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Effects of tree species and N additions on forest floor microbial communities and extracellular enzyme activities

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ABSTRACT

Forest nitrogen (N) retention and soil carbon (C) storage are influenced by tree species and their associated soil microbial communities. As global change factors alter forest composition, predicting long-term C and N dynamics will require understanding microbial community structure and function at the tree species level. Because atmospheric N deposition is increasing N inputs to forested ecosystems across the globe, including the northeastern US, it is also important to understand how microbial communities respond to added N. While prior studies have examined these topics in mixed-species stands, we focused on the responses of different tree species and their associated microbial communities within a single forest type – a northern hardwood forest in the Catskills Mountains, NY. Based on prior studies, we hypothesized that N additions would stimulate extracellular enzyme activities in relatively labile litters, but suppress oxidative enzyme activities in recalcitrant litters, and tested for independent tree species effects within this context. During the 2007 growing season (May–June), we measured enzyme activities and microbial community composition (using phospholipid fatty acid analysis - PLFA) of the forest floor in single-species plots dominated by sugar maple (*Acer saccharum*), yellow birch (*Betula alleghaniensis*), red oak (*Quercus rubra*), American beech (*Fagus grandifolia*) and eastern hemlock (*Tsuga canadensis*), species whose litters range from relatively labile to recalcitrant. Half the plots were fertilized with N by adding NH_4NO_3 ($50\ \text{kg}\ \text{ha}^{-1}\ \text{y}^{-1}$) from 1997 to 2009. Non-metric multidimensional scaling (NMS) and multi-response permutation procedures (MRPP) were used to examine microbial community structure and relationship to enzyme activities.

We found that in response to N additions, both microbial community composition and enzyme activities changed; however the strength of the changes were tree species-specific and the direction of these changes was and not readily predictable from prior studies conducted in mixed-species stands. For example, in contrast to other studies, we found that N additions caused a significant overall increase in fungal biomass that was strongest for yellow birch (24% increase) and weakest for sugar maple (1% increase). Contrary to our initial hypotheses and current conceptual models, N additions reduced hydrolytic enzyme activities in hemlock plots and reduced oxidative enzyme activity in birch plots, a species with relatively labile litter. These responses suggest that our understanding of the interactions between microbial community composition, enzyme activity, substrate chemistry, and nutrient availability as influenced by tree species composition is incomplete. NMS ordination showed that patterns in microbial community structure (PLFA) and function (enzyme activity) were more strongly influenced by tree species than by fertilization, and only partially agreed with the structure–function relationships found in other studies. This finding suggests that tree species-specific responses are likely to be important in determining the structure and function of northeastern hardwood forests in the future. Enhanced understanding of microbial responses to added N in single and mixed-species substrates with varying amounts of lignin and phenols may be needed for accurate predictions of future soil C and N dynamics.

1. Introduction

In the last half century, anthropogenic contributions to atmospheric N deposition has led to increased N inputs to forested ecosystems in most industrialized areas of the world,

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including the northeastern U.S., Europe, and Asia, and this trend is expected to continue into the future (Galloway et al., 1995; Howarth et al., 2002; Janssens et al., 2010). Because primary production in these ecosystems is thought to be N limited (Vitousek and Howarth, 1991), increased N availability has the potential to increase plant productivity, and therefore carbon storage (Thomas et al., 2010). However, given that soil microbial communities regulate decomposition of plant litter and thereby nutrient loss and retention in these systems, it is important to understand the effect of added N on microbial community structure and function .

In a review of N-addition studies, Treseder (2008) found that increased N tends to decrease total microbial biomass, though the response of specific taxa (e.g., fungi or bacteria) varied. Studies have reported that increased N results in reduced or altered fungal populations (Dighton et al., 2004; Frey et al., 2004; Lilleskov et al., 2002), altered populations of N-fixing bacteria (Compton et al., 2004), shifts in Gram-positive and Gram-negative bacteria (Gallo et al., 2004), and decreased fungal:bacterial ratios (Frey et al., 2004; Waldrop et al., 2004a). Other studies have reported no significant changes in microbial community structure with N additions (DeForest et al., 2004). Importantly, the response of the microbial community composition to added N appears to be substrate-specific. For example, microbial communities in sugar maple/basswood and oak-dominated forests differed in their response to added N (Gallo et al., 2004; Myers et al., 2001; Waldrop et al., 2004a).

From a functional perspective, the activity of extracellular enzymes produced by microbes and their response to added N has received considerable attention because these enzymes contribute to the processes controlling decomposition and therefore C storage (Waldrop et al., 2004b; Zak et al., 2008). In response to added N, decomposition of labile (high in cellulose, low in lignin or tannins) plant litter or soil organic matter (SOM) is accelerated while decomposition of recalcitrant litter (high in lignin) or humified SOM is slowed (Carreiro et al., 2000; Fog, 1988), and these opposing responses have been linked to changes in enzyme activity (Janssens et al., 2010). Nitrogen additions to both labile and recalcitrant substrates are thought to allow microbes to invest N into enzyme production, often resulting in increased activity of enzymes responsible for cellulose degradation (e.g., β-glucosidase, cellobiohydrolase), for acquisition of organic N (*N*-acetyl-β-glucoaminidase) or for overcoming increased P limitations (phosphatases). Oxidative enzyme activities (phenol oxidase, peroxidase) are also often stimulated by N in labile substrates. However in recalcitrant substrates where specialized lignolytic fungi may dominate (e.g., white-rot basidiomycetes), N additions may suppress oxidative activity of many enzymes, and therefore slow decomposition (Carreiro et al., 2000; Saiya-Cork et al., 2002; Sinsabaugh et al., 2005; Waldrop et al., 2004b) and potentially suppress respiration (Janssens et al., 2010). Therefore, depending on the nature of the dominant substrates, enzymatic responses to added N (and their concomitant influence on SOM) may be specific to individual plant communities or even plant species. For example, in oak-dominated forests where litter is relatively recalcitrant and white-rot fungi are abundant, N additions result in decreased oxidative enzyme activities and SOM accumulation. In contrast, in sugar maple/basswood forests where litter decomposes more rapidly, N additions have increased enzyme activities and decreased SOM (Waldrop et al., 2004b). These contrasting responses led Waldrop et al. (2004a) to propose a model wherein oxidative enzyme response to added N is determined by the interaction of litter type (labile vs. recalcitrant) and fungal community (those dominated by white-rot basidiomycetes vs. communities dominated by other fungi including soft-rots).

While general responses to added N have been observed the structural and functional responses of microbial communities in specific litter, soil or ecosystem types are difficult to predict (Sinsabaugh et al., 2005). Among other factors, microbial communities can vary beneath different tree species within a single ecosystem (Grayston and Prescott, 2005; Saetre and Bååth, 2000) and so it could be expected that responses to added N may also vary at this scale. Understanding within-stand variability in the microbial community (as well as the linkages between community structure and function and their response to increased N) is important because it may influence both soil C storage (Waldrop et al., 2004b) and forest N retention or loss (Templer et al., 2005). Therefore, as forest composition changes, for example as a result of forest pests and other disturbances, predicting long-term C and N dynamics will require an understanding of how microbial community structure and function are altered at the tree species level (rather than forest stand level) and how those communities respond to N deposition. While previous studies suggest that different mixed-species forest types can have contrasting microbial responses to N additions, the role of individual tree species in contributing to these stand-level effects has not been extensively assessed.

This study was conducted to determine how enzyme activity and microbial community composition differs among tree species of the northern hardwood forest, and how each responds to added N. Previous work has shown that tree species in the northern hardwood forest differ in their microbial biomass, nutrient cycling characteristics and influence on soil chemistry (Lovett et al., 2004; Templer et al., 2003; Weand et al., 2010) and in their nutrient cycling responses to N additions (Templer et al., 2005). Based on these known tree species-level differences, the results from recent meta-analyses (Janssens et al., 2010; Treseder, 2008), and prior studies on mixed-species stand-level analyses of microbial community and enzyme activity responses to N additions (sugar maple/basswood stands and black oak/white oak stands in Michigan) (DeForest et al., 2004; Gallo et al., 2004; Waldrop et al., 2004a), we generated the following hypotheses. We hypothesized that: (A) all tree species would have different microbial communities with distinct enzyme activities, (B) in soils beneath species with relatively labile litter (e.g., sugar maple and yellow birch), N additions would stimulate the activity of hydrolytic and oxidative enzymes, (C) beneath species with more recalcitrant litter (e.g., red oak, American beech, and hemlock), N additions would stimulate hydrolytic enzyme activities but suppress oxidative enzyme activities, and (D) N additions would alter microbial community structure and reduce microbial biomass, particularly fungal biomass, under all tree species.

2. Methods

2.1. Study sites

Our study took place in a northern hardwood forest ecosystem in the Catskill Mountains of southeastern New York. Within the forest, single-species plots dominated by each of the five most dominant tree species in the northern hardwood forest association of the Catskill region (Braun, 1950; McIntosh, 1972): sugar maple (*Acer saccharum* Marsh), American beech (*Fagus grandifolia* Ehrh.), yellow birch (*Betula alleghaniensis* Britton), eastern hemlock (*Tsuga canadensis* (L.) Carr) and red oak (*Quercus rubra* (L.) Britton) were identified and used to test for tree species differences in microbial communities and enzyme activities. This ecosystem is located on thin Inceptisol soils derived from bedrock consisting of flat-lying sandstones, shales and conglomerates of Devonian age, and glacial till of variable depth (Rich, 1934; Stoddard and Murdoch, 1991).