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Relationship between soil enzyme activities, nutrient cycling and soil fungal communities in a northern hardwood forest

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ABSTRACT

Soil fungi are highly diverse and act as the primary agents of nutrient cycling in forests. These fungal communities are often dominated by mycorrhizal fungi that form mutually beneficial relationships with plant roots and some mycorrhizal fungi produce extracellular and cell-bound enzymes that catalyze the hydrolysis of nitrogen (N)- and phosphorus (P)- containing compounds in soil organic matter. Here we investigated whether the community structure of different types of mycorrhizal fungi (arbuscular and ectomycorrhizal fungi) is correlated with soil chemistry and enzyme activity in a northern hardwood forest and whether these correlations change over the growing season. We quantified these relationships in an experimental paired plot study where white-tailed deer (access or excluded 4.5 yrs) treatment was crossed with garlic mustard (presence or removal 1 yr). We collected soil samples early and late in the growing season and analyzed them for soil chemistry, extracellular enzyme activity and molecular analysis of both arbuscular mycorrhizal (AM) and ectomycorrhizal/saprotrophic fungal communities using terminal restriction fragment length polymorphism (TRFLP). AM fungal communities did not change seasonally but were positively correlated with the activities of urease and leucine aminopeptidase (LAP), enzymes involved in N cycling. The density of garlic mustard was correlated with the presence of specific AM fungal species, while deer exclusion or access had no effect on either fungal community after 4.5 yrs. Ectomycorrhizal/saprotrophic fungal communities changed seasonally and were positively correlated with most soil enzymes, including enzymes involved in carbon (C), N and P cycling, but only during late summer sampling. Our results suggest that fine scale temporal and spatial changes in soil fungal communities may affect soil nutrient and carbon cycling. Although AM fungi are not generally considered capable of producing extracellular enzymes, the correlation between some AM taxa and the activity of N acquisition enzymes suggests that these fungi may play a role in forest understory N cycling.

1. Introduction

Many soil fungi in forests form symbiotic associations with plant roots, notably the mutually beneficial relationships with trees, shrubs and herbaceous plants (Leake et al., 2002; Lindahl et al., 2007; Smith and Read, 2008). These mycorrhizal fungi are important for plant acquisition of soil resources, especially soil nitrogen (N) and phosphorous (P) and water (Parniske, 2008) and may inhibit pathogens, increasing overall plant growth and fitness (Smith and Read, 2008). The primary source of carbon (C) for mycorrhizal fungi is provided by the plant host, with arbuscular mycorrhizal fungi completely dependent on plants for C resources (Smith and Read, 2008). Despite the C cost to the plant of forming mycorrhizal relationships, numerous studies have shown the importance of mycorrhizal fungi to plant growth and survivorship, including the survival of young plant life stages (Hetrick et al., 1988; Stinson et al., 2006; Smith and Read, 2008).

Many soil fungi are also saprotrophic and are the primary decomposers of wood, litter and organic matter, obtaining organic C, N and energy from these organic substrates (Rayner and Boddy, 1988; Leake et al., 2002; Lindahl et al., 2007). Saprotrophic fungi are especially important in forests with acidic soils where they are more numerous than bacteria within the decomposer community, particularly in litter and shallow soil layers (Maraun and Scheu, 1996).

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The actions of saprotrophic and mycorrhizal fungi may be largely complementary, since both groups differ in terms of resource utilization, both for C and nutrients such as N and P. The behavior and activity of saprotrophic and mycorrhizal fungi may be spatially separated, with saprotrophic fungi generally confined to fresh litter and the surface of the forest floor where C is mineralized, and mycorrhizal fungi dominating in more decomposed litter and soil where N is mobilized and made available to plants (Lindahl et al., 2007). Although competition for C and nutrients has been shown to occur among fungal species, with different fungal taxa targeting different C resources (Hanson et al., 2008), mycorrhizal fungi are not expected to compete with saprotrophs for C since they are primarily reliant upon the plant host for C. Competition for nutrient resources is more likely to occur among groups of mycorrhizal fungi that co-inhabit forest soils.

Ectomycorrhizal (ECM) fungi that interact with forest trees may have saprotrophic capabilities (Read and Perez-Moreno, 2003; Courty et al., 2007, 2010a; Cullings et al., 2008), but these capabilities may be restricted to recovering nutrients from dying roots and fungal mycelia in soil and not related to the C acquisition strategy of the fungi (Baldrian, 2009). ECM fungi can access organic sources of N and P (Read and Perez-Moreno, 2003), using these resources for their own growth and transferring them to the plant host. Arbuscular mycorrhizal (AM) fungi are also important in forests, forming relationships with some forest trees (e.g. the genus *Acer* and *Fraxinus*) and many herbaceous plants (Smith and Read, 2008). However, AM fungi are not considered to possess saprotrophic capabilities, and it is expected that they rely on inorganic sources of N and P (Read and Perez-Moreno, 2003; Smith and Read, 2008). AM fungi can excrete calcium oxalate which has been shown to be important in liberating P from Ca–P compounds (Walker and Syers, 1976). Calcium oxalate can also form Al–organic complexes possibly immobilizing Al and liberating P that can be bound to Al under acidic conditions (Traina et al., 1986; Koslowsky and Boerner, 1989). However, some studies have found evidence suggesting that AM fungi may be able to access organic sources of P and N (Joner et al., 2000; Koide and Kabir, 2000; Hodge et al., 2001), possibly through facilitation of soil bacterial communities that actively engage in organic matter degradation (Read and Perez-Moreno, 2003). This suggests that organic and inorganic resource partitioning may occur between ECM and AM mycorrhizal fungi in soil, with implications for plant partitioning of resource pools and coexistence based upon mycorrhizal formation in forests. Since soil fungi are important in litter decomposition, nutrient mineralization, and nutrient uptake by plants, fungal community changes could affect C and N cycling in forest ecosystems.

In this study, we investigated the relationship between fungal community structure and soil chemistry and enzyme activity at two points of the growing season in a northern hardwood forest. We predicted that 1) ECM and saprotrophic fungal community structure (i.e. taxa presence and distribution) would be positively correlated with soil enzyme activity, soil organic matter content and dissolved organic C and 2) AM fungal community structure would be positively correlated with inorganic N and P content and would show no relationship to soil enzyme activity. Because the functional activity of soil fungi is compromised by biotic and abiotic environmental change (Egerton-Warburton and Allen, 2000; Lilleskov et al., 2002; Jones et al., 2003), we quantified these relationships in an experimental paired plot study that excluded white-tailed deer (*Odocoileus virginianus*) since 2002 and removed garlic mustard (*Alliaria petiolata*) in half of each plot since 2006. Both abundant deer and allelopathic invasive plant species such as garlic mustard (*A. petiolata*) pose possible threats to North American forest ecosystems and have been shown to decrease the abundance of ectomycorrhizal (Rossow et al., 1997; Wolfe et al., 2008) and arbuscular mycorrhizal fungi in greenhouse studies (Roberts and Anderson, 2001; Stinson et al., 2006; Cipollini and Gruner, 2007), respectively. Together, abundant deer populations and garlic mustard invasion have the potential to dramatically affect communities of soil fungi in forests and we wanted to consider the effects of these threats while exploring the relationships between mycorrhizal communities and soil chemistry. Forest plant and fungal communities perturbed by deer or invaders can require decades to recover (e.g. Rossow et al., 1997; Bassett et al., 2005; Sharrow, 2007). Thus, this experiment permits an examination of the early stages of recovery of soil fungal communities and activity. Fungal communities were described using molecular techniques while enzyme activity was used as a proxy measure of C, N, and P cycling in forests.

2. Materials and methods

2.1. Site description and soil sampling

Our study was conducted at Trillium Trail, a 16 ha mixed deciduous forest located in Allegheny County, Pennsylvania. Trillium Trail has been part of a forest preserve managed by the Borough of Fox Chapel since 1949. The forest overstory is dominated by white and red oak (*Quercus alba* L. and *rubra* L.), sugar maple (*Acer saccharum* Marshall), American beech (*Fagus grandifolia* Ehrh.), and tulip poplar (*Liriodendron tulipifera* L.). The forest also contains a diverse woody and herbaceous understory. Herbaceous plants common in the understory include white trillium (*Trillium grandiflorum*), wild ginger (*Asarum canadense* L.), touch-me-not (*Impatiens capensis* Meerb. and *I. pallida* Nutt.), mayapple (*Podophyllum peltatum* L.), Jack-in-the-pulpit (*Arisaema triphyllum* L.), and false solomon's seal (*Maianthemum racemosum* L.). A complete list of the plant species at Trillium Trail can be found at http://www.pitt.edu/~kalisz/Protocols.html. In the early 1990s, garlic mustard (*A. petiolata* Bieb.) began invading Trillium Trail from road edges and has become common throughout the site, often growing in dense patches within the forest (Knight et al., 2009). The study site is characterized by moderately sloping ground (8–15% slope) and silt loam soils.

Six 14 × 14-m paired plots were established in the autumn of 2002 with one plot from each pair fenced to exclude deer. Fences are 2.5-m tall and constructed from 6-cm wire fencing, effectively eliminating deer but allowing free movement of small mammals, birds and insects (Knight et al., 2009). Each plot was further subdivided into 36 subplots measuring 4-m^2. Beginning in 2006, garlic mustard was removed from half of each plot, resulting in a fully crossed design with four treatments (minus garlic mustard/minus Deer, garlic mustard/minus Deer, minus garlic mustard/Deer, garlic mustard/Deer).

On June 7 and August 28, 2007, we collected two soil cores from the center of 5 subplots located within each treatment. Each paired plot is divided into 36 subplots ($n = 18$ per half plot) and we sampled the 5 subplots located in the middle of each half plot to minimize edge effects. The ten soil cores collected from the subplots were combined for 1 composite soil sample for each plot × treatment to be used for DNA extraction and soil enzyme and chemical analysis ($n = 24$ composite samples, 4 treatments × 6 paired plots). Soil cores (2-cm diameter) were collected to a depth of 5-cm, which corresponds to the A soil horizon. Soil samples were kept on ice until processing. At the time that these samples were collected, deer were excluded for 4.5 years and garlic mustard was removed for 1 year. Because these plots and treatments were established as part of a study examining the long-term effects of deer and garlic mustard on the forest biodiversity, we did not expect to detect a significant signal of the treatments this early in the experiment. Rather, we wanted to establish baseline data across the growing season for future comparison.

2.2. Soil nutrient analyses

Soils were extracted with potassium sulfate (K_2SO_4) and the extracts were analyzed for dissolved organic carbon (DOC) and nitrogen (DON), and ammonium (NH_4^+), nitrate (NO_3^-) and phosphate (PO_4^{3-}). 5-g of soil were combined with 25-ml of 0.5 M K_2SO_4 and shaken on an orbital shaker (~125 rpm) for 1 h. Two soil-free blanks were shaken with each set of analyses. Samples were then vacuum filtered through Pall A/E glass fiber filters, and frozen for subsequent analysis. DOC and total dissolved N (TDN) were determined using a Shimadzu TOC-V total organic C analyzer with a total dissolved N module (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA). DOC is expressed as μg-C per gram dry soil. DON was calculated as TDN−NH_4^+−NO_3^- and is expressed as μg-N per gram dry soil. Microbial biomass C and N were determined using the modification of the fumigation–extraction method (Brookes et al., 1985) described by (Scott-Denton et al., 2006). Microbial biomass C and N are expressed as μg of C or N per gram dry soil. No correction for extraction efficiency (i.e. K_{eC} or K_{eN}) was used.

NH_4^+, NO_3^-, and PO_4^{3-} concentrations in the unfumigated K_2SO_4 extractions were determined using standard colorimetric rapid microplate analyses. Ammonium was determined using a modified Berthelot reaction (Rhine et al., 1998). Nitrate was determined by using a modification of the Griess reaction, which involves the reduction of nitrate to nitrite and subsequent colorimetric analysis of nitrite (Doane and Horwath, 2003). Phosphate was determined using the malachite green-based colorimetric microplate analysis described by D'Angelo et al. (2001).

2.3. Microbial enzyme assays

The enzyme assays were conducted using standard high throughput microplate protocols with soil slurries (Saiya-Cork et al., 2002; Weintraub et al., 2007), which were prepared by homogenizing 1-g (wet weight) of soil with 125-ml of 50 mM sodium acetate buffer using a Biospec Tissue Tearer for 1 min (Saiya-Cork et al., 2002). The mean soil pH (5.25–5.5) of the samples was used for the pH of the sodium acetate buffer for the soil slurries.

Enzyme assays for α-glucosidase (AG), β-glucosidase (BG), N-acetyl-β-glucosaminidase (NAG), β-xylosidase (BX), β-D-cellobiosidase (CBH), leucine aminopeptidase (LAP), and phosphatase (PHOS) were conducted using fluorogenic substrates. These enzymes catalyze the reactions that hydrolyze the terminal linkages of oligomers released by the degradation of larger polymers into monomers (Saiya-Cork et al., 2002; Weintraub et al., 2007). BG and CBH release glucose and cellobiose respectively from cellulose. BX hydrolyzes xylose from xylan, a type of hemicellulose. AG hydrolyzes glucose residues from starch. NAG hydrolyzes N-acetyl glucosamine residues from chitin-derived oligomers. LAP catalyzes the hydrolytic release of leucine and other amino acids from peptides. PHOS catalyzes the release of phosphate by hydrolyzing phosphoric ester bonds of phosphate groups in organic molecules. The fluorometric enzyme assays were conducted in 96-well black fluorescent plates and incubated at 20 °C for 4 h. Following incubation, 10-μl of 1.0 M NaOH was added to each well to increase the fluorescence of the substrates. Fluorescence was determined with a Bio-Tek Synergy HT microplate reader (Bio-Tek Inc., Winooski, VT, USA) with 365 nm excitation and 460 nm emission filters. Enzyme activity was corrected for quench and negative controls, and calculated as nmol reaction product per hour per gram of dry soil (nmol h^{-1} g^{-1}).

Assays for urease, phenol oxidase (PHENOX) and peroxidase (PEROX) activities were conducted using similar high throughput microplate assays, but with chromogenic substrates (Saiya-Cork et al., 2002; Weintraub et al., 2007). Urease catalyzes the release of NH_4^+ from urea. PHENOX and PEROX catalyze oxidative reactions and are involved in lignin decomposition, however PEROX is only functional in the presence of hydrogen peroxide (Saiya-Cork et al., 2002; Weintraub et al., 2007). These assay microplates were incubated as described above. Urease microplates were measured for absorbance at 610 nm, and phenol oxidase and peroxidase were measured for absorbance at 460 nm using a Bio-Tek Synergy HT microplate reader (Bio-Tek Inc., Winooski, VT, USA) and rates are expressed as μmol h^{-1} g^{-1}. Net peroxidase activity was calculated as the phenol oxidase activity subtracted from the peroxidase activity.

2.4. DNA extraction and amplification

DNA was extracted from 500-mg soil fresh weight by bead beating 2× at 6500 RPM for 20 s using a Precellys homogenizer (Bertin Technologies, France) and purified using a Power Soil DNA Isolation Kit (MO BIO Laboratories, CA, USA). To analyze the community of AM fungi in soil we targeted 18S rDNA using universal eukaryotic primer NS31 (Simon et al., 1992) and AM specific primer AM1 following general procedures in Helgason et al. (1998) except that 32 PCR cycles were used. PCR was carried out in 50-μl reaction volumes using 1-μl of purified DNA diluted 1:10 with sterile TE buffer (approximately 100 ng) on a PTC 100 Thermal Cycler (MJ Research, Boston, USA). Primers were labeled with the fluorochromes 6FAM (AM1) and HEX (NS31). Labeled PCR product was digested with restriction enzymes *Alu*I, *Hinf*I and *Hsp*92 (Promega, Madison, WI, USA) and used for terminal restriction fragment length polymorphism (TRFLP) analysis, which was completed through the Cornell Bioresource Center using an Applied BioSystems 3730xl DNA Analyzer and Peak Scanner software version 1 (http://www.appliedbiosystems.com).

TRFLP profiles (*Alu*I, *Hinf*I and *Hsp*92 profiles) were used to determine species identity and proportional abundance within soil samples using a molecular typing protocol including a site-specific database of fungal sequences and fingerprints (Burke, 2008; Burke et al., 2009). In brief, 3 TRFLP profiles were generated for each soil core and used to identify AM species using the program Fragsort (Sciarini and Michel, 2002; http://www.oardc.ohio-state.edu/trflpfragsort/index.php). TRFLP profiles were used as input for Fragsort, which then used our TRFLP database to identify AM species in each core by matching TRFs in the community sample to the species in our database (Burke, 2008; Burke et al., 2009). For a species to be considered present on roots, the appropriate TRF must appear in all three community profiles (Burke, 2008; Burke et al., 2009). The database used to identify AM species was constructed from AM clones recovered from Trillium Trail (Burke, 2008) supplemented by sequences of AM species downloaded from Genbank (Supplemental Table 1).

To analyze the community of ECM and saprotrophic fungi, we targeted the internal transcribed spacer (ITS) 2 region located between the 5.8S and 28S rRNA gene using labeled primers 58A2F (6FAM) and NLB4 (HEX) (Burke et al., 2005, 2006b; Martin and Rygiewicz, 2005). PCR was carried out in 50-μl reaction volumes using 1-μl of purified DNA diluted 1:10 with sterile TE buffer (approximately 100 ng) using conditions previously described (Burke et al., 2005, 2006b). Labeled PCR product was digested with restriction enzyme *Alu*I (Promega, Madison, WI, USA) and used for TRFLP as noted above. Primers 58A2F and NLB4 will detect both ECM and saprotrophic fungi but our analysis is expected to include primarily ECM fungi since the litter layer was removed prior to soil sampling and ECM fungi are expected to dominate soil fungal communities (Lindahl et al., 2007).

Since we do not have a site-specific database of ECM/saprotrophic fungi for Trillium Trail, we were not able to identify fungi to species. Rather, we used terminal restriction fragments (TRFs) as

operational taxonomic units (OTUs) where each TRF is considered a species type and the area under each peak used to calculate proportional abundance of OTUs within a soil core (Burke et al., 2006a, 2006b, 2008).

2.5. Statistical analyses

Three-way ANOVA using the general linear model was used to compare soil chemistry and potential enzyme activity among the samples, with deer exclusion, garlic mustard removal, and sampling time factors in the analysis using SigmaStat 3.5 (Systat Software Inc., CA, USA). Differences in fungal richness, evenness and Shannon diversity between treatments and sampling times were determined through use of Students *t*-Test or Mann–Whitney Rank Sum Test. Richness represents the AM species or ECM/saprotrophic OTUs detected in soil cores. Richness (S), Evenness (equitability = H′/ln(richness)), and Shannon diversity (H′) were calculated for each sample using procedures available through PC-ORD (MjM Software, Gleneden Beach, Oregon). Only TRFLP peaks with >50 fluorescence units (scale of 5000) were included in our analysis (i.e. major TRFs). To further assess the relationship between community structure of soil fungi, soil chemistry and enzyme activity we used non-metric multidimensional scaling (NMS) procedures available through PC-ORD 4 (MjM Software, OR, USA). The Sørenson distance with a random starting configuration was used for these analyses. A maximum of 400 iterations were used for 50 runs with data for the Monte Carlo test randomized. To determine if treatments (deer exclusion, garlic mustard removal, and sampling time) affected fungal community structure, we used multi-response permutation procedures (MRPP) available through PC-ORD 4. MRPP is a non-parametric, multivariate procedure for testing the hypothesis of no difference between groups (McCune and Grace, 2002). The proportional abundance of detected fungal species was used for both MRPP and NMS and all proportional abundance data were transformed before analysis (Burke et al., 2006a, 2008; Burke, 2008).

3. Results

3.1. Analysis of soil chemistry and enzyme activity

We found that soil DOC and DON declined significantly while nitrate and ammonium increased significantly between June and August (Table 1). There were no other significant changes between sampling times for other soil chemical parameters. The potential activity of soil enzymes NAG, CBH, AG, BG, BXYL and PHOS increased significantly between June and August (Table 1). Deer exclusion (4.5 yrs) and garlic mustard removal (1 yr) treatments did not significantly affect either soil chemistry or enzyme activities in our experimental plots.

3.2. ECM/saprotrophic community diversity and structure

ECM/saprotrophic richness ranged from 3 to 21 OTUs per soil sample and diversity ranged from 0.3 to 2.5. OTU richness and diversity were not significantly affected by treatments. However, community evenness was significantly lower (Mann–Whitney Rank Sum Test, U Statistic = 326.5, $P = 0.047$) in deer exclusion plots (fenced plots, 0.73 ± 0.03) than in deer control plots (unfenced, 0.83 ± 0.03). NMS analysis revealed clear separation in ordination space between fungal communities from early June soil samples and those from late August samples (Fig. 1). ECM/saprotrophic communities in early June samples were significantly and positively correlated with soil DON, DOC, and organic matter content, whereas communities in late August samples were significantly and positively correlated with soil ammonium and nitrate concentrations (Table 2, Fig. 1). In addition, potential activities of NAG, CBH, AG, BG, BXYL, and PHOS were strongly correlated with ECM/saprotrophic communities, with positive correlations between these enzymes and soil samples collected in late August. LAP and urease activities, which were significantly correlated with AM fungal communities (Table 2, Fig. 2), were not significantly correlated with ECM/saprotrophic communities, suggesting that potential soil enzyme activities are affected by the presence of certain fungal taxa in forest soils. MRPP analysis indicated that deer exclusion and garlic mustard removal did not significantly affect ECM/saprotrophic community structure. However, MRPP analysis did indicate that these fungal communities were significantly different between sampling times ($A = 0.348$, $P < 0.001$).

Table 1
Results of soil chemical and enzyme analysis for Trillium Trail deer exclusion and garlic mustard removal plots in June and August of 2007. Three-way ANOVA using the general linear model was used to compare soil chemistry and potential enzyme activity among the samples, with deer exclusion, garlic mustard removal, and sampling time factors in the analysis. We found that deer exclusion and garlic mustard removal did not significantly affect soil chemistry and enzyme activity, but sampling time had some significant effects. Means ± standard error of the mean are shown for June and August contrasts. Significant differences between seasons are shown in bold. ND is used to indicate when a parameter was not detectable.

Soil parameter	June	August	*P*
% Moisture	0.28 (0.01)	0.30 (0.01)	0.15
DOC (μg C/g dry soil)	190.7 (25.1)	114.4 (14.9)	**0.01**
DON (μg N/g dry soil)	25.2 (1.9)	10.8 (1.9)	**<0.001**
MB C (μg C/g dry soil)	251.1 (21.7)	238.7 (21.0)	0.68
MB N (μg N/g dry soil)	46.2 (4.1)	41.0 (4.0)	0.36
Nitrate (μg N/g dry soil)	0.14 (0.41)	5.27 (0.39)	**<0.001**
Phosphate (μg P/g dry soil)	0.31 (0.12)	0.48 (0.12)	0.33
Ammonium (μg N/ g dry soil)	0.28 (0.45)	3.78 (0.26)	**<0.001**
% Organic matter	13.3 (0.93)	11.9 (0.90)	0.29
NAG (nmol/h/g)	ND	112.8 (12.2)	**<0.001**
CBH (nmol/h/g)	ND	80.3 (11.8)	**<0.01**
AG (nmol/h/g)	ND	10.3 (2.0)	**0.02**
BG (nmol/h/g)	31.7 (37.3)	225.3 (22.1)	**<0.001**
LAP (nmol/h/g)	4.3 (1.9)	3.9 (1.8)	0.87
BXYL (nmol/h/g)	4.1 (4.9)	42.5 (4.7)	**<0.001**
PHOS (nmol/h/g)	73.3 (42.2)	375.3 (25.0)	**<0.001**
PHENOX (nmol/h/g)	8053 (3229)	3209 (1910)	0.21
NETPEROX (nmol/h/g)	16.6 (2.6)	15.5 (2.5)	0.76
UREASE (nmol/h/g)	1492 (333)	1048 (322)	0.34

3.3. AM community diversity and structure

AM richness ranged from 1 to 7 species per sample, and diversity (H′) ranged from 0 to 1.6. We did not detect significant differences among treatments for species richness, evenness, or diversity. We detected 18 different AM species in soil from the study sites, representing 2 AM genera: *Acaulospora* and *Glomus*. We detected 2 species of *Acaulospora* and 16 species of *Glomus* at the field site. *Glomus* sp 1a and 3a were the mostly frequently detected AM species encountered, occurring in 84 and 98% of soil samples respectively. *Glomus* sp 2d and 4b were the least frequently detected, being found in only 2% of samples (Supplemental Tables 2 and 3). The AM community in soil was significantly correlated with soil DOC, DON, phosphate and ammonium concentrations. LAP and urease activities were also significantly correlated with AM community structure (Table 2, Fig. 2). Specifically, *Glomus* sp 1b and 2b were positively correlated with LAP activity, while *Glomus* sp 3a was positively correlated with urease activity. However, AM communities were not significantly correlated with phosphatase activity (Table 2, Fig. 2). MRPP analysis indicated that treatments (4.5 yrs of deer exclusion and 1 yr of garlic mustard removal) and sampling time did not significantly affect the AM fungal community

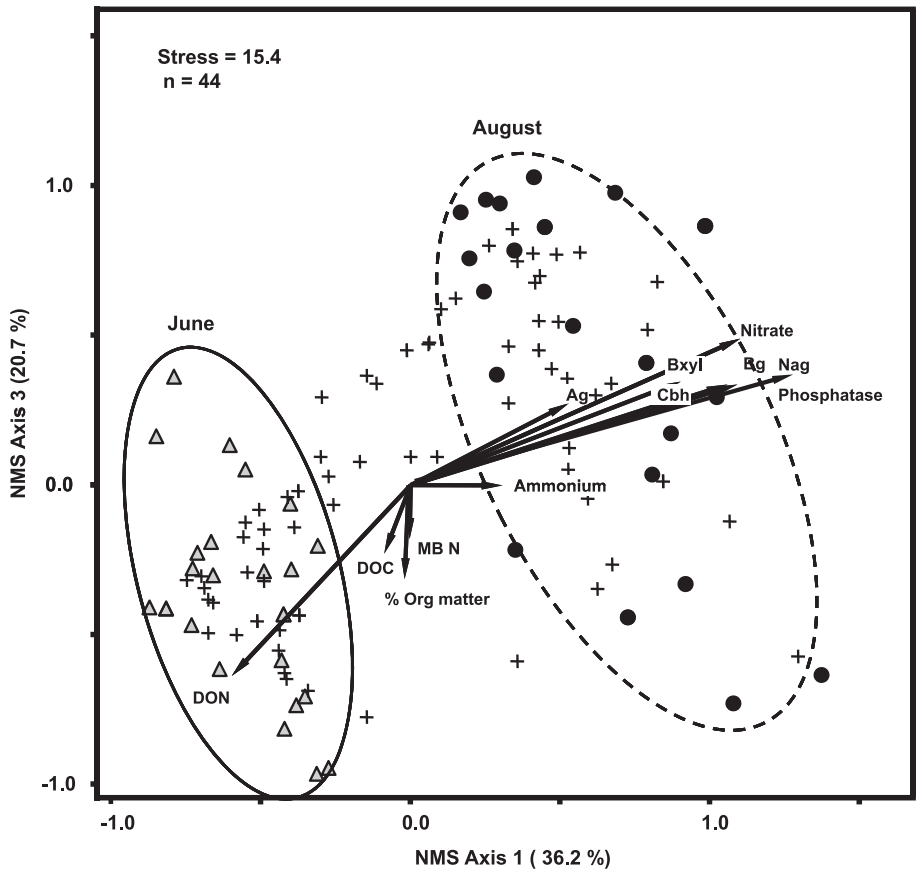

Fig. 1. Non-metric multidimensional scaling ordination based on proportional abundance of identified ECM/saprotrophic terminal restriction fragments (TRFs) in soil cores with joint plots for the most important environmental variables. The joint plot vector lengths indicate the strength and direction of the strongest correlations. The proportion of variance explained by axes 1 and 3 is shown. Cumulative variance was $r^2 = 0.693$. See Table 2 for correlations with all environmental variables. Shaded triangles designate samples collected in early June while closed circles represent samples collected in late August. Specific TRFs are represented by the + symbols.

Table 2
Relationships between soil environmental and vegetation variables and NMS dimensions using Pearson correlation. Significance of correlations was determined using the critical values for correlation coefficients (Zar, 1998). For AMF samples ($n = 45$), $P < 0.05$ for $r > 0.285$ for two tailed test and for ECM/Saprobe samples ($n = 44$), $P < 0.05$ for $r > 0.291$ for two tailed test. Significant correlations are in bold type.

	AMF			ECM/Saprobe		
	Dim1	Dim2	Dim3	Dim1	Dim2	Dim3
% Moisture	−0.265	0.153	0.038	0.194	0.060	−0.229
DOC	−0.128	**−0.458**	−0.008	−0.215	0.066	**−0.352**
DON	−0.051	**−0.325**	−0.024	**−0.564**	0.027	**−0.583**
MB C	−0.250	0.135	−0.022	0.063	0.023	−0.237
MB N	−0.247	0.098	−0.194	−0.050	0.043	**−0.312**
Nitrate	−0.064	0.062	0.004	**0.767**	0.108	**0.511**
Phosphate	−0.137	**−0.315**	0.063	0.198	0.091	0.172
Ammonium	0.041	**0.312**	0.090	**0.406**	0.019	−0.002
% Organic matter	−0.215	−0.048	0.097	−0.084	0.042	**−0.408**
GM adult density	0.069	**0.283**	−0.248	−0.048	−0.038	0.232
NAG	0.101	0.143	−0.071	**0.753**	−0.016	**0.425**
CBH	0.052	0.071	−0.076	**0.688**	0.035	**0.394**
AG	−0.245	0.063	0.192	**0.530**	**0.315**	**0.382**
BG	0.114	0.111	−0.018	**0.764**	0.033	**0.416**
LAP	−0.137	**0.490**	−0.132	0.105	0.133	−0.049
BXYL	−0.136	−0.027	0.141	**0.694**	0.156	**0.432**
PHOS	0.041	0.082	0.027	**0.822**	0.047	**0.438**
PHENOX	0.109	0.145	−0.205	**−0.311**	−0.102	−0.280
NETPEROX	−0.126	0.039	0.215	−0.015	0.001	−0.095
UREASE	0.106	**−0.298**	**−0.299**	−0.030	−0.263	−0.270

in soil. The total number of garlic mustard adults within the 5 sampled subplots per plot ranged from 0 to 127 individuals and it is possible that this large variance in garlic mustard individuals was the reason for the lack of difference in AM abundance between weeded and unweeded plots in MRPP. Interestingly, the NMS analysis indicated that the specific community of AM fungi in soil was significantly correlated with the density of adult garlic mustard plants in each plot (Table 2, Fig. 2).

4. Discussion

4.1. Relationships between ECM/saprotrophic fungal communities and soil chemistry

Although we found evidence to support our hypothesis that communities of ECM and saprotrophic fungi in soil would be associated with enzyme activity, these relationships changed significantly over time. Seasonal changes in ECM and saprotrophic communities are well documented with significant changes possible within even a few months (Avis et al., 2008; Courty et al., 2008, 2010b; Burke et al., 2009). ECM and saprotrophic fungi often dominate the microbial biomass of forest soil and litter, and many ECM fungi and all saprotrophic fungi produce extracellular enzymes that are capable of mineralizing C, N and P from soil organic matter and litter (Leake et al., 2002; Read and Perez-Moreno, 2003; Smith and Read, 2008). Since potential enzyme activity is a useful proxy measure of nutrient cycling in forests, our results indicate that nutrient cycling changed in our study forest between June and late

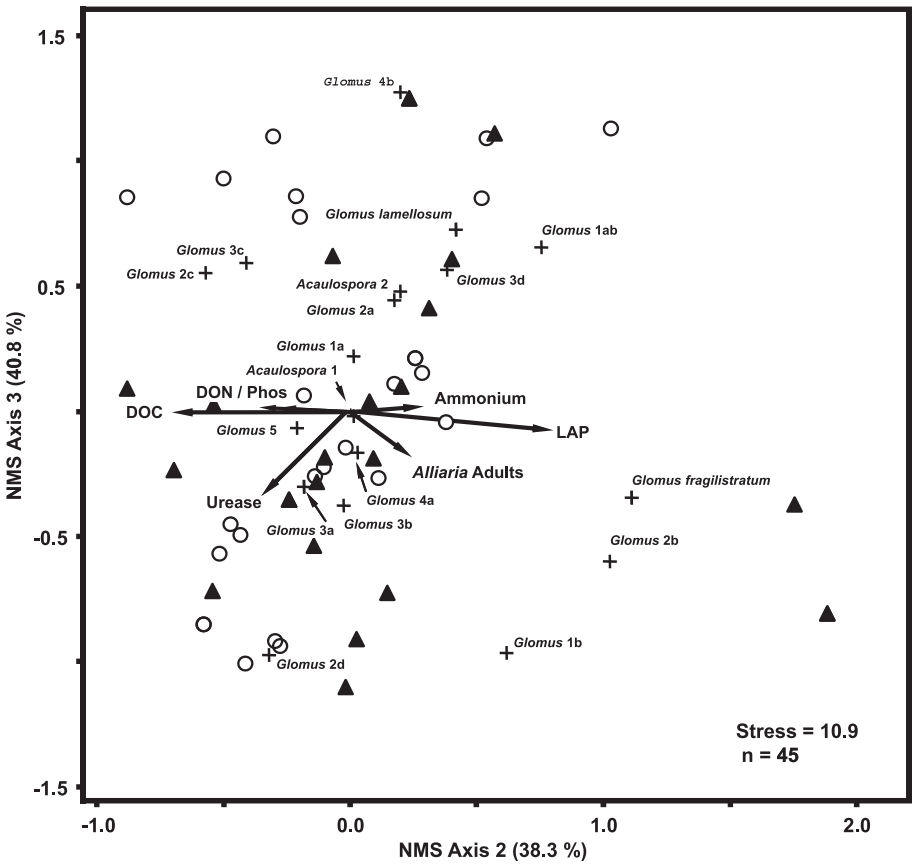

Fig. 2. Non-metric multidimensional scaling ordination based on proportional abundance of identified AM species in soil cores with joint plots for the most important environmental variables. The joint plot vector lengths indicate the strength and direction of the strongest correlations. The proportion of variance explained by axes 2 and 3 is shown. Cumulative variance was $r^2 = 0.919$. See Table 2 for correlations with all environmental variables. Open circles designate samples collected in early June while closed triangles represent samples collected in late August. Specific AM species detected in our samples are represented by the + symbols.

August sampling, and these changes are associated with seasonal changes in ECM/saprotrophic communities.

Seasonal changes in ECM/saprotrophic communities and their function may have been driven by the senescence and decomposition of herbaceous plant litter at our study site. Our field site is marked by a diverse understory of native herbaceous spring and summer flowering species that senesce during late summer. Litter fall from herbaceous plants can comprise as much as 16% of forest litter fall on an annual basis (Gilliam, 2007), and significant positive correlations between herbaceous plants and ECM fungi have been noted in a previous study (Burke et al., 2009). The decomposition of herbaceous litter on the soil surface in late summer could have resulted in the liberation and transport of organic and inorganic compounds from litter into soil and influenced the ECM/saprotrophic community in soil, stimulated its activity and contributed to the seasonal increase in nitrate and ammonium. Significant correlations were found only for enzymes capable of degrading more labile carbon such as starch (AG) and short chain cellulose oligomers (BG), which suggests that the soil fungal communities are not capable of degrading more recalcitrant material, such as lignin. ECM fungi can dominate soil fungal communities (Lindahl et al., 2007), and may not be capable of producing enzymes such as PHENOX or PEROX used to degrade recalcitrant material such as lignin and cellulose (Leake et al., 2002; Smith and Read, 2008; Baldrian, 2009). Yet some ECM fungi can utilize organic C, and may produce enzymes for C acquisition during periods of reduced plant photosynthesis (Cullings and Courty, 2009). Nonetheless, it may be more likely that obligate saprotrophs in soil are responsible for the production of C acquisition enzymes (Leake et al., 2002). It is also possible that our methods were not sensitive enough to detect low activities of these enzymes in our samples.

We observed significant increases in soil nitrate and ammonium concentrations between June and late August, and changes in fungal communities with soil N availability have been observed in fertilization studies (Edwards et al., 2004). ECM fungi are capable of utilizing most organic forms of N and P, and have been shown to use amino acids, peptides, proteins and chitin as N sources (Leake et al., 2002; Read and Perez-Moreno, 2003). We observed significant positive correlations between the ECM/saprotrophic community in late summer and enzymes involved in utilization and degradation of organic N and P; for example NAG, which is involved in chitin degradation, and PHOS, which catalyzes the release of phosphate from organic compounds. These enzyme activities were highest in late summer, and did not appear associated with June soil fungal communities. The relative difference in activities of enzymes involved in C acquisition (e.g. AG, BG, BX) and N acquisition (e.g. LAP, NAG, Urease) can be used to determine whether the microbial community is C or N limited (Sinsabaugh et al., 2009). ECM/saprotrophic communities were correlated with enzymes for C, N and P acquisition, but given the different habit and ecology of these fungi, it is likely that they are utilizing different soil resources. Additional studies that clearly distinguish between ECM and saprotrophic fungi are needed to better assess both resource use and exploitation within these fungal communities.

Although previous studies have observed reductions in ECM colonization of tree roots (Wolfe et al., 2008) and changes in fungal

community structure in soil subjected to garlic mustard extracts (Callaway et al., 2008), we did not observe any effect of garlic mustard abundance or weeding on ECM/saprotrophic fungi in our study. Garlic mustard abundance varied greatly among the subplots sampled and may not have been high enough to consistently affect soil fungi in our study. In addition, garlic mustard treatments had been in effect for only one season prior to our sampling and this may not have been a sufficient period of time for differences among the treatments to manifest.

Deer exclusion also did not significantly influence ECM/saprotrophic fungal community structure, richness or diversity, or enzyme activity. However, we did observe significant increases in fungal evenness in control plots to which deer had access. Trampling and deer browsing significantly affect soil quality and plant community productivity and composition (Côté et al., 2004), and these changes may have resulted in decreased dominance of some fungal species and the increases in evenness we observed. Our sampling focused on the top 5-cm of soil, and litter was excluded from our analysis. Deer could affect herbaceous plant cover, the amount of herbaceous litter, and litter temperature, moisture and dryness through increases in light penetration to the soil. Additional work should be conducted to examine effects of deer on litter decomposition and fungal communities associated with litter.

4.2. Relation between AM fungal communities and soil chemistry

We found evidence to support our hypothesis that AM fungi are positively correlated with soil inorganic N and P content but we also found correlations between AM fungal community structure and some soil enzymes. We found significant correlations between AM communities and both LAP and urease activity. Both of these enzymes are involved in N cycling in soils. LAP catalyzes the release of amino acids from peptides and is produced by bacteria, cyanobacteria, and algae, while urease catalyzes the release of NH_4^+ from urea and is produced by bacteria, yeast and plants. Consequently both enzymes are important for N cycling in soils.

AM fungi are not generally considered capable of saprotrophic ability and it is expected that they rely on inorganic sources of N and P (Read and Perez-Moreno, 2003; Smith and Read, 2008); however, there is some limited evidence to suggest that AM fungi can acquire N from organic sources (Hodge et al., 2001; Read and Perez-Moreno, 2003; Hodge and Fitter, 2010). It is not unusual for AM hyphae to proliferate in patches high in soil organic matter (St. John et al., 1983) and this proliferation may be a consequence of high nutrient availability and enzyme activity within organic microsites. This enzyme activity could be the result of soil bacteria that are key drivers of carbon and nutrient cycling in many systems and could be especially important in areas of high soil organic matter. Although it is probable that AM fungi simply proliferate in soil microsites with high levels of LAP and urease activity, it is possible that AM fungi could produce these enzymes and additional work is needed to explore this possibility. Not surprisingly, we found no relationship between AM fungi and soil enzymes indicative of C acquisition and cycling. AM fungi are obligate plant mutualists, receiving C from the plant host, and they are not expected to produce enzymes involved in C degradation and acquisition. AM fungi are often considered of greater importance in the uptake of soil P (Read and Perez-Moreno, 2003; Smith and Read, 2008), and AM fungi were correlated with soil phosphate. However, we did not find a significant correlation between AM fungi and PHOS activity.

It is well-established that AM communities can affect plant community structure and diversity (van der Heijden et al., 1998; Klironomos et al., 2000; Vogelsang et al., 2006), and these effects may be a consequence of the different functional traits of AM species (Helgason et al., 2002). Helgason et al. (2002) found that AM species varied in their ability to acquire P and soil resources and these differences can affect plant performance. At our study site, some AM species were correlated with soil P content while others were correlated with soil NH_4^+ content suggesting not only that different AM species may be acquiring different soil resources, but that they could affect plant nutrient gain with significant consequences for plant growth and survival.

Although we expected that deer presence would affect AM communities in the long-term, we did not find any significant differences between deer exclusion and deer access plots at the time this study was conducted. Our study site is dominated by AM species in the genus *Glomus* (Burke, 2008), many of which also form relationships with forest trees such as maple and ash (Smith and Read, 2008) present in our study site. Formation of symbioses between adult trees and AM could have buffered these communities from changes associated with the loss of much of the herbaceous plant community through deer browsing (Knight et al., 2009).

Although we found no significant differences in enzyme activities or AM fungal communities between weeded vs. non-weeded plots, NMS analysis indicated that AM community structure was significantly correlated with the number of garlic mustard individuals in the sampling subplots. Our results are in agreement with Roberts and Anderson (2001) who found that the mycorrhizal inoculating potential (MIP) of soil declined as the density of garlic mustard individuals increased. The lack of differences observed between weeded and unweeded subplots could result from the large range of individuals encountered in some of the unweeded areas and that we sampled only one year after the weeding treatment was initiated.

5. Conclusion

We found that AM and ectomycorrhizal/saprotrophic fungal communities in soil were correlated with different enzyme activities, suggesting that these fungal groups play different roles in soil C and nutrient cycling. Of particular interest is the relationship between AM fungi and LAP and urease activities, two enzymes involved in N cycling. AM fungi are not generally considered capable of producing extracellular enzymes and are often thought to be more important to plant P than N acquisition. However, our data suggest that some AM communities may be correlated with the potential activities of N acquisition enzymes in soil and may affect N cycling in the understory. The relationships between AM taxa and soil enzymes will require additional study. Together, our data also suggest fine scale temporal and spatial dynamics of the soil fungal community in our forest understory paired plots and reveal subtle differences in fungal community composition. Ongoing long-term sampling studies will assess the joint responses of the plant understory, the litter layer and the soil fungal community as they recover from the effects of overabundant white-tailed deer and the possible allelopathic effects of *A. petiolata* on soil enzyme activity and nutrient cycling.

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Appendix. Supplementary material

Supplementary material related to this article can be found at doi:10.1016/j.soilbio.2010.12.014.

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