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THE COMMUNITY-LEVEL AND ECOSYSTEM-LEVEL CONSEQUENCES OF
FEEDBACKS BETWEEN THE SOIL ECOSYSTEM AND THE PLANT
COMMUNITY DURING FOREST UNDERSTORY INVASION

by

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ABSTRACT OF THE DISSERTATION

The Community-level and Ecosystem-level Consequences of Feedbacks Between the
Soil Ecosystem and the Plant Community During Forest Understory Invasion

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This dissertation addresses the consequences of reciprocal interactions between the plant and the soil microbial communities, and how those interactions affect nutrient cycling and plant competition during exotic plant invasion. Each chapter is linked by the common theme of evaluating the importance of these feedbacks to the rate of plant invasion in the forest understory.

In the first two chapters, I utilize microcosms to evaluate the importance of leaf litter inputs for plant-soil feedback. The first chapter demonstrates that leaf litter from native and exotic plants create divergent soil microbial communities, altering soil enzyme activities and nitrogen cycling, which in turn affects the growth of native and invasive plants. However, while this plant-soil interaction affects growth rate, it does not change the competitive hierarchy or the success of the invasive plant. The second chapter shows how the effect of an exotic species' leaf litter on soil microbes varies over a range of invasion severity. Using litter mixtures ranging from 0% to 100% exotic litter, I show

that ecosystem-level effects of invasion on carbon and nitrogen cycling are linearly related to the exotic plant density, while community-level effects on soil microbes are non-linear and very sensitive to low levels of invasion.

In the final chapters, I extend these results to examine whole-plant effects in more natural plant communities. The third chapter uses a large-scale field experiment to explore the temporal dynamics of invasion impacts. I show that the short-term impact of native and invasive plants on soil microbes is weak, while long-term effects are much stronger. However, after restoration of native plants, the legacy effect of invasion remains strong. Using experimental litter-removal, I also show that belowground plant litter more strongly influences the soil microbial community than aboveground litter. The fourth chapter examines how the diversity of the native community influences the invasion impact on soils. I show that while diversity has little direct effect, individual native plant species can influence how an exotic invasive shrub affects the soil ecosystem. Together, these results show that the importance of plant-soil feedbacks for exotic invasion is context- and scale-dependent, exhibiting nonlinear dynamics that depend on the native community and the degree of invasion, and vary in strength over time.

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DEDICATION

I dedicate this to my wife, whose unfaltering cheerful attitude and loving support daily
renews my soul.

The world is charged with the grandeur of God.
It will flame out, like shining from shook foil;
It gathers to a greatness, like the ooze of oil
Crushed. Why do men then now not reck his rod?
Generations have trod, have trod, have trod;
And all is seared with trade; bleared, smeared with toil;
And wears man's smudge and shares man's smell: the soil
Is bare now, nor can foot feel, being shod.

And for all this, nature is never spent;
There lives the dearest freshness deep down things;
And though the last lights off the black West went
Oh, morning, at the brown brink eastward, springs—
Because the Holy Ghost over the bent
World broods with warm breast and with ah! bright wings.

—Gerard Manley Hopkins

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Introduction

Feedbacks between plants and soil have long been recognized as an important driver in soil formation (Brady and Weil, 2002) as well as plant community composition and succession (Kardol et al., 2006). Feedback can be positive or negative, and occurs when the plant community both causes and subsequently responds to changes in soil physical, chemical, or biological properties (Ehrenfeld et al., 2005). Agriculturalists have long known that this effect can affect future yields because certain crops increase or decrease specific nutrients and soil-borne pathogens, and they rotate crops accordingly in order to maximize yields. Their work in very simple plant communities consisting of one or two species accentuates plant-soil feedback, and as a result, agriculturalists were the first to recognize and apply an operational understanding of plant-soil feedback (Ehrenfeld et al., 2005).

Given that plant-soil feedback has been a fundamental principle of agriculture for hundreds of years, the application of this principle to a functional understanding of natural plant communities is a surprisingly recent development. A recent meta-analysis of plant-soil feedback (Kulmatiski et al., 2008) noted that 40 of the 45 studies surveyed (89%) were published after 2001. Furthermore, a brief discussion of the communities and biomes where feedbacks are important reveals that feedback is most often demonstrated in extreme environments, where communities are dominated by a few species (Ehrenfeld et al., 2005, Kulmatiski et al., 2008). More thorough investigations of plant-soil feedback are often conducted as greenhouse studies using pure monocultures in pots (Callaway et al., 2004, Klironomos, 2002). Thus, while our understanding of the theory and mechanisms underlying plant-soil feedbacks has progressed, our

understanding of how feedback may function in structuring natural communities is much more limited and is only tested in simplified systems such as agricultural fields, monocultures, or simplified extreme environments.

There are a number of emergent factors of complex natural ecosystems that are not reflected in studies of plant-soil feedback in monocultures and simplified communities. For example, species-rich communities exhibit emergent properties in soil microbial community structure (Zak et al., 2003) that can alter the strength or direction of feedback (Bartelt-Ryser et al., 2005). Coevolution is another emergent property of complex natural systems that might alter feedback, resulting in unique feedbacks during the invasion of non-native plants (Callaway and Aschehoug, 2000, Klironomos, 2002, Ehrenfeld et al., 2001). The feedback effects of living plant tissues might even differ from dead plant tissues fallen from the same plant species (Ehrenfeld et al., 2005), and in complex plant communities composed of a mixture of dead and living plant tissue, it is important to understand how the interplay of these emergent factors results in an overall feedback effect. This dissertation explores in greater detail some of these key emergent properties and components of plant-soil feedback in order to better understand the role of plant-soil feedback in structuring complex plant communities.

Feedback and Exotic Plant Invasion. Plant communities invaded by non-native invasive plants are ideal systems to study the role of plant-soil feedback, and throughout this dissertation I take advantage of plant invasions to explore plant-soil feedback. Exotic species invasions are an important semi-experimental tool that provides unique opportunities to study various aspects of ecology and evolution (Sax et al., 2005). This

approach is particularly germane to the study of plant-soil feedback, as an expanding number of studies suggest that positive plant-soil feedback may be an important mechanism underlying exotic invasions (Callaway et al., 2004, Reinhart and Callaway, 2004, Klironomos, 2002, Ehrenfeld et al., 2001, van Grunsven et al., 2007, Vogelsang and Bever, 2009, Farrer and Goldberg, 2009). Because invasive species frequently dominate local communities, the potential role of feedback may be amplified in invaded communities. Furthermore, while invaded communities are drastically altered by invasion, they are less artificial than agroecosystems and more accurately represent natural communities, providing an opportunity to understand how feedback might structure communities in a diverse natural system.

Klironomos (2002) provided convincing early evidence that positive feedback between plants and soil biota could be a contributing factor in exotic plant invasion. In a study using five non-native invasives and five rare native species, he demonstrated that rare native plants grew poorly in soil where they had previously been grown compared to soil previously occupied by other species, while the five invasive species grew relatively well in their own previously-occupied soil. This study delved further, showing that pathogens and saprobes had relatively little effect on the growth of invasives while having a strong negative effect on rare native species. Since most species benefited from mycorrhizae, the net effect was negative feedback for native species and positive for invasives. Since this pioneering study, many other studies have shown that feedback between invasive plants and soil pathogens plays a strong role in invasion (van Grunsven et al., 2007, van der Putten et al., 2007, Reinhart and Callaway, 2006, Reinhart and Callaway, 2004)

While escape from belowground enemies and pathogens is well-studied, plant-soil feedback can also promote exotic invasion when the invader changes soil abiotic conditions (Vivrette and Muller, 1977, Callaway and Aschehoug, 2000). This is again well-known in agricultural systems, where crop rotation is based not only on avoiding soil pests and pathogens but also on nutritional needs of different crop species. In natural systems, the abiotic component of feedback has not been very well studied, and many studies focus on the role of allelopathy and “novel weapons” in invasion (Bais et al., 2003, Callaway and Aschehoug, 2000, Callaway et al., 2005). While this has been suggested for a few invasive plants, the results of these studies are hotly debated (Blair et al., 2005, Blair et al., 2006, Callaway et al., 2005, Inderjit et al., 2006). Although allelopathy is clearly not a factor in all plant invasions, many plant invasions do affect the abiotic soil environment, often by increasing nutrient availability (Ehrenfeld, 2003, Hawkes et al., 2005). The potential of increased nutrient availability to produce positive plant-soil feedback during invasion has largely been neglected in the literature. The research described in this dissertation explores further this potential pathway of feedback.

To study this pathway for feedback, I utilize two non-native invasive species in New Jersey forests, Japanese barberry (*Berberis thunbergii*) and Japanese stiltgrass (*Microstegium vimineum*). Both exhibit potential for positive plant-soil feedback (Kourtev et al., 1998, Ehrenfeld et al., 2001). Field measurements of soil microbial community composition and function under *Berberis* and *Microstegium* were strikingly different from soils under native *Vaccinium* cover (Kourtev et al., 2003, Ehrenfeld et al., 2001). The two invasive species increased the bacterial : fungal ratio as well as the arbuscular mycorrhizal biomass in rhizosphere soils compared to soil under *Vaccinium*.

These shifts in microbial community structure altered the enzyme activities and substrate-induced respiration rates of the soil (Kourtev et al., 2003). *Berberis* and *Microstegium* increase pH, net nitrification, and net N mineralization relative to soils under native *Vaccinium* shrubs in forests, and these results were replicated with plants grown in pots containing a common initial soil under greenhouse conditions (Ehrenfeld et al., 2001). Thus, *Berberis* and *Microstegium* altered the soil biota, increasing nitrogen availability and creating soil conditions that potentially give the exotics a competitive edge over natives, which would result in a positive feedback loop (Ehrenfeld et al., 2001). Though these data suggest the existence of a positive feedback loop that promotes the success of these two invasive species, the assumption that increased nitrogen availability will give the invasive species a competitive advantage has not yet been tested. In the first chapter, I examine whether different species' leaf litter create a soil environment that alters interspecific competition.

The second chapter explores this question further by examining how one exotic invasive species' leaf litter (*Berberis*) affects the soil biotic and abiotic environment over a range of mixtures with native tree canopy leaf litter. The relationship between dominance of a species and its impact on the system and potential feedbacks is important both from a theoretical and a management perspective (Yokomizo et al., 2009). For example, negative feedback on a competitively dominant species was stronger when it was more abundant in experimental mesocosms (Suter et al., 2007). Despite this evidence, the dominance of native or invasive species has largely been overlooked, both as a factor that controls the effects of invasion (Dietz and Edwards, 2006) and as a measure of invasion success (Lundholm and Larson, 2004). Only a few experimental

studies (Lundholm and Larson, 2004, Dietz and Edwards, 2006, Crall et al., 2006) and one modeling study (Levine et al., 2006) have addressed the relationship between the dominance of exotics and feedback.

Feedback in Diverse Communities. Empirical and theoretical evidence suggest the diversity of a community is important in determining the role of feedback in native plant communities. Strong plant-soil feedbacks are most often demonstrated in species-poor environments and agricultural systems and rarely shown in species-rich systems (Ehrenfeld et al., 2005). Plant-soil feedbacks have also been demonstrated in low diversity, extreme environments, where plants have evolved mechanisms to tolerate environmental stress that contributes to strong plant-soil feedbacks (Chapman et al., 2006). Theory also predicts mutualism, a form of biotic positive feedback, is also more common in simplified extreme environments, where it operates as a mechanism for coping with environmental stress (Bertness and Callaway, 1994, Bruno et al., 2003). Finally, theory also predicts that weak interactions are more common in diverse systems, while the strong interactions required for strong feedback effects are more common in species-poor communities (McCann, 2000). These studies all suggest that feedbacks may be moderated in diverse communities, though empirically-based tests of this are few.

Though the link in the literature between diversity and feedback is poorly studied, a strong connection has been made between diversity and invasibility of natural systems (Gilbert and Lechowicz, 2005, Naeem et al., 2000, Hector et al., 2001, Stohlgren et al., 2003). However, the mechanism for this relationship is not known and is perhaps one of the most contentious topics in the field of invasion biology (Gilbert and Lechowicz,

2005, Hector et al., 2001, Naeem et al., 2000, Stohlgren et al., 1999, Stohlgren et al., 2003). The oldest and leading explanation is that species-rich systems are less invasible because more of the available niche space is occupied, leaving less opportunity and fewer resources for potential invaders to utilize (Elton, 1958). Temporally-fluctuating resources may also be more efficiently sequestered by species-rich communities, reducing the chance of successful invasion (Davis et al., 2000). Short-term experimental evidence tends to support these theories (Naeem et al., 2000, Hector et al., 2001), but observational studies that incorporate long-term dynamics and other extrinsic factors show greater invasion in species-rich communities instead of reduced invasion (Stohlgren et al., 2003, Stohlgren et al., 1999).

Because these studies operate at different spatial and temporal scales, the patterns observed may result from different mechanisms that operate at different scales (Brown and Peet, 2003, Shea and Chesson, 2002, Wu and Loucks, 1995). A better mechanistic understanding of the processes that underlie these patterns is integral to reconciling these conflicting lines of research. Understanding the role of feedback in invasion and how feedback relates to diversity can help clarify the process underlying patterns observed between diversity and invasibility. In the third chapter, I examine the potential for feedback in a more diverse, natural setting by conducting a field experiment that manipulates the dominant understory species. I used a field-based approach in order to reduce possible artifacts that may result from studies that examine feedback in species-depauperate monoculture studies. In this chapter, I also extend the research from the first two chapters, which focused on effects of leaf litter inputs, by examining whole-plant effects as well as isolated effects of belowground and aboveground inputs. Finally, the

fourth chapter explicitly examines the relationship between diversity and feedback effects, using an experimental plant diversity gradient with varying degrees of invasion to determine the how diversity moderates the impacts of invasion on soil microbial community structure and function, and how that affects the success of an invasive species.

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Chapter 1

Microbial-mediated feedbacks of leaf litter on invasive plant growth and interspecific competition

Summary

1 Feedbacks between plants and soil pathogens play an important role in competition, invasion, succession, and community development. However, very little is known about the consequences of feedbacks between leaf litter and saprophytic soil microbes, even though it is this relationship that largely regulates nutrient cycling.

2 We decomposed litter of two native and two exotic invasive species in microcosms to precondition a common soil, then decomposed either the same species' litter or a different species' litter on the preconditioned soil. We measured changes in microbial community structure (phospholipid fatty acids) and function (soil enzyme activities and decomposition) and related those changes to the preconditioning treatment.

3 In a subsequent experiment, we used preconditioned soils to grow seedlings of a native shrub and an exotic invasive shrub, both under intraspecific and interspecific competition.

4 We found significant changes in microbial community structure from the preconditioning treatment that persisted through time. These changes altered soil enzyme activities and subsequent litter decomposition. Furthermore, effects of the preconditioning treatment did not diminish over time, but rather became more significant over time. Litter-driven changes in the microbial community structure and function furthermore impacted the growth rate of two shrub species, but did not directly influence competition between the native and the exotic shrub.

5 Synthesis. Plant-soil feedback driven by interactions between plant leaf litter and the saprophytic microbial community can have important consequences for soil function and plant growth. Further study is needed to determine how these consequences might affect long-term plant community composition, diversity, and invasibility.

Introduction

The interactions between plants and the soil system are increasingly recognized as an important component of competition (Bever, 2003, Casper and Castelli, 2007, Kulmatiski et al., 2008), succession (Kardol *et al.*, 2006), exotic plant invasion (Klironomos, 2002, van der Putten et al., 2007, Farrer & Goldberg 2009) and the maintenance of diversity (Petermann et al., 2008, Packer and Clay, 2000, Augspurger and Wilkinson, 2007). These plant-soil interactions can result in a feedback loop between plants and soil, either through chemical and physical effects on soil, through plant litter inputs (both aboveground and belowground) or through the accumulation of pathogens or symbionts in the soil (Ehrenfeld *et al.*, 2005). Feedback occurs when one or more of these effects on soil differentially influences the growth of different species, therefore influencing intraspecific competition and plant community composition. Much of the research to date on plant-soil feedbacks has focused on species-specific belowground pathogens and pests, but little is known about feedbacks involving litter inputs.

Litter-driven plant-soil feedbacks that involve saprotrophic decomposers rather than pathogens are thought to be weaker due to high levels of redundancy in the saprotrophic microbial community (van der Putten et al., 2007, Wardle, 2006), but this

hypothesis has not been explicitly tested, and little is known about the strength of litter-driven feedbacks. Many litter decomposition studies have shown that different species' litter can significantly alter the soil microbial community (Wardle, 2006), often creating a "home-field advantage" causing litter to decompose more quickly on soils where the same litter type was previously decomposing (e.g., Vivanco and Austin, 2008, Ayers et al., 2009). This demonstrates potential for the microbial community to adapt both in structure and function to unique plant species traits. However, whether or not this change in the microbial community affects plant community composition is not known. Aboveground and belowground litter strongly influence plant community composition in the long term (Wedin and Tilman, 1990, Wedin and Tilman, 1993, Hobbie, 1992, Berendse, 1994); in fact, plants are one of the primary factors that drive soil formation (Jenny, 1941), largely through litter inputs. How these long-term litter effects relate to short-term plant-soil feedback is not fully understood.

While studies on plant-soil feedback often investigate the effects of a single plant grown in isolation or monoculture and ignore competition, initial experiments (Kardol *et al.*, 2007) and preliminary meta-analysis (Kulmatiski *et al.*, 2008) suggests that plant-soil feedbacks operate differently in monoculture and in competitive environments. To understand the strength of feedback effects, it is important to put them in the context of interspecific competition, since natural plant populations do not exist in monoculture. In fact, because plant-soil feedback is a process that by definition impacts competition, it may be difficult or even impossible to realistically consider plant-soil feedback outside of the context of plant competition (Casper and Castelli, 2007).

Here we examine plant-soil feedback in four co-occurring forest understory species. Two are common native shrub species in upland hardwood forests of New Jersey, and two are invasive exotic plants common to the forest understory in the region. Low-bush blueberry (*Vaccinium angustifolium* L., hereafter *Vaccinium*) and maple-leaf viburnum (*Viburnum acerifolium* L., hereafter *Viburnum*) are often dominant understory species in upland forests of New Jersey, and these two genera frequently comprise the majority of the forest understory in uninvaded forests (Ehrenfeld, 1999, Kourtev et al., 1998, pers. obs.). However, these forests are frequently and heavily invaded by Japanese stiltgrass (*Microstegium vimineum* (Trin.) A. Camus, hereafter *Microstegium*), Japanese barberry (*Berberis thunbergii* DC., hereafter *Berberis*) or both (Kourtev et al., 1998). These two species can comprise over 90% of the understory cover in some areas (K.J. Elgersma & J.G. Ehrenfeld, unpublished data). *Berberis* and *Microstegium* have both been shown to dramatically alter the soil microbial community structure, extracellular enzyme activities, soil pH and nitrogen cycling in greenhouse microcosm experiments (Kourtev et al., 2003) and field observational studies (Ehrenfeld et al., 2001). It has further been suggested that these altered conditions may favour further invasion by these species, creating a positive feedback loop (Kourtev et al., 2003), but this hypothesis has not been tested. Further research has shown that the distribution of *Berberis* is also influenced by past land use history (DeGasperis and Motzkin, 2007, Flory and Clay, 2006), suggesting that plant-driven alteration of soil conditions may be less important than human-engineered change. Understanding the importance of plant-driven feedback is therefore an important step in understanding the controls over invasion for these species.

In this study, we examine how plant leaf litter inputs from these four species affect the soil microbial community structure and function, and how this feeds back to influence plant growth and competition. We focus on leaf litter in order to isolate feedbacks operating through the saprophytic microbial community rather than species-specific pathogen interactions. We test specifically whether litter-driven changes in the microbial community have functional consequences, both for the function of the microbial community itself, and for plant growth. Finally, we compare the effects of litter on plant growth in monoculture and under interspecific competition.

Materials and methods

Phase I: Soil pre-conditioning phase

Senesced leaf litter from each of the four study species was collected in fall 2004 from various locations in and around New Brunswick, NJ. During the period of leaf senescence for each species, plants of each species were shaken vigorously to simulate a strong wind, and the freshly-fallen litter was collected from the forest floor around the base of the plant. When necessary, this litter was supplemented with fully senescent leaves hand-picked from the plant. Only leaves that detached easily were used. For *Microstegium*, leaves and culms were gathered from densely-invaded areas by cutting fully-senesced aboveground biomass with a scissors. All leaves were air-dried and stored at room temperature until needed.

Soil was collected in January 2005 from an uninvaded area of mixed hardwood forest (Kourtev *et al.*, 1998) at Allamuchy State Park (Allamuchy, NJ, USA). Mineral soil from the top 10 cm was collected, passed through a 2-cm sieve to remove rocks and coarse woody debris, and packed into 10-cm pots, using approximately 900 g soil per pot.

Pots were placed in a greenhouse, and 3 g dried leaf litter from one of the four species was placed on the soil surface. Pots were kept moist by regular watering, and the decomposing litter in all pots was removed and replaced with more litter after a majority of the litter had decomposed (in December 2005). At the end of this preconditioning phase, three pots from each treatment were destructively sampled to measure the microbial community structure and soil enzyme activities (see methods detailed below), while the remaining pots were used for the next phase of the experiment.

Microbial community structure

To examine how soil pre-conditioning affected the microbial community, we collected 4 grams soil from the top 5 cm of the pot and extracted phospholipid fatty acids (PLFAs). This method quantifies the amount of phospholipid fatty acids from the cell membranes of different microbial functional groups, and is commonly used in ecological studies to estimate the relative abundances of different microbial groups (Joergensen and Wichern, 2008). We used a method modified from White et al. (1979). Soil was extracted twice with 15 mL single-phase chloroform: methanol: phosphate buffer (1:2:0.8) solution. Supernatant from the two extractions were combined, mixed with DI water and chloroform (2.5 mL each) and separated overnight. The chloroform was then removed, evaporated to <1mL, and fractionated through a silicic acid column (100-200 mesh) by eluting with chloroform, acetone, and methanol into neutral-, glyco-, and phospho-lipids, respectively. The phospholipids were retained and concentrated, and an internal standard (nonadecanoic acid, 19:0) was added. The phospholipids were then saponified and methylated using the protocol from the Sherlock Microbial Identification System (MIDI Inc., Newark, DE, USA). The resulting fatty acid methyl esters (FAMES)

were identified using the MIDI Sherlock system with an HP gas chromatograph with flame ionization detector (GC-FID, Hewlett Packard 5890 Series II, Palo Alto, CA, USA) utilizing an HP Ultra 2 phenyl methyl silicone fused capillary column (25 m×0.2 mm i.d., film thickness 0.33 µm). The temperature was increased from 170 °C to 250 °C at 5 °C / min, and FAMES were identified and quantified based on peak areas and retention times using the MIDI Sherlock system. All peak areas were standardized by the internal standard (19:0) peak area.

Soil enzyme activity

The activities of six extracellular enzymes (acid phosphatase, β-glucosidase, chitinase, aminopeptidase, phenol oxidase and urease) were assayed at the end of the preconditioning period. These enzymes were chosen because of their roles in carbon (β-glucosidase, chitinase, phenol oxidase), nitrogen (chitinase, aminopeptidase, urease), and phosphorus (acid phosphatase) cycling. Soil from the top 5 cm was collected from three replicate pots for each treatment and stored at 4 °C until analysis, which was conducted within 24 hours. A microplate assay method was adapted from Sinsabaugh et al. (2000) and Waldrop et al. (2000), and analyses were conducted using a robotic pipet system and spectrophotometric plate reader (Biomek Laboratory Automation Workstation, Beckman Coulter Inc., Fullerton, CA, USA) in the High-Throughput Screening Laboratory at Rutgers University. Soil slurries were made from 20 g fresh soil and 200 mL acetate buffer solution (50 mM, pH 5.0), and eight 50-µL subsamples from each soil slurry were pipetted into 96-well plates. Separate 400-µL aliquots were dried overnight at 70 °C to determine the mass of suspended soil in the 50-µL subsamples. Acid phosphatase, β-glucosidase, chitinase and aminopeptidase were assayed using 10

mM p-nitrophenyl-linked substrates dissolved in acetate buffer (pNP-phosphate, pNP- β -D-glucopyranoside, p-NP-N-acetyl-B-D-glucosaminide and glycine-p-nitroanilide, respectively), while phenol oxidase and urease were assayed using 10 mM L-dihydroxyphenylalanine (L-DOPA) and 25 mM urea as substrates, respectively. Each well received 150 μ L substrate and was incubated at room temperature for 4 hours (p-NP substrates) or 18 hours (phenol oxidase and urease). Soil controls were established by adding 150 μ L acetate buffer solution instead of the substrate. Substrate controls were also established by adding 50 μ L buffer (instead of 50 μ L soil slurry) to 150 μ L of each substrate.

At the end of the incubation, 50 μ L supernatant was transferred to a new 96-well plate for measurement of absorbance. Absorbance was measured directly for phenol oxidase at 450 nm. For the p-NP-linked substrates, 50 μ L 0.1 M NaOH was added before measuring absorbance at 420 nm, while for urease, an ammonium test kit (Hach, Loveland, CO, USA) was used. 40 μ L salicylate reagent was added to the urease supernatant and allowed to react for 3 minutes, then 40 μ L cyanurate reagent was added. Absorbance was measured after 20 minutes at 600 nm. There were a large number of outliers caused by interference from soil particles when measuring absorbance, so the median absorbance value from among the eight analytical replicates was used instead of the mean. Absorbances were converted to concentrations (i.e., mM p-NP) using a standard curve relating known concentrations to absorbance. All concentrations were then converted to enzyme activity (i.e., mM p-NP / kg soil / hr).

Phase II: Litter decomposition

To determine the effect that soil preconditioning had on litter mass loss, litter in half of the preconditioned pots was replaced with another 3 g litter of the same species

after the 18-month preconditioning phase (in July 2006), while litter in the remaining pots was replaced with 3 g of a different species' litter. This resulted in litter of each species decomposing on soil in pots that either contained the same litter previously ("home" treatment) or litter of a different species ("away treatment"). Approximately 35 pots were used for each treatment combination (Fig. 1).

The rate of mass loss was measured twice during this second phase of the experiment; once after an 8-month decomposition period (in March 2007) to determine short-term effects of the "home" and "away" treatments, and again after a subsequent 9-month decomposition period (in December 2007) to determine if effects were attenuated in the longer term. The first measurement was done by carefully removing all pieces of decomposing litter from the soil surface, which were then dried at 70°C and weighed to determine mass loss. Three grams of fresh senescent litter from the same species was then placed in the pots and allowed to decompose until the second measurement in December 2007, which was performed in the same manner. In addition, three pots from each treatment were destructively sampled shortly before both sampling times (in March 2007 and November 2007) to determine microbial community structure (PLFA) using the same methods previously described. Enzyme activities were also analyzed during the final sampling of this phase of the experiment.

Phase III: Plant growth

To determine how soil preconditioning affected plant growth and competition, the pots used in the litter decomposition experiment that remained after the end of Phase II were planted with either two *Berberis thunbergii* seedlings, two *Viburnum dentatum* seedlings, or one seedling of each species. To simplify interpretation of the results for

this experiment, only pots that had contained a single species of decomposing litter (“home treatment”) during the previous litter decomposition experiments (Phases I and II) were used for the Phase III experiment (Fig. 1). *Berberis thunbergii* seedlings were grown from seed collected the previous fall, and *Viburnum dentatum* seedlings were obtained from Pinelands Nursery (Jackson, NJ, USA). We used *Viburnum dentatum* seedlings in place of *Viburnum acerifolium* because the latter species was not available from commercial nurseries and because *V. dentatum* is a closely-related congener that is readily available and grows intermixed with *V. acerifolium* at the site the soils came from. Seedlings of both species were less than 6 months old and grown from seeds collected within New Jersey. The soil was gently shaken from the seedlings’ roots, then the roots were gently washed in water to remove all soil before planting. Seedlings were weighed before planting to obtain live mass. After 10 days, seedlings that had died from transplant shock were replaced, and after an additional 10 days, the initial height of each plant was measured. The initial dry mass of each plant was also estimated from a regression of dry mass versus live mass obtained from a separate set of *Berberis* and *Viburnum* seedlings. These seedlings were weighed fresh, then oven-dried at 70°C to obtain dry mass.

The height of each planted seedling was measured after 7 weeks and again after 11 weeks, when each plant was harvested, dried at 70°C and weighed to determine the final dry mass. Root:shoot ratio was determined by separating roots from shoots and weighing roots and shoots from each plant separately. Plant growth (height increment over 7 and 11 weeks, and biomass increment over 14 weeks) was log-transformed and expressed as a log response ratio.

Statistical analysis

The microbial community structure was summarized using principal components analysis (PCA) to reduce the dimensionality of the PLFA data. Most of the variation in the PLFA profiles could be accounted for with two dimensions. Multivariate analysis of variance (MANOVA) on the principal component scores was used to test for significance differences between means in the ordination space. All other analyses were performed using linear models, and all analyses were implemented in R version 2.9.2 (R Development Core Team 2009). Nested analysis of variance (ANOVA) was used for Phases I and II because the experimental design was not fully factorial, while factorial mixed-model ANOVAs were used to analyze the factorial design of Phase III. The mixed model included a random block effect for each pot to account for non-independence of the two plants grown in the same pot. Normality of all data was assessed using normal probability plots. Most enzyme and plant growth data were log-transformed to meet assumptions of normality and homoscedasticity, but back-transformed values are presented in the figures.

Results

Phase I: Initial effects of litter on the soil microbial community

Litter used for the decomposition pre-treatment differed strongly in tissue chemistry and decomposition rate (Table 1). Applying these different litters to the soil surface during the pre-treatment period resulted in different microbial communities at the end of the pre-conditioning period. Principal components analysis of the individual PLFAs showed marginally significant differences ($p=0.068$) among litter treatments on principal components axis 2 (Fig. 2). PC2 was negatively correlated with most PLFAs

and highly correlated to the total PLFA ($r = -0.976$, $p < 0.0001$). Axis 2 can therefore be described as a metric of total microbial biomass. The biomass of broad microbial taxonomic groups also reflected this trend; actinomycete biomass differed significantly between litter treatments ($p = 0.021$), while treatments had a marginally significant effect on Gram positive ($p = 0.087$) and Gram negative ($p = 0.083$) bacterial biomass (Fig. 3).

Though microbial community structure changed during the initial litter preconditioning, no significant effects were detected on soil enzyme activities during this initial phase (data not shown).

Phase II: Effects of pre-conditioning on subsequent litter decomposition

To test whether litter decomposition rate was affected by the pre-conditioning treatment, litter of different species decomposed in pots from each pre-conditioning treatment. Nested analysis of variance on the percent mass loss during this 7-month decomposition period shows a strong and significant effect of the type of decomposing litter ($F_{3,351} = 254.27$, $p < 0.0001$, Fig. 4) as well as a significant effect of the type of litter previously present ($F_{6,351} = 2.68$, $p = 0.015$). Single degree-of-freedom contrasts were used to test more specifically whether litter decomposed more quickly on “home” soil compared to “away”. These contrasts showed that only *Viburnum* litter decomposed significantly slower on “home” soil than “away” soil ($p = 0.0019$). While differences due to preconditioning were also seen in the other litter types, these differences could not be explained by contrasts between “home” and “away” soil. Similar results were observed during the final sampling of this phase of the experiment, but are not reported further here.

Phase II: Effects of pre-conditioning on subsequent soil enzyme activity

The litter treatments significantly altered five of the six extracellular enzyme activities. Urease activity was not significantly affected by any treatments. It also exhibited inherently high variation compared to the other five enzymes (CV = 2.9 compared to CV <1 for all other enzymes), and results from this enzyme are not presented further. For the remaining five enzymes, the type of decomposing litter at the time of sampling significantly affected enzyme activity in all cases ($p < 0.01$) except phenol oxidase, which was marginally different ($p=0.084$). There was significant additional variation due to the preconditioning treatment for acid phosphatase, β -glucosidase, chitinase and phenol oxidase (Table 2, Fig. 5). For β -glucosidase and phenol oxidase, this additional variation from preconditioning was explained by significant differences between “home” and “away” treatments (contrast analysis; $p = 0.022$ and $p = 0.001$ for β -glucosidase and phenol oxidase, respectively).

Phase II: Effects of pre-conditioning on subsequent soil microbial community

The soil microbial community was characterized 8 months after the start of the second phase of decomposition in order to determine the initial response of the microbial community after replacing the pre-treatment litter. Principal components analysis of the microbial community structure (PLFAs) summarized 79.5% of the variance on two axes (56.2% and 23.3% of the variation on axis 1 and axis 2, respectively). Axis 1 was positively correlated with most of the identified phospholipid fatty acids, resulting in a strong correlation ($r = 0.970$, $p < 0.001$) to total microbial biomass. Axis 2 correlated positively to most markers for gram + bacteria and negatively with many markers for fungi, actinomycetes, and protozoans, resulting in a significant positive correlation to the Gram + : Gram- ratio ($r = 0.656$, < 0.001) and the bacteria:fungi ratio ($r = 0.758$, $p <$

0.001). Axis 1 can then be summarized as a measure of total microbial biomass, while axis 2 represents a measure of community composition. Nested ANOVAs demonstrated a marginally significant influence of the pretreatment on axis 1 ($p = 0.056$).

To determine if this response persisted over time, the microbial community was characterized again after an additional 8-month decomposition period (in November 2007). The effects observed at the first sampling date strengthened at the second sampling date. There was a significant effect of the current litter type ($p = 0.015$) and a significant effect of the pretreatment ($p = 0.021$) on the first principal components axis (Fig. 6). The first axis summarized 63.6% of the variation and was negatively correlated to most PLFAs, resulting in a significant correlation to total microbial biomass ($r = -0.979$, $p < 0.001$). Axis 2 explained an additional 24.2% of the variation and was positively correlated with most saturated PLFAs but negatively correlated with most other PLFAs. This caused a significant positive correlation ($r = 0.717$, $p < 0.001$) to the saturated:unsaturated fatty acid ratio, a measure of physiological stress (Kieft et al., 1994, Fierer et al., 2003, Bossio and Scow, 1998). However, axis 2 was not significantly related to any treatments.

Phase III: Effects of pre-conditioning on subsequent plant growth

The initial height increment measured after 7 weeks showed that overall, *Berberis* had marginally more aboveground growth than *Viburnum* ($p = 0.091$, Table 3), and this effect was highly significant at the end of the experiment (11 weeks), with *Berberis* growing approximately 30% faster than *Viburnum* overall ($p = 0.005$). Initially, there were no effects of the litter preconditioning on height growth, but by the end of the experiment this effect was highly significant ($p = 0.006$), with *Berberis* growing least in

pots pretreated with *Viburnum* litter and *Viburnum* plants growing most in pots pretreated with *Berberis* litter (Fig. 7a). There was also a significant but different effect of interspecific competition for *Berberis* and *Viburnum* heights after 7 weeks (Table 3, competition * species interaction, $p = 0.004$) that persisted to the end of the experiment ($p = 0.017$). *Berberis* grew approximately 21% more when grown in monoculture with another *Berberis* plant than when grown with *Viburnum*, while *Viburnum* grew approximately 20% less when grown in monoculture, indicating that although *Berberis* increased in height more rapidly than *Viburnum*, *Viburnum* was a stronger interspecific competitor. This competitive hierarchy was not significantly affected by the litter preconditioning treatment either after 7 weeks or at the end of the experiment (non-significant litter*competition interaction, Table 3).

While height was affected by many of the treatments, the shoot : root ratio also varied strongly by species, litter preconditioning treatment, and indirectly by competition ($p < 0.001$, Table 3). In general, *Berberis* allocated more biomass aboveground ($p = 0.001$), but this also depended on the competitive environment. When grown in monoculture, *Berberis* allocated significantly more of its biomass to shoots than when grown under interspecific competition, while *Viburnum* allocated significantly more to roots in monoculture than in interspecific competition (species*competition interaction). Furthermore, both species tended to allocate more aboveground when grown in soil preconditioned with *Berberis* litter. *Berberis* shoot : root ratio also increased slightly but significantly with increasing biomass ($R^2 = 0.071$, $p = 0.003$) while *Viburnum* did not ($R^2 = 0.022$, $p = 0.116$). However, accounting for the ontogenetic increase in shoot : root

ratio that occurred as biomass increased did not qualitatively change the effects of any experimental treatments on the shoot : root ratio (Table 4).

Since differences in aboveground growth might be explained by these differences in shoot : root ratio rather than differences in total growth, dry biomass increment was also measured at the end of the experiment. Overall, *Viburnum* increased in biomass significantly more than *Berberis* ($p < 0.001$, Table 3, Fig. 7b), even though *Berberis* increased in height more than *Viburnum*. However, both *Viburnum* and *Berberis* increased in biomass most when grown in soil preconditioned by *Berberis* leaf litter (Table 3, Fig. 7b). Preconditioning affected growth of both species in the same way and the competitive hierarchy was not affected by preconditioning since soil preconditioned with *Berberis* litter was best for both species, while the other three preconditioning treatments had little effect on either *Berberis* or *Viburnum* growth.

Discussion

Feedback effects on microbial community structure and function (Phase II)

Both the microbial community structure and its function were strongly influenced by the type of leaf litter decomposing on the soil surface, as well as the type of leaf litter that was previously decomposing. However, this “legacy” of the past leaf litter revealed itself in an unexpected way. Although the initial leaf litter preconditioning apparently had relatively weak immediate effects on microbial community structure and function, those effects were persistent through time, and strongly affected the microbial community later on. This was most clearly demonstrated by the extracellular enzyme activities. Immediately following the pretreatment period, there was no significant difference between the four pretreatment groups for any of the 6 extracellular enzymes. However,

16 months after the pretreatment litter was replaced, there was not only an obvious effect of the current litter type, there was also a significant effect of the previous (pretreatment) litter type for four of the six enzymes. The microbial community structure, as measured by PLFAs, responded in a similar manner. After the initial pretreatment stage, there was only a marginally significant effect of the pretreatment litter on the microbial community, largely driven by a change in total microbial biomass. However, these marginally significant effects of the pretreatment litter type not only persisted through time, the effects strengthened and were most significant 16 months after the pretreatment litter had been replaced.

It was surprising that the pretreatment effects on both the microbial community structure and resulting enzyme activity were more significant 16 months after pretreatment, rather than immediately following the pretreatment. It is possible that the microbial community may have responded less to the individual types of litter available than to the diversity of substrates available for decomposition. After preconditioning, the preconditioning litter was removed as completely as practically feasible; however, small bits of particulate organic matter and any soil organic matter resulting from the decomposition of the pretreatment litter was left behind on the soil surface. When the litter was replaced with a new type of litter, it essentially created a mixture of substrates available for decomposition. Numerous studies have shown that microbial communities in litter mixtures can vary non-additively in both structure and function (Wardle, 2006, Kubartová et al., 2009, Hoorens et al., 2003, Hättenschwiler et al., 2005, Gartner and Cardon, 2004). Though this is not a very likely explanation given the small amount of mixing, it may account for some of the interactions seen.

It is also possible, however, that this interaction over time represents an effect of the initial conditions on microbial community assembly. Previous work has shown that the trajectory of microbial community assembly can be altered by the species that are initially present (Sloan et al., 2006, Fukami and Morin, 2003). If the litter preconditioning slightly altered the microbial community, it is possible that those altered initial conditions could result in different trajectories of microbial community assembly during the treatment time period. This would explain why microbial communities under the same type of litter differed depending on what type of litter was previously present.

It is furthermore important to note that the change in microbial community structure also affected the function of the microbial community. In addition to significant differences in extracellular enzyme activities, there were significant differences in the rate of litter decomposition due to the pretreatment. These differences suggest the distinct microbial communities were not simply functionally redundant, as is often suggested for soil microbes (Wardle, 2006). Instead, the changes in microbial community structure influenced the function of the community; a result that can arise when mutualistic interactions are important. Quorum sensing (Camilli and Bassler, 2006, Keller and Surette, 2006) and the formation of consortia to decompose organic matter (Brenner et al., 2007, Peterson et al., 2006) are two examples of mutualistic interactions that could be important to decomposition. If the pretreatment caused a loss or decrease in abundance of any given microbial group vital to the function of the consortium, then the potential for a consortium to decompose specific organic molecules would have been affected.

Feedback effects on plant competition (Phase III)

This study also tested whether the effects of litter on soil microbial communities influenced plant competition. We found that although the litter pretreatment strongly affected overall plant growth, altering growth by up to 45% in *Berberis* and 75% in *Viburnum*, it did not affect the outcome of competition between these two species. Instead, pretreatments that benefited one plant species benefited the other species as well (Table 3, Fig. 7). Thus, the effect of nitrogen-rich *Berberis* litter on overall plant growth was strong, but plant-soil feedback was relatively weak.

Our finding that relatively strong effects on plant growth had little effect on competition differed from the findings of many feedback studies on other plant species (e.g., Kardol et al., 2006, Petermann et al., 2008, Vogelsang and Bever, 2009), as well as previous suggestions for feedback in this species (Kourtev *et al.*, 2003). These studies all demonstrated or suggested the potential for soil feedbacks to alter competition through changes in the soil microbial community. While these, along with most feedback studies, examine feedback in soil which is influenced by both aboveground and belowground plant inputs, the current study isolated the potential for aboveground leaf litter inputs to drive feedback. The finding that leaf litter-driven feedback operates differently from that of previous studies suggests that different mechanisms may drive feedback from aboveground inputs and from belowground inputs.

Van der Putten et al. (2007) suggested that pathogen-mediated feedbacks belowground have stronger effects on competition than aboveground litter-mediated feedback. We are not aware of any explicit test of this hypothesis since most feedback studies do not separate the aboveground and belowground feedback mechanisms. The current study examines aboveground leaf litter inputs alone, and the results are consistent

with the hypothesis suggested by van der Putten et al. (2007). The higher-quality *Berberis* litter in our study likely increased nutrient availability in the soil, which benefited both *Berberis* and *Viburnum*, leading to a strong increase in plant growth but a weak feedback to plant competition.

Despite this weak short-term effect on competition, leaf litter can strongly influence competition in the long term by changing soil nutrient availability and altering competition for belowground resources (Berendse, 1994, Wedin and Tilman, 1993, Hobbie, 1992, Chapman et al., 2006). How long-term feedbacks from litter relate to shorter-term feedback has not been well-studied though, and few studies distinguish between belowground and aboveground inputs (Ehrenfeld *et al.*, 2005). Meier *et al.* (2009) found that root extracts from *Geum rossii* reduced growth of a co-occurring competitor, but whether or not this affected competition was not assessed. Dorrepaal *et al.* (2007) decomposed litter from 21 separate species while simultaneously growing a phytometer (*Poa alpina*) in the soil with the decomposing litter. They then examined the short-term (9.5 months) and mid-term (21.5 months) feedback effects on the growth of the phytometer. They found large short-term differences between species in the litter-driven feedback effects that attenuated in the medium-term, resulting in only small differences between the control treatment and the different litter treatments. In contrast, the present study found strong effects on plant growth even after 3.5 years. However, we did not simultaneously grow plants during the litter decomposition period, and we periodically replaced litter after most of it decomposed. As a result, while the present study examined the development of feedback over time with successive generations of litter, Dorrepaal *et al.* (2007) examine the persistence of this effect over time. Further

research into both the persistence and development of feedback effects over time would help in understanding how short-term feedbacks relate to the strong long-term feedbacks on competition suggested by Hobbie (1992) and others.

Of further interest in this study was whether *Berberis*, an invasive understory shrub, was a stronger competitor on soil pretreated with *Berberis* litter, since this type of positive feedback might be linked to invasiveness (Klironomos, 2002). The strong competitive ability of *Viburnum* in this study was surprising since *Berberis* is invasive and forms dense thickets in areas where the understory was formerly dominated by *Viburnum* and *Vaccinium* (Ehrenfeld, 1999). *Viburnum* consistently outcompeted *Berberis* in this study though, accumulating on average 13.2% more biomass when growing with *Berberis* than when growing with another *Viburnum*. In contrast, *Berberis* grew 25.6% less when growing with *Viburnum* than with another *Berberis*.

These two species also responded quite differently to inter-specific competition; *Berberis* increased aboveground allocation when grown with *Viburnum*, resulting in higher aboveground growth and shoot : root ratio than *Viburnum*. *Viburnum* on the other hand increased belowground allocation when grown with *Berberis*, resulting in less aboveground growth but more total biomass increase. *Berberis*, in other words, responded primarily to the increased aboveground competition, while *Viburnum* responded to the increased belowground competition. A “snapshot” view of shoot : root ratio can be difficult to interpret because this ratio changes with the age and size of the plant in many species, resulting in “apparent plasticity” (Casper et al., 1998, Weiner, 2004, Geng et al., 2007). However, we observed only a weak relationship between biomass and shoot : root ratio in *Berberis* ($R^2 = 0.071$) and no relationship in *Viburnum*,

and all plants were approximately the same age. Therefore, we believe differences in shoot : root ratios most likely represent real shifts in allocation in response to environmental conditions. These shifts in shoot : root ratio do not demonstrate a change in competitive ability and we did not explicitly test the response of these two species to aboveground or belowground competition. However, the plastic shift in shoot : root ratio strongly suggest that *Berberis* may respond more readily to aboveground competition, while *Viburnum* may respond more to belowground competition. Further experiments are needed to test this explicitly.

It is likely that in this greenhouse experiment where light was readily available and soils were unfertilized, belowground resources were more limiting than aboveground resources. *Viburnum* responded to competition by increasing belowground allocation, possibly explaining *Viburnum*'s stronger competitive ability overall. However, under field conditions where light is more limiting, stronger aboveground competitors (such as *Berberis*) may be at more of an advantage. While not conclusive, this may suggest that where *Berberis* is invasive, aboveground competition may be more important than belowground competition. This could be compounded by the intense deer herbivory in the region that might exasperate aboveground competition (Côté *et al.*, 2004) and the high regional levels of nitrogen deposition that might reduce shift competition from belowground to aboveground (Wilson and Tilman, 1991). Furthermore, easily-decomposed litter such as *Berberis* leaf litter that increases nutrient availability might favour *Berberis* by shifting competition from belowground to aboveground. While this was not observed in the current study, it would be more likely to occur under field conditions where light is more limiting and aboveground competition is stronger. This

could therefore be an important effect of *Berberis* invasion, creating the potential for positive plant-soil feedback in the long term. Long-term field studies are needed to test this hypothesis, as the current study only raises this possibility but does not test this hypothesis.

In conclusion, we found strong effects of litter type on microbial community structure and function. A succession of litter types separated over time had non-additive effects on both microbial community structure and decomposition rates as well. However, whether or not those effects influence plant competition in the short or long term is a largely unexplored question, since most studies do not differentiate belowground and aboveground effects and often focus on pathogen-mediated feedback. We found that despite large effects on plant growth, plant competition was not strongly affected by leaf litter inputs in our short-term study, though our findings suggest that *Berberis* litter may affect competition by shifting competition from belowground to aboveground. This finding highlights the need for more studies that explicitly test feedback effects on intraspecific competition rather than using monoculture-based studies. Future research is also needed to understand how this and other short-term feedback studies relate to the long-term feedback effects of litter on plant community composition.

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Table 1. Leaf litter chemistry and decomposition rate of four species used to precondition soil.

Species	<i>Berberis thunbergii</i>	<i>Microstegium vimineum</i>	<i>Vaccinium corymbosum</i>	<i>Viburnum acerifolium</i>
Tissue % carbon	44.1	42.4	46.9	51.6
Tissue % nitrogen	2.41	0.7	1.27	0.92
C:N ratio	18.3	60.6	36.9	56.1
% soluble compounds	59.6	23.3	63.3	60.5
% hemi-cellulose	11.6	31.3	8.0	5.6
% cellulose	13.4	37.3	13.6	10.9
% lignin	15.4	8.0	15.0	23.0
Lignin:N ratio	6.37	11.44	11.86	25.05
Decomposition constant (k)	1.942	0.762	0.393	1.545

Table 2. Nested analysis of variance for 5 extracellular enzyme activities. Data were log-transformed to satisfy the assumption of normality.

Source	num d.f.	den d.f.	Acid phosphatase		β -glucosidase		Chitinase		Amino peptidase		Phenol oxidase	
			F	p	F	p	F	p	F	p	F	p
Litter type	3	20	9.537	< 0.001	48.613	< 0.001	7.646	0.001	9.634	< 0.001	2.554	0.084
Preconditioning litter type	6	20	3.133	0.025	10.711	< 0.001	3.909	0.010	1.322	0.293	9.083	< 0.001

Table 3. ANOVA results for the growth of *Berberis* and *Viburnum*.

Source	num d.f.	den d.f.	7-week height increment		Final height increment		Final biomass increment		Final shoot:root ratio	
			F	p	F	p	F	p	F	p
Species	1	97	2.910	0.091	8.198	0.005	322.9	< 0.001	11.023	0.001
Competition	1	110	0.003	0.958	1.568	0.213	0.324	0.571	0.652	0.421
Preconditioning	3	110	1.609	0.192	4.364	0.006	3.588	0.016	6.485	< 0.001
Species*competition	1	110	8.832	0.004	5.900	0.017	3.505	0.064	12.241	< 0.001
Species*preconditioning	3	97	1.613	0.191	1.059	0.370	1.386	0.252	1.491	0.222
Preconditioning*competition	3	110	1.036	0.380	1.012	0.391	0.404	0.751	1.119	0.345
Species*comp.*precondition.	3	110	1.240	0.299	2.077	0.107	0.777	0.510	1.817	0.148

Table 4. ANCOVA for the shoot : root ratio of *Berberis* and *Viburnum* accounting for the ontogenetic shift in shoot : root ratio as biomass increases.

Source	num d.f.	den d.f.	F	p
Species	1	95	9.725	0.002
Competition	1	110	0.6112	0.436
Preconditioning	3	110	6.302	<0.001
Species*competition	1	110	13.362	<0.001
Species*preconditioning	3	95	1.341	0.266
Preconditioning*competition	3	110	1.142	0.335
Species*comp.*precondition.	3	110	1.866	0.140
Biomass	1	95	1.672	.199
Species*biomass	1	95	14.624	<0.001

Figure Legends

Fig. 1. Experimental design and number of replicate pots (Phase I and II) or plants (Phase III) that were measured for decomposition (Phase I and II) or growth (Phase III). For enzyme and PLFA analyses, $n = 3$ for all treatments. Arrows indicate which pots were used for each treatment in the subsequent phases of the study. Replicate numbers are not necessarily the same from one stage to the next, since not all pots were sampled in all phases.

Fig. 2. Principal components analysis of phospholipid fatty acids from soil beneath the four species' litter after the 18-month pretreatment.

Fig. 3. Biomass of microbial functional groups after the 18-month pretreatment with four different litter types. Bars represent means \pm standard error ($n = 3$).

Fig. 4. Litter mass loss after decomposing on soil that previously had the same species' litter or a different species. Bars are grouped by the type of decomposing litter, while shading indicates the previous litter pretreatment. Bars represent means \pm standard error ($34 \leq n \leq 37$).

Fig. 5. Enzyme activity of 5 extracellular enzymes. Data shown are untransformed but were log-transformed for analysis. The few negative phenol oxidase values observed resulted from high background activity measured in the control soils.

Fig. 6. Principal components analysis of the microbial community structure 18 months after the pretreatment stage finished. Data points are shown averaged by pretreatment type (a) and by the type of currently decomposing litter (b). Bars represent standard error of the mean ($n = 3$).

Fig. 7. Change in height (A) and total biomass (B) of *Berberis* and *Viburnum* growing in soil preconditioned by four different kinds of leaf litter. Different letters indicate significantly different groups (Tukey's HSD comparisons).

Fig. 1. Experimental design and number of replicate pots (Phase I and II) or plants (Phase III) that were measured for decomposition (Phase I and II) or growth (Phase III). For enzyme and PLFA analyses, $n = 3$ for all treatments. Arrows indicate which pots were used for each treatment in the subsequent phases of the study. Replicate numbers are not necessarily the same from one stage to the next, since not all pots were sampled in all phases.

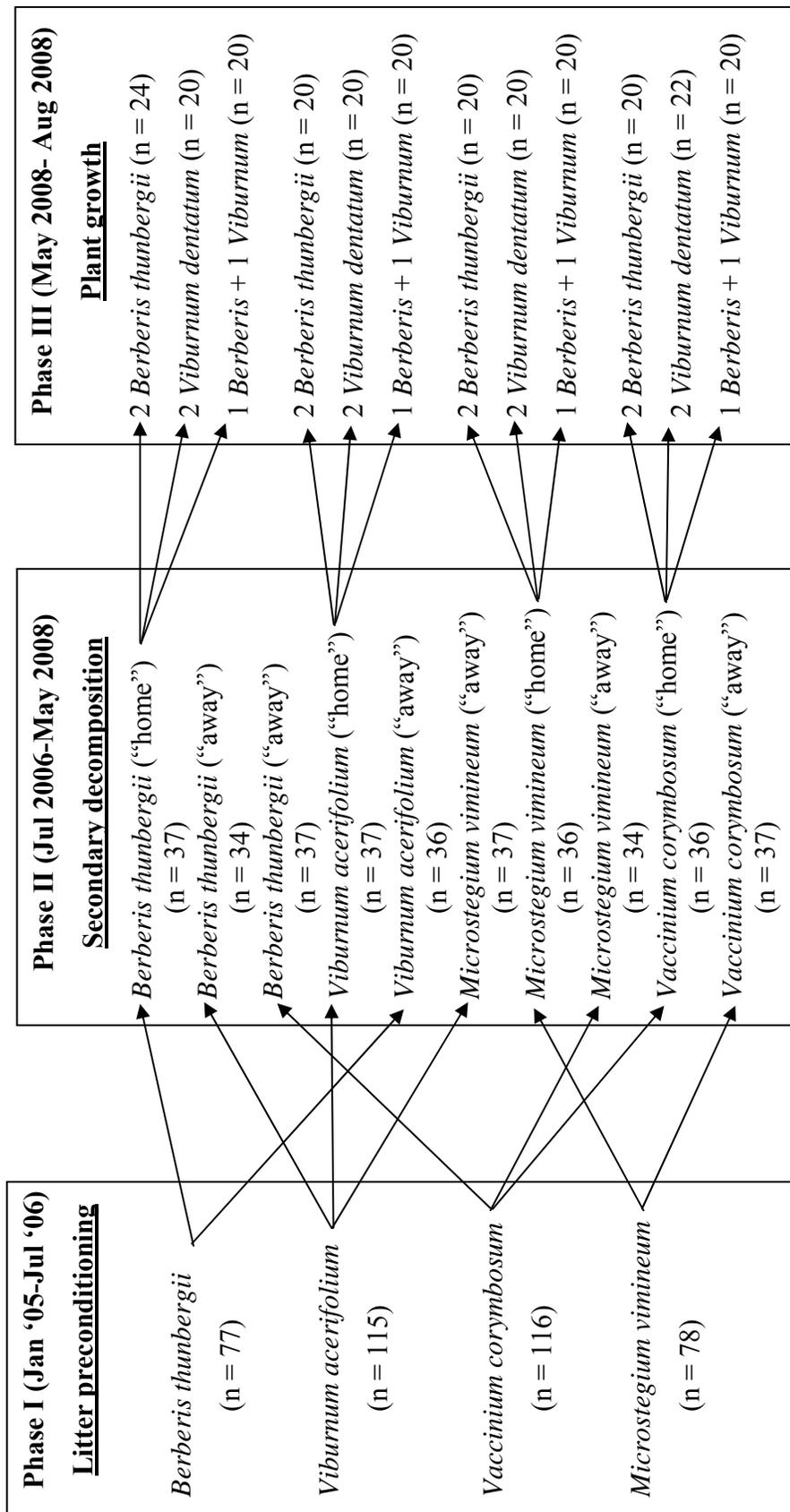


Fig. 2. Principal components analysis of phospholipid fatty acids from soil beneath the four species' litter after the 18-month pretreatment.

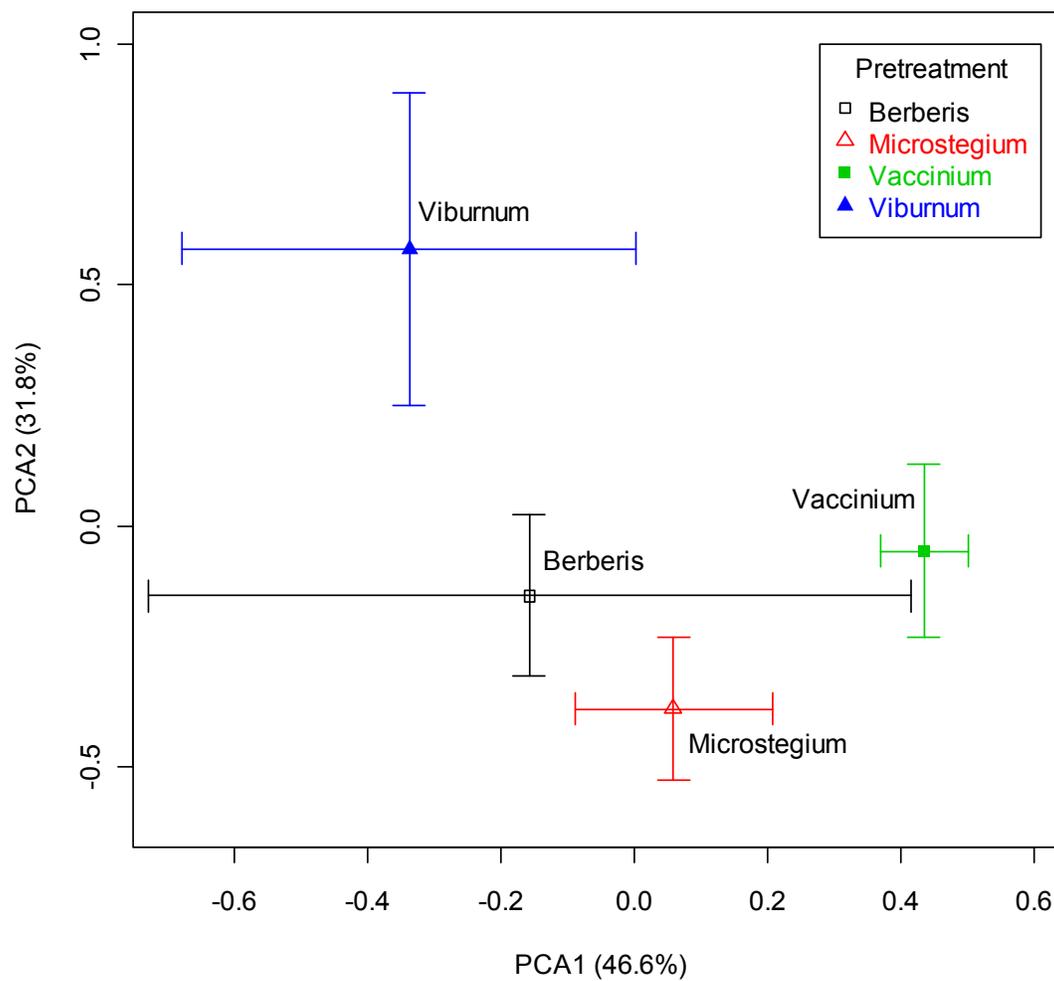


Fig. 3. Biomass of microbial functional groups after the 18-month pretreatment with four different litter types. Bars represent means \pm standard error (n = 3).

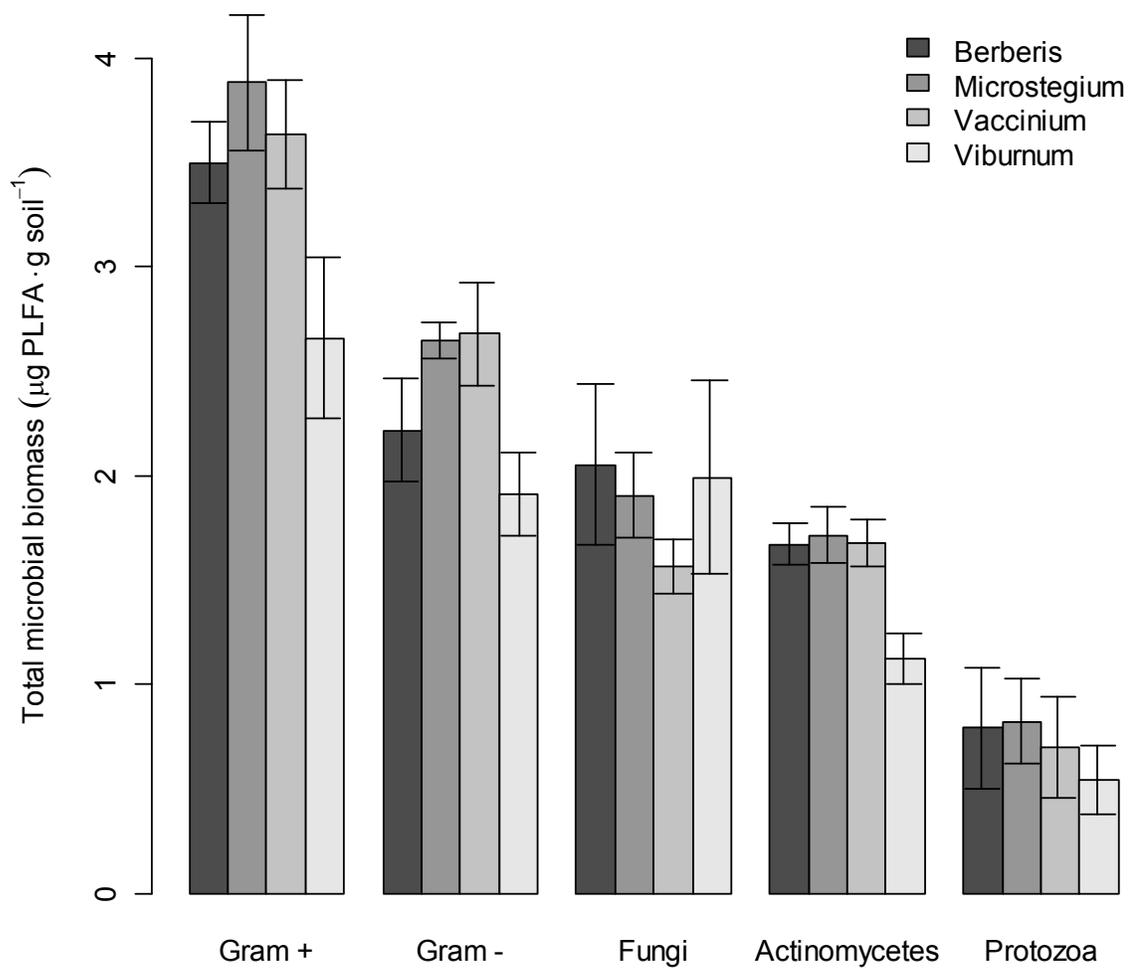


Fig. 4. Litter mass loss after decomposing on soil that previously had the same species' litter or a different species. Bars are grouped by the type of decomposing litter, while shading indicates the previous litter pretreatment. Bars represent means \pm standard error ($34 \leq n \leq 37$).

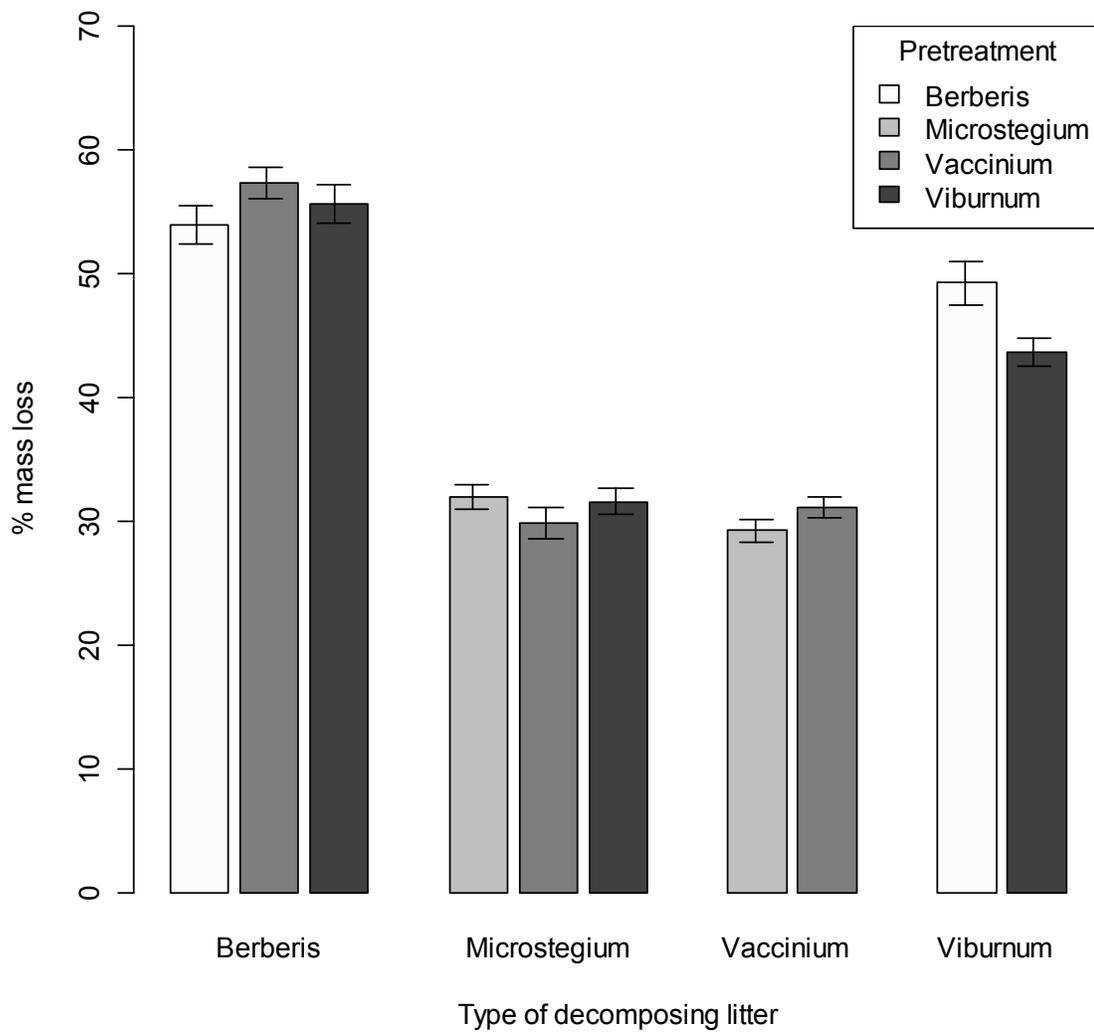


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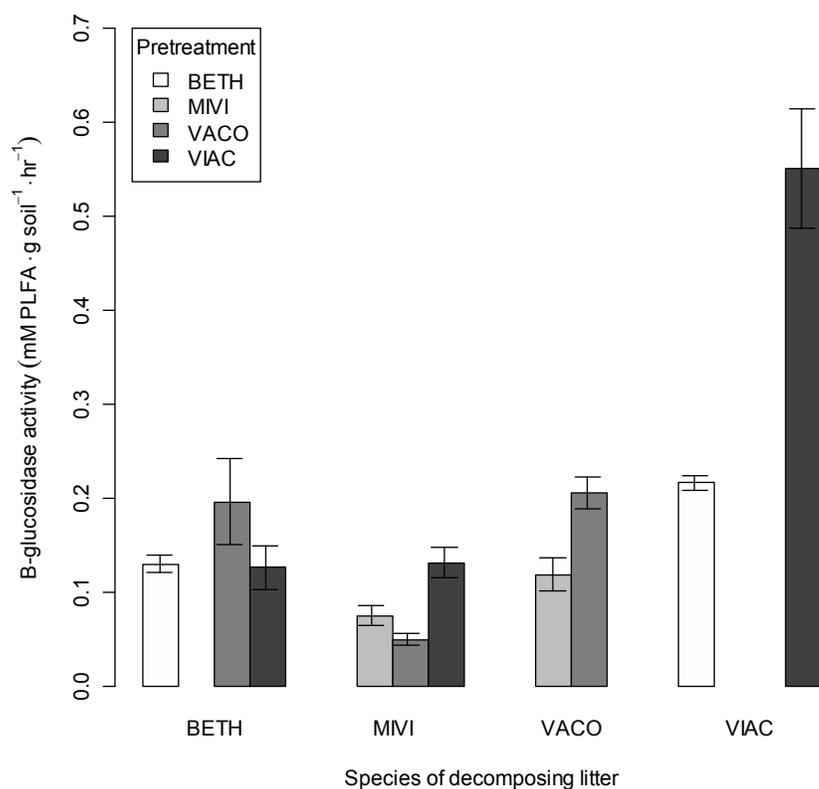
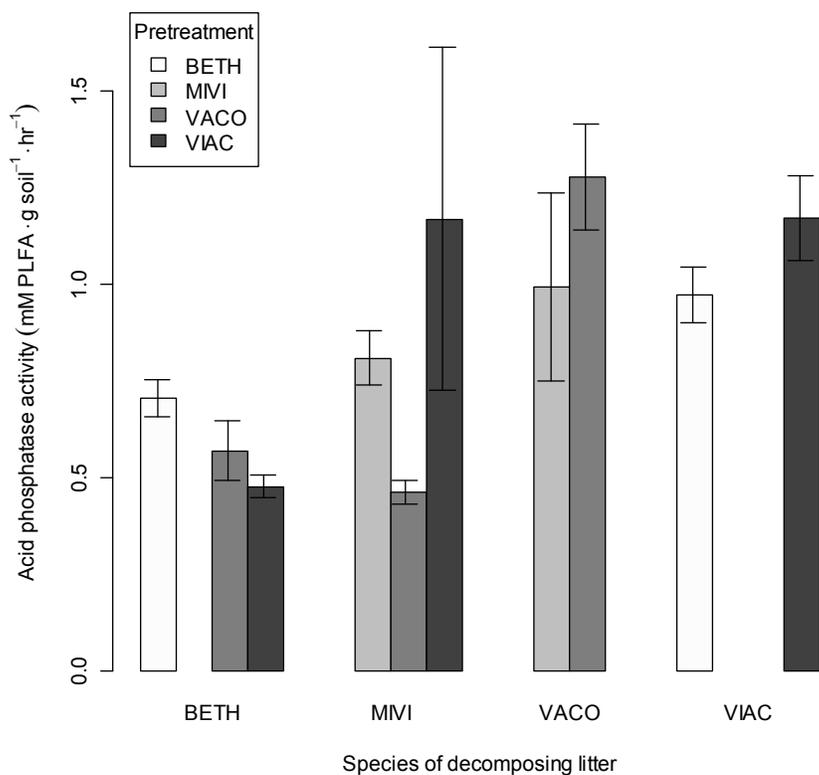


Fig. 5, continued

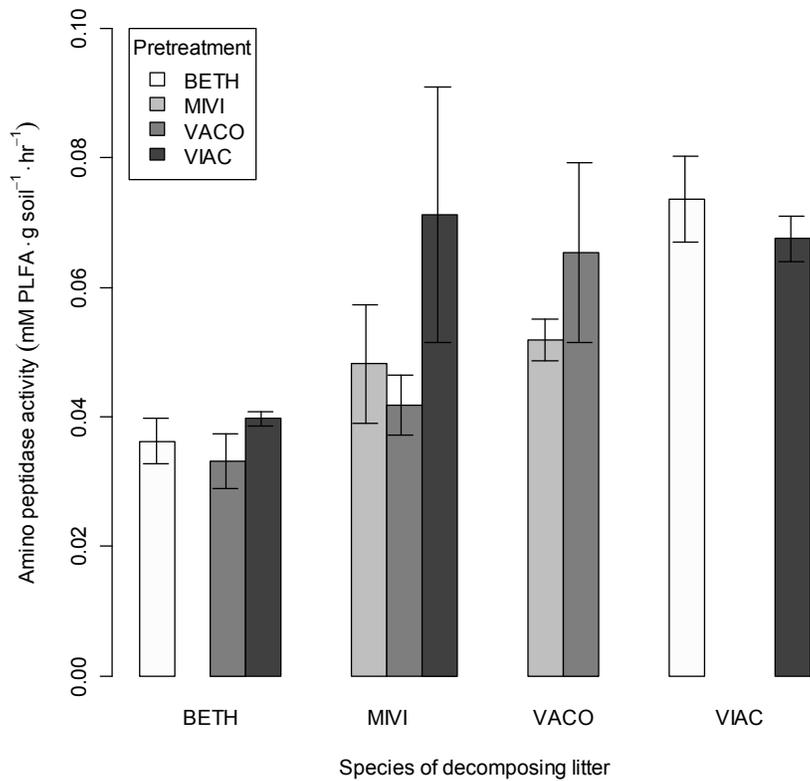
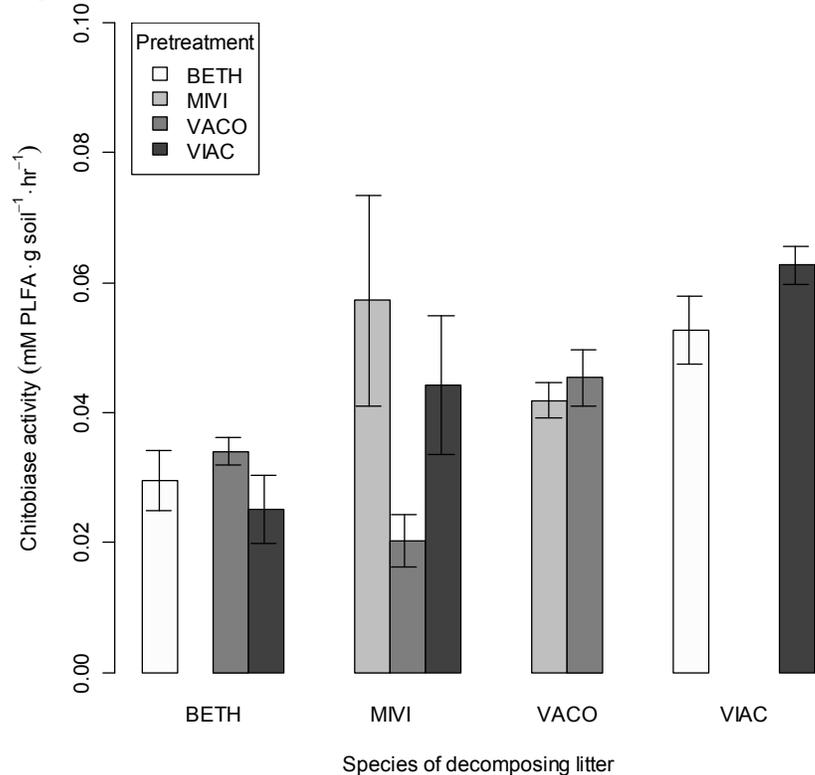


Fig. 5, continued

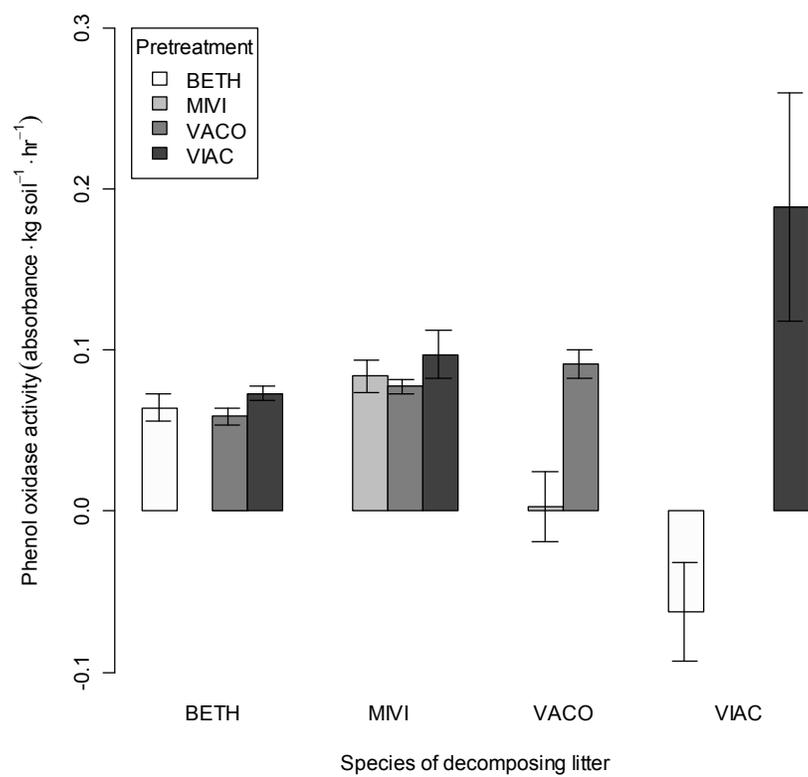


Fig. 6. Principal components analysis of the microbial community structure after 34 months of litter decomposition. The pretreatment stage lasted 18 months, and the second phase lasted a subsequent 16 months. Data points are shown averaged by pretreatment type (a) and by the type of currently decomposing litter (b). Bars represent standard error of the mean ($n = 3$).

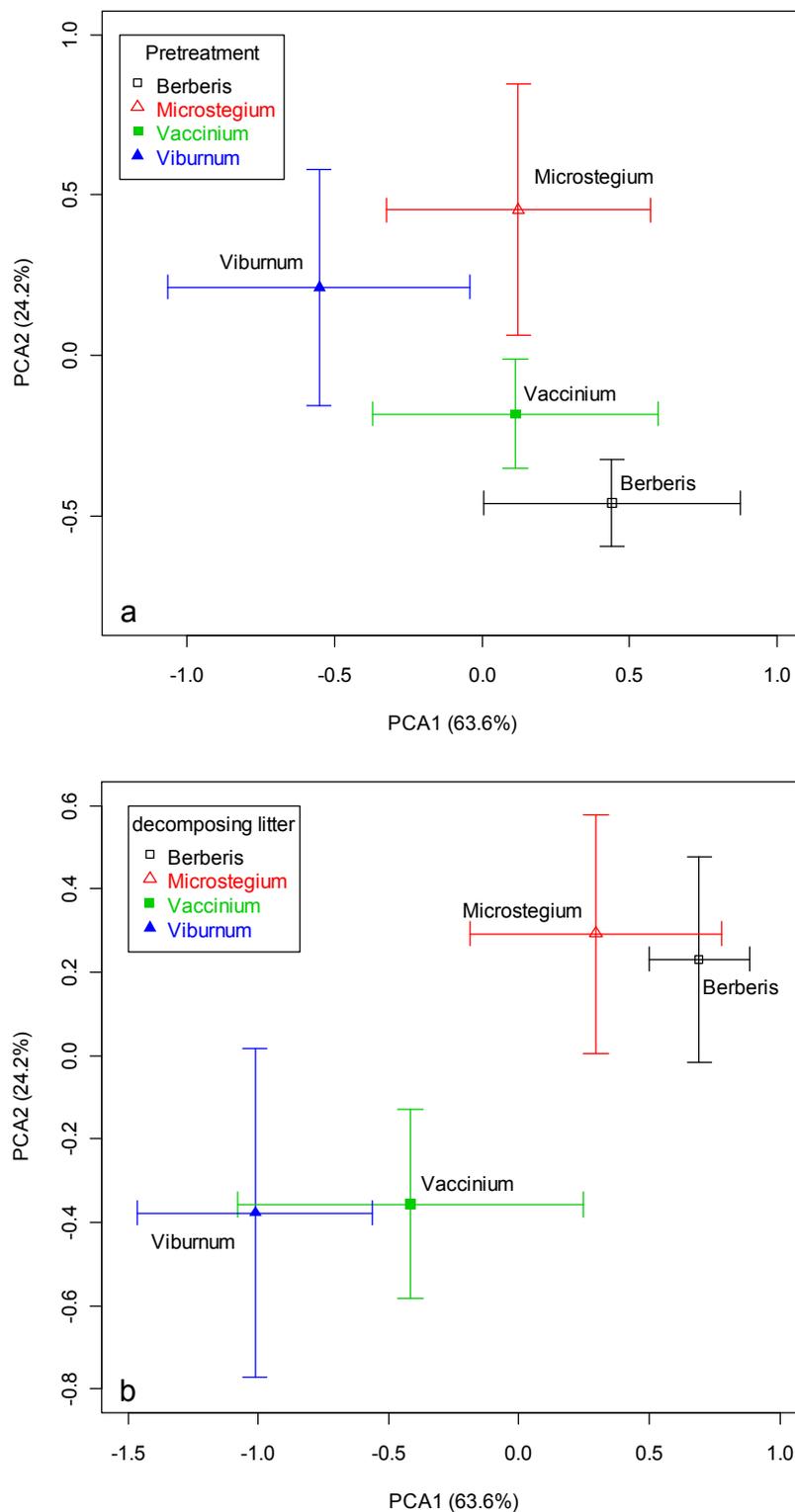
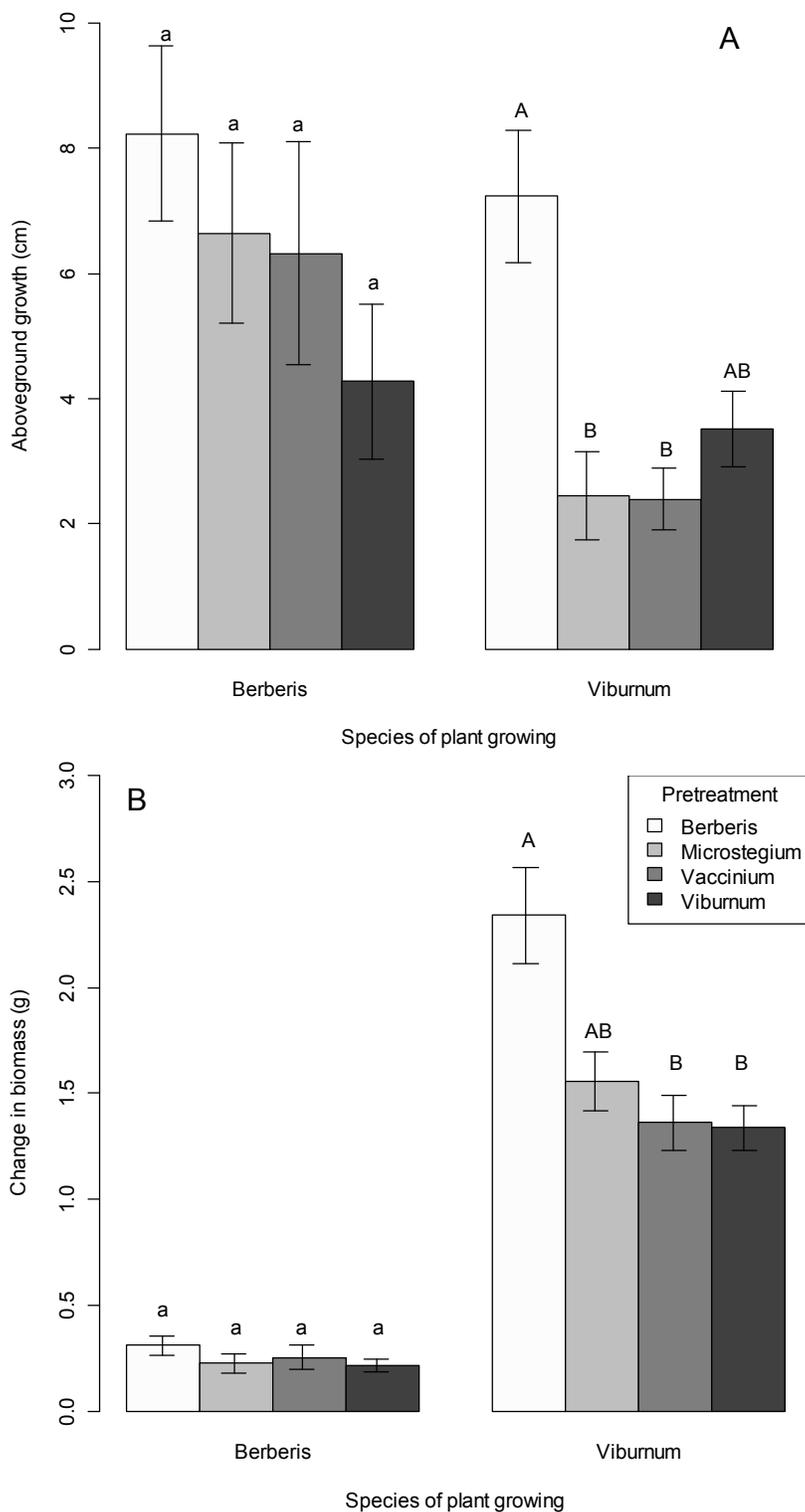


Fig. 7. Change in height (A) and total biomass (B) of *Berberis* and *Viburnum* growing in soil preconditioned by four different kinds of leaf litter. Different letters indicate significantly different groups (Tukey's HSD comparisons)



Chapter 2

Linear and non-linear impacts of a non-native plant invasion on soil microbial community structure and function

Abstract

Biological invasions can alter ecosystem functions such as litter decomposition and nutrient cycling, but little is known about how rapidly these changes occur. It is often assumed that impacts are proportional to invasion density, but this assumption has never been tested and has little theoretical justification. We tested the hypothesis that microbial community structure and function changed with invasion and were proportional to the density of an invasive shrub. We constructed microcosms with mixtures of native and invasive leaf litter, and measured microbial community structure (phospholipid fatty acids) and function (litter decomposition). Decomposition was linearly related to the degree of invasion ($R^2 = 0.945$), but the ratio of bacteria to fungi exhibited a strong non-linear response ($R^2 = 0.513$). These results indicate that impacts are not always proportional to invasion density. This finding has theoretical implications for the study of biological invasions as well as practical implications for the management of exotic invasive species.

Introduction

Exotic invasive species can have dramatic ecosystem-wide effects, especially when they are extremely abundant or represent novel functional forms in the ecosystem (Vitousek & Walker 1989; Crooks 2002). The ecological impacts of these abundant invaders can be very high (Strayer *et al.* 2006; Kenis *et al.* 2009), and often result in high

economic impacts as well (LeMaitre *et al.* 1996; Pimentel *et al.* 2005). However, the impacts of low-density invasions are poorly characterized, and little research has been done on impacts during the early stages of invasion.

Although ecosystem effects may be obvious when an invasive species is well established and integrated into the ecosystem, little is known about ecosystem impacts during the early stages of invasion, when abundance is low. Exotic invasive species often go unnoticed in early stages of invasion, either because they are misidentified or because they are relatively rare (Lockwood *et al.* 2007). Even when invasives are noticed, it is often most prudent to focus on rapid eradication rather than monitoring impacts (Simberloff 2009). Furthermore, quantifying the impacts of invasion can be a challenging task even for dense, large-scale invasions (Parker *et al.* 1999), but quantifying the impacts of sparse, early-stage invasions is seldom even attempted. As a result, scarce resources available for research and monitoring are often focused on dense invasions (Strayer *et al.* 2006), leaving very little known about the impacts of invasive species that occur in low densities.

Understanding the impacts of low-density invasions is important to successfully manage invasive species. In many cases, the economic resources or labor needed to completely eradicate an invader are not available to the manager, even when complete eradication is within the realm of possibility. In these cases, management that succeeds in reducing an invasive species' population size to a relatively low density is often considered a success (Simberloff 2009). However, implicit in this strategy of "maintenance management" is an assumption that reducing the level of invasion will also proportionately reduce the impacts of invasion, a widespread assumption that has not

been tested. Furthermore, assuming that impacts are proportional to density can have very high management costs when this assumption is false (Yokomizo *et al.* 2009).

Despite the potential cost of this untested assumption, it is very common for scientists and managers to assume impacts are proportional to density because the only data available on the impacts of invasion are from high-density invasions (Yokomizo *et al.* 2009). Using this assumption, impact is quantified in a high-density invasion, then divided by the density to estimate the per capita effect. Impacts of low-density invasion can then be estimated, assuming the per capita impact is constant. Parker *et al.* (1999) formalized this approach in a conceptual model in which impact is equal to the product of the spatial extent of invasion, the abundance per unit area, and the effect per individual. This widely-cited conceptual model assumes a linear relationship between the degree of invasion, or in other words, that the per-capita effect of an invasive species is independent of density. Ricciardi (2003) built on this model by accounting for the composition of the recipient community, but retained the assumption of density-independence. Lockwood *et al.* (2007) recognized that impact may differ between stages of invasion (transport, establishment, and spread) as different biological characteristics become important to invasion success, but assume constant per capita effects within a given stage of invasion. Despite these improvements in the conceptual model of invasion impacts, all of these models assume that per capita effects are density-independent.

While the simplifying assumption that per capita impacts are constant may be reasonable given the lack of data to suggest otherwise, ecological theory strongly suggests that the assumption of a linear relationship may be invalid. Nonlinear dynamics are common and important in ecological communities, and accounting for these nonlinear

dynamics can greatly improve model predictions (Hsieh *et al.* 2005). Nonlinear dynamics can arise from feedbacks between organisms and the environment, which are common in plant communities (Ehrenfeld *et al.* 2005; Kulmatiski *et al.* 2008) and may contribute to plant invasiveness (Klironomos 2002; Reinhart *et al.* 2003). Indirect effects can also contribute to nonlinear community dynamics (reviewed in Wootton [1994]), which can change the pattern of invasion (Lau & Strauss 2005). Finally, ecological communities are sufficiently complex that unexpected results are common, and extrapolation based on simple relationships is frequently misleading (Doak *et al.* 2008).

Empirical evidence to support this body of theory is limited, but research examining the relationship between density and impact of crop pest species in agricultural and forestry systems has shown both linear (Poché *et al.* 1982; Parsons *et al.* 2005) and nonlinear relationships (Liebhold *et al.* 1993; Nava-Camberos *et al.* 2001; Brown *et al.* 2007). Together, this body of ecological theory and the empirical results from agricultural and ecological studies suggest that per capita impacts are not constant, and that the utility of this framework for assessing impacts of early-stage invasion is severely limited.

Japanese barberry (*Berberis thunbergii* DC., hereafter referred to as *Berberis*) is a common exotic invasive shrub in eastern deciduous forests of the United States that can form dense, nearly monospecific stands in the forest understory, but can also occur in sparse populations that appear to be relatively stable (Ehrenfeld 1999, pers. obs.). Dense invasion by *Berberis* causes a dramatic shift in soil microbial community structure from a fungal-dominated community to a more bacterial-dominated one (Kourtev *et al.* 2003). This shift in the ratio of bacteria to fungi is an important metric of the soil microbial

community because in general, bacterial-dominated communities are thought to represent early-succession microbial communities (Ohtonen *et al.* 1999; Bardgett *et al.* 2005) with an open, fast-cycling food web with high rates of inputs and outputs. In contrast, fungal-dominated communities are thought to represent slower, more conservative food webs associated with lower rates of nutrient mineralization and leaching (Bardgett & McAlister 1999; Zeller *et al.* 2001; Boyle *et al.* 2008; Coleman 2008). Thus, a shift in the bacterial to fungal ratio often results in alteration of nutrient cycles and strong ecosystem impacts.

These ecosystem impacts of *Berberis* invasion were in fact previously demonstrated by differences between highly-invaded and uninvaded areas, both in the field and in greenhouse microcosms. *Berberis* induced an increase in net nitrogen mineralization and nitrification (Ehrenfeld *et al.* 2001) as well as a change in soil enzyme activities (Kourtev *et al.* 2002). The goal of the current study is to quantify the relationship between the density of *Berberis* and its impact on soil microbial community structure and function. By doing so, we aim to test if the per capita effect of *Berberis* is density dependent. Specifically, we test the hypothesis that the initial invasion by *Berberis* causes a shift in microbial community structure and function. Second, we test the hypothesis that the impact of *Berberis* on soil microbial community structure and function is proportional to the density of *Berberis*.

Materials and methods

Litter decomposition

Berberis litter was collected by stripping senescent leaves from branches during litterfall in October 2005. This method was employed in order to collect adequate quantities of pure *Berberis* litter. Mixed-species tree canopy litter was collected from a

mature oak-maple-beech forest (basal area: 16% *Quercus velutina*, 32% *Quercus alba*, 26% *Acer rubrum*, 13% *Fagus grandifolia*, 10% *Carya glabra*; Rutgers University Helyar Woods, New Brunswick, NJ) in December 2005 by raking fallen leaves from the forest floor. All litter was air-dried for 1 month. Tree canopy litter was cut into 2-3 cm pieces (approximately the size of *Berberis* leaves) to ensure different proportions of *Berberis* and canopy litter did not also differ in the size distribution of litter pieces.

Soil was collected from the top 10–15 cm of an uninvaded mature beech-oak forest (Rutgers University Kilmer Woods, Piscataway, NJ) in late December 2005 by removing the litter layer and sieving soil through a 1-cm mesh screen to remove debris and homogenize the soil. Microcosms were constructed in mid-January 2006 by filling 10-cm diameter plastic cups with approximately 5 cm layer of soil. A circular piece of 1.5-mm mesh window screen was placed on top of the soil layer to aid in future recovery of the decomposed litter, which was placed on top of the mesh. *Berberis* and tree canopy litter was then weighed and mixed in varying proportions (0%, 2.5%, 5%, 10%, 25%, 50%, 75%, and 100% *Berberis* litter), with each microcosm receiving a total of 4 grams of the litter mixture. We chose these litter proportions in order to determine whether a small proportion of exotic litter (typical of very early stages of invasion) would cause changes in microbial (and ecosystem) function, or whether changes in function are only observed after some threshold level of invasion has occurred. Four replicate microcosms were constructed for each treatment; however, three microcosms were contaminated during the microbial community extraction and were excluded from the data set, for a total of 29 microcosms.

Microcosms were loosely covered to prevent rapid drying and kept at 30 °C in a dark incubator for two months. Adequate moisture was maintained by spraying microcosms with a fine mist three times weekly. After this initial rapid decomposition period, microcosms were removed from the incubator and kept uncovered in a greenhouse at 25 °C for an additional 5 months. Litter was then carefully collected, dried at 70 degrees C, and weighed. All litter samples were then ground up and analyzed for %C and %N using a Costech Analytical ECS 4010 elemental analyzer (Valencia, CA, USA) at the University of Nebraska – Lincoln’s Ecosystem Analysis Lab. A subsample of soil from each microcosm was dried at 105 °C for 4 days to measure soil moisture, and the remaining soil was kept frozen (-20°C) until phospholipid fatty acid (PLFA) analysis was conducted.

Microbial community structure

Soil microbial community structure was quantified using the PLFA technique. This method extracts phospholipid fatty acids from microbial cell membranes in soil and quantifies them, giving an approximate measure of the relative abundances of different microbial groups. PLFA is the most commonly used approach in ecological studies of soil microbial community structure (O'Donnell *et al.* 2005) because it provides a rapid, flexible, and ecologically relevant metric of soil microbial community structure (reviewed in (Joergensen & Wichern [2008]).

PLFAs were extracted using a method modified from White *et al.* (1979). Approximately 4 grams soil was extracted twice using 15 mL one-phase chloroform: methanol: phosphate buffer solution (1:2:0.8). Extractant from the first and second extractions were combined, mixed with equal parts DI water and chloroform, and allowed

to separate overnight. The chloroform layer was then removed, concentrated, and fractionated through a silicic acid column (100-200 mesh) into neutral-, glyco-, and phospho-lipids by eluting with chloroform, acetone, and methanol, respectively. The phospholipid fraction was retained and evaporated to < 1 mL (40°C, 200hPa). An internal standard (19:0, nonadecanoic acid) was added and the phospholipid fraction was then saponified and methylated following the protocols of the Sherlock Microbial Identification System (MIDI Inc., Newark, DE, USA).

The resulting fatty acid methyl esters (FAMES) were analyzed using the MIDI Sherlock system connected to an HP gas chromatograph with flame ionization detector (GC-FID, Hewlett Packard 5890 Series II, Palo Alto, CA, USA) and an HP Ultra 2 phenyl methyl silicone fused capillary column (25 m×0.2 mm i.d., film thickness 0.33 µm). The temperature was ramped from 170 °C to 250 °C at 5 °C / min. FAMES were identified and quantified by the MIDI Sherlock system based on retention times and peak area. Peak areas were standardized using the internal standard (19:0) peak area, and reported as relative abundances (mol%).

Statistical analysis

Litter decomposition (expressed as percent mass loss) was related to the initial percentage of *Berberis* in the litter mixture using linear, log-linear, quadratic, and cubic models. These models were chosen to represent a range of possible responses to increasing effects of invasion (see Fig. 1). In addition, the linear and quadratic models were further tested for a threshold effect caused by the presence or absence of *Berberis*. Akaike's Information Criterion corrected for small sample sizes (AICc) was used to select the model that best fit the data, and a lack-of-fit test was implemented to explicitly

test the adequacy of the linear model. No transformations were needed to meet the assumptions of normality and homoscedasticity.

The microbial community structure was categorized into broad microbial groups (Gram + bacteria, Gram – bacteria, fungi, actinomycetes, and protozoans) by summing up PLFAs that are signature biomarkers of each group, as reported in the literature (White & Ringelberg 1998; Zelles 1999). This was used as a metric of biomass for each microbial group. The effects of *Berberis* on the biomass of each microbial group was tested using the same set of linear and nonlinear candidate models that was used to analyze the litter decomposition. Data were log-transformed when necessary to satisfy the assumption of homoscedasticity.

To examine overall microbial community structure, the microbial groups were ordinated onto two axes using nonmetric multidimensional scaling (NMDS) based on a Bray-Curtis similarity index. Principal Coordinates Analysis (PCoA) was used to supply the initial configuration for the NMDS. Multivariate analysis of variance (MANOVA) was used to test for differences between treatments in the two-dimensional ordination space. Furthermore, to test for differences between treatment groups along each of the two NMDS axes, the same set of linear and nonlinear models used to analyze litter decomposition were employed to analyze differences in community structure. Finally, linear models were used to determine if differences in microbial community structure explained differences in the rate of litter decomposition. All statistical analyses were conducted using R version 2.7 (R Development Core Team 2009). Ordinations were performed using the “vegan” package in R (Oksanen *et al.* 2009).

Results

Litter Decomposition

Mass loss after 29 weeks increased linearly with the initial percentage of *Berberis* in the litter mixture, and 94.5% of the variation in mass loss was explained by the initial litter composition ($p < 0.0001$, Fig. 2). AICc scores for a subset of possible linear and nonlinear models suggested the linear fit was best (Table 1), and a lack-of-fit test showed insufficient evidence to reject the linear model ($F_{6,21} = 0.354$, $p = 0.900$). Furthermore, there was insufficient evidence to show any threshold effect resulting from any particular percentage of *Berberis* litter or the presence or absence of *Berberis*. Similarly, nitrogen mineralized from the litter after 29 weeks was linearly related to the initial percentage of *Berberis* in the mixture (Fig. 3), and there was again no evidence for a nonlinear relationship ($F_{6,21} = 0.373$, $p = 0.888$).

Microbial Community Structure

The proportion of *Berberis* litter had no significant effect on the total biomass of the microbial community (data not shown); however, it did significantly affect the relative abundances of the microbial groups (Wilks' λ , $p = 0.030$). Specifically, the relative abundances of Gram + bacteria ($p = 0.047$), fungi ($p < 0.001$), actinomycetes ($p = 0.001$), and protozoans ($p = 0.006$) were significantly affected by *Berberis* (Table 2). For all of the microbial groups except one (protozoans), the top candidate model was nonlinear and included a threshold effect, indicating the microbial community was significantly different when *Berberis* was absent compared to when it was present, even in a small amount.

Changes in microbial community structure were also examined by ordinating communities into a two-dimensional space. Distances along the first NMDS axis explained a large portion of the variation in distances in the original Bray-Curtis similarity matrix ($r^2 = .786$), and the second NMDS axis explained most of the remaining variation (combined $r^2 = 0.98$). Two axes were used because including additional axes did little to improve this correlation or to reduce stress (final stress = 7.7).

The NMDS ordination revealed a strong nonlinear effect of the litter composition on the microbial community structure (Wilks' λ , $p = 0.005$, Fig. 4a). The top-ranked candidate model for NMDS axis 1 was a unimodal curve (quadratic model) that explained a significant portion of the variation in community structure along the first axis ($p = 0.014$, Fig. 4b). This relationship was driven by the high abundance of protozoans in the 0% *Berberis* and 100% *Berberis* treatments and low abundance in the other treatments. Most of the variation among litter treatments, however, was along NMDS axis 2. This axis represented the gradient from fungal-dominated communities (e.g., 0% *Berberis*) to bacterial- and actinomycete-dominated communities (e.g., 100% *Berberis*). The top-ranked model for the second axis was nonlinear (quadratic with threshold) and explained 54.5% of the variation along the second axis ($p < 0.001$, Fig. 4c, Online Resource 1). To explore this relationship further, we explicitly examined the effect of litter composition on the bacterial : fungal ratio. We found a strong nonlinear relationship between this ratio and litter composition ($F_{3,25} = 8.77$, $p < 0.001$) that explained 51.3% of the variation. The AICc scores of the candidate models strongly favored the quadratic model with a threshold effect caused by the presence of *Berberis* litter (Table 2). The bacterial : fungal ratio was much lower when *Berberis* litter was

absent than when it was present ($p < 0.0001$), even when *Berberis* litter was present in an extremely small quantity (Fig. 5). When *Berberis* was present, the relationship was unimodal, with the highest bacterial : fungal ratios observed in soils with either a low or high proportion of *Berberis* (Fig. 5).

Although the proportion of *Berberis* litter significantly affected both the microbial community structure and litter decomposition rate, no significant relationship was found between these two response variables (data not shown).

Discussion

We designed this study to determine whether the soil microbial community responded linearly to changes in litter inputs resulting from the invasion of a non-native understory shrub. The results showed that the microbial community function (decomposition) responded linearly, while microbial community structure responded non-linearly (Fig. 4). In particular, the response of the bacterial : fungal ratio was strongly non-linear (Fig. 5). These nonlinearities were surprisingly consistent; microbial groups consistently and drastically changed when *Berberis* litter was added into the mixture, even when added in an extremely small quantity (2.5%). These drastic shifts in microbial community structure were unexpected and could not have been predicted based on the microbial community response along the rest of the litter gradient.

The nonlinear changes in microbial community structure were exemplified by the effects of *Berberis* on the bacterial : fungal ratio. This ratio is an important metric of the soil microbial community because the relative abundance of these two microbial groups can affect the rates of carbon cycling (Demoling *et al.* 2008), soil organic matter storage and decomposition (Six *et al.* 2006), and nitrogen cycling (Högberg *et al.* 2007; Boyle *et*

al. 2008). Fungi and bacteria also have different growth rate responses to changes in litter quality, resulting in different proportions of soil organic matter being converted into microbial biomass (Rousk & Bååth 2007), which has implications for soil carbon storage and sequestration (Six *et al.* 2006). Many studies have demonstrated an increase in bacterial biomass relative to fungal biomass after addition of nitrogen (Bardgett & McAlister 1999; Demoling *et al.* 2008) or nitrogen-rich litter (Kourtev *et al.* 2003; Rousk & Bååth 2007) and along natural fertility gradients (Högberg *et al.* 2003). A shift from fungi to bacteria, as seen in this study after addition of *Berberis* litter, can indicate a shift from a closed-cycle, oligotrophic food web to an open, rapid-cycling food web (Bardgett *et al.* 1999; Coleman 2008).

Previous studies have also shown a similar increase in the bacterial : fungal ratio in soils dominated by *Berberis* (Ehrenfeld *et al.* 2001; Kourtev *et al.* 2003), but these studies do not tell us how sensitive the microbial community is to a small amount of invasion. The surprising result from this study was that this shift toward a bacterial-dominated system occurred when *Berberis* was present in any quantity. In fact, replacing only 2.5% of the canopy litter with *Berberis* litter nearly doubled the amount of bacteria relative to the amount of fungi. This suggests a rapid and dramatic change in the soil microbial community after only a small amount of *Berberis* invades an area, with a sudden shift from a fungal- to a bacterial-dominated community.

Managers dealing with invasive plants on a limited budget may treat eradication as an unreasonable goal for restoration, and instead try to keep the abundance of invasive species at a reasonably low level (Simberloff 2009). Implicit in this strategy, however, is an underlying assumption that the effect of invasion diminishes proportionally as

abundance decreases. This study shows that at least for some types of impacts, this assumption may not be justified and should be examined when making management decisions. While we did not examine a suite of invasive species and cannot generalize our results beyond invasion by *Berberis*, our findings do provide evidence that the degree of impact and the density of invasion may not always correspond to one another.

This conclusion was further supported by the strongly non-linear change in bacterial : fungal ratios in litter mixtures that did contain *Berberis* (Fig. 5). As the proportion of *Berberis* increased, the bacterial : fungal ratio decreased in mixtures with less than 50% *Berberis*. However, in mixtures with greater than 50% *Berberis*, the bacterial : fungal ratio increased as the proportion of *Berberis* increased. This relationship was unexpected and might have been driven by predator-prey dynamics or other trophic relationships in the soil. The only other study we are aware of that examined soil microbial communities under a range of invasion levels (0%, 38%, 80%, and 100% invaded) also showed a similar unimodal response of the bacterial : fungal ratio to invasion by *Mikania micrantha* (Li *et al.* 2006). Though this study did not explicitly look at the shape of the microbial response to the level of invasion, their results were strikingly similar to our findings, lending support to the conclusion that the effects of invasion can be nonlinearly related to the degree of invasion. Though one can only speculate on what might have caused such a result, this unpredictability highlights the complexity of soil food web structures and suggests that one cannot assume the effects of *Berberis* invasion will gradually and linearly increase as *Berberis* dominance increases.

While the response of microbial community structure to *Berberis* was nonlinear, microbial community function (litter decomposition) responded in a strictly linear

manner (Figs. 2, 3). In field studies, *Berberis* litter decomposes much faster than oak or birch litter (Ehrenfeld *et al.* 2001; Kourtev *et al.* 2002). It was not surprising then to find that increasing the amount of this highly-decomposable litter rapidly increased the overall decomposition rate. It was surprising, however, that this relationship was strictly linear. The potential for non-linear per-capita effects on litter decomposition is particularly likely in view of the many studies demonstrating decomposition rates in litter mixtures are not simple linear combinations of the decomposition rates of the component species (reviewed in Hättenschwiler *et al.* 2005), and studies have found that litter mixtures may decompose faster than, slower than, or equal to the expected rate based on a simple mixing model of the component species (Hättenschwiler *et al.* 2005, and references therein). Because of the large differences in litter chemistry and decomposition rates between *Berberis* and canopy litter, we expected to observe non-additive effects. *Berberis* litter is chemically very different from the tree canopy litter that we collected, containing a large amount of nitrogen (2.41% N), and a low C:N ratio (18.3) when compared to canopy litter (KJE and JGE, unpublished work). However, our results were similar to other studies which have found predominantly linear effects of species mixtures on decomposition and nutrient mineralization rates (Rothe & Binkley 2001), and a lack of relationship between the chemistry of component litters and the decomposition rate of their mixtures (Hoorens *et al.* 2003).

Few other studies have examined the effects of invasion across a gradient of invasion strength, and studies on the effects of invasion most commonly contrast “heavily invaded” and “uninvaded” sites (but see Li *et al.* 2006; but see Li *et al.* 2007). Experimental (greenhouse or common garden) studies often use monocultures of either

native or exotic invasive species to provide strong contrasts between the two. While this is a useful method to ensure results and examine the “worst-case scenario”, it leaves unanswered the question of how communities and ecosystems respond to low levels of exotic invasion.

Understanding how systems respond across a gradient of invasion would aid land managers in making decisions about exotic invasive species (Yokomizo *et al.* 2009). In a recent modeling effort, Yokomizo and colleagues (2009) demonstrated that the shape of the density-impact curve, which describes the level of impact at any given invasion density, can strongly affect the optimal strategy for managing an invasive species. Furthermore, the cost of falsely assuming density-independence can be very substantial in terms of wasted management resources, especially when the true density-impact curve exhibits a threshold effect at a low density of invasion, as seen in this study. Our study is, to our knowledge, the first to explicitly show that such a density-impact curve exists in a real invasion. This highlights the need, raised by Yokomizo and colleagues, to measure density-impact curves in real invasions, and examine the assumption of density-independent impacts.

Finally, this study highlights the importance of understanding the impacts during the early, low-density stage of invasion. Despite its seemingly innocuous nature, impacts during this stage may in fact be high, as shown in this study. Many populations of non-native species are considered “naturalized” but not “invasive,” and while some of these species remain naturalized, others eventually become invasive after a “lag phase” (Williamson & Fitter 1996). Identifying which species are incipient invaders is an important step in the management of biological invasions. This study shows that in

addition to identifying these incipient invaders, future research should also focus on identifying invasive species that have strong ecosystem-level effects at low population densities.

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Table 1. Model fitting of the relationship between the initial litter composition and mass loss after 7 months. The hypothesized threshold divides treatments with *Berberis* litter present or *Berberis* absent.

Model	R^2_{adj}	F	p	AICc	$\Delta AICc$
Linear	0.943	463.4	<0.0001	161.20	
Quadratic	0.942	228.2	<0.0001	163.29	2.092
Linear with threshold	0.941	223.2	<0.0001	163.89	2.699
Quadratic with threshold	0.940	147.2	<0.0001	166.05	4.856
Cubic	0.940	146.6	<0.0001	166.16	4.964
Log-linear	0.931	376.0	<0.0001	166.89	5.696

Table 2. Δ AICc scores for the set of candidate models testing the relationship between litter composition and the biomass of microbial groups. The top-ranked model is indicated by asterisks, representing the significance of that model

(*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, NS: $p > 0.05$).

Model	Gram+	Gram-	Fungi	Actinomycetes	Protozoans	Bacteria : Fungi
Quadratic + threshold	0.129	2.941	***	2.874	3.483	***
Linear + threshold	*	NS	5.645	***	9.427	3.491
Log-linear	2.023	1.362	15.048	12.495	8.508	14.293
Cubic	3.218	5.337	17.415	15.280	**	18.352
Quadratic	0.732	2.487	18.550	15.285	0.598	17.619
Linear	3.495	0.105	16.501	13.848	7.459	15.178
R ² for the top model	0.193	NS	0.5403	0.393	0.387	0.513

Figure Legends

Figure 1. Examples of theoretical responses of ecosystem function to the degree of invasion.

Figure 2. Response of litter decomposition rate (% mass loss after 7 months) to the initial proportion of *Berberis* litter. The regression line represents the best fit model.

Figure 3. Nitrogen content (%) remaining in decomposing leaf litter after 7 months. The regression line represents the best fit model.

Figure 4. NMDS ordination plot of microbial community structure after 7 months (plot a). Points represent the mean \pm 1 standard error, and color indicates the initial proportion of *Berberis* litter. Each microbial group name indicates its position in the ordination space. Plots b and c depict the relationship between litter composition and the NMDS1 (plot b) and NMDS2 (plot c) axis scores. The fitted curve represents the predicted response of the top-ranked model.

Figure 5. Response of the bacteria : fungi ratio to the initial proportion of *Berberis* litter. The line indicates the best fit model.

Figure 1

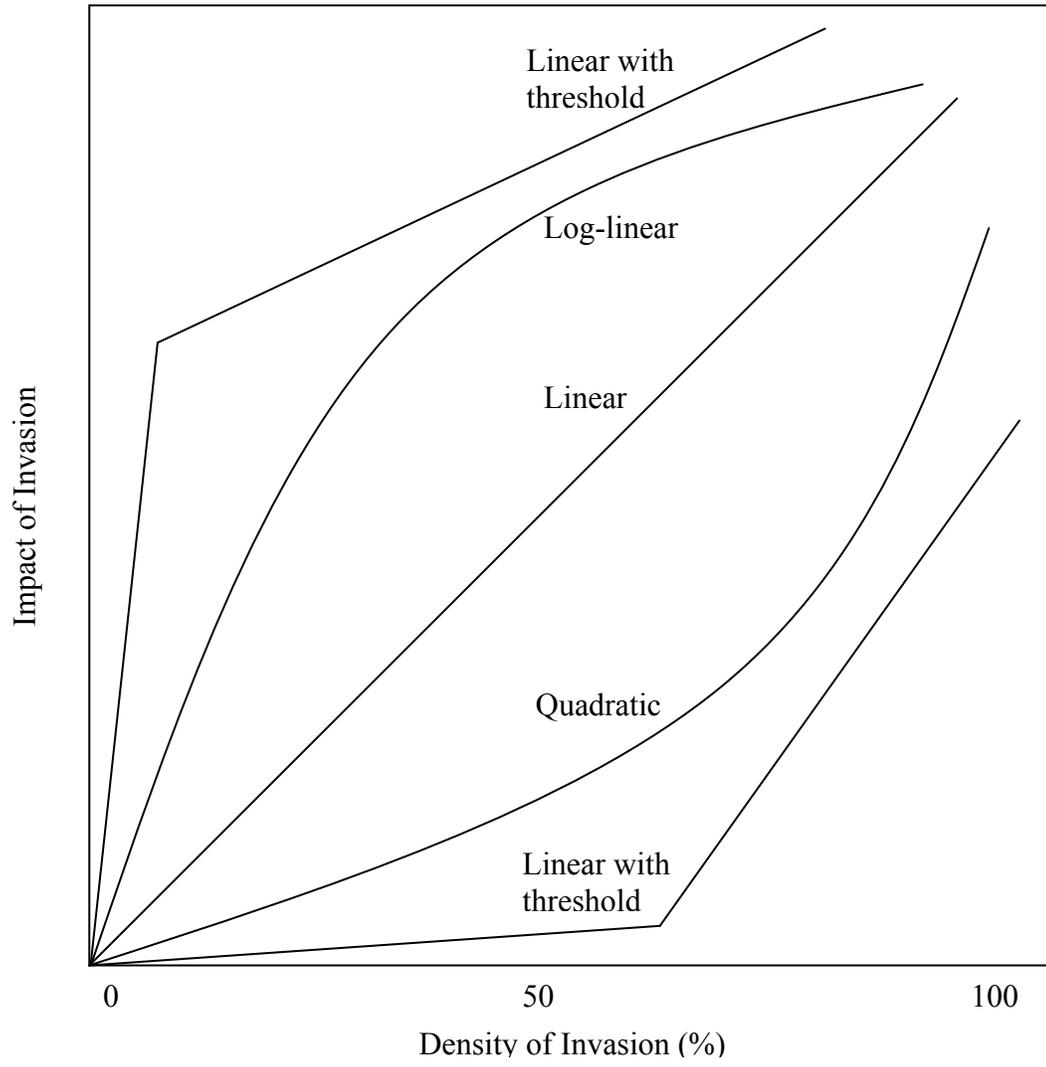


Figure 2

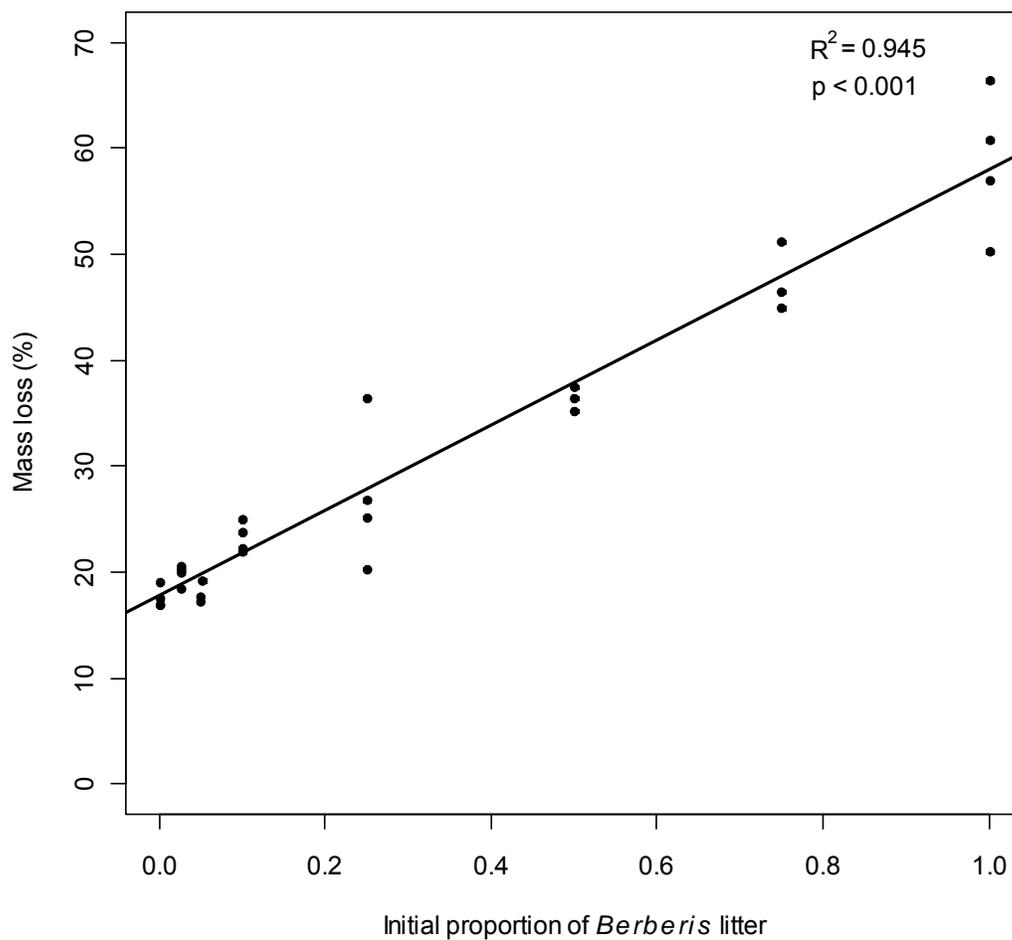
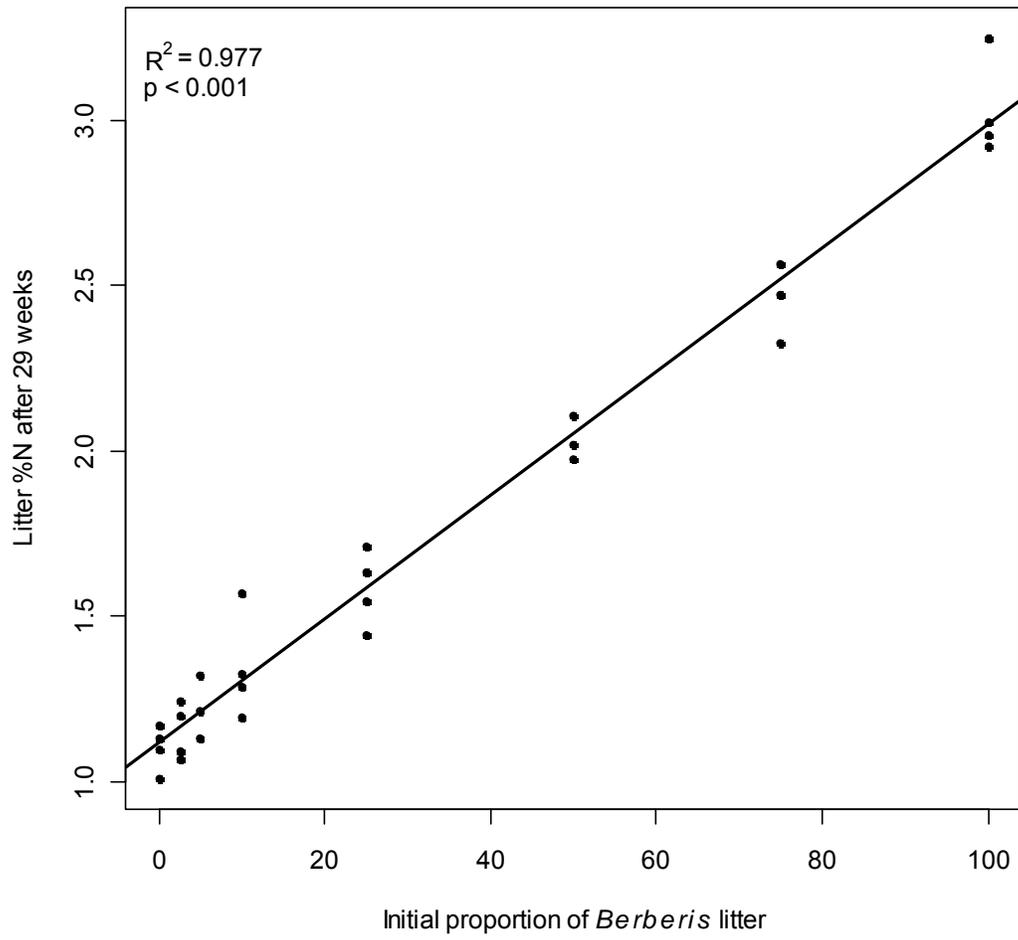


Figure 3



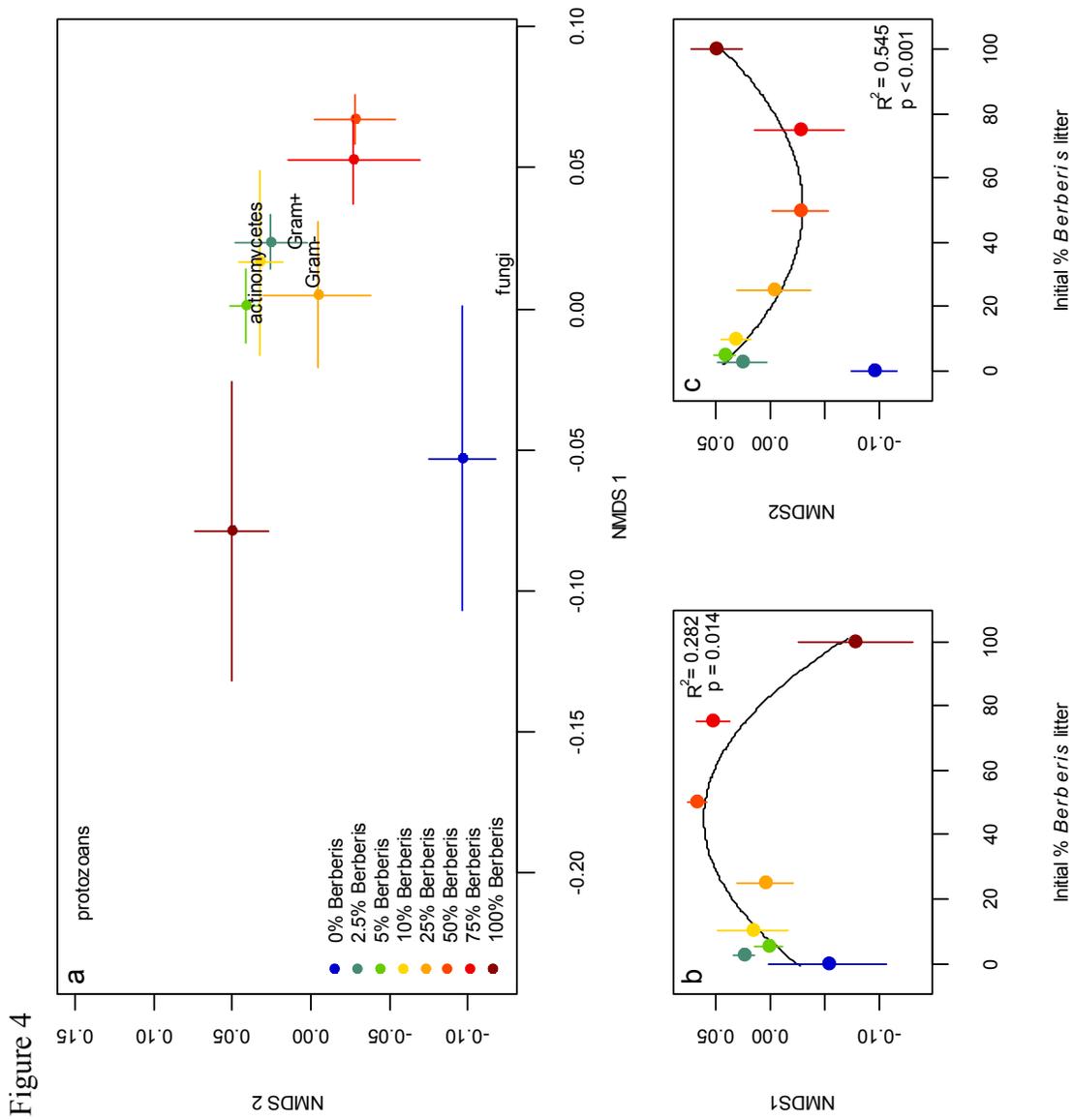
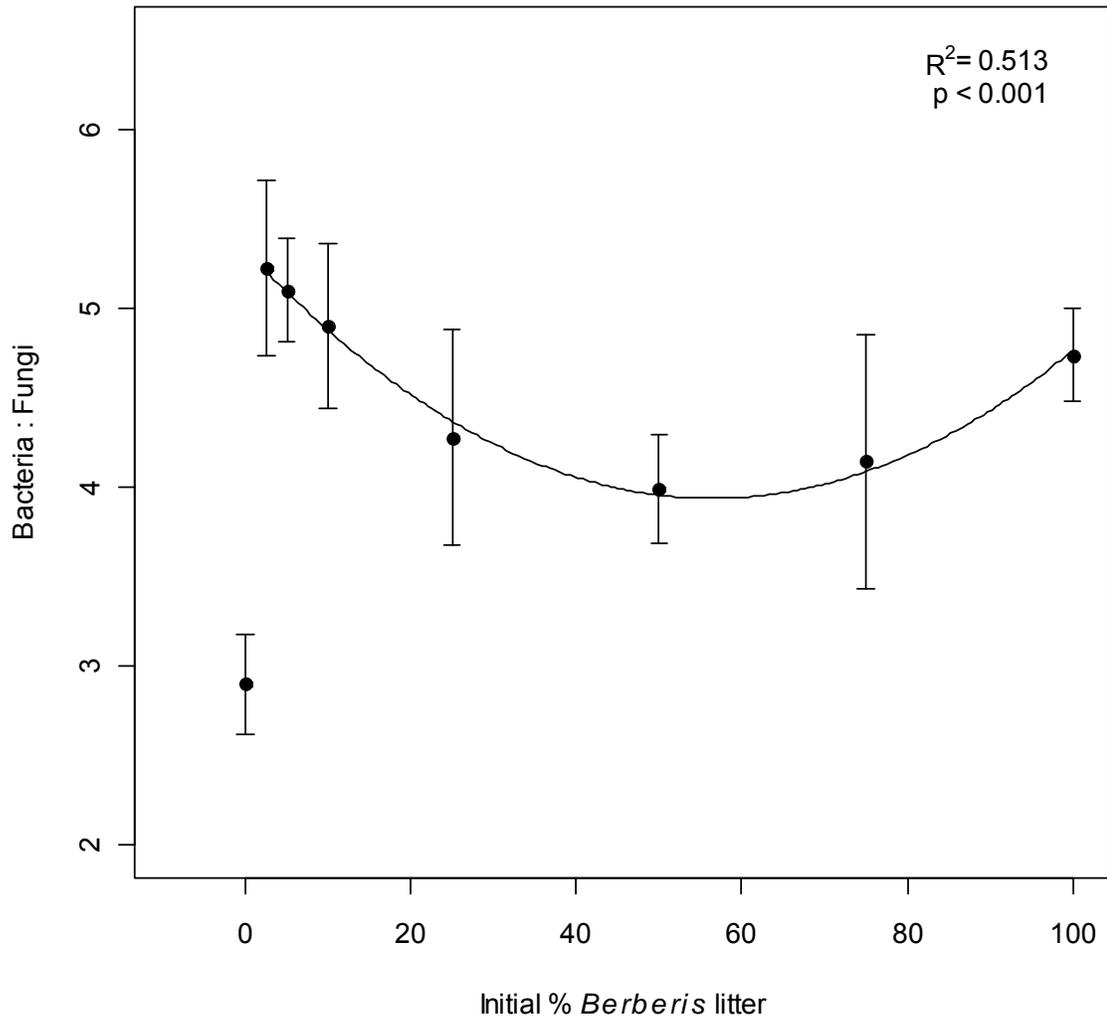


Figure 5



Chapter 3

Short- and long-term impacts of exotic shrub invasion on soil microbes, enzyme activities, and nitrogen cycling: a field manipulation

Abstract

Plant invasions can have substantial consequences for the soil ecosystem, with large changes in microbial community structure and nutrient cycling that can profoundly alter the ecosystem. While large impacts have been shown in many existing invasions, relatively little is known about the mechanisms of these impacts, making it difficult to predict impacts for future plant invasions. In addition, because most studies compare soils from long-established dense invasions to uninvaded areas, little is known about the spatial or temporal dependence of invasion impacts. We examined the short-term impacts and long-term legacies of invasion by Japanese barberry (*Berberis thunbergii*) into the native understory vegetation in eastern deciduous forests by experimentally manipulating vegetation in the forest understory. We selected two replicate sites in each of three understory forest communities (dominated by exotic *Berberis*, native *Viburnum* species, or native *Vaccinium* species) and experimentally manipulated vegetation in a full-factorial design by removing the existing aboveground biomass and planting each of the species missing from the site in separate plots. In addition, we replaced aboveground litter in half of the plots with an inert substitute to determine if invasion impacts were driven by aboveground or belowground plant inputs. We found that two years after experimental invasion of *Berberis* into native-dominated sites or restoration of natives into *Berberis*-dominated sites, the structure and function of the soil microbial community in both previously invaded and uninvaded areas was largely determined by the legacy

effect of the previous vegetation type, and was not affected by the current vegetation type. Aboveground litter removal had only weak effects on the microbial community, suggesting that impacts were driven largely by belowground processes. These results suggest that impacts of both invasion and restoration on the soil ecosystem do not occur quickly, but rather exhibit long-lasting legacy effects from previous belowground plant inputs. Further studies are needed to address the temporal scale of impacts on the soil microbial community and the duration of legacy effects.

Introduction

Exotic invasive plants can have large and variable impacts on soil microbial communities (van der Putten et al. 2007) and soil nutrient processes (Vitousek and Walker 1989, Ehrenfeld 2003, Liao et al. 2008), and these alterations can have major consequences for ecosystem function and ecosystem services (Pimentel et al. 2005, Vilà et al. 2010). While one of the goals of invasion biology is predicting impacts, at least two major challenges prevent accurate prediction of the impacts on soil microbial community structure and function; poor understanding of the mechanisms underlying plant influences on soil microbial communities, and a limited understanding of the spatio-temporal scale on which these mechanisms operate.

Understanding the mechanisms of invasion impacts on the soil ecosystem is a major challenge for invasion biology that is necessary to predict impacts of future invasions. Field observations, or “natural” experiments (Sax et al. 2005, Yoshida et al. 2007), are one of the most common ways of measuring impacts, but such comparative studies often lack the ability to test mechanistic hypotheses since causality cannot be determined. Greenhouse and common garden studies are often employed to answer

mechanistic questions, but this approach can introduce other experimental artifacts. Manipulative field studies avoid the artifacts of a greenhouse study, but these studies are relatively rare because of the ethical considerations of creating small-scale invasions, as well as the difficulties imposed by inherent site-to-site variability present in natural systems. However, a mechanistic understanding of invasion impacts is important to predicting future invasion impacts (Parker et al. 1999, Ricciardi 2003, Lockwood et al. 2007).

One particular question of interest in invasion ecology and ecology in general is determining the relative importance of aboveground (leaf litter) and belowground inputs (root litter & exudates) in controlling belowground nutrient cycling and microbial communities (Ehrenfeld, in press). While this does not specifically point to the mechanisms driving invasion impacts, understanding the relative importance of aboveground and belowground impacts does begin to address how impacts occur. Aboveground inputs are episodically deposited on the soil surface, while belowground inputs are widely distributed within the soil, in close proximity to soil biotic communities, and are continuously present. These fundamental differences in input types suggest they may differentially affect both microbial communities and the nutrient cycling processes that depend on these communities. This question is therefore of broad interest in ecology because of its importance to understanding and modeling the effects of community composition and diversity on ecosystem functions like nutrient cycling (Hobbie 1996, Lajtha et al. 2005, Hobbie et al. 2010), carbon sequestration (Hobbie 1996, Fahey et al. 2005, Crow et al. 2009), and productivity (Sayer 2006).

Despite the importance of understanding relative aboveground and belowground litter effects, there is little consensus on the relative effects on the microbial community or nutrient cycling, in part due to differences between different methods used to deal with the challenging task of manipulating belowground inputs. Root-trenching and tree-girdling studies, for example, have sometimes found large effects on microbial communities (Högberg et al. 2001, Siira-Pietikäinen et al. 2001, Weintraub et al. 2007) and nitrogen cycling (Ehrenfeld et al. 1997, Ross et al. 2001, Zeller et al. 2008), but similar studies have also found little effect (Hart and Sollins 1998, Holub et al. 2005, Hannam et al. 2007). Litter-addition studies often reveal significant species effects of litter on microbial community structure and nutrient cycling, especially when those species have strongly contrasting litter chemistry (Knops et al. 2002, Kraus et al. 2003, Chapman et al. 2006), but these studies tend to focus on aboveground litter effects and only indirectly address the relative roles of aboveground and belowground litter. A few studies have combined these approaches in order to examine the relative importance of aboveground and belowground litter. While most have found that belowground litter has a dominating role (Siira-Pietikäinen et al. 2001, Brant et al. 2006, Pollierer et al. 2007, Keith et al. 2009), others have found weak effects of both litter types (Holub et al. 2005, Hannam et al. 2007) or even interactive effects (Subke et al. 2004).

In studies that focus specifically on exotic invasions, numerous field and lab studies have shown strong effects of invasive plant litter on soil microbial communities and nutrient cycling, and field observations and common garden or greenhouse studies have shown whole-plant effects as well (reviewed in Ehrenfeld 2003). However, studies that examine both aboveground and belowground inputs in a way that can separate these

effects are very rare (Farrer and Goldberg 2009). As a result, there is still a great need for studies that address the relative roles of different plant inputs in affecting the soil ecosystem during invasion. Examining which aboveground and belowground inputs drive ecosystem change is difficult in an observational field study, but it is important to understanding and predicting invasion impacts.

A further challenge to predicting the impact of invasion is the lack of data on the temporal dependence of impacts. Because most studies that address the impacts of invasion on soil microbial communities use field comparisons of existing invaded and uninvaded areas, the results of these “natural experiments” are often not related to the time since invasion, and it is often not known how long ago invaded areas were invaded. As a result of this common approach, little is known about the temporal dependency of invasion impacts. Similarly, there are few data on the density dependence of impacts. Field observational studies, as well as greenhouse and common garden studies, often compare uninvaded to heavily-invaded sites, but do not relate impacts to invasion density (Ehrenfeld, in press), although this relationship is important for management decisions (Yokomizo et al. 2009). The experimental design often used for invasion impact studies has thus resulted in a lack of data on the spatio-temporal dependence of impacts.

In this study, we differentiated the short-term and long-term effects of invasion by manipulating vegetation in sites that are uninvaded and sites with long-established invasion. We furthermore differentiated between effects of aboveground and belowground litter inputs by removing aboveground litter and by comparing legacy effects of decaying belowground litter from the previous vegetation type to effects of inputs from the actively-growing vegetation. We conducted the study using Japanese

barberry (*Berberis thunbergii* DC., hereafter *Berberis*), which is an invasive understory shrub that grows under a wide range of conditions, but is most successful under high nitrogen, high light conditions (Silander and Klepeis 1999) and is most often found in disturbed or previously-disturbed early successional forest (Lundgren et al. 2004, Flory and Clay 2006, DeGasperis and Motzkin 2007, Mosher et al. 2009). Field observational studies (Kourtev et al. 1998, Kourtev et al. 2002) and greenhouse experiments (Ehrenfeld et al. 2001, Kourtev et al. 2003) have shown it alters soil microbial community structure, resulting in changes in extracellular enzyme activities in the soil and increasing nitrification rates relative to native understory species. Our aim was to elucidate the mechanisms by which *Berberis* impacts soil microbial community structure and function, and to assess how rapidly these impacts occur by comparing short-term experimental invasions to long-term naturally-occurring invasions. Based on results of previous greenhouse experiments, we hypothesized that *Berberis* would rapidly change the microbial community structure and function, and that changes would be most apparent when *Berberis* contributed both aboveground and belowground inputs.

Methods

Experimental Design

In August 2004, plots were established at 6 sites in Allamuchy State Park in northwestern New Jersey (40°54'13"N, 74°48'05"W). Sites were chosen based on the dominant understory vegetation type (sitetype factor), with two sites dominated by *Vaccinium* spp., two dominated by *Viburnum* spp., and two dominated by *Berberis thunbergii*. *Berberis* sites have been invaded for at least 20 years, and most likely more than 35 years (J.G. Ehrenfeld, unpublished data). Because past land use history can

influence *Berberis* invasion as well as soil characteristics (DeGasperis and Motzkin 2007, Mosher et al. 2009), we selected sites that were contained within a former hunting reserve. These areas were never tilled or cleared for agriculture and had no apparent indicators of past agricultural land use. The soils at all sites were Rockaway series (typic fragiudults) located on the pre-Cambrian gneisses of the Highlands Province. Forest canopy vegetation was relatively constant between sites, and was dominated by a mixture of oaks and hickorys (see Kourtev et al. [1998] for further site details). All sites were between 0.25 and 2 km from neighboring sites, ensuring that sites were relatively functionally decoupled.

Within each site, we constructed twelve 2m X 2m plots, with each plot approximately 2 m from any neighboring plot. The understory vegetation of each plot was surveyed by counting and identifying all woody and herbaceous stems within the plot. In 8 of the 12 plots, all vegetation was then clipped at the ground level, and young shrubs (~2 years old) of either *Berberis thunbergii*, *Vaccinium angustifolium*, or *Viburnum dentatum* were planted after gently rinsing all soil from their roots. In the remaining 4 plots per site, vegetation was left undisturbed as a control, resulting in 4 plots containing the dominant vegetation type from that site, 4 plots with another vegetation type, and 4 plots with the other remaining vegetation type (vegetation factor). The growth and survival of the planted shrubs are not reported because aboveground biomass increased very little during the experiment, and mortality was not explained by any treatment factors or any other measured variables. Finally, each plot was divided into two subplots, and leaf litter was removed from one of the subplots once each spring and twice each fall to maintain one litter-free subplot per plot (litter factor). To avoid the

confounding effects on microclimate that litter removal might have, we placed Styrofoam packaging peanuts in the litter removal subplots, and contained the peanuts inside the plot using a 30 cm tall plastic fence. Preliminary experiments showed that the Styrofoam was inert (did not leach nitrogen) and could mimic the effects of litter on soil temperature and moisture. This design resulted in a total of 144 subplots (3 site types * 2 sites per vegetation type * 12 vegetation plots * 2 litter subplots) with 4 levels of nesting (subplot | plot | site | sitetype).

Soil measurements

Soils from each subplot were sampled in mid-July 2006, approximately two years after plots were established. Three adjacent 5-cm deep cores, each 5 cm in diameter, were taken from the top 5 cm of mineral soil near the base of a plant in each subplot using bulk density corers. Two cores were used for measurement of nitrogen mineralization. One of the nitrogen mineralization cores was covered with aluminum foil loosely to allow gas exchange and replaced in the ground to incubate for one month, while the other was placed on ice in a cooler for transport to the lab, where it was extracted within 24 hours to measure initial N (see methods below). The third core was taken from each subplot one week later and transported in a cooler to the lab, where one half of the core was used within 24 hours to measure enzyme activities, while the other half was frozen until processing for phospholipid fatty acid (PLFA) analysis.

Inorganic nitrogen was extracted from both the initial (July) and final (August) soil cores by shaking 10 grams wet soil in 40 mL 2M KCl for 1 hour and filtering the extract through Whatman #42 filter paper. Extracts were frozen prior to analysis for both NH_4^+ and $\text{NO}_3^-/\text{NO}_2^-$ on a Lachat Multichannel Injection Analyser (Lachat Instruments,

Loveland, CO, USA). A separate subsample was dried at 105°C for 96 hours to determine the soil moisture of the 10-gram sample. Nitrogen mineralization rates were calculated as the difference between the initial and final samples, and reported as milligrams N per kg dry soil per day.

Six soil enzyme activities (Table 1) were assayed using a microplate method (adapted from Waldrop et al. [2000]) and Sinsabaugh et al. [2000]) as a measure of potential cycling rates of carbon (β -glucosidase, chitinase, phenol oxidase), nitrogen (chitinase, urease, aminopeptidase), and phosphorus (acid phosphatase). Soil slurries were made from 20 g wet soil suspended in 200 mL buffer solution (50 mM sodium acetate adjusted to pH 5.0) by stirring 1 min. on a stir plate. While stirring, eight 50- μ L aliquots were pipetted from each slurry into 96-well microplates as analytical replicates. 150 μ L of the appropriate enzyme substrate was added into each well, and plates were incubated at room temperature for 4 or 20 hours to allow color development (see Table 1). All substrates were 10 mM solutions dissolved in sodium acetate buffer, except urea, which was a 25 mM solution. Controls for background absorbance of each substrate solution were also prepared by adding 50 μ L buffer solution instead of soil slurry to 150 μ L of each substrate in separate wells. Controls for the background absorbance of the soil were also prepared by adding 150 μ L of acetate buffer solution instead of 150 μ L substrate to soil slurries in separate wells. All controls were incubated and measured simultaneously with the samples. At the end of the incubation period, 50 μ L of supernatant was pipetted out of each well into a new plate. For acid phosphatase, β -glucosidase, chitinase, and amino peptidase, 50 μ L 0.1M NaOH was added to each well to terminate the reaction before reading absorbance at 420 nm. Absorbance of phenol

oxidase was read directly at 450 nm. For urease, an ammonium test kit (Hach Co., Loveland, CO, USA) was used to measure ammonium production, detected by absorbance at 600 nm. All absorbances were converted to enzyme activity using standard curves of ammonium for urease and p-nitrophenol for all other enzymes. Activities were converted to a dry-soil mass basis by drying two 400- μ L aliquots of soil slurry at 105°C to determine dry soil mass per 50- μ L analytical replicate. Enzyme activities for acid phosphatase, β -glucosidase, chitinase, and aminopeptidase were expressed in mg pNP / kg soil / hr, while phenol oxidase was expressed directly as absorbance / g soil / hr, and urease as mg NH_4^+ -N / kg soil / hr. Enzyme analyses were done using a pipette robot with spectrophotometric plate reader (Biomek® Laboratory Automation Workstation, Beckman Coulter Inc. Fullerton, CA, USA) in the Rutgers University High-Throughput Screening Laboratory.

Phospholipid fatty acids were used to measure the soil microbial community structure and determine the relative biomasses of different microbial groups. PLFAs are one of the most commonly used methods for characterizing the soil microbial community in ecological studies (O'Donnell et al. 2005) in part because the method provides a relatively simple, reliable, and ecologically relevant measure of microbial community structure (Joergensen and Wichern 2008). We used a protocol from White et al. [1979], modified during preliminary work for optimal extraction for this soil texture. One 4-g soil subsample was dried at 105°C for 96 hours to determine soil moisture, while a second 4 g subsample was shaken in 15 mL one-phase buffer solution (1:2:0.8 chloroform: methanol: phosphate buffer) overnight. We then centrifuged the resulting slurry and removed the supernatant and shook the soil in an additional 15 mL one-phase

buffer solution for one hour. After centrifuging again, the supernatant from the second extraction was combined with the first, shaken with 2.5 mL DI water and 2.5 mL chloroform, and allowed to separate overnight. The chloroform layer was retained, concentrated in an evaporator (40°C, 200hPa), and fractionated in a 100-200 mesh silicic acid column into neutral-, glyco-, and phospho-lipids by elution with 5 mL chloroform, 10 mL acetone, and 5 mL methanol, respectively. The phospholipid fraction was then concentrated and an internal standard (nonadecanoic acid, 19:0) was added to standardize peak areas.

The extracted PLFAs were then saponified, methylated, extracted into hexane, and washed following the Sherlock Microbial Identification System protocols (MIDI Inc., Newark, DE, USA). The resulting fatty acid methyl esters were quantified by peak area produced by an HP gas chromatograph with flame ionization detector (GC-FID, Hewlett Packard 5890 Series II, Palo Alto, CA, USA) and an HP Ultra 2 phenyl methyl silicone fused capillary column (25 m×0.2 mm i.d., film thickness 0.33 µm) and identified using the Sherlock Microbial Identification system (MIDI Inc., Newark, DE, USA) based on retention time as the temperature of the GC-FID was ramped from 170°C to 250°C at 5°C / min. All peak areas were standardized to the internal 19:0 standard peak area and known amount (5 µg), and reported as mg PLFA / kg soil. PLFAs that are characteristic biomarkers of broad microbial groups (Gram + bacteria, Gram – bacteria, fungi, actinomycetes, or protozoans) were grouped together by their characteristic microbial group and summed to obtain a measure of the relative biomass of each microbial group in each soil sample (White and Ringelberg 1998, Olsson 1999, Zelles 1999). Only the relative biomasses of these 5 microbial groups are reported in this analysis; PLFAs that

are not characteristic biomarkers for any particular group are not analyzed further here for clarity and simplicity of interpretation.

Statistical analyses

Because of the complex hierarchical structure of both the mean and variance structures in the data, all soil measurements were analyzed using a set of Bayesian hierarchical linear models. Bayesian hierarchical modeling is a highly flexible technique that can easily accommodate complex hierarchical data structures that are often found in ecological studies (Qian and Shen 2007, Cressie et al. 2009). Because we had little prior knowledge about how our experimental treatments would influence soil parameters, we used non-informative priors for both the mean and variance structure following Congdon (2001). For the univariate measures of nitrogen mineralization, we used normal priors for the mean structure with mean 0 and variance 1×10^6 , and a gamma prior for the precision with shape and scale parameters both equal to 1×10^{-3} . For the multivariate measures (microbial community structure and enzyme activities), we used similar priors for the mean and a proper Wishart prior with the minimum degrees of freedom for the precision (Congdon 2001, p.43). We felt these choices of prior distributions were further justified when exploratory analyses showed that results were relatively insensitive to a wide range of prior distributions for both univariate and multivariate analyses.

Nitrification and ammonification were modeled separately as univariate normal random variables with mean and variance parameters that both depended on the hierarchical linear structure. For example, nitrification was modeled as a random normal variable with a separate mean for each sitetype (*Vaccinium*, *Viburnum*, and *Berberis*), each site nested within sitetype, plot nested within site, and subplot nested within plot. It

also had a separate variance for each sitetype (*Vaccinium*, *Viburnum*, and *Berberis*), plot type (*Vaccinium*, *Viburnum*, and *Berberis*), and subplot type (litter, no litter). This is similar to the frequentist approach of fitting a nested ANOVA model with unequal variances between treatments. Further details and WinBUGS code used to fit this model are included in Appendix 1. Normal probability plots were used to assess normality, which was adequately achieved after log-transformation of both variables. Log-transformed data were used for all analyses, but back-transformed values are reported in all tables and figures.

Similarly, normal probability plots were used to assess normality of the six enzyme measurements, which was adequately achieved after log-transforming acid phosphatase and phenol oxidase. Due to a high degree of correlation between different enzyme activities, these variables were modeled simultaneously by first collapsing the 6 enzyme activities down to 3 variables with principal components analysis, then modeling the 3 principal component scores as a multivariate random normal variable with mean and variance-covariance parameters that depended on the hierarchical linear structure. The model used was therefore similar to the frequentist approach of fitting a nested MANOVA model with unequal variances between treatments.

Log-transformation was also used to satisfy the assumption of normality for the biomass of all 5 microbial groups identified by the PLFA analysis. These response variables were also modeled simultaneously as a single multivariate random normal variable. Log-transformed values were used for analysis, but only back-transformed values are reported here.

While statistical models frequently assume constant variance within and between treatments, it was apparent from exploratory data analysis that this assumption was not valid and the variance differed between many of the experimental treatments. As a result, the mean and variance structures for each soil response variable were determined in a two-step model selection process. First, we selected the appropriate variance structure by assuming the “full model” (Table 2) for the mean structure, and fitting a set of candidate models for the variance structure to determine which experimental treatments caused differences in variance. Candidate models allowed for differences in variance between sitiesypes, sites, vegetation treatments, and litter treatments, and all possible combinations thereof. The deviance information criterion (DIC, Spiegelhalter et al. [2002]) was used to identify the top model for the variance structure. Then, using the top model for the variance structure, we fit a set of 24 candidate models to determine the mean structure (see Table 2 for list of candidate models). These candidate models were chosen because they loosely parallel the null hypothesis testing process used in frequentist ANOVAs with Type II sums of squares, in which the effect of a particular term in the model is adjusted for all same- or lower-order terms. We used DIC to select the best-fitting model(s), following the guidelines that models with $\Delta\text{DIC} < 2$ cannot be ruled out, and models with $\Delta\text{DIC} < 5$ have fair support. DIC is a Bayesian measure of model adequacy and is related to Akaike’s Information Criterion (Spiegelhalter et al. 2002). Both AIC and DIC rank models based on the likelihood of each model after penalizing them for the complexity of the model. The penalty terms used for AIC and DIC differ in form but are similar in function, with both penalizing more complex models more severely. We based inferences on the posterior distributions from the top model(s), and assessed all models

with $\Delta\text{DIC} < 2$. All Bayesian analyses were conducted in Winbugs version 1.4.3 (Gilks et al. 1994) using the R2WinBUGS package (Sturtz et al. 2005) in R version 2.9.1 (R Core Development Team 2009). During model selection, two MCMC chains were run for 10,000 iterations each after a 1,000 iteration burn-in period. Production chains for top models were run for 50,000 iterations. Convergence of all models was assessed using the CODA package (Plummer et al. 2009) in R, and posterior distributions were analyzed using R 2.9.1.

Results

The single largest factor by far that affected microbial community structure, enzyme activities, and nitrogen mineralization was the long-term legacy effect of the dominant understory vegetation. This factor alone explained 65% of the variation in nitrate production, 37% of variance in ammonium production, 28% of the microbial community structure, and 20% of variation in enzyme activities. All of the highly-ranked models included the sitetype effect (Table 2), and removing this effect from the main effects model (analogous to Type II sums of squares hypothesis testing) caused an overwhelming drop in the DIC score (i.e., $\Delta\text{DIC} = 168.3$ for PLFAs).

This long-term legacy effect greatly altered the microbial community, mostly through differences between sitetypes in total microbial biomass (Figure 1). The microbial biomass, as measured by PLFAs, was highest for all microbial groups at the *Vaccinium* sites, and lowest for most groups at *Viburnum* sites. Sites invaded by *Berberis* had intermediate microbial biomass. While the effect of the dominant vegetation was strong, the vegetation manipulations had virtually no effect on the microbial community. However, the litter removal did change the community structure, slightly increasing total

bacterial biomass (the sum of Gram+ and Gram- bacteria) and doubling the biomass of protozoans in the soil (Figure 2). The top-ranked model for microbial community structure was ranked much higher than all other models, and included sitetype and litter effects and a random site factor. Only one other model was ranked with $\Delta\text{DIC} < 5$. However, since ΔDIC for the second-ranked model was greater than 2, we concluded the top-ranked model was well-supported.

The changes in microbial community structure were paralleled by changes in soil enzyme activities. Principal components analysis reduced the dimensionality of the enzyme data from 6 enzymes to 3 principal components which together explained 88.4% of the variation in enzyme activity. The first axis explained 50.9% of variance, and was positively correlated with acid phosphatase and phenol oxidase and negatively associated with beta glucosidase, chitinase, and aminopeptidase (Figure 3). The second axis explained 21.0% of variance, and because it was negatively correlated to most enzymes, it can be summarized as a measure of overall activity. The third axis, which explained 16.5% of the variance, could be summarized as a measure of urease activity, since it was strongly correlated to urease and relatively uncorrelated to the other 5 enzymes (Figure 3).

Like the microbial community structure, soil enzyme activity was strongly related to the long-term legacy effects of the dominant understory vegetation in the top-ranked model, which included sitetype and litter effects as well as a random site blocking factor. On both axis 1 and axis 2, *Berberis*-invaded sites differed strongly from the *Vaccinium*- and *Viburnum*-dominated sites (Figure 4). This reflected large differences between native and invaded sites in both the relative magnitudes of the different enzyme activities

(axis 1) and the overall magnitude of enzyme activity (axis 2), which was highest in the invaded sites. While *Vaccinium* and *Viburnum* sites were very similar on axis 1 and 2, they differed on axis 3 due to a higher rate of urease activity in the *Viburnum* sites. Soil enzyme activity was also affected to a lesser extent by the litter manipulation. There were no noticeable effects on axis 1 or axis 2, but on axis 3 there was a clear difference between plots with and without litter (Figure 5). Litter removal caused a marked increase in axis 3 scores, reflecting an increase in urease activity in the litter-removal plots. The top-ranked model was strongly supported, and no competing models were ranked with $\Delta\text{DIC} < 5$, providing strong evidence against any competing models.

The response of ammonium and nitrate mineralization rates to the treatments was similarly dominated by the long-term effects of the understory vegetation type. All highly-ranked models for these two response variables included the sitetype factor. In the top model for ammonification, the rate of ammonification in *Berberis*-invaded sites was only 18% and 23% that of *Vaccinium* and *Viburnum* sites, respectively (Figure 6a). *Berberis*-invaded sites also exhibited an approximately 65-fold higher nitrification rate than the *Vaccinium* and *Viburnum* sites (Figure 6b). As a result, while the sitetype had little influence on the total amount of N mineralized, it greatly changed the form of nitrogen being made available. The relative nitrification index (RNI, nitrification rate / total N mineralization rate), was dramatically different in *Berberis*-invaded sites compared to *Vaccinium* and *Viburnum* sites (Figure 6c).

While the top model for both nitrification and ammonification included only the sitetype effect and a random block effect for the site, a few competing models were also well-supported by the data. For example, the model for nitrification that included an

additional litter effect was ranked almost as highly, with $\Delta\text{DIC} = 0.2$ (Table 2). This indicates essentially identical support for the models with and without the litter effect. The model that included a litter effect for ammonification also had some support ($\Delta\text{DIC} = 1.8$). However, the effect of litter removal was small, with only a slight (10%) decrease in the rate of ammonification (Figure 7a) and a 26% increase in the amount of nitrate production (Figure 7b). The effect of litter removal was therefore similar (though smaller) to the effect of *Berberis* invasion, creating an approximately 50% increase in the relative nitrification index (Figure 7c).

Discussion

Despite manipulating the vegetation in this experiment, the legacy effect from the previous vegetation type was the overwhelmingly most important factor that structured the soil microbial community (measured by PLFAs) and its function (enzyme activity and N mineralization). Even 2 years after manipulating the vegetation, there was essentially no effect of the current vegetation on the soil microbial community. This is a striking result, especially since previous studies have shown *Berberis* strongly affects the soil microbial community, enzyme activities, and nitrogen mineralization within a matter of months in the greenhouse (Kourtev et al. 2003) and in previous field observational (non-experimental) studies (Ehrenfeld et al. 2001), as well as concurrently-conducted greenhouse studies (Yu et al., unpublished manuscript). We experimentally invaded plots in the field, but did not observe any of these effects previously documented, although our data do support the findings of previous studies showing that the long-term presence of *Berberis* results in altered nitrogen cycling.

There are at least three possible reasons that explain the lack of short-term effects from *Berberis* invasion in manipulated plots and the presence of long-term effects in the different sitetypes. First, it is possible that effects of *Berberis* invasion previously observed and seen in this study are not driven by invasion, but instead the differences pre-date and cause invasion. Plant invasions are often considered to be “natural” experiments (Sax et al. 2005), and it is extremely common to compare invaded sites with nearby uninvaded sites to determine invasion impacts on soil. The assumption of this method is that invasion causes the differences between invaded and uninvaded sites, even though the causality of the relationship can not be directly established. Conclusions from “natural” experiments must therefore be viewed cautiously. Many pre-existing factors can affect the invasibility of a particular site, and site history and past land use has been linked to invasibility in many other invasions (e.g., Parks et al. 2005, Kulmatiski et al. 2006) as well as *Berberis* invasions (Lundgren et al. 2004, Flory and Clay 2006, DeGasperis and Motzkin 2007).

Given that all sites in our study had similar documented past land-use histories and no evidence in the field of different land-use histories, had little past anthropogenic impact, and short-term experimental results in the greenhouse corroborate these longer-term field observations, we find it unlikely that the differences in soil conditions we observed existed prior to invasion by *Berberis*. A second explanation is that the impacts of invasion that have been previously seen are related to the density or time since invasion, and our experimental manipulation was not conducted at the appropriate density or length of time. Based on previous greenhouse studies, we expected to see effects within two years, and we limited the length of the experiment to prevent the *Berberis*

from reproducing and causing further invasion. It is possible though that a longer time was needed for noticeable impacts to occur. Our plots may also have been too sparsely vegetated during manipulation to have strong effects, although we planted at a density that was realistic for typical restoration projects and approximates the natural density of plants in the forest understory (1 plant per m^2). However, the aboveground density may not be a good indicator of belowground impacts if impacts are driven by root production and turnover or root exudation, rather than aboveground litter inputs. When mature, the root systems of these shrub species can be very dense beneath the plant canopy (J. Ehrenfeld, unpublished data), but our plantings may not have been established long enough to fill the belowground soil volume to that extent. In contrast, plants growing in pots under optimal greenhouse conditions can fill the soil volume relatively quickly. Furthermore, very little is known about how either the density of invasion or the time since invasion relates to impacts. *Berberis* populations can be found at many levels of density, from very sparse ($\ll 1$ bush per m^2) to greater than 4 per m^2 , where each bush may have up to 40 aboveground stems (Ehrenfeld 1999). It is not known how long it takes for the sparse populations to reach these high densities, but the slow rate of accretion of stems (1 stem per year) suggests that the dense invasions used in comparative field studies represent several decades of population growth.

The relationship between impact on soil properties and either plant density or time since invasion may be linear or nonlinear, or there may be a threshold of invasion density at which impacts occur. Although this relationship is extremely important for management of invasions (Yokomizo et al. 2009), this is seldom studied (Elgersma & Ehrenfeld, unpublished manuscript). Similarly, very little is known about the temporal

development of invasion impacts, and it is possible that 2 years is not long enough for invasion to have an impact in the field. However, since the *Berberis* sites differed strongly from the two native sites, this leads to the question of why the impacts after 2 years do not reflect longer-term impacts seen at the *Berberis* sites.

The third possible explanation for observing significant differences between sitetypes but not between vegetation treatments may help to answer this question. Strong differences between sitetypes could arise if invasion effects are driven primarily by belowground litter rather than aboveground litter or root exudates. We removed aboveground vegetation from plots before re-vegetating them, but to minimize soil disturbance and mimic restoration practices, we did not remove belowground biomass from the soil. If the effects from decomposition of that remaining belowground biomass overwhelmed the current vegetation's effects, we would expect to see strong sitetype effects and weak effects of vegetation manipulation, as in fact seen in this study. In uninvaded forest systems, aboveground litter inputs have relatively weak effects on belowground soil microbial community structure, food webs, and energy flow, which tend to be dominated by belowground inputs rather than aboveground litter (Pollierer et al. 2007, Keith et al. 2009), although aboveground inputs can dominate in some cases (Crow et al. 2009). Furthermore, the relative importance of belowground litter for dissolved organic matter production increases during succession, and dissolved organic matter is strongly influenced by belowground litter in mature forest (Uselman et al. 2009), such as the sites in this study. Unfortunately, little is known about the relative importance of root turnover, exudation, and litterfall for invasion impacts. The vast majority of studies either manipulate litter or live plants but seldom both (Nilsson et al.

2008), and to our knowledge, this is the only study comparing aboveground to belowground impacts of invasion in a forest ecosystem. Farrer and Goldberg (2009) found that in wetlands, aboveground litter from an invasive cattail had a much stronger effect on soil nutrient cycling than actively-growing plants did; however, in this case, the invasive cattail created an unusually thick litter layer. The dominating importance of aboveground litter in this case may reflect the large difference between native and invasive litter layer thickness, rather than a generalizable effect of litter on soil. In contrast, Coleman and Levine (2007) found that aboveground litter from invasive grasses in California grasslands had relatively similar impacts on the plant community compared to actively-growing invasive grasses. Our study suggests that belowground litter can have a dominant effect on forest soils during invasion that produces a long-lasting legacy.

Because restoration actions typically involve killing or removing the aboveground but not belowground parts of invasive plants, our results suggest that because invasion impacts occur primarily through belowground biomass, the impact of an invasion may persist for long periods of time despite restoration efforts. Long-lasting soil-mediated anthropogenic legacies are well-documented in forest systems, with some legacies that are even apparent after 1700 years (Dambrine et al. 2007, Plue et al. 2008). The duration of legacies from the dominant vegetation has not received as much attention as anthropogenic legacies, but our results suggest that these legacies can strongly outweigh the influence of the current vegetation on microbial community structure and function at least on the timescale that is relevant to restoration efforts.

We observed smaller but significant effects of aboveground litter removal on the microbial community. Litter removal increased protozoan biomass and urease activity in

the soil, and there was evidence suggesting some effect on nitrogen mineralization as well, mostly through a change in the form of nitrogen available rather than the total amount. These effects were surprisingly small however, considering the large amount of carbon and nitrogen removed through litter. This finding is consistent with the explanation that invasion impacts in forests are driven by belowground rather than aboveground processes, at least in the short to medium term. Similar results were obtained in a large multi-site study, where aboveground litter removal had a smaller impact on the belowground food web than belowground litter (Keith et al. 2009), and neither aboveground nor belowground litter removal had noticeable effects on nitrogen mineralization (Holub et al. 2005). These studies suggested that in mature forests, soil organic matter (SOM) controls short to medium term dynamics rather than litter inputs, which influence soil processes through SOM formation processes (Quideau et al. 2001, Crow et al. 2009). We do note, however, that litter can influence soil processes directly by changing soil physical conditions, an effect that we eliminated by using an inert litter replacement. If litter impacts operate indirectly through SOM, then SOM functions as “memory” in soils and may create a long delay between invasion and its impacts, as well as a delay between plant community restoration and restoration of the soil ecosystem. This “memory” is largely ignored in greenhouse studies, which typically use a common or homogenized mineral soil for all treatments, allowing for fast results, but this effect of SOM may be important to understanding invasion impacts. This difference between field and greenhouse experiments highlights the importance of validating greenhouse results in a natural setting.

In conclusion, our results show strong long-term impacts of invasion but very little short- to mid-term impact. We also observed strong legacy effects from invasion. These results suggest that invasion impacts from aboveground litter inputs are less severe than impacts that occur through belowground inputs and changes in soil organic matter formation. This may delay the impact of the plant community on the soil ecosystem, either after invasion or after native plant restoration. This also reveals the importance of long-term monitoring of the soil ecosystem after restoration. Finally, we emphasize the need for further studies that address aboveground and belowground mechanisms of invasion impacts, as well as studies to determine how impacts relate to the density and age of invasion.

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Table 1. Descriptions of the six enzyme activities measured.

Enzyme name	IUBMB EC [§] nomenclature	Primary nutrient	Substrate	Incubation time (hrs)	Λ^{\ddagger}
Acid phosphatase	EC 3.1.3.2	P	p-nitrophenyl-phosphate	4	420
β -glucosidase	EC 3.2.1.21	C (labile)	p-nitrophenyl- β -D-glucopyranoside	4	420
Chitinase	EC 3.2.1.52	C, N	p-nitrophenyl-N-acetyl- β -D-glucosaminide	4	420
Amino-peptidase	EC 3.4.11.1	N	glycine-p-nitroanilide	4	420
Phenol oxidase	EC 1.10.3.2	C (lignin)	L-3,4-dihydroxy-phenylalanine	20	450
Urease	EC 3.5.1.5	N	urea	20	600

[§]International Union of Biochemistry and Molecular Biology enzyme commission

[‡]Absorbance wavelength

Table 2. List of candidate models and their DIC scores relative to the top model.

Shading indicates model rank (Dark = top model; intermediate, $\Delta\text{DIC} < 2.0$; light, $\Delta\text{DIC} < 5$). The vegetation and litter treatments are indicated by “veg” and “litter”, respectively. All models for NO_3^- allowed the variance to differ among vegetation and litter treatments. Models of NH_4^+ allowed variance to differ between sitetypes, vegetation, and litter treatments. The variance-covariance matrix was constant between treatment groups for PLFAs and for enzymes.

Model	ΔDIC			
	PLFAs	Enzymes	NH_4^+	NO_3^-
Full model [§]	58.9	52.5	14.5	19.5
Full model minus 3-way interaction	39.1	31.9	4.5	15.0
Full model minus 3-way and litter*veg 2-way int.	27.1	27.2	7.2	10.7
Full model minus 3-way and litter*sitetype 2-way int.	42.5	25.7	1.0	10.9
Full model minus 3-way and sitetype*veg 2-way int.	14.4	19.4	6.2	12.5
Main effects plus veg*sitetype interaction	30.6	21.1	3.1	6.7
Main effects plus litter*sitetype interaction	2.4	14.6	7.7	8.3
Main effects plus litter*veg interaction	17.9	13.1	4.4	8.4
Sitetype + site sitetype + veg + litter (Main effects)	6.1	8.3	5.5	4.4
Sitetype + veg + litter	61.4	69.2	29.5	18.7
Sitetype + site sitetype + litter	0	0	1.8	0.2
Site sitetype + veg + litter	174.5	99.7	45.9	137.5
Sitetype + site sitetype + veg	17.2	20.5	3.4	4.0
Sitetype + litter	55.2	61.2	25.6	14.6
Veg + litter	199.6	133.2	58.9	143.4
Sitetype + veg	69.9	79.9	27.4	18.2
Sitetype + site sitetype	10.9	12.2	0	0
Site sitetype + Veg	180.1	109.5	43.7	136.0
Site sitetype + Litter	168.3	91.1	44.3	134.2
Litter	193.0	125.1	56.4	139.9
Sitetype	63.4	71.9	23.8	14.3
Veg	204.6	142.0	56.7	141.8
Site sitetype	10.9	101.0	42.4	132.6
intercept only	197.8	133.9	54.4	138.4

[§]Full model: $Y = \text{sitetype} + \text{site}|\text{sitetype} + \text{veg} + \text{litter} + \text{sitetype}*\text{veg} + \text{sitetype}*\text{litter} + \text{veg}*\text{litter} + \text{sitetype}*\text{veg}*\text{litter} + \varepsilon(\text{litter}|\text{veg}|\text{site}|\text{sitetype})$

Figure Legends

Figure 1. Legacy effects of different sitetypes on microbial community structure (as measured by PLFA). Each sitetype is indicated by the dominant vegetation that was present before experimental manipulation. Error bars indicate 95% credible intervals.

Figure 2. Effects of litter removal on microbial community structure. Error bars indicate 95% credible intervals, and asterisks indicate the 95% credible interval for the difference between treatments exceeds zero.

Figure 3. Correlations between principal component scores and the six enzyme activities measured.

Figure 4. Legacy effects of the dominant vegetation on enzyme activities summarized by principal components axis 1 vs. axis 2 (b), and axis 1 vs. axis 3 (a). Ellipses indicate 95% credible intervals. Vectors show correlations between PC axes and individual enzymes (AP = acid phosphatase, BG = β -glucosidase, CH = chitobiase, AM = aminopeptidase, PO = phenol oxidase, UR = urease).

Figure 5. Effects of litter removal on soil enzyme activities, summarized by 3 principal component axes. Ellipses indicate 95% credible intervals, and vectors indicate correlations between PC axes and individual enzymes (AP = acid phosphatase, BG = β -glucosidase, CH = chitobiase, AM = aminopeptidase, PO = phenol oxidase, UR = urease).

Figure 6. Rate of ammonium production (A), nitrate production (B), and relative nitrification index (C) in each of the 6 sites. “Vac”, “Ber”, and “Vib” indicate *Vaccinium*, *Berberis*, and *Viburnum* sitetypes, respectively. Error bars are 95% credible intervals, and lowercase letters indicate comparisons between sitetypes.

Figure 7. Average rates of ammonification (A), nitrification (B), and the relative nitrification index (C) in litter-present and litter-absent plots. Note the order of magnitude difference in scales for ammonification and nitrification. Error bars indicate 95% credible intervals.

Figure 1

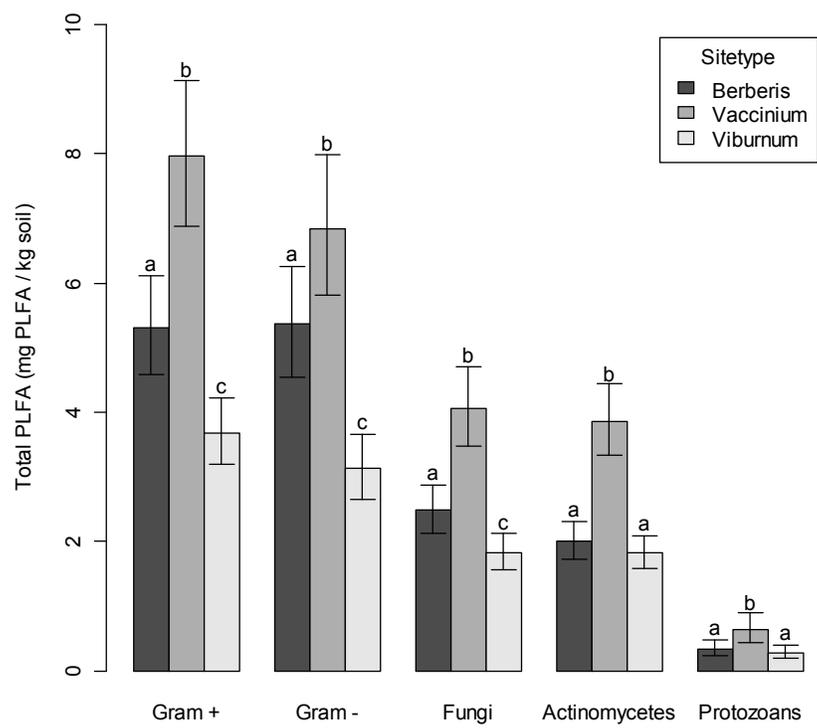


Figure 2

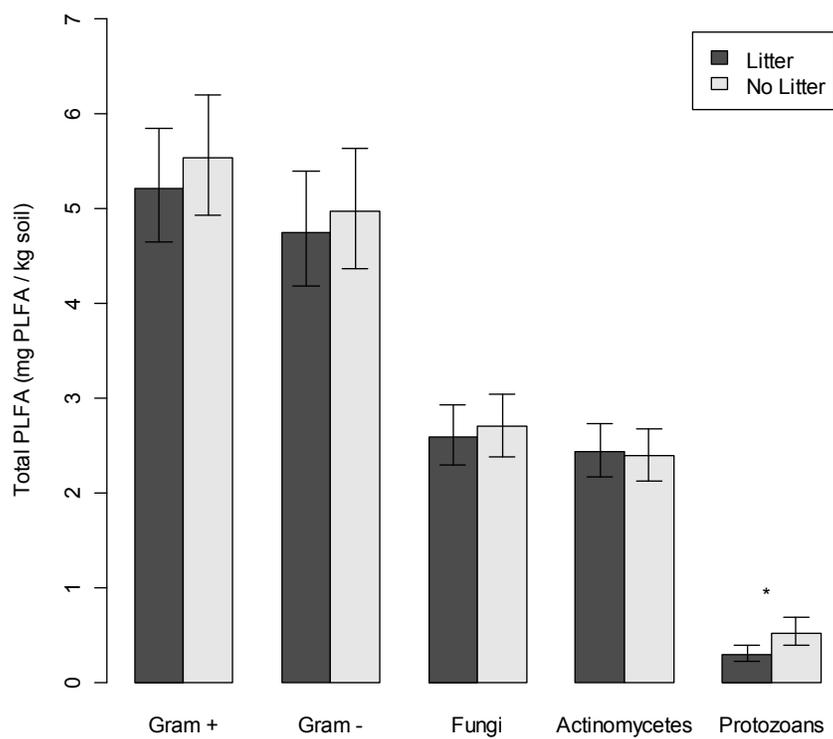


Figure 3

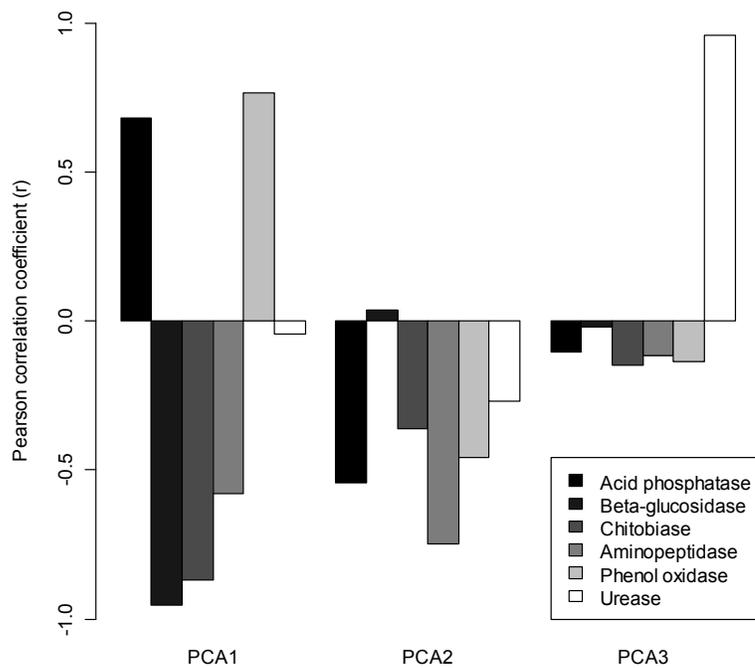


Figure 4

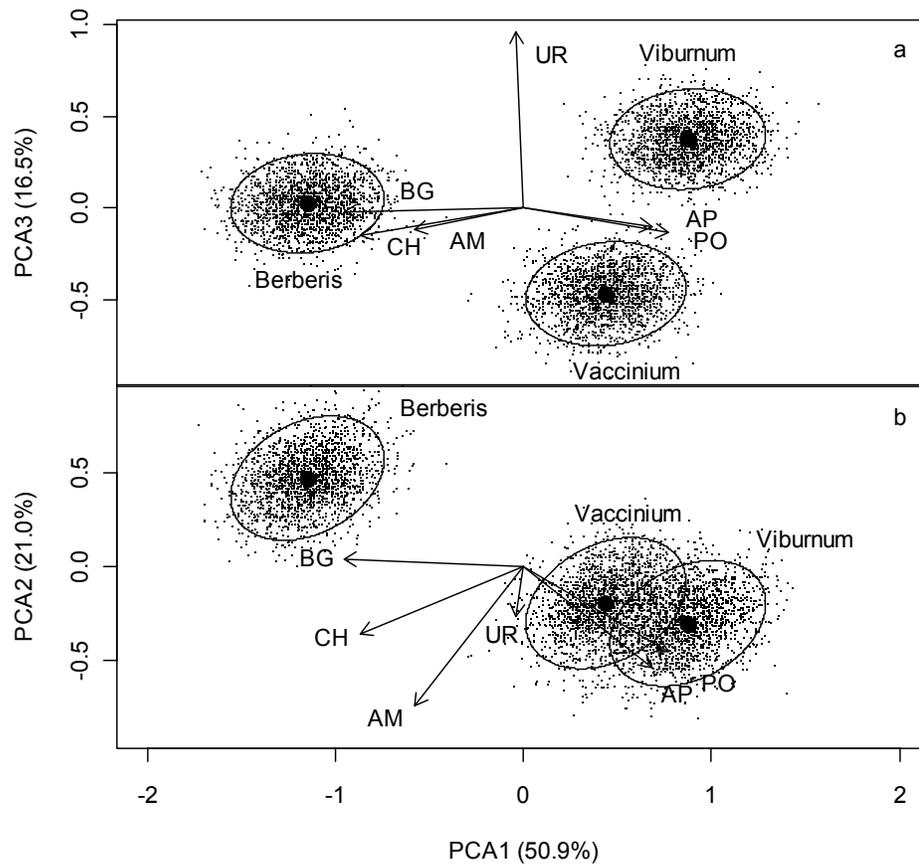


Figure 5

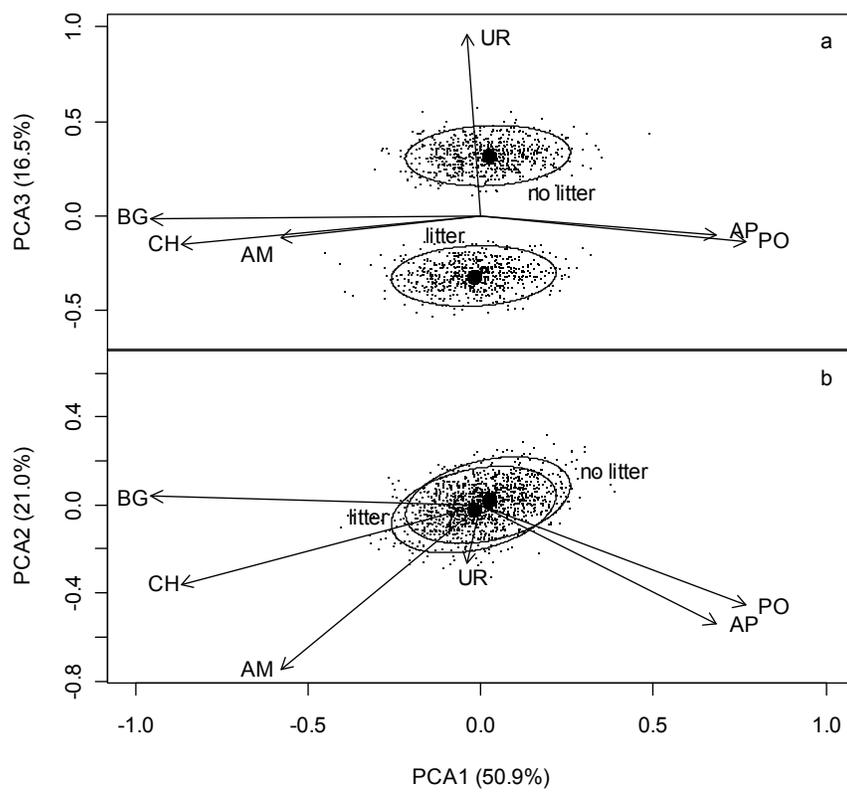


Figure 6

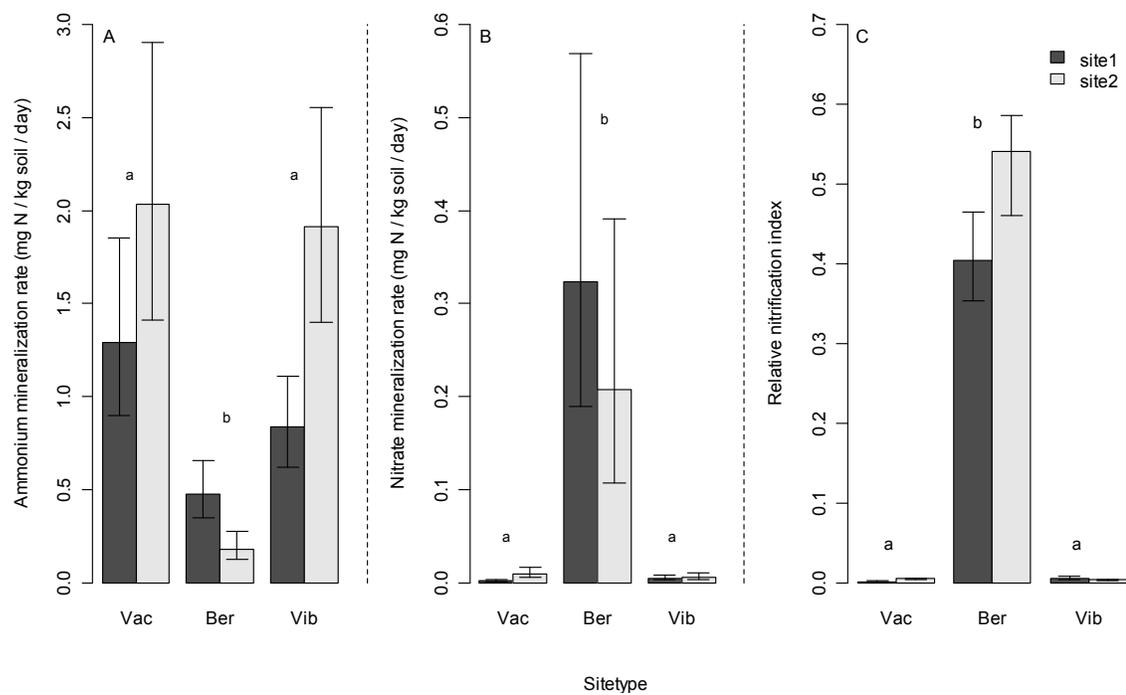
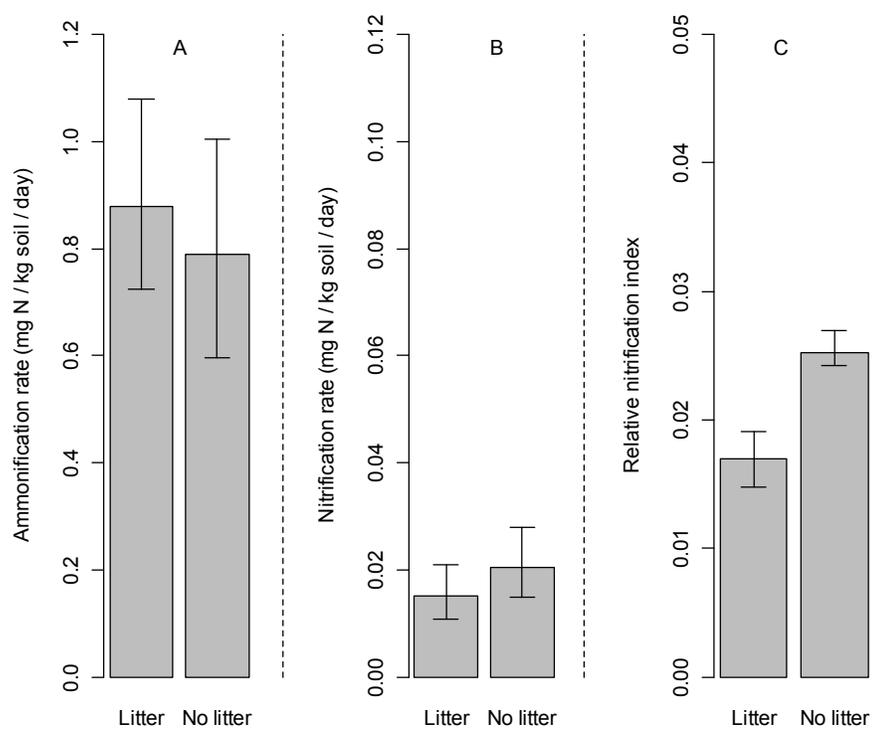


Figure 7



Appendix 1

Below is WinBUGS code used to estimate treatment effects on nitrate mineralization.

“n” is the data line, “NMIN” is the response variable (nitrification rate), “mu.1” and “tau.1” are the estimated mean and variance, respectively, for each treatment combination. The “type”, “site”, “veg”, and “subplot” nodes are indicator variables from the data for which sitetype, site, vegetation type, and subplot each data point comes from, respectively. The nodes “diff.4”, “diff.3”, “diff.2”, and “diff.1” are the effects due to different sitetypes, sites nested within sitetype, vegetation treatment types, and litter treatments, respectively. The “vegint” and “litterint” nodes are the sitetype*vegetation type and sitetype*vegetation type*litter type interactions, respectively.

```

model{
  for (n in 1:144)
    {NMIN[n] ~ dnorm (mu.1[type[n],site[n],veg[n],subplot[n]],
      tau.1[type[n],veg[n],subplot[n]])}
  for (i in 1:3)  #for 3 sitetypes
    {mu.4[i] <- mu.5 + diff.4[i]  #sitetype = grand mean + sitetype effect
  for (j in 1:2)  #for 2 sites
    {mu.3[i,j] <- mu.4[i] + diff.3[i,j]    #site = sitetype mean + site effect
  for (k in 1:3)  #for 3 veg treatments
    {mu.2[i,j,k] <- mu.3[i,j] + diff.2[k] + vegint[i,k] #plot = site + veg effect + interaction
      #plot = site + vegetation effect + interaction
  for (l in 1:2)  #for 2 litter suplots
    {mu.1[i,j,k,l] <- mu.2[i,j,k] + diff.1[l] + litterint[i,k,l] } } } }

```

```

#subplot = plot + subplot effect + interaction

#uninformative priors

diff.1[1]<- diff.1[2] * -1

diff.1[2] ~ dnorm(0, 1.0E-6)

diff.2[1]<- (diff.2[2] + diff.2[3]) * -1

for (i in 2:3){diff.2[i] ~dnorm(0, 1.0E-6)}

diff.3[1,1]<-diff.3[1,2] * -1

diff.3[1,2]~dnorm(0, 1.0E-6)

diff.3[2,1]<-diff.3[2,2] * -1

diff.3[2,2]~dnorm(0, 1.0E-6)

diff.3[3,1]<-diff.3[3,2] * -1

diff.3[3,2]~dnorm(0, 1.0E-6)

diff.4[1]<- (diff.4[2] + diff.4[3]) * -1

for (i in 2:3){diff.4[i] ~dnorm(0, 1.0E-6)}

litterint[1,1,1]<-(litterint[2,3,2]+litterint[2,2,2]+litterint[3,3,2]+litterint[3,2,2]) * -1

litterint[1,1,2]<-litterint[2,3,2]+litterint[2,2,2]+litterint[3,3,2]+litterint[3,2,2]

litterint[1,2,1]<-(litterint[2,2,1] + litterint[3,2,1]) * -1

litterint[1,2,2]<-(litterint[2,2,2] + litterint[3,2,2]) * -1

litterint[1,3,1]<-(litterint[2,3,1] + litterint[3,3,1]) * -1

litterint[1,3,2]<-(litterint[2,3,2] + litterint[3,3,2]) * -1

litterint[2,1,1]<-(litterint[2,3,1] + litterint[2,2,1]) * -1

litterint[2,1,2]<-(litterint[2,3,2] + litterint[2,2,2]) * -1

litterint[2,2,1]<-litterint[2,2,2] * -1

```

```

litterint[2,2,2]~dnorm(0, 1.0E-6)
litterint[2,3,1]<-litterint[2,3,2] * -1
litterint[2,3,2]~dnorm(0, 1.0E-6)
litterint[3,1,1]<-(litterint[3,2,1] + litterint[3,3,1]) * -1
litterint[3,1,2]<-(litterint[3,2,2] + litterint[3,3,2]) * -1
litterint[3,2,1]<-litterint[3,2,2] * -1
litterint[3,2,2]~dnorm(0, 1.0E-6)
litterint[3,3,1]<-litterint[3,3,2] * -1
litterint[3,3,2]~dnorm(0, 1.0E-6)
vegint[1,1]<-vegint[3,2] + vegint[3,3] + vegint[2,2] + vegint[2,3]
vegint[1,2]<-(vegint[2,2] + vegint[3,2]) * -1
vegint[1,3]<-(vegint[2,3] + vegint[3,3]) * -1
vegint[2,1]<-(vegint[2,2] + vegint[2,3]) * -1
vegint[3,1]<- (vegint[3,2] + vegint[3,3]) * -1
for (i in 2:3){for (j in 2:3){vegint[i,j]~dnorm(0, 1.0E-6)}}
for (i in 1:3){for (k in 1:3){for (l in 1:2){tau.1[i,k,l]~dgamma(0.001, 0.01) }}}
mu.5~dnorm(0, 1.0E-6)
}

```

Chapter 4

Dominance of an exotic invasive species and the diversity of the invaded community
alters impacts of invasion on soil enzyme activity

Abstract

Exotic invasive species can have large effects on soil biotic, abiotic, and physical factors, resulting in large changes in the function of the soil ecosystem. There is an increasing recognition that these changes in soil ecosystem function can feed back to influence plant community composition and the rate of exotic invasion. While many studies have shown evidence for these plant-soil feedbacks with individual species grown in monoculture, little is known about how plant-soil feedbacks operate in a diverse plant community, or how diversity (both species richness and evenness) can influence plant-soil feedback. In this study, I manipulated the diversity of a native shrub community and the dominance of an invasive shrub species (*Berberis thunbergii*) in a greenhouse microcosm experiment. I then measured the effects of plant diversity and of the dominance of the invasive shrub on six soil enzyme activities to quantify the effects on soil function. Then, in a second phase of the experiment, I grew a single *Berberis thunbergii* plant in soil from each microcosm to test whether changes in soil function had any effect on subsequent growth of the invasive species. I found that microcosms with higher plant diversity had higher rates of enzyme activities, which resulted in higher rates of *Berberis thunbergii* growth during the second phase of the experiment. In contrast, while a higher dominance of *Berberis thunbergii* in the first phase of the experiment also led to increased enzyme activities, this did not result in higher rates of *Berberis thunbergii* growth during the second phase. These results suggest that while the diversity

and degree of invasion strongly influence the impact of the plant community on soil ecosystem function, this does not always feed back in a way that influences the rate of exotic invasion. Future studies that investigate the role of diversity and plant community structure in plant-soil feedback are needed to determine if these results are generalizable to other plant communities and other exotic invasive species.

Introduction

Exotic invasive species are among the most economically damaging components of anthropogenic global change (Pimentel et al. 2005, Vilà et al. 2010), and despite a massive body of research on the impacts of invasions, our ability to predict the impact of an invasive species is still extremely limited (Lockwood et al. 2007). This may be due in part to poorly-understood feedbacks that are altered by invasive species, leading to complex non-linear dynamics and threshold effects (Peters et al. 2004, Ehrenfeld et al. 2005, Kulmatiski et al. 2008). For plant invasions in particular, feedback between plants and the soil ecosystem can strongly influence the rate of exotic species invasion (Klironomos 2002, van der Putten et al. 2007b) and the rate of native species invasion during natural succession (van der Putten and Peters 1997, Kardol et al. 2007). Plant-soil feedback may therefore be an important aspect of plant invasion that influences the impact of an invasive species.

Research on plant-soil feedback has increased rapidly during the past decade as the importance of this mechanism is increasingly recognized and as the technology to characterize soil biota has become more accessible (Ehrenfeld et al. 2005, Kulmatiski et al. 2008). Despite the rapid increase in research, however, virtually all studies have focused on the feedback effects of plants grown in monoculture, and we have an

extremely limited understanding of the role of feedback when the dominance of the invasive species varies or when the diversity of the native plant community varies. A recent meta-analysis suggests that feedback may operate differently in a diverse competitive environment compared to monocultures (Kulmatiski et al. 2008), highlighting the need for studies that put plant-soil feedback into the context of diverse natural communities.

Empirical and theoretical evidence suggest the diversity of a community is important in determining the role of feedback in native plant communities. Strong plant-soil feedbacks are most often demonstrated in species-poor environments and agricultural systems and rarely shown in species-rich systems (Ehrenfeld et al. 2005). Plant-soil feedbacks have also been demonstrated in low diversity, extreme environments, where plants have evolved mechanisms to tolerate environmental stress that contributes to strong plant-soil feedbacks (Chapman et al. 2006). Theory also predicts mutualism, a form of biotic positive feedback, is also more common in simplified extreme environments, where it operates as a mechanism for coping with environmental stress (Bertness and Callaway 1994, Bruno et al. 2003). Finally, weak interactions are more common in diverse systems, while the strong interactions required for strong feedback effects are more common in species-poor communities (McCann 2000). These studies all suggest that feedbacks may be moderated in diverse communities, though empirically-based tests of this are few.

Though the link between diversity and feedback is poorly studied, strong positive (Naeem et al. 2000, Hector et al. 2001) and negative (Stohlgren et al. 2003, Gilbert and Lechowicz 2005) relationships between diversity and invasibility of natural systems have

been demonstrated. However, the mechanism for this relationship is not known and is perhaps one of the most contentious topics in the field of invasion biology (Naeem et al. 2000, Hector et al. 2001, Gilbert and Lechowicz 2005, Stohlgren et al. 2005, Flather et al. 2006, Fridley et al. 2006, Jarnevich et al. 2006, Fridley et al. 2007). The oldest and leading explanation is that species-rich systems are less invasible because more of the available niche space is occupied, leaving less opportunity and fewer resources for potential invaders to utilize (Elton 1958). Temporally-fluctuating resources may also be more efficiently sequestered by species-rich communities, reducing the chance of successful invasion (Davis et al. 2000). Short-term and small-scale experimental evidence tends to support these theories (Naeem et al. 2000, Hector et al. 2001), but observational studies that incorporate large areas, long-term dynamics, and other extrinsic factors show greater invasion in species-rich communities instead of reduced invasion (Stohlgren et al. 2003, Stohlgren et al. 2005). Community diversity and plant-soil feedback may also interactively influence the rate or success of invasion.

The dominance of native or invasive species has similarly been largely overlooked, both as a factor that controls the effects of invasion (Dietz and Edwards, 2006) and as a measure of invasion success (Lundholm and Larson, 2004). Only a few experimental studies (Lundholm and Larson, 2004, Dietz and Edwards, 2006, Crall et al., 2006) and one modeling study (Levine et al., 2006) have addressed the relationship between the dominance of exotics and invasion. However, since dominance affects the evenness of a community and therefore its diversity, dominance could also interactively influence the role of plant-soil feedback in plant community dynamics.

In this study, I tested how the dominance of an invasive species and the diversity of the native plant community influence the role of plant-soil feedback. I conducted the study using Japanese barberry (*Berberis thunbergii* DC., hereafter referred to as BETH), a common exotic invasive shrub in eastern deciduous forests of the United States that often forms dense, nearly monospecific stands in the forest understory. These dense stands dramatically change the nitrogen cycle in the soil, increasing the net nitrogen mineralization rate and nitrification rate (Ehrenfeld et al. 2001) and altering soil enzyme activities (Kourtev et al. 2002). Because nitrogen is a limiting factor for BETH under natural forest conditions, these changes in soil function could favor the growth of BETH and result in a positive feedback. The goal of this study was to test whether BETH invasion into a native community leads to positive plant-soil feedback, and how the diversity of the native community and the dominance of the invasive plant changes the effects on soil and the subsequent feedback effects on BETH growth.

Methods

Pretreatment phase. Soil was collected from Allamuchy State Park (Allamuchy, NJ) in November 2006 from an uninvaded area by sieving soil from the top 10 cm of the mineral soil layer (measured after removing the organic layer). Soils in this area are Rockaway series typic fragiudults on the pre-Cambrian gneisses of the Highlands Province (see Kourtev et al. [1998] for site details). Soil was passed through a 2-cm sieve to remove large stones and homogenize the soil. All soil was covered and stored over winter in a large container in a storage shed at ambient (outside) temperature. The following spring, the soil was mixed with a small amount of commercial-grade sand to

improve drainage (7:1 soil:sand ratio), filled into 4-L pots, and transported to a greenhouse where they were planted in June 2007.

To create a range of soil conditions characteristic of areas dominated by BETH or native species, field-collected soil was “pretreated” by growing combinations of six different plants in each pot for approximately 2 years. Six native understory shrub species were used in this experiment during the pretreatment phase to create a gradient of diversity: *Cornus racemosa* Lam. (hereafter CORA), *Hamamelis virginiana* L. (HAVI), *Lindera benzoin* (L.) Blume (LIBE), *Vaccinium angustifolium* Aiton (VAAN), *Viburnum dentatum* L. (VIDE), and *Viburnum prunifolium* L. (VIPR). These species were chosen because they are commercially available, relatively common native understory species in the region and often co-occur in the same habitat. All native plants except VAAN were purchased from Pinelands Nursery (Columbus, NJ) as first-year seedlings grown from locally-collected seed sources. VAAN plants were purchased as newly-rooted cuttings from DeGrandchamps Farms (South Haven, MI). All native-species mixtures were also planted with varying numbers of BETH to create a gradient of invasion density. All BETH used for the pretreatment phase were either collected as seedlings from Allamuchy State Park (Allamuchy, NJ) in June 2007 or were planted from newly-rooted cuttings that were rooted in perlite in April 2007.

Pots were planted according to an experimental design that decoupled the effects of native species richness and invasion density (Table 1). In approximately half of the pots, native species richness was held constant while invasion density was varied, and in the rest of the pots, invasion density was held constant while richness was varied. These were set up as separate experimental gradients; however, due to large numbers of plants

that died, these separate gradients were no longer effective in decoupling richness and invasion density. The data from these two experiments were therefore pooled to examine both dominance and diversity effects, and will not be discussed separately. Before planting, all plants were bare-rooted and gently washed, then weighed to attain each plant's initial live mass. Live biomass was related to dry biomass using regression equations developed from a subset of plants that were weighed before and after drying at 70° C for one week.

After planting, pots were placed on two adjacent greenhouse benches, watered three to four times weekly, and randomly rotated monthly to avoid any possible microclimatic effects. At approximately 7 days, 30 days, and 60 days after planting, dead plants were removed from pots and replaced with new plants; however, after 2 months, any plants that died were left in place. After approximately 90 days, some plants began to show signs of nutrient stress, and a weak dose of fertilizer (Miracle Gro) was applied to each pot, resulting in an addition of approximately 0.014 g N/pot. Fertilization was repeated occasionally when plants began to exhibit signs of nutrient stress. All pots were kept in the greenhouse during the 2007-2008 winter, but were placed outdoors to promote winter dormancy between October 2008 and March 2009. Pots were maintained for two years until July 2009, when soil was sampled from the pots and analyzed for extracellular enzyme activity (see methods below). All plants were then harvested by gently overturning the pot, disentangling individual plants' roots, and drying all plants in a drying oven for one week at 70° C before weighing individual plants. Plants that were dead at the time of harvest were also dried and weighed, but recorded as dead. After

harvesting the plants, the soil was returned to the pot and used for the next phase of the experiment (see “feedback phase”, below)

Soil enzyme activities

Six soil enzyme activities (Table 2) were assayed at the end of the pretreatment phase using a microplate method (adapted from Waldrop et al. [2000] and Sinsabaugh et al. [2000]) as a measure of potential cycling rates of carbon (β -glucosidase, chitinase, phenol oxidase), nitrogen (chitinase, urease, aminopeptidase), and phosphorus (acid phosphatase). Because of the large number of samples, the order of the analysis of the samples was determined using a stratified random sampling, with replicates as strata and treatments randomized within strata. Soil slurries were made from 20 g wet soil suspended in 150 mL buffer solution (50 mM sodium acetate adjusted to pH 5.0) by stirring 1 min. on a stir plate. While stirring, eight 50- μ L aliquots were pipetted from each slurry into 96-well microplates as analytical replicates. 150 μ L of the appropriate enzyme substrate was added into each well, and plates were incubated at room temperature for 24 or 20 hours to allow color development (see Table 2). All substrates were 10 mM solutions dissolved in sodium acetate buffer, except urea, which was a 25 mM solution. Controls for the background absorbance of the soil were also prepared by adding 150 μ L of acetate buffer solution instead of 150 μ L substrate to soil slurries in separate wells. All controls were incubated and measured simultaneously with the samples.

At the end of the incubation period, 50 μ L solution was pipetted out of each well into a new plate. For acid phosphatase, β -glucosidase, chitinase, and amino peptidase, 50 μ L 0.1M NaOH was added to each well to terminate the reaction before reading

absorbance at 420 nm. Absorbance of phenol oxidase was read directly at 450 nm. For urease, an ammonium test kit (Hach Co., Loveland, CO, USA) was used to measure ammonium production, detected by absorbance at 600 nm. All absorbances were converted to enzyme activity using standard curves of ammonium for urease and p-nitrophenol for all other enzymes. Activities were converted to a dry-soil mass basis by drying two 400- μ L aliquots of soil slurry at 105°C to determine dry soil mass per 50- μ L analytical replicate. Enzyme activities for acid phosphatase, β -glucosidase, chitinase, and aminopeptidase were expressed in mg pNP / kg soil / hr, while phenol oxidase was expressed directly as absorbance / g soil / hr, and urease as mg NH_4^+ -N / kg soil / hr. Enzyme analyses were done using a pipette robot with spectrophotometric plate reader (Biomek® Laboratory Automation Workstation, Beckman Coulter Inc. Fullerton, CA, USA) in the Rutgers University High-Throughput Screening Laboratory. All data analyses used the average enzyme activity per sample (averaged across analytical replicates). Because large soil particles occasionally interfered with absorbance measurements, microplate wells were visually examined and data from wells with large soil particles were removed before calculating any averages.

Feedback Phase

To examine the effect of soil pretreatment on subsequent BETH growth, all plants were removed from the pretreated soil, and each pot was replanted with a single BETH plant. Because mortality during the pretreatment phase was high in many pots, only 4 of the 8 replicate pots (Table 1) were kept for the feedback phase, and data from the 4 replicates per treatment with the highest mortality are not reported here. These pots were then planted with one BETH rooted cutting per pot in August 2009. Cuttings were from

field-collected branch segments dipped in rooting hormone and rooted in pure perlite 2 months prior to planting. Each BETH was weighed before planting to obtain initial mass, which was related to initial dry mass using regression equations developed on a separate subset of BETH cuttings. BETH cuttings were then grown for 19 weeks before harvesting the plants by gently rinsing away all soil and drying the plants in a drying oven for one week at 70° C.

Data analysis

I used measures of the pot-level native species diversity and BETH dominance to test for effects on soil enzyme activity and on the second-generation BETH growth and survival. However, because many of the plants during the pretreatment phase died during the pretreatment and therefore represent decomposing litter rather than actively growing plants, I treated dead plants and live plants separately, and separately quantified the diversity (species richness and Shannon diversity index) of both live and dead plants, as well as the biomass of live and dead BETH plants as a percent of the total live plant and dead plant biomass, respectively. The Shannon diversity index was calculated by using the total biomass of each species as an estimate of its abundance. These four predictor variables (live & dead diversity, live & dead BETH dominance) were then used to predict enzyme activity and second-generation BETH growth and survival (Figure 1, arrows 1-4). I tested the hypothesis that the pretreatment-phase plant community could directly affect BETH growth and survival (Figure 1, arrows 2 & 4) because the plant community changes nutrient availability, which could influence subsequent BETH growth. I tested the hypothesis that the plant community would influence enzyme activity (Figure 1, arrows 1 & 3) because the soil microbial community both influences soil enzyme activity

and is influenced by the plant community. Furthermore, I tested whether enzyme activity was a good predictor of BETH growth and survival because enzyme activity regulates nutrient cycling, which could affect subsequent BETH growth (Figure 1, arrow 5). The normality of all data was assessed using normal quantile plots and data were transformed when necessary using the Box-Cox method for transformation. This resulted in log-transformation of the plant growth data.

To test the strength of these hypothesized relationships, I used the deviance information criterion (DIC, Spiegelhalter et al. 2002) to select the best models from a set of candidate Bayesian generalized linear models. DIC is a Bayesian measure of model adequacy and is related to Akaike's Information Criterion (Spiegelhalter et al. 2002). Both AIC and DIC rank models based on the likelihood of each model after penalizing them for the complexity of the model. The penalty terms used for AIC and DIC differ in form but are similar in function, with both penalizing more complex models more severely.

The form of the candidate models I ranked depended on the type of predictor and response variable (Appendix 1). The effects of diversity and BETH dominance on second-generation BETH growth were simple linear models. The effects of diversity and BETH dominance on BETH survival were modeled as a logistic regression, with second-generation BETH survival as a binomial random variable dependent on BETH dominance or diversity. The six soil enzyme activities were highly correlated, so principal components analysis was used to summarize the variation in enzyme activity on 2 axes (see results). I therefore modeled the response of soil enzymes to plant diversity and BETH dominance as a bivariate normal random variable. When enzyme activity was

used as a predictor variable for second-generation BETH growth and survival, the two principal components axes were included as separate independent predictor variables in the model.

For all models, I used relatively uninformative priors on all parameters. Predictors in all linear models were given normal prior probabilities with mean 0 and precision $1 * 10^{-6}$. Predictors in logistic regression models were given normal prior probabilities with mean 0 and precision 1.5. The results of all models were therefore strongly influenced by the data and very weakly influenced by the prior probabilities. Further details and WinBUGS code for example models are included in Appendix 1.

Results

Overall, 46.2% of the BETH planted during the feedback phase survived to the end of the feedback phase. Prediction of the survival rate of the second-generation BETH was not strongly improved by any of the possible predictor variables (data not shown). Furthermore, neither BETH growth nor BETH survival during the feedback phase was predicted by enzyme activities (data not shown). Therefore, only the relationships between diversity and enzyme activities, first-generation diversity and second-generation BETH growth, first-generation BETH dominance and enzyme activities, and first-generation BETH dominance and second-generation BETH growth (Figure 1, arrows 1 - 4) will be discussed further.

Effects of diversity and invasion density on soil enzyme activities

Principal components analysis summarized 98.7% of the variance of the six enzymes on two axes, with most of the variance explained by the first axis (Figure 2).

Principal components axis 1 (PCA1) was positively correlated to most enzyme activities, and therefore is a measure of overall activity in the soil. PCA2 was negatively correlated to β -glucosidase, an enzyme involved in the breakdown of labile C, and positively correlated to phenol oxidase, which is involved in the breakdown of recalcitrant C. PCA2 therefore can be summarized as a measure of the relative ability to break down recalcitrant C versus labile C.

Soil enzyme activities were influenced by the species identities of both live and dead plants present at the end of the pretreatment phase, and all of the best-fitting models included random species effects for both live and dead species (Table 3). In order to examine how much of the variance is accounted for by the species identities, I plotted the estimated variance explained by dead and live species, as well as the unexplained variance (random error, Figure 3). Random species effects of the dead species were strongest for PCA1 and accounted for nearly as much variance as the random error accounted for, while the live species accounted for nearly as much of the variance as error accounted for on PCA2 (Figure 3). When species richness was used as a measure of diversity (Table 3, “Species Richness” column), the number of species of dead plants was a significant predictor of soil enzyme activities, with a weak but significant increase in enzyme activity on PCA1 as the number of dead species increased (Figure 4). Species richness had no noticeable effect on PCA2 (data not shown).

When diversity was measured using the Shannon diversity index (Table 3, “Shannon Diversity Index” column), the most parsimonious model indicated that in addition to random species effects on soil enzyme activity, there was a positive effect of the live plant diversity on PCA1 as well. However, this model was not supported by the

data much better than a model that also included an effect for the diversity of dead species, nor was it supported much better than the model including neither live nor dead diversity (Table 3). In addition, DIC scores were consistently higher when Shannon diversity index was used as a measure of diversity, indicating none of these models were supported as well as when species richness was used as a measure of diversity. Enzyme activity was thus more strongly related to species richness than to the Shannon diversity index.

The density of BETH invasion during the pretreatment phase, measured as BETH biomass / total biomass, significantly improved prediction of soil enzyme activities (Table 4). Increasing dominance of dead BETH biomass at the end of the pretreatment period resulted in an overall increase in soil enzyme activity (Figure 5). However, only the dominance of dead BETH plants was an important predictor; the dominance of live BETH plants at the end of the pretreatment stage did not improve the model fit (Table 4). Furthermore, the dominance of dead BETH plants had only small effects on the overall enzyme activity.

Effects of diversity and invasion density on subsequent BETH growth

All of the best-supported models for BETH growth during the feedback phase of the experiment included random species terms for both live and dead species of the pretreatment phase, indicating the species present during the pretreatment phase influenced the growth rate of BETH during the feedback phase (Table 5). These random effects were furthermore similar in magnitude to each other, and each accounted for approximately the same amount of variation as random error did (Figure 6).

When species richness was used as a measure of diversity (Table 5, “Species Richness” column), the top model included terms for the richness of both live and dead species in addition to the random species effects, reflecting an increase in BETH growth in pots that previously contained more live species (Figure 7a) or more dead species (Figure 7b). The top model included both live and dead species richness, although the models that included only one of the two terms had nearly as much support as the full model. Similar results were obtained when the Shannon diversity index was used to measure diversity rather than species richness (Table 5, “Shannon diversity index” column).

The density of invasion during the pretreatment phase had very little effect on the subsequent growth of BETH during the feedback phase. The candidate models that included effects of pretreatment invasion density were ranked either lower than or nearly the same as the model without those effects (Table 6). There was, however, substantial evidence in support of the model that included random species effects, suggesting again that the species present during the pretreatment stage influenced subsequent BETH productivity.

Discussion

The most consistently important attribute of the pretreatment-phase plant community throughout these experiments was the species identities of the plants that were present during the pretreatment. This attribute was what most influenced soil enzyme activities and the growth of BETH during the feedback phase (Tables 3-6). It is often recognized that functional attributes of the soil microbial community such as

extracellular enzyme activities are related to plant community composition because of chemical differences in the aboveground and belowground litter inputs (Kourtev et al. 2002, Güsewell and Freeman 2005, Dornbush 2007, Mahaney 2010). These species-driven effects on soil function can have a large effect on ecosystem-level factors such as carbon and nitrogen cycling (Wardle et al. 2009). However, it is not known whether these species-specific legacies on soil function persist through time, thereby affecting subsequent plant community composition or invasibility (Berendse 1994, Knops et al. 2002, Chapman et al. 2006). The results of our study show species-specific effects on both soil function (enzyme activity) and on subsequent BETH invasion; however, enzyme activity and subsequent invasion were not directly related. This suggests that species-specific effects on soil influenced invasion through some other mechanism besides enzyme activity. There are many possible ways that species could influence soils in a way that might affect BETH invasion, such as by changing soil pathogens, altering soil structure, or changing nutrient levels either by altering plant nutrient uptake or by changing nutrient supply rates (Ehrenfeld et al. 2005). Thus, while plant community composition affects enzyme activity and BETH growth rate, the effect on BETH growth rate is not due to the effect on enzyme activity.

The diversity of the pretreatment-phase plant community also had a positive effect on both soil enzyme activity (Figure 4) and BETH growth (Figure 7), though effects on BETH were small. These effects of diversity were in addition to the random species effects that were included in the model, meaning they were not simply driven by an increasing probability of including a species with a positive effect (Doak et al. 1998, Tilman et al. 2006). Plant diversity influences soil microbial community structure and

subsequently soil enzyme activity and nitrogen cycling (Zak et al. 2003, Fornara and Tilman 2009), and therefore increased nutrient cycling may have explained the greater growth rate of BETH in more diverse communities. Increasing the diversity of dead plant species (and therefore the diversity of litter types) also increased enzyme activities, probably due to the greater diversity and availability of substrates for decomposition (Scherer-Lorenzen 2008). This could also contribute to increased nutrient cycling and a greater growth rate of BETH.

Unexpectedly, higher diversity of both live and dead plants during the pretreatment phase led to increased BETH growth during the feedback phase of the experiment. Since most small-scale experimental studies show a negative relationship between diversity and invasibility (Fridley et al. 2007), I hypothesized that high diversity would lead to lower BETH growth, perhaps through greater nutrient depletion. An increased diversity of dead plants may have instead increased nutrient supply rates through litter turnover and thereby allowed greater BETH growth, and when species richness was used as a measure of diversity, there was evidence for this effect of dead species diversity. A higher diversity of live plants also led to greater growth of BETH though, even though higher live plant diversity might be expected to deplete more soil resources (Hooper and Vitousek 1998). When either species richness or the Shannon diversity index was used as a measure of diversity, the top-ranked model indicated that the live species richness increased subsequent BETH growth. Although most small-scale studies find the opposite effects of diversity on invasibility, some studies have found either no effect or similar effects to this study (Hector et al. 2001, Fridley et al. 2007).

Because of high plant mortality, the range of live-plant diversity in this study was quite limited, which may limit the robustness of these results.

The dominance of BETH during the pretreatment phase of this study increased overall soil enzyme activities, a result that was consistent with previous studies on soils where BETH was present and absent (Kourtev et al. 2002, Kourtev et al. 2003). The current study furthermore demonstrated that the increase in enzyme activity associated with BETH invasion has a linear relationship with the density of BETH invasion. Previous studies have only compared the effects of invasion where BETH is either absent or dominant, an approach that only reveals whether or not differences occur between invaded and uninvaded areas, but fails to reveal the type of relationship between invasion density and its impact on soil. The linear relationship between invasion density and impact shown in this study can greatly simplify management decisions about areas that exhibit a range of invasion densities (Yokomizo et al. 2009).

This study tested the hypothesis that changes in soil function following BETH invasion creates a positive feedback loop, thereby contributing to the invasiveness of BETH. The results, however, do not provide sufficient evidence to support this hypothesis. Although greater BETH invasion led to greater soil enzyme activities, this did not have any noticeable effect on subsequent BETH growth. While a number of studies have found evidence for positive plant-soil feedback during invasion of a non-native species (Reinhart et al. 2003, Reinhart and Callaway 2004, Stinson et al. 2006, van der Putten et al. 2007b, Mangla et al. 2008), most of these studies focus on feedbacks that operate through species-specific soil pests and pathogens and community-level processes. Very few studies have shown evidence of an ecosystem-level change in nutrients or soil

characteristics that drive plant-soil feedback during exotic invasion (but see Vivrette and Muller [1977]). This may be in part because plant-pathogen relationships are more species-specific than plant-saprotroph relationships (van der Putten et al. 2007a). As a result, BETH has strong impacts on the soil ecosystem, but this may not be a factor that contributes to its invasiveness.

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Table 1. Experimental design used to create gradients in invasion density and the native species richness. All pots contained 6 plants total; numbers below indicate how many individuals of each species were included in each pot.

Gradient	n	Richness	#BETH	CORA	LIBE	HAVI	VAAN	VIDE	VIPR
BETH dominance	8	2	1	5					
	8	2	1		5				
	8	2	1					5	
	8	2	2	4					
	8	2	2		4				
	8	2	2					4	
	8	2	3	3					
	8	2	3		3				
	8	2	3					3	
	8	2	4	2					
	8	2	4		2				
	8	2	4					2	
	8	2	5	1					
	8	2	5		1				
8	2	5						1	
Native species richness	8	5	2		1	1	1	1	
	8	5	2	1	1	1			1
	8	5	2	1			1	1	1
	8	5	2		1	1	1		1
	8	5	2	1	1			1	1
	8	5	2	1		1	1		1
	8	3	2			2	2		
	8	3	2			2			2
	8	3	2				2	2	
	8	3	2		2				2
	8	3	2	2	2				
	8	2	2			4			
	8	2	2						4
	8	2	2				4		
	8	2	2	4					
	8	2	2					4	
	8	2	2		4				

Table 2. Descriptions of the six enzyme activities measured.

Enzyme name	IUBMB EC [§] nomenclature	Primary nutrient	Substrate	Incubation time (hrs)	Λ^{\ddagger}
Acid phosphatase	EC 3.1.3.2	P	p-nitrophenyl-phosphate	24	420
β -glucosidase	EC 3.2.1.21	C (labile)	p-nitrophenyl- β -D-glucopyranoside	24	420
Chitinase	EC 3.2.1.52	C, N	p-nitrophenyl-N-acetyl- β -D-glucosaminide	24	420
Amino-peptidase	EC 3.4.11.1	N	glycine-p-nitroanilide	24	420
Phenol oxidase	EC 1.10.3.2	C (lignin)	L-3,4-dihydroxy-phenylalanine	20	450
Urease	EC 3.5.1.5	N	urea	20	600

[§]International Union of Biochemistry and Molecular Biology enzyme commission

[‡]Absorbance wavelength

Table 3. Effects of the diversity of both live and dead species on soil enzyme activities. Diversity is measured either as species richness or using the Shannon Diversity Index. The top candidate model is indicated by dark shading, and additional well-supported models ($\Delta DIC < 2.0$) are indicated by lighter shading.

Live species random effects	Model effects			Species Richness		Shannon Diversity Index	
	Dead species random effects	Live species diversity effects	Dead species diversity effects	DIC*	ΔDIC	DIC	ΔDIC
	✓	✓	✓	823.520	4.157	821.994	0.589
✓	✓		827.820	8.457	821.405	0	
✓	✓	✓	819.363	0	824.083	2.678	
✓	✓		821.979	2.616	821.979	0.574	
✓	✓	✓	829.737	10.374	822.616	1.211	
	✓		845.507	26.144	849.664	28.259	
✓			824.955	5.592	824.955	3.550	
	✓		847.050	27.687	847.050	25.645	
		✓	829.287	9.924	828.940	7.535	
		✓	835.967	16.604	831.183	9.778	
			844.716	25.353	850.290	28.885	
			853.262	33.899	853.262	31.857	

*Deviance Information Criterion

Table 4. Effects of the dominance of both live and dead BETH on soil enzyme activities. Dominance is measured as the percentage of the total live or dead biomass in the pot that is represented by BETH. The top candidate model is indicated by dark shading.

Live species random effects	Model effects			DIC*	ΔDIC
	Dead species random effects	Live %BETH	Dead %BETH		
✓	✓	✓	✓	689.724	4.402
✓	✓	✓		694.172	8.850
✓	✓		✓	685.322	0
✓	✓			690.083	4.761
✓		✓		697.592	12.270
	✓		✓	703.217	17.895
✓				692.465	7.143
	✓			707.450	22.128
		✓	✓	711.262	25.940
		✓		707.297	21.975
			✓	716.516	31.194
				713.402	28.080

* Deviance Information Criterion

Table 5. Effects of the diversity of both live and dead species on BETH growth. Diversity is measured either as species richness or using the Shannon Diversity Index. The top candidate model is indicated by dark shading, and additional well-supported models ($\Delta DIC < 2.0$) are indicated by lighter shading.

	Model effects				Species Richness		Shannon Diversity Index	
	Live species random effects	Dead species random effects	Live species diversity effects	Dead species diversity effects	DIC*	ΔDIC	DIC	ΔDIC
✓	✓	✓	✓	✓	244.859	0	244.832	1.758
✓	✓	✓			246.466	1.607	243.074	0
✓	✓	✓		✓	246.7	1.841	249.795	6.721
✓	✓				248.938	4.079	248.938	5.864
✓			✓		262.232	17.373	260.962	17.88
		✓		✓	262.473	17.614	263.082	20.008
✓					262.570	17.711	262.570	19.496
	✓				261.154	16.295	261.154	18.080
			✓	✓	265.349	20.490	263.381	20.307
			✓		263.749	18.890	261.536	18.462
				✓	265.232	20.373	265.409	22.335
					263.438	18.579	263.438	20.364

*Deviance Information Criterion

Table 6. Effects of the dominance of both live and dead BETH on subsequent growth of BETH. Dominance is measured as the percentage of the total live or dead biomass in the pot that is represented by BETH. The top candidate model is indicated by dark shading, and additional well-supported models ($\Delta DIC < 2.0$) are indicated by lighter shading.

Live species random effects	Model effects			DIC*	ΔDIC
	Dead species random effects	Live %BETH	Dead %BETH		
✓	✓	✓	✓	226.507	3.810
✓	✓	✓		226.570	3.873
✓	✓		✓	222.697	0
✓	✓			224.670	1.973
✓		✓		233.725	11.028
	✓		✓	236.528	13.831
✓				236.971	14.274
	✓			234.893	12.196
		✓	✓	237.548	14.851
		✓		235.947	13.250
			✓	239.555	16.858
				237.669	14.972

*Deviance Information Criterion

Figure 1. Conceptual diagram of the hypothesized relationships in the data. Arrows point from independent predictor variables to dependent response variables.

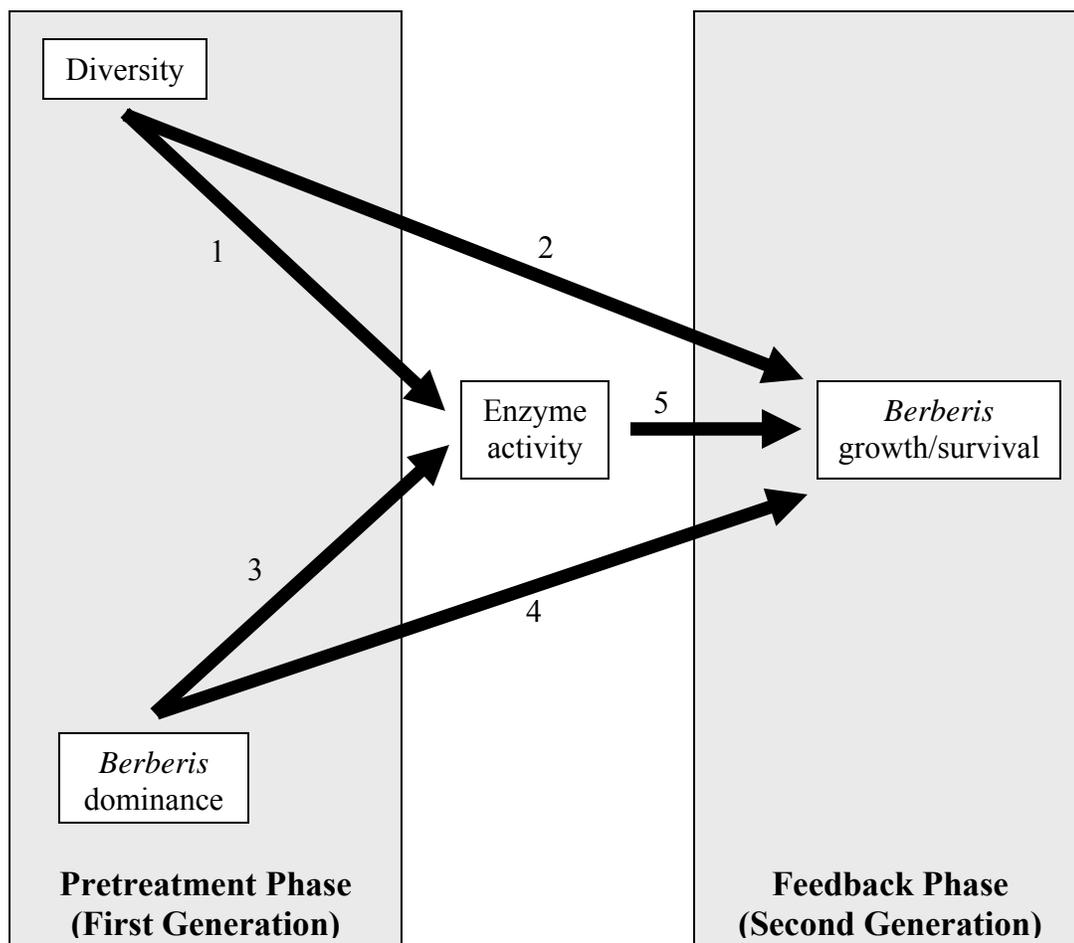


Figure 2. Correlations between six soil enzyme activities and the two principal component axes used to summarize the activities. Axis 1 explained 90.1% of the variance and axis 2 accounted for an additional 8.6%.

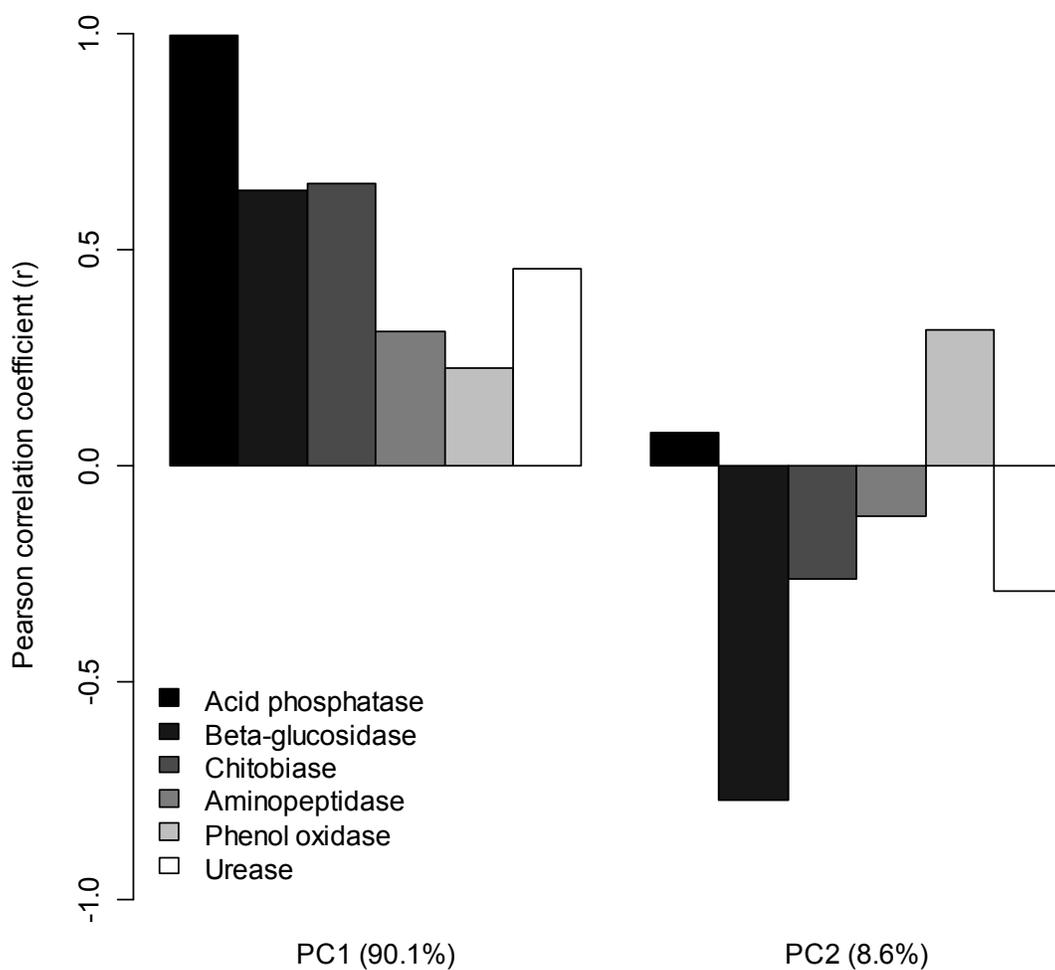


Figure 3. Estimated variance components of the random effects of live species, dead species, and error on PCA1 and PCA2. Displayed are the mean estimates from the top candidate model of the amount of variation due to each factor, and 95% credible intervals for the mean.

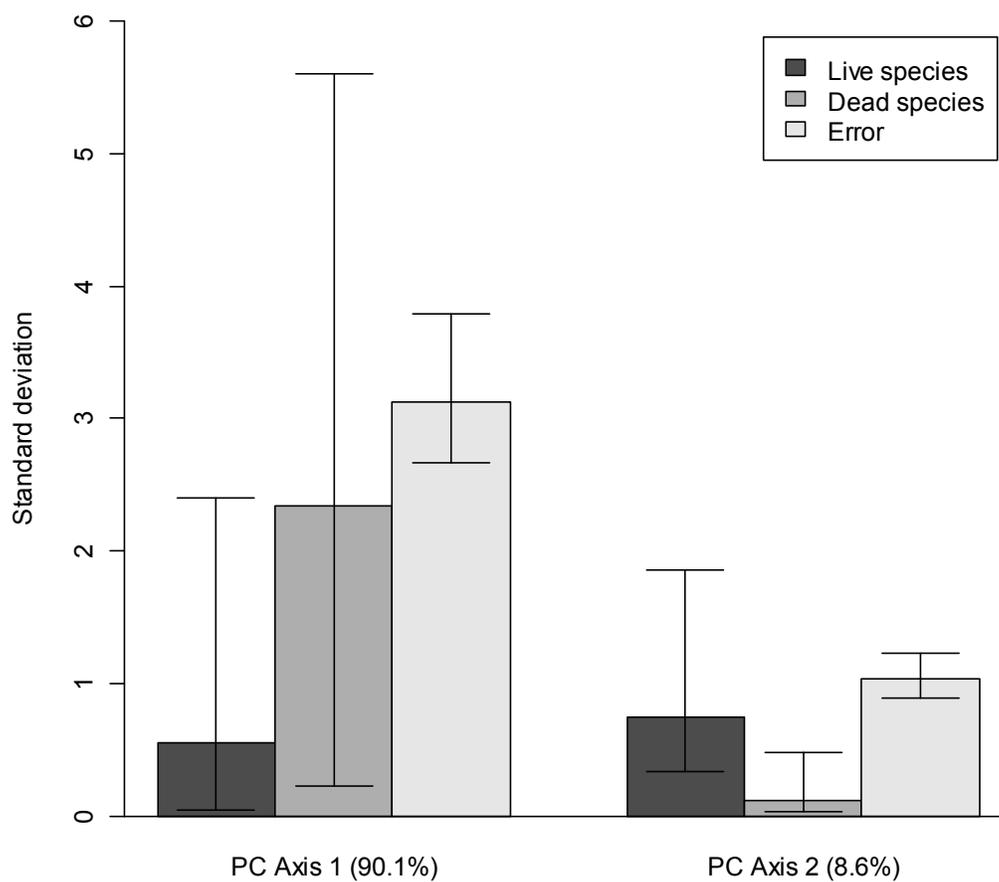


Figure 4. Relationship between the number of dead species and overall enzyme activity (PCA1). Data points show raw data, uncorrected for random species effects. The solid and dotted lines indicate the best fit line and 95% credible intervals, respectively, for the regression after accounting for random species effects.

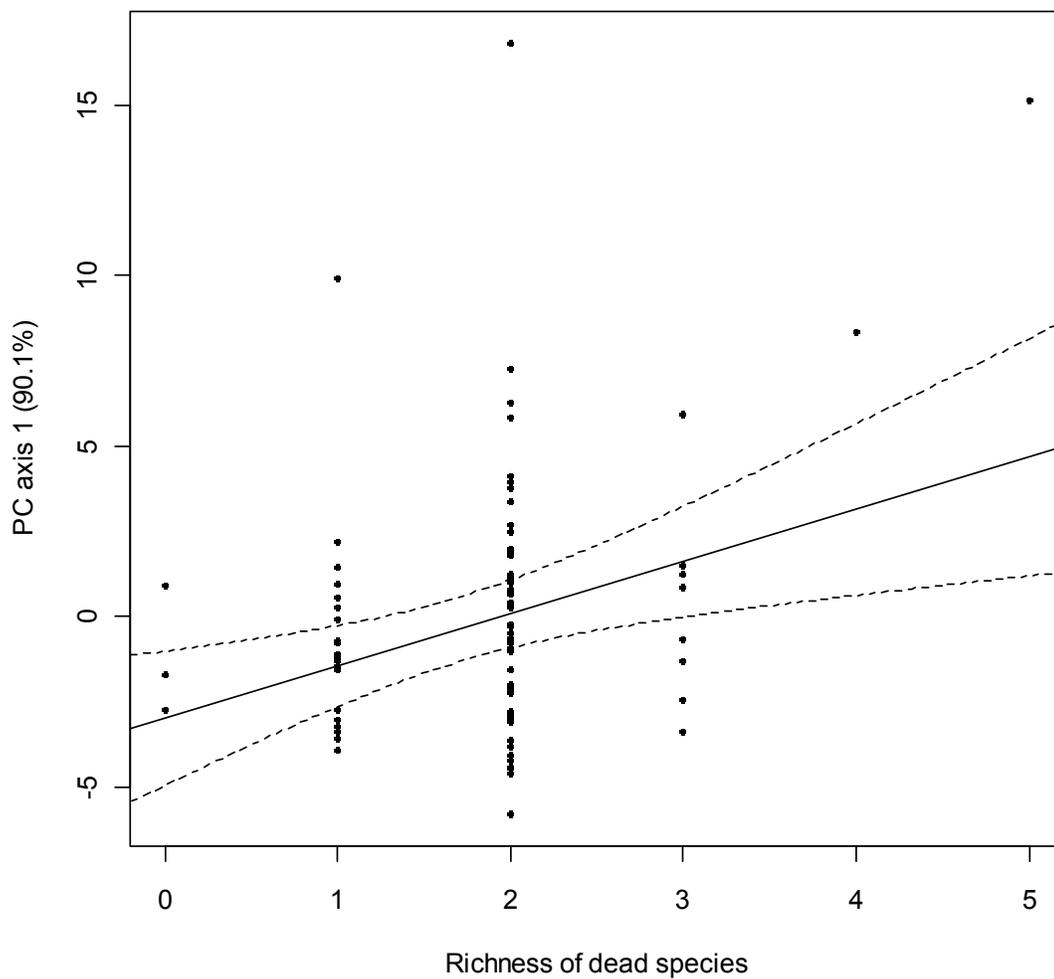


Figure 5. Relationship between the dominance of dead BETH and overall enzyme activity (PCA1). Data points show raw data, uncorrected for random species effects. The solid and dotted lines indicate the best fit line and 95% credible intervals, respectively, for the regression after accounting for random species effects.

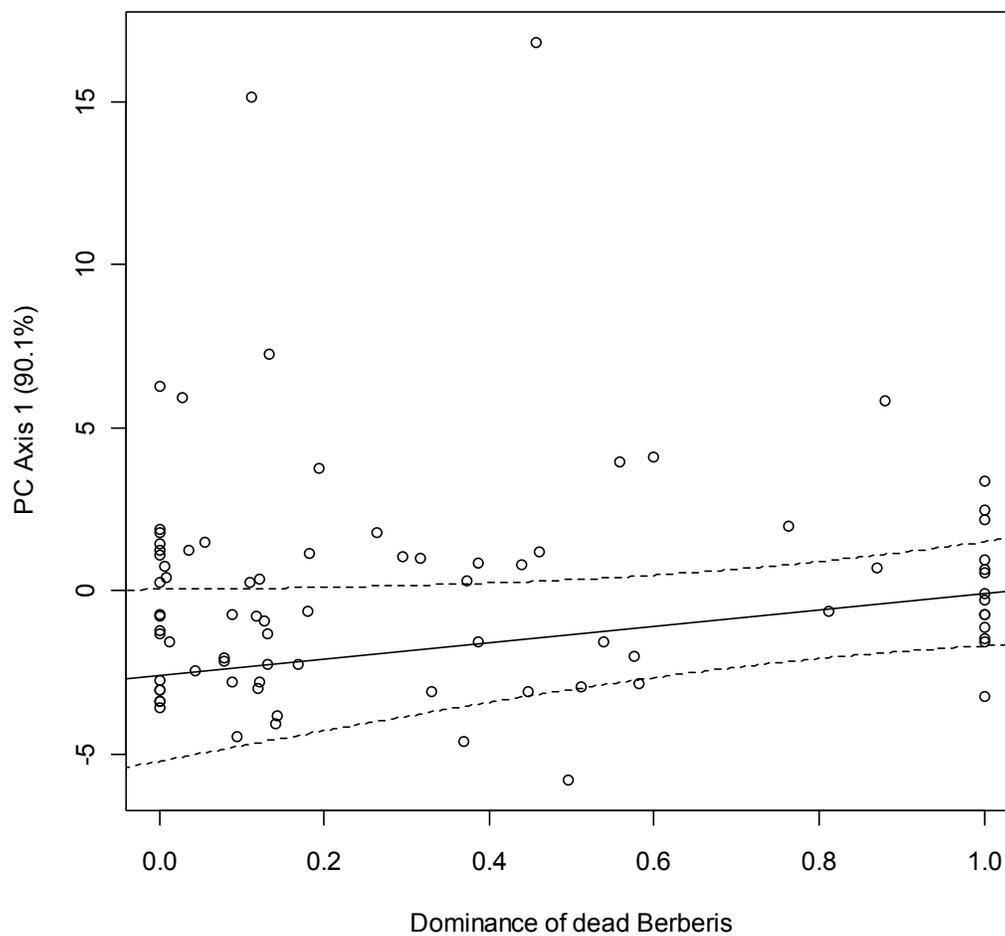


Figure 6. Estimated variance components of the random effects of live species, dead species, and error on the growth of BETH. Displayed are the mean estimates from the top candidate model of the amount of variation due to each factor, and 95% credible intervals for the mean.

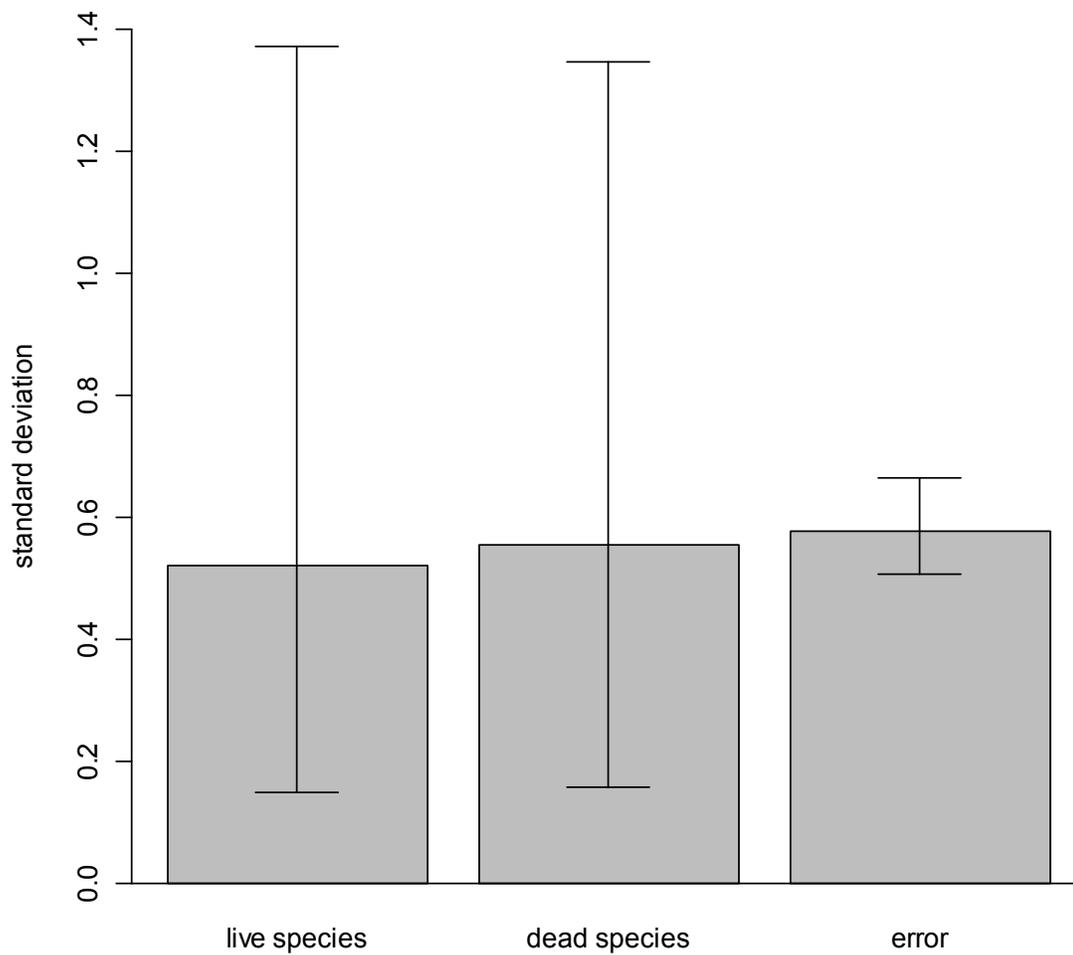
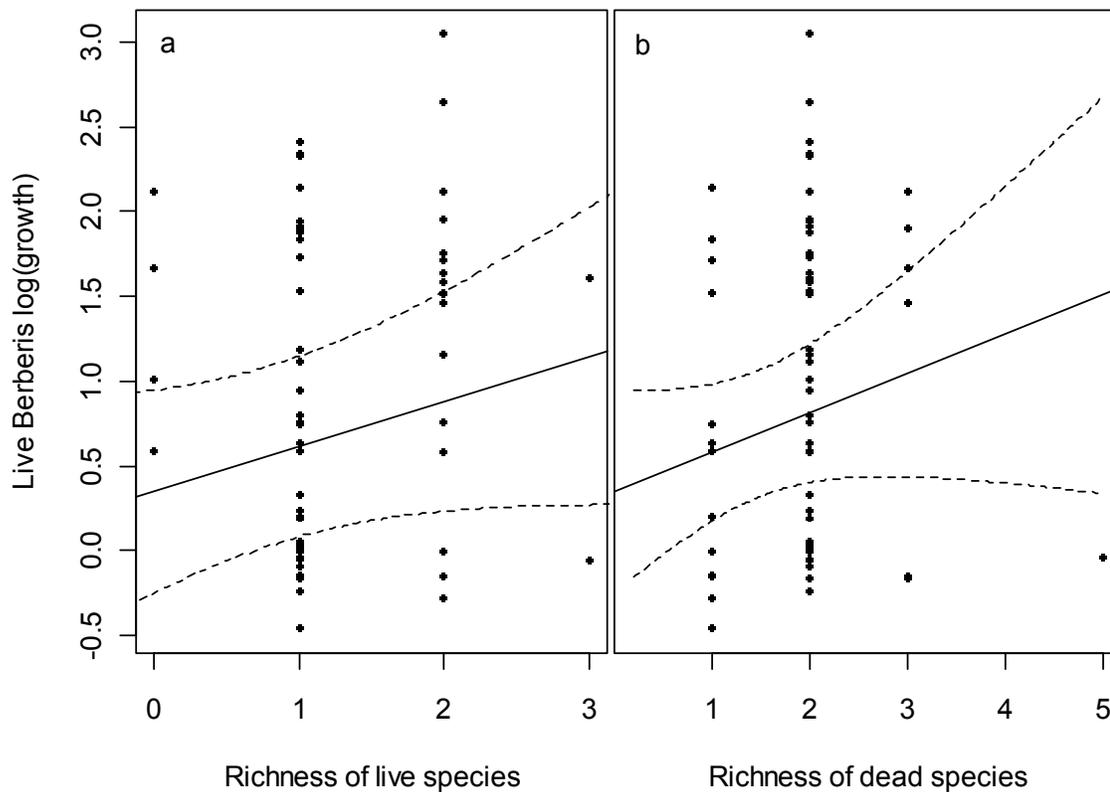


Figure 7. Effects of live species richness (a) and dead species richness (b) on the subsequent growth of BETH. Data points show raw data, uncorrected for random species effects. The solid and dotted lines indicate the best fit line and 95% credible intervals, respectively, for the regression after accounting for random species effects.



Appendix 1

The forms of all Bayesian models are shown below. “BETHgrowth” is the log-transformed biomass accumulation of BETH plants during the feedback phase. The “species.combo” indicates which random combination of species are used, “live.richness” is the number of species alive at the end of the pretreatment phase, “dead.richness” is the number of species dead at the end of the pretreatment, “liveBETH.dominance” is the percent of live biomass at the end of the pretreatment that is living BETH biomass, “deadBETH.dominance” is the percent of the dead biomass that is dead BETH biomass, and BETHsurv is the survival rate of BETH during the feedback phase. The “enzymes” variable is a vector of length 2, containing principal components axis 1 and axis 2 scores.

Model of treatment effects on BETH growth

$$\text{BETHgrowth} \sim \text{species.combo} + \text{live.richness} + \text{dead.richness} + \text{liveBETH.dominance} + \text{deadBETH.dominance}$$

Model of treatment effects on BETH survival

$$\text{logit(BETHsurv)} \sim \text{species.combo} + \text{live.richness} + \text{dead.richness} + \text{liveBETH.dominance} + \text{deadBETH.dominance}$$

Model of treatment effects on soil enzyme activities

$$\text{enzymes[1:2]} \sim \text{species.combo} + \text{live.richness} + \text{dead.richness} + \text{liveBETH.dominance} + \text{deadBETH.dominance}$$

Model of soil enzyme activities' effects on BETH growth

$$\text{BETHgrowth} \sim \text{species.combo} + \text{enzymes[1]} + \text{enzymes[2]} + \text{enzymes[1] : enzymes[2]}$$

Model of soil enzyme activities' effects on BETH survival

$$\text{logit(BETHsurv)} \sim \text{species.combo} + \text{enzymes[1]} + \text{enzymes[2]} + \text{enzymes[1] : enzymes[2]}$$

Conclusion

This dissertation explores the reciprocal interactions between plants and the soil microbial community during exotic plant invasion in the forest understory. I have shown how these interactions influence soil enzyme activities and nutrient cycling, and how that affects plant growth and interspecific competition. This work has shed light on the roles of both plants and microbes in the soil ecosystem, but clearly much remains to be explored. It is my hope that this research will generate interest in this important area, generating new hypotheses and research and helping us to further understand the important relationships between plants and soil. In a time of both increasing dependence on the earth's ecosystem services to support an increasing human population, as well as massive and often disruptive global change, it is more vital than ever to clarify how plants and soils interact, and how exotic plant invasion might alter the ecosystem.

Plants interact with the saprotrophic microbial community through aboveground (leaf) and belowground (root) litter inputs, as well as through root exudation. Leaf litter in particular is an important input of organic matter into the soil ecosystem and therefore strongly influences the microbial community, nutrient cycling, and the soil ecosystem as a whole (Brady & Weil 2002, this dissertation). The long-term effects of leaf litter on soil through soil organic matter formation and other soil formation processes in turn influences the plant community as well (Brady & Weil 2002, Chapman et al. 2006). When exotic plant species invade the forest understory, they alter the leaf litter inputs and thereby influence the soil microbial community. Since exotic invasive species frequently have higher leaf nutrient concentrations (Baruch & Goldstein 1999, Leishman et al. 2007)

and often possess unique leaf litter chemistry with novel secondary chemicals (Ehrenfeld 2006), there is substantial potential for exotic plant invasion to alter the soil microbial community structure and function.

These changes were demonstrated in the first two chapters of this dissertation. The first chapter showed that soil microbial communities that developed under Japanese barberry and Japanese stiltgrass (two common invasive understory species) differed from microbial communities beneath litter of two common co-occurring native shrubs (highbush blueberry and mapleleaf viburnum) after only 3 years. This short-term change in the microbial community furthermore affected soil ecosystem function, strongly altering soil enzyme activities in the soil and weakly affecting the decomposition rate of leaf litter on the soil surface. Japanese barberry leaf litter had especially strong effects on the soil ecosystem, perhaps due to its high nitrogen content and unique secondary phytochemistry. Japanese barberry contains high concentrations of alkaloids, with up to 3.5% of its mass comprised of nitrogen-containing alkaloids (Villinski et al. 2003). Approximately one third of the alkaloid content is berberine, a biologically active alkaloid with anti-microbial properties found in members of the *Berberis* genus (Villinski et al. 2003).

The effects of Japanese barberry leaf litter on the soil ecosystem were further explored in the second chapter, where I used microcosms to explore the response of the soil to a wide range of barberry invasion densities. I found that even small percentages of barberry litter mixed in with tree canopy litter had strong effects on the soil microbial community structure. Compared to pure tree canopy litter, the addition of only 2.5% barberry litter to tree canopy litter nearly doubled bacterial biomass relative to fungal

biomass. While the change in soil microbial community structure did not translate into a change in litter decomposition rate above the soil surface in this short-term experiment, previous research suggests that changes in the bacterial : fungal ratio in soil can heavily impact nutrient and carbon cycling (Romani et al. 2006, Myrold and Posavatz 2007, Rousk and Bååth 2007, Strickland et al. 2009). We furthermore did not measure carbon or nitrogen cycling within the soil profile, where changes would likely occur more quickly than in the litter layer. Though we did not directly test for mechanisms by which barberry litter alters microbial community structure, the strong response to small amounts of barberry and the nonlinear response to increasing amounts suggests that the unique phytochemistry and antimicrobial properties of barberry litter play an important role in barberry's effects on soil.

In the second half of the dissertation, I explored how emergent properties of the whole plant community influence the effect of barberry on soil microbial communities. In the third chapter, I showed that the long-term (decade-scale) effects of a well-established plant community on the soil microbial community structure and function are much stronger than the short-term (2-year) effects of actively growing vegetation. When plots dominated by one vegetation type in the field were re-planted with a new vegetation type, the long-term effects of the previous vegetation type overwhelmed any short-term effects from the current vegetation type two years after re-vegetation. In this study, the microbial community structure from barberry-dominated sites was again significantly different compared to two native vegetation types, and this difference in microbial community structure translated into a difference in soil ecosystem function, measured by soil enzyme activities and nitrogen mineralization rates.

I further explored in the fourth chapter how the emergent property of plant community diversity influences the effect of barberry on the soil microbial community. In this experiment, I found that even in diverse plant communities, soil microbial community function (measured in terms of enzyme activities) increased as the dominance of barberry increased. I also found a direct effect of diversity on the soil microbial community function (measured by enzyme activities), with increasing plant diversity resulting in higher soil enzyme activities. This means that as barberry invades the forest understory, it may influence the microbial community directly as well as indirectly through changing the diversity of the forest understory.

Finally, a major objective of this dissertation was to determine if plant-driven effects on the soil ecosystem feed back to further influence plant growth and competition. In the first chapter, I showed that leaf litter-driven changes in the soil microbial community changed the rate of plant growth. Soil microbial communities that developed beneath barberry litter increased the growth rate of both barberry and a co-occurring native shrub (*Viburnum dentatum*). Because soils beneath barberry promoted growth of both species equally, this did not change the competitive hierarchy, and therefore in this experiment there was no strong evidence for positive plant-soil feedback beneath barberry. However, it is important to keep in mind that barberry litter is more likely to be found beneath actively-growing barberry rather than beneath native shrubs. This spatial aspect will result in a greater effect of barberry litter on itself than on other co-occurring shrubs, which likely results in positive plant-soil feedback in the field. Furthermore, as seen in the third chapter of the dissertation, the short-term effects seen in this experimental study are probably magnified greatly over a longer time period, resulting in

stronger plant-soil feedback over the long term than what is seen in a short-term greenhouse experimental setting. The final chapter also suggested that plant-soil feedback in the field may be stronger than in the greenhouse. Barberry dominance and diversity both increased soil enzyme activities, indicating that nutrient cycling rates can be increased both by barberry and by a diverse background plant community. Thus, in a diverse field setting, the potential for feedback is greater. Barberry grew faster when it invaded soil previously occupied by a diverse plant community than when it invaded soil previously influenced by a depauperate plant community. This suggests that as barberry invades a previously-diverse plant community, it grows more rapidly and therefore influences the microbial community more rapidly by producing more litter above- and below-ground.

This dissertation has shown that understanding the success of exotic invasive plants and plant communities in general requires an understanding not only of the characteristics of exotic plants (van Kleunen et al. 2010) and the characteristics of the native plant community (Kennedy et al. 2002, Levine et al. 2004), but also the soil microbial community. Plants depend on the soil microbial community for resources (Knops et al. 2002), but the microbial community also depends on resources from plants, which can radically alter the structure of the microbial community over the short term and long term, thereby changing the competitive environment of the plant community (Chapman et al. 2006). While these complex relationships are as yet not fully understood, this dissertation makes clear that any comprehensive theory of exotic or native plant invasion must consider the soil microbial community in addition to the plant community.

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Curriculum Vitae

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