatment on the amount of nitrogen or carbon leached or on denitrification rates. Ammonium accounted for the majority of DIN in leachate, regardless of diversity or nitrogen treatment (roughly 99% or DIN was ammonium, data not shown). Since neither DON nor DOC were affected by nitrogen deposition, we averaged those treatments when searching for mechanisms to explain observed diversity effects (below).

There were no effects of diversity on the starting values of litter chemistry (lignin content, C:N ratio, condensed tannin content, hydrolysable tannin content, or total phenolic content). However certain litter traits were correlated strongly with carbon and nitrogen fluxes. The average amount of DON leached from soil increased strongly with lignin content ($r^2 = 0.65, P = 0.0016$, Fig. 3.3) while average soil respiration decreased with lignin content ($r^2 = 0.40, P = 0.0261$, Fig. 3.3). Average soil respiration also decreased with hydrolysable tannin content ($r^2 = 0.337, P = 0.0479$, Fig. 3.4). No other relationships between litter chemistries and nitrogen or carbon fluxes were found. Since lignin measurements are sometimes thought to be confounded by high tannin concentrations (Hammel 1997), we tested for correlations between total phenolics, condensed tannins, and hydrolysable tannins and lignin content and found none ($P>0.1$ in all cases).

**DISCUSSION**

Our results demonstrate that intraspecific diversity of leaf litter is positively related to soil respiration. In general, biodiversity has been thought not to affect soil
respiration because so many microorganisms perform that function (Schimel 1995). However, in accordance to the complementary niche hypothesis (Tilman et al. 1997), more diverse litters may have provided substrate for a more diverse assemblage of metabolic pathways which may have led to an increase in soil respiration. Magill and Aber (2000) suggested a similar effect of species diversity during decomposition, whereas others have reported no change in respiration in response to diversity treatments (McTiernan et al. 1997, Maly et al. 2000, Ekschmit et al. 2001).

The effects of intraspecific litter diversity on soil respiration were most pronounced under high levels of nitrogen deposition (Figure 3.2). The interactive effects of nitrogen deposition and litter diversity may explain why some studies have failed to find an effect of diversity on respiration (McTiernan et al. 1997, Maly et al. 2000, Ekschmit et al. 2001). In treatments without added nitrogen, microbes may have been nitrogen limited and unable to take advantage of diverse litter substrate. Under higher nitrogen deposition, microbes may have been released from nitrogen limitation and able to take advantage of more diverse substrate. This may point to a relatively unexplored facet of the complementary niche theory. In this case, it seems that the addition of a limiting nutrient (nitrogen) allowed the complementary niche mechanism to function. Changes in resource availability may influence how biodiversity affects ecosystem function.

Contrary to our previous field results (Madritch and Hunter 2002), there were no effects of intraspecific litter diversity on nitrogen fluxes. The amount of inorganic nitrogen leached increased with nitrogen deposition treatment as expected, but was unaffected by diversity treatments. The amount of DON leached was independent of
diversity and nitrogen deposition treatments. Total nitrogen leached remained unaffected by nitrogen deposition, probably since most (63%) of the nitrogen leached was organic. Similar to Magill and Aber (2000), we found that the relative amount of DON in leachate decreased slightly with nitrogen deposition. Perakis and Hedin (2002) suggest that nitrogen deposition in temperate regions has shifted the form of nitrogen loss towards dissolved inorganic nitrogen, whereas tropical systems remain dominated by DON loss. However, Qualls et al. (2000) report DON as the major form of nitrogen loss through leaching in temperate systems also.

Previous work (Madritch and Hunter 2002) has shown that genetically-mediated variation in litter chemistries can differentially affect nutrient cycling during decomposition. Following this lead, we searched for diversity affects on litter chemistries and for correlations between litter chemistries and soil respiration and nutrient retention. We found no effect of intraspecific litter diversity on any litter chemistry indices measured. However both lignin and hydrolysable tannin content were negatively correlated with soil respiration ($r^2 = 0.64, P=0.0261$ and $r^2 = 0.58, P= 0.048$, respectively). We found a strong positive correlation between lignin content and the amount of DON leached from the soils ($r^2 = 0.65, P= 0.0016$), whereas similar work by Magill and Aber (2000) found a strong positive correlation between DOC released and initial litter lignin content. No other phenolic indices proved to be correlated to any measured aspect of soil respiration or nutrient retention. Nor were there any correlations between lignin and any of the phenolic measurements, suggesting that DON release is under partial control by litter lignin and is not confounded by potential errors in lignin content analysis. Low lignin litters may permit the growth or activity of microbes which
retain DON, whereas high lignin litters may inhibit microbial use of DON and/or immobilize inorganic nitrogen, resulting in higher amounts of DON loss.

Our measurements of soil respiration lead us to conclude that reductions in intraspecific diversity have the potential to interact with other facets of global change to affect ecosystem processes. Similarly, Reich et al. (2001) showed that plant species diversity interacts with elevated carbon dioxide and nitrogen deposition to increase total plant biomass. Our previous field study (Madritch and Hunter 2002) demonstrated that intraspecific diversity can influence ecosystem processes. Here, we show that intraspecific diversity can interact with nitrogen deposition to affect belowground processes. As previously stated by other workers (Qualls et al. 2000, Perakis and Hedin 2002), nitrogen deposition appears to decrease the relative amount of DON in soil leachate. In our study, initial litter lignin content had more influence over DON than did litter diversity or nitrogen deposition. Lignin, as well as hydrolysable tannin, content also affected soil respiration. However, neither of these litter chemistry indices were affected by diversity treatments. The direct effects of phenotypic diversity on soil respiration observed here (Figures 3.1 and 3.2) appear to be independent of the indices of litter chemistry that we measured. Despite an increasing frequency of biodiversity and ecosystem function studies, much more work is needed to examine a) effects of genetic biodiversity, and b) interactions between biodiversity and other global biogeochemical changes (Bengtsson 1998).
ACKNOWLEDGMENTS

This work was supported by grants from the Andrew W. Mellon Foundation and the National Science Foundation (Grants DEB-9906366 to M.D.H. and DEB-0104804 to M.D.M.). We also thank the Savannah River Site for site use permission and M. Cabrera, D. Coleman, J. Hamrick, P. Hendrix, R. Pulliam, R. Sharitz, B. Nuse, and J. Rogers for comments and/or laboratory assistance. Two anonymous reviewers greatly improved a previous version of this manuscript.

LITERATURE CITED

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SAS version 8.2 for Windows TS2M0. 1998. SAS Institute, Inc. Cary, NC.


**Figure 3.1.** Effects of intraspecific litter diversity on soil respiration. Respiration increased with intraspecific diversity ($\chi^2 = 9.62$, d.f. = 2, $P = 0.0082$). Data are averages across all N deposition treatments and replicates. Bars represent +/- 1 S.E.
Figure 3.1

![Bar chart showing the respiration (g CO₂/m²/hr) for different numbers of phenotypes.](image)

- Number of Phenotypes: 1, 3, 6
- Respiration: 0.35, 0.37, 0.41, 0.43, 0.45, 0.47, 0.49, 0.51, 0.53

**P** = 0.0082
Figure 3.2. Interactive effects of intraspecific litter diversity and nitrogen deposition on soil respiration. Nonparametric analysis of non-normal respiration data shows a significant date*diversity* N deposition effect ($\chi^2 = 47.26$, d.f. = 6, $P < 0.0001$) indicating that intraspecific diversity and nitrogen deposition interact to effect ecosystem function. Intraspecific diversity increased soil respiration under high nitrogen treatment. Data are averages of 6 (single phenotype) or 3 (3 and 6 phenotypes) replicates.
Figure 3.2

Date * diversity * N deposition effect P <0.0001
**Figure 3.3.** The effects of litter lignin concentration on A) DON leached and B) CO₂ content \( (r^2 = 0.65, P = 0.0016) \). The average amount of soil respiration decreased with initial lignin content \( (r^2 = 0.40, P = 0.0261) \). Each point represents the mean of 3 N deposition treatments.
Figure 3.4. The effect of litter hydrolysable tannin concentration on CO$_2$ resired from soil microcosms. The average amount of soil respiration decreased with initial hydrolysable tannin content ($r^2 = 0.34, P = 0.0479$). Each point represents the mean of 3 N deposition treatments.
Figure 3.4

![Graph showing the relationship between % Hydrolysable Tannin and Avg Respiration (g CO2/m²/hr).](image-url)
CHAPTER 4
PHENOTYPIC DIVERSITY AFFECTS NUTRIENT CYCLING
THROUGH VARIATION IN LITTER CHEMISTRY\textsuperscript{1}

\textsuperscript{1}Madritch, M.D. and M.D. Hunter. To be Submitted to Oecologia.
ABSTRACT: Habitat fragmentation and declining population sizes lead to losses in the intraspecific genetic diversity of forest species. While reductions in genetic diversity are known to affect population processes, we have shown previously that reductions in intraspecific diversity of a forest tree, *Quercus laevis*, also have the potential to affect ecosystem functioning through leaf litter decomposition. We expand upon our previous short-term experiment to consider the longer-term impact of phenotypic diversity on ecosystem processes. Specifically, we investigate the effects of litter phenotypic diversity on carbon and nutrient fluxes in the litter and soil, and on populations of microarthropods. Over a three year period, reductions in litter diversity significantly affected carbon and nitrogen fluxes. Fluxes within single phenotype treatments were significantly, but unpredictably, different from a mixed phenotype treatment. Fluxes were associated with variation in litter chemistry which has a strong genetic component. We found no effects of phenotype diversity on soil microbial or microarthropod communities. However, litter diversity effects upon carbon and nitrogen fluxes support our previous suggestion that losses in genetic diversity may influence ecosystem processes.

**Key words:** biodiversity, ecosystem functioning, intraspecific diversity, decomposition, nutrient cycling, phenotypic diversity, litter chemistry, tannin
INTRODUCTION

Humans continue to dominate an increasing percentage of the earth’s surface and resources (Vitousek et al. 1997). One consequence of this human domination is a 100-1000 fold increase in species extinction rates above background levels (Pimm et al. 1995). This current decline in biodiversity, and the promise of further losses in the future, is of major ecological concern for several reasons. Two primary concerns are the loss of evolutionary potential (Woodruff 2001) and the alteration of basic ecosystem functioning (Ehrlich and Ehrlich 1981).

The current state of biodiversity and ecosystem functioning research has been reviewed by several authors (e.g., Chapin et al. 2000, McCann 2000, and Loreau et al. 2001). In general, biodiversity is an important determinant of ecosystem functioning, but the exact relationship varies with the type of system studied and the taxonomic level of biodiversity altered. In particular, belowground responses to changes in biodiversity are highly variable (Loreau et al 2001). For instance, increasing plant litter diversity may increase (Blair et al. 1990), decrease (McTiernann et al. 1997), or randomly affect litter nitrogen release (Wardle et al. 1997). Likewise, soil respiration can respond positively (Briones and Ineson 1996, McTiernann et al. 1997), randomly (Chapman et al. 1988), or not at all (Bardgett and Shine 1999) to increased litter diversity. The mechanisms through which litter diversity may affect ecosystem functioning are not clearly understood. Litter chemistry can be important (Treseder and Vitousek 2001, Madritch and Hunter 2002), as well as soil fauna and flora including microbial (Degens 1998) and microarthropod communities (Hansen and Coleman 1998). Although not major constituents of soil fauna biomass, microarthropods can make large contributions to
nutrient cycles via microbial grazing (Coleman and Crossley 1996) and have been shown
to increase in diversity with plant litter diversity treatments (Hansen 2000).

Previous biodiversity and ecosystem functioning studies have focused primarily
on the effects of species diversity. However, one consequence of human land use is a
reduction in plant and animal population sizes and, in some cases, the extinction of some
species. Since intraspecific genetic diversity is proportional to population size, declines
in genetic diversity precede species extinctions (Lande 1999). Ledig (1992) reviewed the
particularly drastic reduction of genetic diversity in forest ecosystems caused by
anthropogenic forces. Despite trends of declining genetic diversity in natural
populations, very little research has focused on the importance of intraspecific diversity
to ecosystem functioning. While we know that intraspecific genetic diversity can
potentially be important for species viability, pathogen resistance, and overall fitness (see
Amos and Balmford 2001 for a review), we know very little about how losses in genetic
diversity affect large-scale ecosystem functions such as carbon and nutrient cycling.

Intraspecific diversity in forest ecosystems may affect nutrient cycling through
leaf litter decomposition. Roughly ninety percent of all primary production enters the
detrital pathway (Coleman and Crossley 1996), and much of this is through leaf fall in
deciduous forests. Many of the leaf litter chemistry traits that have large impacts on
ecosystem processes are genetically-mediated. Polyphenolics, for instance, are
particularly relevant to ecosystem functioning because they are the most widely
distributed class of secondary metabolites and interact strongly with several aspects of
carbon and nitrogen cycling (Haettenschwiler and Vitousek 2000). Some polyphenolics
remain biologically active after leaf senescence, and in general, depress decomposition
rates and retard carbon and nitrogen mineralization (Horner et al. 1988). Polyphenolic concentrations can also vary widely within a single species due, in part, to genetic differences (Hunter and Hull 1993). For instance, after surveying all known tree species of the *Leucaena* genus grown in a common garden, Dalzell and Shelton (2002) found significant variation in the production of condensed tannins, both among species of the same genus (0-336 g tannin/ kg litter) and within single species (145 fold difference in total condensed tannin content for *L. trichandra*). Several studies also demonstrate genetically-mediated polyphenolic variation in oak species (Rossiter et al. 1988, Klaper et al. 2001). Other litter chemistry traits such as lignin content (Meentemeyer 1978) and C:N ratio (Melillo et al. 1982) also influence nutrient cycling (Horner et al. 1988) and can vary within a single species.

The genotypic identity of leaf litter has been linked to nutrient cycling previously by Driebe and Whitham (2000) and Treseder and Vitousek (2001). In two cottonwood species and their hybrids, genetically-mediated variation in condensed tannin concentrations differentially affected in-stream litter decomposition rates (Driebe and Whitham 2000). Likewise, genetically distinct populations of *Metrosideros polymorpha* trees exhibited considerable variation in genetically-mediated litter chemistries important to decomposition and nitrogen cycling (Treseder and Vitousek 2001). Since differences in genetically-mediated litter chemistries can affect nutrient cycling, it follows that a loss in genetic variation in litter traits important to decomposition could affect nutrient and energy cycling.

We have shown previously that a loss in intraspecific diversity of turkey oak (*Querce laevis*) litter can have ecosystem consequences during litter decomposition
(Madritch and Hunter 2002). Our initial results covered an 18 month period and it was unclear whether or not long term effects on nutrient cycling would persist. In addition, it was unclear whether effects of litter phenotypic diversity were due solely to variation in litter chemistry, or due to associated variation in the soil microbial and/or microarthropod communities. Here, we present nutrient cycling data from a three-year litter decomposition experiment in which we varied the intraspecific diversity of *Q. laevis* litter. We also analyze the microbial and microarthropod communities relevant to ecosystem functioning.

**METHODS**

During leaf fall in 1998, we collected leaves from 9 individual *Q. laevis* trees (phenotypes) and established a decomposition study at the Department of Energy’s Savannah River Site (SRS) in Aiken, SC. *Q. laevis* is endemic to the southeastern U.S. coastal plain, often growing on poor, sandy soils. Our field site is a 60-hectare old growth stand of *Q. laevis*, (85% canopy cover) and long leaf pine, *Pinus palustris*, (10% canopy cover) and lies on a gently sloping (5% grade) south-facing sandhill undisturbed since 1943 according to aerial photographs. The 9 individuals were chosen randomly from a pool of 1,572 adult *Q. laevis* trees previously genotyped by Berg and Hamrick (1994) using 9 polymorphic allozyme loci. Since we were unable to locate replicate clones using microsatellite markers (Klaper *et al.* 2001), we can only interpret results as effects of phenotypic diversity and not genetic diversity per se. All litter was collected by hand from known trees after senescence but before leaf-fall; turkey oaks generally hold their leaves for some weeks after senescence.
Following litter collection, we established sets of 10 litter boxes, 9 with single phenotypes and 1 with an equal mix of all 9, at each of 9 sites within a 3.2-ha plot (n=90 boxes total). Litter boxes were open-bottomed, meter square boxes covered with vinyl coated hardware mesh to exclude non-treatment litter. Ten litterbags, 1 mm² nylon mesh and containing 10 g of dry litter were introduced into each box at the start of the experiment. We added 150 g of loose litter to each box (equivalent to average litter fall per m²) at the beginning of the study and at the beginning of the second and third years. The loose litter added was the same phenotype (or a mix of all 9) as in the litterbags in each box. Bags of litter were therefore imbedded within boxes of litter of the same phenotype.

After an initial three month collection, litter bags were collected each summer and winter (3, 6, 12, 18, 24, 30, and 36 months) and analyzed for carbon, nitrogen, litter chemistry, and microbial carbon content. Bags were also removed for microarthropod extractions using Tullgren funnels. On the first four collection dates, a single litter bag was used for all analyses, while two bags were removed on each of the 3 remaining collection dates, one for microbial analysis, and one for microarthropod and chemical analysis. Since litter mass declines over time, it was necessary to collect two bags during the last three collection periods to ensure ample litter material for proper analysis. Soil samples were taken on each collection date using a 2 cm diameter, 10 cm long soil corer at three different locations within each box. Soil cores were divided into 0-5 cm and 5-10 cm depth categories, coinciding roughly with organic and mineral soil layers. All three cores were then bulked, mixed and sieved of root matter before analyses for total carbon and nitrogen, microbial carbon, microbial nitrogen, and pH measurements. Soil
moisture was also monitored every 3 months, and soil temperature measured hourly by HOBO data loggers at each of the 9 sites.

Bacterial diversity was estimated at the end of year two using Biolog ECO microplates which estimate bacterial diversity by measuring microbial catabolism of 32 different carbon substrates. Functionally different bacterial communities have different patterns of substrate use as indicated by a colorimetric reaction in 96-well microtiter plates. We extracted soil bacterial communities and followed dilution protocols of Zak et al. (1994) and recorded plate absorbances every 12 hours for 72 hrs at 550nm. At the end of year three, we extracted soil microbial DNA using Qiagen DNeasy kits and employed T-RFLP techniques (Liu et al. 1997) to estimate microbial diversity. PCR products were digested with the restriction enzymes CfoI and HaeIII, and final product lengths and quantities were determined by electrophoresis with an ABI 310 automated DNA sequencer.

Soil microarthropods were collected in modified Tullgren funnels (Mallow and Crossley 1984) and stored in 70% ethanol. We sorted microarthropods into three suborders of Acari (Oribatida, Asitgmata, and Prostigmata), the order Collembola, and “others”. Microbial biomass carbon for both the soil and litter was determined by fumigation extraction (Ross and Sparling 1993) using published $k_{ec}$ values (Voroney et al. 1993). Microbial biomass nitrogen for the soil was determined by fumigation extraction (Vance et al. 1987) followed by persulfate digestion (Cabrera and Beare 1993) using published $k_{em}$ values (Voroney et al. 1993). Soil respiration was monitored monthly in each box with a portable infrared gas analyzer (EGM-2 PPM Systems).

Litter total phenolic, condensed tannin, and hydrolysable tannin contents were
estimated using previously established techniques (Rossiter et al. 1988, Madritch and Hunter 2002). Litter and soil total carbon and nitrogen values were determined using a Carlo Erba NA 1500 CHN analyzer. Litter lignin content was measured using an ANKOM automated fiber analyzer. Ion exchange resin bags were used to estimate nitrate and ammonium availability in the soil (Binkley 1984). Two resin bags were buried at a depth of 8 cm within each box and left in the field for approximately 3-month intervals. Resins were extracted in 1M KCl and analyzed for NH$_4^+$ and NO$_3^-$ using an Alpkem RFA 300 analyzer.

**Statistics**

Assumptions of normality were tested for all data using a Shapiro-Wilk W test and non-normal data were transformed as necessary. We used repeated measures ANOVA procedures to estimate phenotypic effects over time using the 9 sites as replicates while local environmental effects were estimated using the 9 phenotypes as replicates. By comparing the variance explained by phenotype and site, we were able to estimate the relative contributions of litter phenosytype and local environment to variation in decomposition and nutrient fluxes. The mixed litter treatment served as a reference with which to compare single phenotype litters. To test for non-additive effects of mixing litter phenotypes, we compared the mixed litter treatment responses to the mean response of the 9 single phenotype litters using repeated measures ANOVA procedures. Decomposition experiments are not amenable to robust partitioning of sampling and complementarity effects as is possible for aboveground biomass production (Loreau and Hector 2001). Therefore, we compared the mean of the single phenotypes
(expected response) with the mixed litter treatment (observed response), similar to analysis suggested for decomposition experiments by Loreau (1998) and demonstrated by Wardle et al. (1997).

When estimating the effects of litter chemistry on carbon and nitrogen fluxes, we accounted for changes in litter chemistry over time by employing methods similar to the analyses of population time series (Royama 1992, Berryman 1999). Briefly, we assumed that the rate of change in nutrient concentration between time \( t \) and \( t+1 \) would be related to the chemistry of litter at time \( t \). We therefore calculated the change in the nutrient under consideration (X) as \( \ln \left( \frac{X_{t+1}}{X_t} \right) \), repeated this for each of the time steps (0, 3, 6, 12, 18, 24, 30, and 36 months), combined data across all sites, and performed a stepwise multiple regression between the nutrient flux of interest and our estimates of litter chemistry (Berryman 1999, Madritch and Hunter 2002).

Biolog ECO-plate absorbance data were analyzed using principal components analysis (PCA, PCord v. 4). Axes with broken-stick eigenvalues less than the actual eigenvalues were kept for further analysis (Jackson 1993). Litter boxes were plotted along the retained axes and subsequent MANOVA procedures (Proc GLM, SAS v. 8) were used to test for significant site and phenotype effects. Microarthropod data were non-normal and therefore analyzed using non-metric multidimensional scaling (NMS, PCord v. 4). NMS provides a more robust analysis of non-normal, heavily skewed data, whereas PCA is preferred for data sets that approach normality (McCune and Grace 2002). MANOVA procedures (Proc GLM, SAS v. 8) were also used to test for site and phenotype effects on NMS ordinations. T-RFLP results were analyzed using Genescan 3.1.2. software. Electropherograms of T-RFLP data indicate microbial diversity by the
number and size of fragment groups after restriction enzyme digestion, while population sizes of each group are indicated by relative peak height. We tested for litter phenotype effects on soil microbial diversity by overlaying electropherograms from different litter phenotype treatments and searching for differences in peak location and peak height.

Given that variation in litter phenotype has both genetic and environmental components (Klaper and Hunter 1998), we previously used allozyme data (Berg and Hamrick 1994) and a Mantel test (Mantel 1967) to estimate the contribution of tree genotype to variation in litter phenotype (Madritch and Hunter 2002). The genetic distance matrix consisted of 8 published allozyme markers (Berg and Hamrick 1994), while the litter chemistry matrix included day zero estimates of condensed tannin, hydrolysable tannin, phenolics, lignin, and C:N ratio. This prior analysis is also relevant to data presented here, and shows that 34% of the variation in litter chemistry can be explained by genetic variation among phenotypes (Madritch and Hunter 2002).

**RESULTS**

Changes in the concentration of litter nitrogen and carbon over time were dominated by phenotype effects ($P<0.0001$) and unrelated to the site of decomposition (Table 4.1, Figure 4.1). Litter mass loss over time was affected by both litter phenotype treatment and site location (Table 4.1), with marked differences in decomposition rates among phenotype treatments (Figure 4.2). The amount of microbial biomass present in the litter was affected only by site and not by phenotype treatment (Table 4.1).

Soil ammonium availability, as measured by resins bags, also experienced significant phenotype effects over time (Table 4.1, Figure 4.3). However, other soil
responses including respiration, percent carbon and nitrogen, microbial biomass, microbial nitrogen, nitrate availability, and pH were all dominated by site effects (Table 4.1). Neither soil temperature nor soil moisture varied by litter treatment or site (P>0.05, data not shown).

Whereas litter nutrient responses were dominated by phenotype effects, and soil responses were dominated by site effects, all indices of litter chemistry during decomposition were affected by both litter phenotype and site location, with the exception of litter C:N ratio which was only affected by litter phenotype (Table 4.1).

Nutrient fluxes in the litter and soil of single phenotype treatments often differed from the mixed litter treatment (Figures 4.1-4.3). However, there were no significant differences between the mean of single phenotype responses and the mixed litter response for metrics reported in Table 4.1 (all P>0.5). Thus, we found no long-term, non-additive effects of phenotype diversity on litter or soil nutrient dynamics.

Soil microbial and microarthropod communities were unaffected by litter phenotype treatment, but significantly influenced by site. PCA analysis of Biolog ECO-plate absorbance data yielded two axes which described significant differences among sites but no effects of litter phenotype treatments (MANOVA P=0.0459 and P>0.05, respectively, Figure 4.4). Likewise, T-RFLP representative electropherograms of soil microbial profiles did not differ significantly among litter phenotype treatments (Figure 4.5). Similar to PCA analysis of microbial communities, NMS analysis of microarthropod data averaged over time showed significant differences among sites, but no effect of litter phenotype treatments (MANOVA P<0.0001 and P<0.05, respectively,
Figure 4.6). The average counts of microarthropods over the 3 year period are given in Table 4.2.

Table 4.1 shows that all litter chemistries were affected by litter phenotype, and previous work estimated that 34% of the variation in initial litter chemistry was due to variation in genotype (Madritch and Hunter 2002). We hypothesized that genetically-mediated variation in litter chemistries would influence nutrient cycles, and therefore performed stepwise multiple regressions of litter chemistries and changes in nutrient concentrations. Because microarthropod communities can also influence nutrient dynamics (Blair et al. 1992), we included the microarthropod community NMS axes of each collection date as independent variables in the stepwise regression analysis. Microbial diversity was not repeatedly sampled over time and was therefore excluded from the stepwise regression analysis.

All litter nutrient fluxes were influenced by litter chemistries as indicated by stepwise regression results (Figure 4.7). Litter total phenolic concentrations had the most influence over nutrient dynamics in the litter, and both hydrolysable tannin concentrations and C:N ratios also exhibited strong influence over litter carbon and nitrogen change, respectively (Figure 4.7). Similarly, all nutrient fluxes in the soil, with the exception of soil carbon change at 5-10cm, were affected by litter chemistries (Figure 4.8). Again, litter phenolic concentrations were most influential on soil nutrient dynamics in general, and litter lignin concentration was strongly correlated with soil pH change (Figure 4.8). Microarthropod communities only slightly influenced changes in soil nitrogen, soil microbial carbon and soil pH, all at the 5-10 cm depth (Figure 4.8).
DISCUSSION

Our data indicate that the phenotypic diversity of leaf litter can influence long-term carbon and nitrogen fluxes during decomposition. The transfer of carbon and nitrogen to and from litter, litter decomposition rate, and the availability of ammonium in the soil beneath litter treatments, were all significantly affected by litter phenotype treatment (Figs 4.1-4.3). Variation in litter chemistry among phenotype treatments seemed to play a major role in ecosystem responses, as stepwise regression results showed that litter chemistry was important to virtually all measured aspects of nutrient cycling (Figs 4.7-4.8). Repeated measures ANOVA showed significant phenotype effects on all litter chemistries and previous work estimated that 34-40% of the variation in chemical phenotype is due to genetic variation among individual trees (Madritch and Hunter 2002, Klaper and Hunter 1998). Thus, phenotypic litter diversity is most likely affecting nutrient dynamics via genetically-mediated variation in litter chemistries.

Several researchers have found evidence that microbial communities can adapt to the quality of leaf litter input (McClaugherty et al. 1985, Hunt et al. 1988, Clein and Schimel 1995). However, we found no effects of litter phenotype on microbial community diversity (as measured by Biolog and T-RFLP assays) (Figures 4.4-4.5). Instead, only strong site effects were detected by the Biolog assay. Both of the microbial analyses we used were limited to bacterial diversity, and it is possible that fungal communities played major roles during decomposition. Unfortunately our attempts to use T-RFLP assays with fungal DNA were unsuccessful. Though microarthropod communities are also known to shift with changes in leaf litter species diversity (Hansen 2000), in our study, microarthropod community structure was influenced only by site and
not by litter phenotype (Figure 4.6). In general, soil fauna was not affected by litter phenotype, nor was variation in the community composition of either bacterial or microarthropod communities responsible for differences in nutrient cycling.

Given that nutrient cycling differed among litter phenotypes whereas the community structure of biota did not, it is likely that the same biotic community was sufficiently flexible to process variable litter inputs. While essential for decomposition and nutrient cycling during leaf litter decomposition, soil microbial communities may be fairly redundant and even simple communities appear able to process most litter (Andren et al. 1995, Lawton et al. 1996, Wardle et al. 1997). However, our community analyses were fairly coarse, well above the species resolution level, and it is possible that our inability to detect community changes at a finer taxonomic resolution precluded detection of litter diversity effects on soil biota.

Litter chemistries are known to be major drivers of terrestrial nutrient cycles (Haettenschwiler and Vitousek 2000) and genetic variation in the concentration of secondary metabolites in leaf litter has been cited by others as a potential determinant of ecosystem functioning. For instance, Driebe and Whitham (2000) showed that genetically-mediated variation in condensed tannin concentrations in two cottonwood species and their hybrids affected both terrestrial and aquatic decomposition rates. Likewise, genetically differentiated M. polymorpha populations grown in a common garden exhibited variation in litter lignin and nitrogen content that predicted differences in overall decomposition rates (Treseder and Vitousek 2001).

In our study, litter chemistry influenced virtually all measured aspects of carbon and nitrogen flux, as well as soil properties such as soil pH, both at the soil surface and
below the organic layer over a three year period. The concentration of total phenolics in litter had a particularly strong influence over both litter and soil nutrient dynamics (Figures 4.7-4.8). Negative partial R-squared values indicate either nutrient loss, or a decrease in the rate of nutrient gain, with increasing litter chemistry concentration. In the litter, phenolics may have decreased the accumulation rate of microbial carbon, and therefore decreased the amount of nitrogen and carbon imported into the decaying litter (Figure 4.7). This also coincides with an overall decrease in decomposition rate, indicated by the positive correlation between litter phenolic concentration and litter mass remaining (Figure 4.7). In the soil, lignin content had a particularly large, negative influence on soil pH (Figure 4.8), presumably through the activity of acidic phenol groups. Other relationships among litter chemistries and litter and soil nutrient dynamics were not as amenable to explanation. Nonetheless, while the precise mechanisms may vary, litter chemistry metrics as a whole explained a large amount of variation in litter and soil nutrient dynamics.

Individual phenotype litter treatments elicited different ecosystem responses than did the mixed litter treatment. These differences were sometimes large, but always unpredictable. Thus, losses in intraspecific diversity may have significant, yet unpredictable effects on basic ecosystem functions. Contrary to our short-term study (Madritch and Hunter 2002), no non-additive effects of litter diversity were detected on any aspect of nutrient cycling. Here, phenotypic diversity affected decomposition and nutrient cycling because of individual litter identities and no evidence was found for non-additive effects. As such, we found no evidence of niche complementarity (Tilman et al. 1996), but rather idiosyncratic effects which appeared to be the result of selection effects
Similar idiosyncratic effects of species litter diversity have also been reported (Wardle et al. 1997), while others have failed to find any effects of litter diversity on ecosystem functioning (Hector et al. 2000).

While we can be certain that the trees from which we collected litter were of different genotypes (Berg and Hamrick 1994, Klaper et al. 2001), we were unable to find replicate clones of these genotypes within the stand. Consequently, interpretation of our results is limited to a discussion of phenotypic effects. Nonetheless, previous work on the same stand of Q. laevis used here (Klaper and Hunter 1998, Klaper et al. 2001, Madritch and Hunter 2002) and work on other species (Driebe and Whitham 2000, Treseder and Vitousek 2001) has shown a genetic component to phenotypic variation in litter chemistries important to decomposition. However, if different tree genotypes establish preferentially under specific soil nutrient conditions, soil chemistry could be the sole cause of variation in litter chemistries. We have previously addressed this issue (Madritch and Hunter 2002) and concluded that a lack of soil nutrient patterns (Klaper et al. 2001), the small-scale random dispersal of adults (Berg and Hamrick 1994), and the random selection of individuals for inclusion into our experiment effectively eliminate the possibility that all litter chemistry differences were caused only by growing environment. Therefore, our results are relevant to the current decline of genetic diversity within forest ecosystems (Ledig 1992).

Intraspecific litter diversity can influence carbon and nitrogen fluxes, and the effects shown here are the result of litter identity. In addition, differences in ecosystem functioning were attributable only to variation in litter chemistry and not any induced changes to microbial or microarthropod communities. Leaf chemistries have long been
recognized as important to plant-herbivore interactions, but increasing evidence suggests they play an equally important role in nutrient cycling (Haettenschwiler and Vitousek 2000). Our work suggests that genetically-mediated variation in secondary metabolites can have important ecosystem consequences when intraspecific diversity is lost. In the turkey oak sandhills system, sufficient variation exists within a single species such that losses in intraspecific diversity elicit ecosystem responses. Whether speciose communities respond likewise remains to be seen. However, without knowing exactly when and where intraspecific diversity is important to ecosystem functioning, it would seem prudent to include the maintenance of intraspecific diversity within conservation plans.

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Table 4.1. Repeated-measures ANOVA of site and phenotype effects on litter and soil responses.

<table>
<thead>
<tr>
<th>Litter Responses</th>
<th>Phenotype</th>
<th>Date x phenotype</th>
<th>Site</th>
<th>Date x site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter carbon change</td>
<td>0.2871</td>
<td>0.0368</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td>0.0408</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Litter nitrogen change</td>
<td>0.0404</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Litter microbial carbon</td>
<td></td>
<td></td>
<td></td>
<td>0.2768</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Percent Litter Remaining</td>
<td>0.0201</td>
<td>0.0215</td>
<td>0.0088</td>
<td>0.0179</td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td>0.0093</td>
<td>0.011</td>
<td>0.0278</td>
</tr>
</tbody>
</table>

| Soil Responses                    |           | 0.065           | 0.0826|               |
|                                   |           |                  | 0.0282| 0.0101       |
| Soil carbon 0-5 cm                |           | 0.0827          | 0.0826|             |
|                                   |           | 0.0282          | 0.0101|             |
| Soil carbon 5-10 cm               |           | 0.097           | 0.1387|             |
|                                   |           | 0.097           | 0.1387|             |
| Soil nitrogen 0-5 cm              |           | 0.0597          | 0.1009|             |
|                                   |           | 0.0597          | 0.1009|             |
| Soil nitrogen 5-10 cm             |           | 0.0313          | 0.1402|             |
|                                   |           | 0.0313          | 0.1402|             |
| Soil microbial carbon 0-5 cm      |           | 0.1038          |       | 0.1038      |
|                                   |           |                  |       |             |
| Soil microbial carbon 5-10 cm     |           | 0.1038          |       |             |
|                                   |           |                  |       |             |
| Soil microbial nitrogen           |           | 0.0708          | 0.143 |             |
|                                   |           |                  | 0.0708| 0.143       |
| Soil microbial C:N                |           | 0.0012          | 0.0006|             |
|                                   |           |                  | 0.0012| 0.0006      |
| Ammonium availability             | 0.0653    | 0.0410          | 0.0798|             |
|                                   | 0.0074    | <0.0001         | <0.0001|            |
| Nitrate availability              | 0.0409    |                 | 0.0798|             |
|                                   | 0.0025    |                 | <0.0001|            |
| Soil Ph 0-5 cm                    |           | 0.0439          |       | 0.0439      |
|                                   |           |                  |       |             |
| Soil Ph 5-10 cm                   |           | 0.0064          | 0.0257|             |
|                                   |           |                  | 0.0064| 0.0257      |
|                                   |           |                  | 0.0064| 0.0257      |
### Litter Chemistry

<table>
<thead>
<tr>
<th></th>
<th>Current</th>
<th>Initial</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignin</td>
<td>0.0085</td>
<td>0.0322</td>
<td>0.0511</td>
</tr>
<tr>
<td></td>
<td>0.0013</td>
<td>0.0003</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total phenolics</td>
<td>0.0859</td>
<td>0.0977</td>
<td>0.0363</td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0064</td>
</tr>
<tr>
<td>Condensed tannins</td>
<td>0.0757</td>
<td>0.0848</td>
<td>0.0414</td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Litter C:N</td>
<td>0.0326</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hydrolysable tannins</td>
<td>0.0434</td>
<td>0.0688</td>
<td>0.0164</td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td>0.0016</td>
<td>0.0156</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**Notes:** Litter carbon and nitrogen changes were calculated as (current concentration – initial concentration)/(initial concentration). R-squared values are given for significant responses, and *P* values are reported directly below in italics.
Table 4.2. Average microarthropod counts over three-year period

<table>
<thead>
<tr>
<th>Table 4.2 Sites</th>
<th>Oribatids</th>
<th>Prostigs</th>
<th>Mesostigs</th>
<th>Collembola</th>
<th>Other microarthropods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>7.96</td>
<td>1.33</td>
<td>0.14</td>
<td>1.05</td>
<td>1.23</td>
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<tr>
<td></td>
<td>0.93</td>
<td>0.22</td>
<td>0.03</td>
<td>0.12</td>
<td>0.15</td>
</tr>
<tr>
<td>Site 2</td>
<td>11.50</td>
<td>1.80</td>
<td>0.23</td>
<td>1.54</td>
<td>1.38</td>
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<td></td>
<td>1.49</td>
<td>0.16</td>
<td>0.04</td>
<td>0.19</td>
<td>0.15</td>
</tr>
<tr>
<td>Site 3</td>
<td>11.15</td>
<td>1.44</td>
<td>0.20</td>
<td>0.81</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>0.63</td>
<td>0.12</td>
<td>0.03</td>
<td>0.13</td>
<td>0.14</td>
</tr>
<tr>
<td>Site 4</td>
<td>6.62</td>
<td>0.77</td>
<td>0.09</td>
<td>0.75</td>
<td>1.05</td>
</tr>
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<td></td>
<td>0.75</td>
<td>0.18</td>
<td>0.01</td>
<td>0.14</td>
<td>0.33</td>
</tr>
<tr>
<td>Site 5</td>
<td>9.46</td>
<td>1.19</td>
<td>0.16</td>
<td>1.73</td>
<td>1.64</td>
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<td>1.00</td>
<td>0.12</td>
<td>0.03</td>
<td>0.29</td>
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<tr>
<td>Site 6</td>
<td>9.85</td>
<td>1.28</td>
<td>0.26</td>
<td>1.25</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>1.17</td>
<td>0.13</td>
<td>0.04</td>
<td>0.20</td>
<td>0.11</td>
</tr>
<tr>
<td>Site 7</td>
<td>5.92</td>
<td>1.07</td>
<td>0.28</td>
<td>1.06</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>0.10</td>
<td>0.09</td>
<td>0.14</td>
<td>0.15</td>
</tr>
<tr>
<td>Site 8</td>
<td>9.41</td>
<td>1.59</td>
<td>0.42</td>
<td>1.52</td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td>0.63</td>
<td>0.21</td>
<td>0.12</td>
<td>0.18</td>
<td>0.15</td>
</tr>
<tr>
<td>Site 9</td>
<td>9.01</td>
<td>1.12</td>
<td>0.32</td>
<td>1.11</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>1.04</td>
<td>0.10</td>
<td>0.04</td>
<td>0.23</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Litter Treatments

| Phenotype 1     | 8.97      | 1.07     | 0.30      | 1.27       | 0.98                  |
|                 | 1.76      | 0.14     | 0.10      | 0.28       | 0.16                  |
| Phenotype 2     | 9.31      | 1.54     | 0.21      | 0.99       | 1.72                  |
|                 | 1.14      | 0.19     | 0.04      | 0.16       | 0.42                  |
| Phenotype 3     | 9.34      | 1.38     | 0.18      | 1.39       | 0.90                  |
|                 | 0.71      | 0.17     | 0.03      | 0.25       | 0.13                  |
| Phenotype 4     | 10.27     | 1.29     | 0.28      | 1.19       | 1.29                  |
|                 | 1.11      | 0.09     | 0.05      | 0.13       | 0.18                  |
| Phenotype 5     | 10.21     | 1.51     | 0.24      | 1.39       | 1.27                  |
|                 | 0.99      | 0.16     | 0.02      | 0.22       | 0.11                  |
| Phenotype 6     | 8.95      | 1.17     | 0.17      | 1.31       | 1.31                  |
|                 | 0.77      | 0.15     | 0.03      | 0.23       | 0.21                  |
| Phenotype 7     | 7.84      | 1.28     | 0.28      | 1.16       | 1.21                  |
|                 | 0.94      | 0.21     | 0.09      | 0.15       | 0.14                  |
| Phenotype 8     | 8.10      | 1.20     | 0.15      | 1.10       | 0.93                  |
|                 | 0.93      | 0.26     | 0.03      | 0.23       | 0.12                  |
| Phenotype 9     | 8.45      | 1.22     | 0.24      | 1.08       | 1.30                  |
|                 | 1.11      | 0.14     | 0.04      | 0.25       | 0.39                  |
| Mix of all 9    | 8.41      | 1.19     | 0.28      | 1.16       | 0.96                  |
|                 | 1.13      | 0.21     | 0.12      | 0.14       | 0.14                  |
Notes: Numbers indicate 3-year average counts of microarthropods per gram of dry leaf litter. Standard error values are in italics below averages. Sites N=10, Litter treatments N=9.
Figure 4.1 Litter Carbon and Nitrogen Content. Changes in litter nitrogen and carbon were calculated as (current concentration – initial concentration)/ (initial concentration). The graph shows the response of each single phenotype litter and the mixed litter treatment. Positive values indicate a net gain of carbon or nitrogen; negative values indicate a net loss. While there were significant differences among litter phenotypes ($P<0.0001$), they did not deviate in any predictable manner from the mixed litter treatment. Bars are ±1 SE, n=9.
Figure 4.1
**Figure 4.2 Litter Decomposition Rates.** The decomposition rates calculated from three years of decomposition differed among litter phenotype treatments \((P<0.001)\), but did not deviate from the mixed litter treatment in any predictable way. Decomposition rates \((k)\) were calculated as \(y = e^{kt}\), where \(y\) is the percent remaining and \(t\) time in days. Bars are ±1 SE, \(n=9\).
Figure 4.2

![Graph showing decomposition rate (k) for different litter phenotypes.](image)

Litter Phenotype: 1, 2, 3, 4, 5, 6, 7, 8, 9, Mix

Decomposition rate (k): P<0.001
Figure 4.3 Soil Ammonium Availability  Ammonium availability in the soil under phenotype litters as measured with ion exchange resin bags. The graph shows the response of each single phenotype litter and the mixed litter treatment. Ammonium in the soil varied under single phenotype treatments over time ($P=0.0074$), but did not deviate in any predictable manner from the mixed litter treatment. Bars are ±1 SE, n=9.
Figure 4.3

**Litter Phenotype**

Ammonium Availability (mgNH₄⁺/resin bag)

- 3 month
- 6 month
- 9 month
- 12 month
- 15 month
- 20 month
- 22 month
- 24 month
- 27 month
- 30 month
- 34 month
- 36 month

**P = 0.0074**
Figure 4.4 PCA Analysis of Biolog Bacterial Diversity Estimates  

Biolog plates estimate bacterial diversity by testing for differential use of 32 carbon substrates. PCA analysis resulted in two axes with broken stick eigenvalues less than actual eigenvalues. MANOVA analyses of PCA axes revealed a significant effect of site on microbial community ($P=0.0459$), but no effect of litter phenotype treatment ($P>0.05$). Points are ±1 SE, n=9.
Figure 4.4

**Site Effect MANOVA**
P = 0.0459

**Litter Treatments MANOVA**
P > 0.05
Figure 4.5 Representative T-RFLP Electropherograms Results of bacterial DNA amplification and Hae III digest of DNA from soil under phenotype treatment 9 and from under the mixed phenotype treatment. Peak position along the x-axis indicates bacteria group identity as determined by fragment length, whereas peak height indicates relative abundance as determined by fragment counts. In all electropherograms, there were no differences in peak position indicating no difference in bacterial diversity and only slight differences in relative abundances.
Figure 4.5

Terminal restriction fragment length (bases)

Frequency

Hae III Phenotype 9

Hae III Phenotype Mix
Figure 4.6 NMS Analysis of Microarthropod Community  

NMS analysis of microarthropod data resulted in two axes: one that explains variance in overall mite abundance and one that explains variance in collembolan and other microarthropod abundance. MANOVA analysis of NMS axes indicate a significant effect of site on soil microarthropods ($P<0.0001$), but no effect of litter phenotype treatment ($P>0.05$). Points are ±1 SE, n=9.
Figure 4.6

Site effects MANOVA $P<0.0001$

Litter treatment effects MANOVA $P>0.05$
Figure 4.7 Litter Chemistry Effects on Litter Nutrient Dynamics  Litter chemistry at time t was correlated with change in litter nutrients from time step t to time step $t+1$ [calculated as $\ln(\text{conc}_{t+1}/\text{conc}_t)$] using back-stepped multiple regressions. All aspects of litter nutrient change were significantly affected by some aspect of litter chemistry. Partial R-squared values are indicated by differently shaded areas. Total model R-squared values for nutrient changes are given to the right of bars. All partial regressions were significant at $P<0.05$, and final regressions at $P<0.001$. 

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Figure 4.7

Litter Responses

- Litter Carbon: $R^2 = 0.2336$
- Litter Nitrogen: $R^2 = 0.4374$
- Litter Microbial Carbon: $R^2 = 0.2528$
- Litter Percent Remaining: $R^2 = 0.1859$

Legend:
- Lignin
- Phenolics
- Condensed tannin
- Litter C:N
- Hydrolysable tannin
Figure 4.8 Litter Chemistry Effects on Soil Nutrient Dynamics  Litter chemistry at time $t$ was correlated with change in soil nutrients from time step $t$ to time step $t+1$ [calculated as $\ln(\text{conc}_{t+1}/\text{conc}_t)$] using back-stepped multiple regressions. All aspects of soil nutrient change were significantly affected by some aspect of litter chemistry, with the exception of soil carbon change at 5-10cm deep. Partial R-squared values are indicated by differently shaded areas. Total R-squared values for nutrient changes are given to the right of bars. All partial regressions were significant at $P<0.05$, and final regressions at $P<0.001$. 
Figure 4.8

Soil responses at time t+1

- △ Respiration
  - R² = 0.1411
- △ Soil Carbon 0-5cm
  - R² = 0.1125
- △ Soil Carbon 5-10cm
- △ Soil Nitrogen 0-5cm
  - R² = 0.1671
- △ Soil Nitrogen 5-10cm
  - R² = 0
- △ Soil Microbial Carbon 0-5cm
  - R² = 0.1875
- △ Soil Microbial Carbon 5-10cm
  - R² = 0.2963
- △ Soil pH 0-5cm
  - R² = 0.3486
- △ Soil pH 5-10cm
  - R² = 0.4304

R-squared
CHAPTER 5

PHENOTYPIC DIVERSITY AFFECTS NUTRIENT DYNAMICS DURING DECOMPOSITION IN THE PRESENCE OF ANOTHER SPECIES

Madritch, M.D. and M.D. Hunter. To be Submitted to Global Change Biology.
ABSTRACT: We have previously demonstrated that intraspecific phenotypic diversity of leaf litter can influence ecosystem functioning during litter decomposition in the field. It is unknown whether the effects of phenotypic diversity persist when litter from additional species is present. We used laboratory microcosms to determine whether the intraspecific diversity effects of turkey oak leaf litter on nutrient dynamics are masked by the presence of naturally co-occurring longleaf pine litter. We varied the phenotypic diversity of oak litter (1, 3, and 6 phenotype combinations) in the presence and absence of pine litter and measured fluxes of carbon and nitrogen over a 42 week period. Soil C:N ratio peaked at intermediate levels of oak phenotypic diversity whereas the total amount of DOC leached from microcosms declined as oak phenotypic diversity increased. In no case did the presence or absence of pine litter influence the response of nutrient fluxes to changes in oak litter diversity. Our results indicate that phenotypic diversity can be important even in the presence of another species, and suggest that conservation efforts should consider both inter- and intraspecific diversity.

Keywords: biodiversity, ecosystem functioning, intraspecific diversity, interspecific diversity, decomposition, nutrient cycling.
INTRODUCTION

Research exploring links between biodiversity and ecosystem functioning has typically concentrated on the effects of species diversity on carbon and nitrogen fluxes. Initial experiments considered only aboveground effects of reduced biodiversity, but studies have expanded to include responses belowground and during decomposition (see Chapin et al. 2000, McCann 2000, and Loreau et al. 2001 for reviews). Early on, it was recognized that the species level may not be the only appropriate taxonomic level for diversity manipulations (Lawton and Brown 1993). Consequently, diversity experiments were designed to vary the diversity of functional groups (i.e. N-fixer, forb, grass, etc.) while simultaneously varying species diversity (Tilman et al. 1997a). Diversity manipulations at multiple taxonomic levels yield extremely variable results. In some cases, functional diversity is more important than species diversity (Tilman et al. 1997a), whereas in others, species composition, and not diversity, explains more variation than does functional group diversity (Hooper and Vitousek 1997). In addition, it is also possible for plant species diversity, and not functional group diversity or species identity, to influence litter decomposition (Hector et al. 2000). These varied results highlight the importance of manipulating diversity at multiple taxonomic levels in order to fully understand the biodiversity and ecosystem function relationship (Bengtsson 1998).

Although diversity manipulations at taxonomic levels coarser than species diversity are fairly common, very few studies have investigated the potential effects of fine scale, intraspecific diversity on ecosystem functioning. In light of declining genetic diversity of forest species due to anthropogenic disturbances (Ledig 1992), it is important to determine whether losses in intraspecific genetic diversity will impact carbon and
nitrogen cycling. If genetic diversity influences ecosystem functioning, there follow potential conservation implications in addition to the population level consequences usually associated with losses in genetic diversity. Several studies have suggested that carbon and nitrogen cycles can be influenced by genetically-mediated variation in litter chemistries (Driebe and Whitham 2000, Treseder and Vitousek 2001). Leaf litter chemistries are particularly relevant to intraspecific diversity and ecosystem functioning studies because 1) chemical variation is, in part, genetically-mediated, often displaying wide variation within single species (for instance, 145 fold variation in condensed tannin concentration in the leaves of *Leucaena trichandra*, Dalzell and Shelton 2002), and 2) they are important to carbon and nitrogen cycles (Haettenschwiler and Vitousek 2000). Therefore, variation in genetically-mediated traits important to decomposition could have significant ecosystem level impacts.

We have previously demonstrated that the phenotypic diversity of leaf litter can affect ecosystem functioning during litter decomposition in the field (Madritch and Hunter 2002). We varied the intraspecific genetic diversity of turkey oak (*Quercus laevis*) litter and monitored fluxes of carbon and nitrogen in the litter and soil. Low genetic diversity treatments yielded idiosyncratic nutrient cycles that differed significantly from high diversity treatments. While this project was unique in its focus on intraspecific, and not interspecific diversity, its implications need to be considered in relation to interspecific diversity. It is important to quantify the relative importance of diversity at different taxonomic levels, just as species diversity has been tested in relation to functional group diversity (Bengtsson 1998). Specifically, we investigated whether the effects of decreased intraspecific diversity would be masked by increasing
interspecific diversity through the addition of a single commonly co-occurring litter species. We replicated our previous field experiment in laboratory microcosms with and without the addition of naturally co-occurring longleaf pine (*Pinus palustris*) litter. By comparing carbon and nitrogen dynamics of turkey oak litter in the presence and absence of longleaf pine litter, we assessed the effects of an additional species on the ecosystem-level consequences of reduced intraspecific genetic diversity.

**METHODS**

As in our previous work (Madritch and Hunter 2002), we collected leaf litter from a 60 hectare old growth stand of turkey oak, *Q. laevis*, (85% canopy cover) and long leaf pine, *P. palustris*, (10% canopy cover) at the Department of Energy’s Savannah River Site (SRS) in Aiken, SC. *Q. laevis* and *P. palustris* are endemic to the southeastern US coastal plain, and often grow on poor, sandy soils. All adult *Q. laevis* individuals (N=1,572) in a 3.2 ha plot within the stand were genotyped previously using 9 polymorphic allozyme loci (Berg and Hamrick 1994) and we chose 9 genetically-distinct *Q. laevis* trees as sources of litter. Since we were unable to locate replicate clones using microsatellite markers (Klaper *et al.* 2001), we can only interpret the results of the following experiments as effects of phenotypic diversity and not genetic diversity per se. We collected all *Q. laevis* litter by hand from the individual trees after senescence but before leaf-fall; turkey oaks generally hold their leaves for some weeks after senescence. Freshly senesced and abscised *P. palustris* litter was collected across the site and pooled to avoid introducing qualitative variation in pine litter into the experiment.
We established laboratory soil microcosms with turkey oak litter, and turkey oak plus longleaf pine litter, and monitored decomposition and nutrient dynamics. By comparing the effects of the phenotypic diversity of oak litter on decomposition processes with and without the addition of pine litter, we were able to determine if the effects of an additional species in the community changed the ecosystem-level consequences of reduced intraspecific diversity. We initiated a 42 week microcosm experiment (Table 5.1) in September 2001. Previous studies have demonstrated that laboratory microcosms can provide representative estimates of in situ nutrient cycling (McLean and Parkinson 1998).

Microcosms consisted of 15 x 5-cm clear acrylic tubes with 150 grams of mixed and sifted soil collected from across the field site at SRS. Litter was ground to 2mm fragments to facilitate decomposition and mixing. All treatment tubes received a total of 2 grams of litter regardless of diversity treatment (1, 3, or 6 genotypes): turkey oak treatments without pine litter received 2-g of turkey oak litter, while turkey oak and longleaf pine treatments received 1.7-g turkey oak litter and 0.3 g pine litter, roughly corresponding to in situ litter layer composition. We also ran 4 replicates each of bare soil and pine litter alone to monitor fluxes in the absence of oak litter. These controls are not considered further here. Microcosms were incubated at 30 C, with a 12 hour photoperiod at 90-100% relative humidity.

Random litter combinations of 1, 3, and 6 genotypes were picked from the pool of 9 known genotypes as previously determined by 9 allozyme markers (Berg and Hamrick 1995). Single genotype treatments were replicated six times while the three, and six genotype treatments were replicated four times. Each replication was a novel random
selection of a genotype, or a mixture of genotypes. Identical genotype combinations within treatments were disallowed in an effort to test diversity itself, and not the identity of the litter treatments; the identity of the specific trees varied with each replication.

We increased the replicates of low diversity treatments in an effort to offset the sampling effect bias (Huston 1997). Briefly, the sampling effect is based on the fact that a functionally significant individual is more likely to be included in high diversity treatments than in low diversity treatments by chance alone. The sampling effect has been cited as both a statistical error (Huston 1997) and as a valid mechanism by which diversity affects ecosystem functioning (Tilman et al. 1997b). Although our design did not allow for statistical partitioning of selection effects as described by Loreau and Hector (2001), we attempted to minimize selection effect bias by increasing the number of low diversity treatments. For the same reason, we excluded a “9 phenotype” high diversity treatment, which would necessarily have included all individuals and could have been misinterpreted to show a false diversity effect.

Initial concentrations of condensed tannins, hydrolysable tannins, total phenolics, lignin, carbon, and nitrogen were measured in the treatment litters using established techniques (Rossiter et al. 1988, Madritch and Hunter 2002). Litter lignin was analyzed using an ANKOM A200 Fiber Analyzer, whereas polyphenolic analyses included a combination of wet chemistry and colorimetric reactions. Litter carbon and nitrogen, as well as soil carbon and nitrogen at weeks 0, 22, and 42, were all measured using a Carlo Erba 1500N total CHN analyzer. We calculated litter chemistry values for the medium and high oak litter diversity treatments by averaging constituting litter chemistry values.
The litter chemistries of oak plus pine litter treatments were calculated as a weighted average of oak constituents and pine litter chemistries.

Each microcosm was leached with 50 ml of 0.01 mM CaCl$_2$ on the following weeks: 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 26, 34, and 42. Measurement intensity was highest during the initial stages of decomposition to better track the movement of labile nitrogen and carbon sources. Monitoring frequency declined with time since more recalcitrant fractions take longer to decompose (Coleman and Crossley 1996). Leachate was analyzed for nitrate and ammonium on an Alpkem analyzer, and for total dissolved organic carbon (DOC) on a Shimadzu 500TOC. Denitrification rates were measured during microcosm flush periods. Head space was sampled immediately after microcosms were flushed, and then 24 hr afterward and analyzed for N$_2$O on a Varian 3600 gas chromatograph. Soil respiration was measured with an IRGA (PP Systems) every other day for the first 12 weeks, then weekly for the remaining 30 weeks.

STATISTICAL ANALYSES

All data were tested to meet assumptions of normality using the Shapiro-Wilk test and non-normal data were transformed as necessary. Prior to analysis, all nutrient leachate data were converted to milligrams flushed per week.

Data were analyzed in two complementary fashions. First, we ran repeated measures ANOVA to investigate the effects of oak litter diversity on nutrient dynamics over time in the presence or absence of pine litter. We observed no significant treatment effects nor interactions among oak diversity, the presence of pine litter, and time. These results are not discussed further here.
Consequently, we analyzed total outputs of nitrate, ammonium, CO$_2$, N$_2$O, and DOC over the entire experimental time period. This essentially integrates all of the nutrient dynamics over the entire 42 week period. Nutrient totals were analyzed by simple two-way ANOVA using oak litter diversity and the presence of pine litter as main effects. In such a model, a significant interaction term between oak litter diversity and the presence of pine would suggest that the addition of a second species (pine) can influence the effect of phenotypic diversity on ecosystem function.

Because litter chemistries are often important drivers of nutrient cycling, we estimated the influence of initial litter chemistries on total nutrient outputs using stepwise multiple regression analyses and each microcosm as an independent sample.

**RESULTS**

Overall, we observed relatively few effects of phenotypic diversity on nutrient fluxes in our microcosm experiment (Table 5.2) however some responses are worthy of note. The average soil C:N ratio was highest at intermediate levels of oak litter diversity and unaffected by the presence or absence of pine litter ($P=0.0195$ and $P=0.3188$, respectively, Figure 5.1). There was no interactive effect of pine litter and oak diversity on soil C:N ($P=0.1136$, Table 5.2) demonstrating that the phenotypic diversity effect is independent of the presence of pine litter.

The presence of pine litter significantly decreased the total amount of DOC leached from the microcosms ($P=0.0083$, Figure 5.2) as did an increase in the phenotypic diversity of oak litter ($P=0.0703$, Figure 5.2). As before, there was no interaction between oak diversity and the pine presence on DOC leached ($P=0.0901$, Table 5.2).
There were no effects of oak litter diversity or pine litter treatments on the quantities of nitrate and ammonium leached from the microcosms (Table 5.2, Figure 5.3). Likewise, total respiration and denitrification were unaffected by litter treatments (Table 5.2).

Litter treatments differed significantly in their initial C:N ratios, hydrolysable tannin concentrations, and total phenolic concentrations. Average litter C:N ratios increased with oak litter diversity ($P=0.0358$, Figure 4) and with the presence of pine litter ($P=0.0058$, Figure 5.4). Hydrolysable tannin concentrations decreased when pine litter was present ($P=0.0200$, Figure 5.4), as did total phenolic concentrations ($P<0.001$, Figure 5.4).

The initial chemistry of litter had a significant impact on the nutrient dynamics within individual microcosms. For example, losses of nitrate and ammonium from microcosms were both positively correlated with the initial concentrations of hydrolysable tannin in litter ($P=0.0001$ and $P=0.0149$, Figure 5.5). The initial concentration of total phenolics in litter treatments was also positively correlated with DOC loss and with the average soil carbon content ($P=0.0198$ and $P=0.0368$, Figure 5.5). Condensed tannin concentration and the C:N ratio of initial litter treatments negatively influenced the loss of ammonium and N$_2$O, respectively ($P=0.0023$ and $P=0.0248$, Figure 5.5).

**DISCUSSION**

We have shown that intraspecific diversity can influence ecosystem functioning independent of species diversity. In our study the phenotypic diversity of turkey oak
litter influenced ecosystem functioning regardless of longleaf pine litter presence. Specifically, the intraspecific diversity of oak litter affected soil C:N ratios (Figure 5.1) independent of pine litter presence, since there was no oak diversity * pine effect. Similarly, total DOC leached was reduced by pine litter presence, and marginally so by oak litter diversity (Figure 5.2), but with no significant interaction. All other nutrient responses, including respiration, denitrification, and the amount of mineral nitrogen leached were unaffected by oak litter diversity or pine presence treatments, but influenced by litter chemistries (Figure 5.5).

Given previous results (Madritch and Hunter 2002), we expected to find significant nutrient responses to oak litter diversity in the absence of pine litter. We then planned to test if the presence of pine litter masked the effects of intraspecific oak diversity. However, oak diversity treatments did not influence the majority of nutrient fluxes in the current study. Loreau and Behera (1999) suggested that biodiversity, and phenotypic diversity in particular, is likely to be more important to ecosystem stability in systems where environmental variation is great. In previous work, intraspecific oak litter diversity was important under field conditions (Madritch and Hunter 2002) and when considered across a range of nitrogen deposition simulations (Madritch and Hunter, submitted). Both of these responses indicate that intraspecific biodiversity may be more important when other environmental factors are varied.

Previous field work showed that litter phenotype treatments affect the ammonium availability in the soil beneath litters (Madritch and Hunter 2002). However, in our present study, there were no effects of any litter treatment on either ammonium or nitrate leached (Figure 5.3). In general, litter diversity effects on the amount of nitrogen
released are extremely variable. Studies in which the species diversity of litter was manipulated have resulted in increased (Blair et al. 1990), decreased (McTiernann et al. 1997), and idiosyncratic (Wardle et al. 1997) patterns of nitrogen release with increasing litter diversity.

No effects of litter treatment on denitrification or respiration were evident. The resistance of soil respiration to litter diversity manipulations is most likely due to high functional redundancy of respiring soil biota (Schimel 1995). Our results corroborate those of Bardgett and Shine (1999) who showed no effect of species litter diversity on soil respiration. In contrast to the number of respiring soil biota, the number of microbial denitrifiers is a smaller subset of the soil microbial community (i.e., less functionally redundant) (Schimel 1995), and the lack of any litter treatment affects was unexpected.

As in previous work (Madritch and Hunter 2002), litter chemistry metrics influenced most aspects of nutrient cycling (Figure 5.5). However, most litter chemistries did not differ among oak diversity treatments, with the exception of litter C:N ratios (Figure 5.4). The soil C:N responses to oak diversity treatments shown in Figure 5.1 are not likely due to the different C:N ratios of the initial litter since soil responses are unimodal (Figure 5.1) whereas litter C:N ratios increase with oak diversity (Figure 5.4). Apparently, the phenotypic diversity of oak litter influences soil carbon and nitrogen through mechanisms not dependant on the litter chemistries measured here. Conversely, the decrease in the amount of DOC leached in the presence of pine litter (Figure 5.3) can be partially attributed to differences in litter chemistry. Litter treatments without pine had higher levels of both hydrolysable tannins and total phenolics (Figure 5.4). While these litter chemistries retard decomposition (Haettenschwiler and Vitousek
they are also fairly water soluble and could contribute to the total amount of DOC
leached. Although litter chemistries are important to carbon and nitrogen cycling, they
do not explain all of the variation caused by litter diversity treatments shown here.

We conclude that variation in intraspecific diversity can remain important to
ecosystem functioning even in the presence of additional species. Coarse variation in
biodiversity does not necessarily prevent the influence of fine scale diversity. Although
phenotypic diversity of turkey oak litter influenced only a few nutrient responses, these
effects of phenotypic diversity were not influenced by the presence or absence of longleaf
pine litter. Nor were the effects of phenotypic diversity on nutrient responses
predictable. Therefore, we encourage the conservation of intraspecific, as well as
interspecific, diversity of forest communities since reductions in genetic diversity may
influence ecosystem functioning even if addition species are present.

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Table 5.1 presents the experimental design of a microcosm experiment in which the phenotypic diversity of oak litter was varied in the presence and absence of pine litter. Diversity replicates are novel combinations of oak phenotypes.

<table>
<thead>
<tr>
<th>Diversity Reps</th>
<th>Pine litter treatment Reps</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Oak Diversity (1 oak genotype)</td>
<td>6 x 2 x 2</td>
<td>= 24</td>
</tr>
<tr>
<td>Medium Oak Diversity (3 oak genotypes)</td>
<td>4 x 2 x 2</td>
<td>= 16</td>
</tr>
<tr>
<td>High Oak Diversity (6 oak genotypes)</td>
<td>4 x 2 x 2</td>
<td>= 16</td>
</tr>
<tr>
<td>Controls (0 oak genotypes)</td>
<td>1 x 2 x 4</td>
<td>= 8</td>
</tr>
<tr>
<td>Total microcosms</td>
<td></td>
<td>= 64</td>
</tr>
</tbody>
</table>
Table 5.2. Simple two-way ANOVA results of oak litter diversity and pine litter effects on nutrient responses. Nutrient fluxes were calculated as the total amount lost from each microcosm, while soil C:N was averaged over time.

<table>
<thead>
<tr>
<th>Table 5.2</th>
<th>Oak Phenotype Diversity</th>
<th>Pine</th>
<th>Oak * Pine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total DOC leached</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>2.800</td>
<td>7.560</td>
<td>2.530</td>
</tr>
<tr>
<td>d.f.</td>
<td>2, 50</td>
<td>1, 50</td>
<td>2, 50</td>
</tr>
<tr>
<td>P</td>
<td>0.070</td>
<td>0.008</td>
<td>0.090</td>
</tr>
<tr>
<td><strong>Total Nitrate Leached</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>1.540</td>
<td>1.990</td>
<td>1.620</td>
</tr>
<tr>
<td>d.f.</td>
<td>2, 50</td>
<td>1, 50</td>
<td>2, 50</td>
</tr>
<tr>
<td>P</td>
<td>0.224</td>
<td>0.165</td>
<td>0.209</td>
</tr>
<tr>
<td><strong>Total Ammonium Leached</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>2.030</td>
<td>1.100</td>
<td>1.690</td>
</tr>
<tr>
<td>d.f.</td>
<td>2, 50</td>
<td>1, 50</td>
<td>2, 50</td>
</tr>
<tr>
<td>P</td>
<td>0.142</td>
<td>0.299</td>
<td>0.195</td>
</tr>
<tr>
<td><strong>Total CO₂ Respired</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>2.040</td>
<td>0.330</td>
<td>0.250</td>
</tr>
<tr>
<td>d.f.</td>
<td>2, 50</td>
<td>1, 50</td>
<td>2, 50</td>
</tr>
<tr>
<td>P</td>
<td>0.141</td>
<td>0.569</td>
<td>0.783</td>
</tr>
<tr>
<td><strong>Total N₂O Denitrified</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>0.350</td>
<td>1.340</td>
<td>1.730</td>
</tr>
<tr>
<td>d.f.</td>
<td>2, 50</td>
<td>1, 50</td>
<td>2, 50</td>
</tr>
<tr>
<td>P</td>
<td>0.703</td>
<td>0.253</td>
<td>0.187</td>
</tr>
<tr>
<td><strong>Average Soil C:N</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>4.260</td>
<td>1.010</td>
<td>2.270</td>
</tr>
<tr>
<td>d.f.</td>
<td>2, 50</td>
<td>1, 50</td>
<td>2, 50</td>
</tr>
<tr>
<td>P</td>
<td>0.019</td>
<td>0.319</td>
<td>0.114</td>
</tr>
</tbody>
</table>
**Figure 5.1 Litter treatment effects on average soil C:N ratio.** The average soil C:N ratio was highest under intermediate oak litter diversity treatments ($P=0.0195$). Different letters indicate significantly different responses within groups. 1 oak phenotype $N=24$, 3 oak phenotypes $N=16$, 6 oak phenotypes $N=16$, Pine treatments $N=28$. Bars represent +/- 1 S.E.
Figure 5.1

The figure shows the relationship between soil C:N ratio and oak phenotype diversity in the presence of pine. The bars represent different levels of oak phenotype diversity (1, 3, 6) under conditions of no pine and pine presence. The error bars indicate the variability in the data.

- For oak phenotype diversity levels 1, 3, and 6 in the no pine condition, the soil C:N ratio is lower compared to the pine presence condition.
- The presence of pine significantly increases the soil C:N ratio compared to the no pine condition ($P=0.0195$).
- There is no significant difference in soil C:N ratio between the no pine and pine presence conditions after adjusting for oak phenotype diversity ($P=0.3188$).

The bars are labeled with letters (A, B) to indicate statistical significance, with A indicating no significant difference from the previous category.
**Figure 5.2 Litter treatment effects on total DOC leached.** The total amount of DOC leached from microcosms decreased in the presence of pine litter ($P=0.0083$) and declined with increases in oak phenotype diversity ($P=0.0703$). Different letters indicate significantly different responses within groups. 1 oak phenotype $N = 24$, 3 oak phenotypes $N = 16$, 6 oak phenotypes $N=16$, Pine treatments $N = 28$. Bars represent +/- 1 S.E.
Figure 5.2

Total DOC Leached (mg)

Oak phenotype diversity

Pine presence

No Pine  Pine

1  3  6

P=0.0703  P=0.008

$P=0.0703$

$P=0.008$
**Figure 5.3 Mineral Nitrogen Leached.** Neither the total amount of nitrate nor ammonium leached was affected by any litter treatments. Different letters indicate significantly different responses within groups. 1 oak phenotype N = 24, 3 oak phenotypes N = 16, 6 oak phenotypes N = 16, Pine treatments N = 28. Bars represent +/- S.E.
Figure 5.3

![Bar chart showing total nitrate and ammonium leached with different levels of oak phenotype diversity and pine presence.](image-url)
Figure 5.4 Litter Chemistry Variation Among Litter Treatments. Average litter C:N ratios increased with oak diversity treatment ($P=0.0358$) and pine litter presence ($P=0.0058$). Hydrolysable tannin concentrations decreased with pine litter presence ($P=0.0200$), as did total phenolic concentrations ($P<0.001$). Different letters indicate significantly different litter chemistries within groups. 1 oak phenotype $N = 24$, 3 oak phenotypes $N = 16$, 6 oak phenotypes $N=16$, Pine treatments $N = 28$. Bars represent $+/-$ 1 S.E.
Figure 5.4

Oak phenotype diversity

NP = No pine, P = Pine
Figure 5.5 Litter Chemistry Influence on Nutrient Dynamics. Litter chemistries influenced most nutrient responses except for average soil nitrogen content and the total amount of carbon respired. Total R-squared values are given to the right of bars. All R-squared values were significant at P<0.05.
Figure 5.5

![Figure 5.5](image-url)

- Total Ammonium leached: $R^2 = 0.2505$
- Total Nitrate leached: $R^2 = 0.2408$
- Total DOC leached: $R^2 = 0.0965$
- Average Soil C: $R^2 = 0.0783$
- Average Soil N: $R^2 = 0$
- Total Respiration: $R^2 = 0$
- Total Denitrification: $R^2 = 0.0899$

Legend:
- □ Condensed Tannin
- □ Total Phenolics
- □ C:N Ratio
- □ Hydrolysable Tannin

$P < 0.05$
CHAPTER 6

CONCLUSIONS

In general, our data show that intraspecific litter diversity can be important to ecosystem functioning. At the species and functional group level, it is becoming increasingly apparent that communities are not functionally redundant: losses in species and functional group diversity result in altered ecosystem functioning (Naeem et al. 1994, Tilman et al. 1994, Chapin et al. 2000, McCann 2000, Loreau et al. 2001). We add to the conclusions of others, and suggest that losses in genetic diversity within a single species can also elicit changes in ecosystem functioning.

We examined the effects of intraspecific litter diversity on nutrient cycling during leaf litter decomposition. Since variation in litter chemistry is both genetically mediated and important to ecosystem functioning (Chapter 1), we suspected that genetically mediated variation in litter chemistry of turkey oak, *Quercus laevis*, litter would influence nutrient cycles. While our work was in progress, two other groups also suggested that reductions in intraspecific diversity may affect ecosystem functioning through genetically-mediated variation in litter chemistries (Driebe and Whitham 2000, Treseder and Vitousek 2001).

Despite being a clonal species, our stand of turkey oak trees did not have any genotype replicates (Klaper et al. 2001), and hence our interpretation is largely limited to a discussion of phenotypic diversity effects. However, over a three-year period, we were able to make a strong link between genetic variation and the variation of chemical
phenotype of litter treatments. Variation in litter chemistry, in turn, caused significant
differences in virtually all aspects of nutrient cycling. In short, we showed for the first
time that reductions in intraspecific diversity can influence ecosystem functioning. In
addition, these influences were significant, but not predictable. Litter diversity did not
influence ecosystem functioning through any measured aspects of soil biota. Only the
influence of litter chemistry was apparent. The soil biota displayed enough plasticity to
remain unchanged under different litter treatments, and nutrient cycles differed according
to substrate inputs and not the processing communities.

In our field experiment, nitrogen cycles were particularly influenced by
intraspecific litter diversity treatments. Since reductions in biodiversity occur in concert
with nitrogen deposition, we tested for an interaction between intraspecific litter diversity
and nitrogen deposition using laboratory microcosms (Chapter 3). Intraspecific litter
diversity treatments interacted with simulated nitrogen deposition treatments to influence
ecosystem functioning. Soil respiration increased with oak litter diversity only under
high nitrogen deposition treatments, suggesting that complementary resource use (Tilman
1997) occurred only after a limiting nutrient (nitrogen) was supplied.

While results from our field and laboratory experiments both showed that
intraspecific litter diversity can influence ecosystem functioning, neither considered the
effects of adding another species. Simply put, the presence of litter from another species
could potentially mask the effects of intraspecific oak litter diversity. To address this, we
used laboratory microcosms and varied the phenotypic diversity of turkey oak litter in the
presence and absence of naturally co-occurring longleaf pine litter (Chapter5). The
phenotypic diversity of turkey oak litter influenced ecosystem functioning regardless of
longleaf pine litter presence. Specifically, the intraspecific diversity of oak litter affected soil C:N ratios independent of pine litter presence. This suggests that reductions in intraspecific diversity can potentially influence ecosystem functioning even if species diversity is maintained. However, intraspecific diversity may be more important in forest systems dominated by only a few species.

We have shown that genetic biodiversity may influence ecosystem functioning and that fine scale diversity can have large, ecosystem level effects. Moreover, these effects are unpredictable in magnitude and direction. Our work adds to the growing number of studies that show effects of biodiversity on ecosystem functioning (Naeem et al. 1994, Tilman et al. 1994, Chapin et al. 2000, McCann 2000, Loreau et al. 2001). More importantly, our results highlight the importance of conserving biodiversity at finer scales not previously supported by biodiversity and ecosystem function studies. We repeatedly found significant, yet unpredictable effects of intraspecific litter diversity on ecosystem functioning. This supports the hypothesis of an idiosyncratic relationship between biodiversity and ecosystem functioning (Lawton 1994. Wardle et al.1997), and more importantly, stresses the conservation of biodiversity as insurance for future ecosystem functioning (Yachi and Loreau 1999).

LITERATURE CITED


