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Root carbon inputs to the rhizosphere stimulate extracellular enzyme activity and increase nitrogen availability in temperate forest soils

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Abstract The exudation of carbon (C) by tree roots stimulates microbial activity and the production of extracellular enzymes in the rhizosphere. Here, we investigated whether the strength of rhizosphere processes differed between temperate forest trees that vary in soil organic matter (SOM) chemistry and associate with either ectomycorrhizal (ECM) or arbuscular mycorrhizal (AM) fungi. We measured rates of root exudation, microbial and extracellular enzyme activity, and nitrogen (N) availability in samples of rhizosphere and bulk soil influenced by four temperate forest tree species (i.e., to estimate a rhizosphere effect). Although not significantly different between species, root exudation ranged from 0.36 to 1.10 g C m^{-2} day^{-1}, representing a small but important transfer of C to rhizosphere microbes. The magnitude of the rhizosphere effects could not be easily characterized by mycorrhizal associations or SOM chemistry. Ash had the lowest rhizosphere effects and beech had the highest rhizosphere effects, representing one AM and one ECM species, respectively. Hemlock and sugar maple had equivalent rhizosphere effects on enzyme activity. However, the form of N produced in the rhizosphere varied with mycorrhizal association. Enhanced enzyme activity primarily increased amino acid availability in ECM rhizospheres and increased inorganic N availability in AM rhizospheres. These results show that the exudation of C by roots can enhance extracellular enzyme activity and soil-N cycling. This work suggests that global changes that alter belowground C allocation have the potential to impact the form and amount of N to support primary production in ECM and AM stands.

Keywords Rhizosphere · Root exudation · Extracellular enzymes · Organic N cycling · Amino acids · Temperate forests · Mycorrhizal fungi

Introduction

Soil microbes control the processes of decomposition and the mobilization of nitrogen (N) through the release of extracellular enzymes that attack soil organic matter (SOM) (Burns 1982). Numerous studies have shown that the addition of labile carbon (C) to soils stimulates SOM decomposition (i.e., the priming effect; e.g., Kuzyakov et al. 2000; De Nobili et al. 2001; Fontaine et al. 2004). Labile C inputs to soils by roots are thought to increase microbial exo-enzyme

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production, which increases SOM decomposition (Asmar et al. 1994), and often but not always increases the supply of N to plants (e.g., Robinson et al. 1989; Herman et al. 2006; Dijkstra et al. 2009; Phillips et al. 2011; Drake et al. 2011). The feedback to microbial enzyme production and nutrient supply is often inferred, however, and not measured. Thus, it remains unclear exactly how the belowground flux of C affects the activity of microorganisms, exo-enzyme production and the depolymerization of N (Frank and Groffman 2009).

Rates of C exudation by tree roots and their impact on the depolymerization of N in temperate forest soils is likely to be influenced by SOM chemistry and the type of mycorrhizal association. Ectomycorrhizal (ECM) fungi, for example, synthesize many different hydrolytic enzymes (e.g., protease, chitinase, glucosidase) in addition to oxidative enzymes that attack recalcitrant forms of SOM (e.g., lignin, polyphenolic compounds) (Chalot and Brun 1998; Talbot et al. 2008), which also tend to occur in greater abundance in ECM soils (Finzi et al. 1998a). By contrast, arbuscular mycorrhizal (AM) fungi are often found in soils with labile SOM chemistry (i.e., low C to N ratios) and produce a narrow range of hydrolytic enzymes and no oxidative enzymes (Veresoglou et al. 2012).

It also appears that ECM tree roots release more exudates than AM tree roots (Smith 1976; Phillips and Fahey 2005), and in a recent study, rates of C and N mineralization were higher in rhizosphere compared to bulk soils of ECM stands, whereas there was little difference in microbial activity between rhizosphere and bulk soils in AM dominated stands (Phillips and Fahey 2006). Recalcitrant forms of SOM often bind N (Fog 1988) and their catabolism requires the activity of both oxidative and hydrolytic enzymes (Sinsabaugh 2010). Hence ECM trees may invest more C belowground than AM trees in an effort to promote microbial enzyme synthesis and the depolymerization of N from recalcitrant SOM (Allison and Vitousek 2005; Chapman et al. 2006; Sinsabaugh 2010). Consistent with this idea, Brzostek and Finzi (2011) used in-growth cores to show that the presence of ECM but not AM roots had a stimulatory effect on proteolytic, chitinolytic, and ligninolytic enzyme activity.

The objective of this research was to compare rates of root exudation as well as exo-enzyme activity and nutrient availability in rhizosphere relative to the bulk soil (herein termed the "rhizosphere effect") in plots dominated by species that vary in mycorrhizal association and SOM chemistry. This builds on our previous work where we examined only the impacts of roots on enzyme activity by determining the extent to which rates of microbial and enzyme activity in the rhizosphere differ from those in the bulk soil. We studied five enzymes that are involved in the release of N from labile (protease, chitinase) or recalcitrant components of SOM (phenol oxidase, peroxidase) and the release of phosphorus (acid phosphatase) from SOM. We tested two hypotheses: (1) ECM roots have higher rates of root exudation than AM roots and (2) the rhizosphere effect on enzyme activity and nutrient availability is greater in high C to N ratio soils influenced by ECM trees than in low C to N ratio soils influenced by AM trees.

Methods

Site description

This research was conducted at two sites, the Harvard Forest in Petersham, MA (herein HF, 42.5°N, 72.18°W), and the Pisgah State Forest in Chesterfield, NH (herein PSF, 42.87°N, 72.45°W). The sites have similar land use history and stand age (Foster 1988, 1992). Soils at both sites are inceptisols classified as Typic Dystrochrepts derived from glacial till overlying granite-schist-gneiss bedrock (USDA National Resource Conservation Service; http://websoilsurvey.nrcs.usda.gov/).

Study plots dominated by one of four target tree species were identified at each site. Plots of sugar maple (*Acer saccharum*) and American beech (*Fagus grandifolia*) were located in the PSF. Plots of eastern hemlock (*Tsuga canadensis*) and white ash (*Fraxinus americana*) were located in the HF. These four species differed in mycorrhizal association, with ash and sugar maple supporting AM fungi and hemlock and beech supporting ECM fungi. Further, hemlock and beech have recalcitrant leaf litter and SOM that is characterized by higher ratios of C-to-N and lower pH than sugar maple and ash (Brzostek and Finzi 2011). At each site we located six replicate, 8-m radius, monodominant plots within larger mixed hardwood/conifer stands where the target tree species constituted 100 %

of standing basal area in the inner 5-m core and >80 % in the entire 8-m radius plot (Lovett et al. 2004). After plot establishment, we also measured the fine root biomass in the top 15 cm of mineral soil in August of 2008. Fine roots were quantified in three replicate 5 cm diameter cores from each plot.

Root exudation and scaling

In June, and August of 2010, we collected root exudates from one root system in each plot for a total of 24 measurements for each sampling date. We used a method modified from Phillips et al. (2008) that allows for the collection of root exudates from live tree roots that remained attached to the parent tree. Terminal fine roots were carefully excavated from the upper mineral soil horizon by hand and extensively washed to remove adhering soil particles. After equilibration overnight in a sand-soil mix, the root system was placed in a 30 ml glass cuvette and the remaining volume was filled with sterile glass beads as a mechanical substrate. Then, 15 ml of a C and N free nutrient solution was added to buffer the root system. The roots incubated for 24 h and then exudates were collected by flushing the cuvette three times with 15 ml of the nutrient solution. The flushes were stored at −20 °C until further analysis. The total non-particulate organic C accumulated in the flushes from each cuvette was analyzed on a TOC analyzer (Shimadzu Scientific Instruments). We scaled the rate of C exudation by the fine root surface area (FRSA; cm^{-2}) and the mass (g) of each root system. FRSA for each excavated root system was measured using WinRhizo (Regents Instruments Inc.). We also scaled our mass-specific rates of root exudation (g C g $root^{-1}$ day^{-1}) to the plot scale (g C m^{-2} day^{-1}) by taking the product of species-specific fine root biomass (g root m^{-2}) and mass-specific rates of exudation (g C g $root^{-1}$ day^{-1}).

Soil sampling protocol

Soil samples were collected in May, June, and August of 2010 from each of the six replicate plots for each species ($n = 24$). We chose the May, June and August time-points because they span the majority of the growing season and are coincident with major phenological events, including leaf out, peak leaf area index, and the start of the seasonal decline in C uptake at the HF, respectively (Urbanski et al. 2007). At each sampling point, we collected the top 15 cm of mineral soil using a 5 cm diameter soil bulk-density sampler from each plot.

The samples were processed within 24 h of collection in the laboratory. We separated the mineral soil horizons into rhizosphere and bulk soil fractions. Fine roots were removed from each sample. Soil adhering to fine roots was operationally classified as rhizosphere soil (sensu Phillips and Fahey 2006). The remaining soil was defined as the bulk soil fraction. After separation, the bulk soil fraction was then sieved through a 2-mm mesh.

Amino acid concentrations

The 2 M KCl extractable pool size of amino acids was determined for every soil sample across the three sampling dates. Eight grams of each mineral soil fraction were extracted in 30 ml of 2 M KCl. The concentration of amino acids for all extracts was quantified using the *o*-phthaldialdehyde and β-mercaptoethanol (OPAME) method (Jones et al. 2002). Concentrations of amino acid N were determined by comparing the fluorescence of the samples relative to a standard curve composed of glycine.

Extracellular enzyme activity

We assayed proteolysis following a method modified from Watanabe and Hayano (1995) and Lipson et al. (1999) for every soil fraction and sample date. Initial and incubated subsamples (2–3 g) received 10 ml of a 0.5 mM sodium acetate buffer (pH 5.0) with a small volume of toluene (400 µl) added to inhibit microbial uptake. After the reagent addition, the initial samples were immediately terminated and the incubated subsamples were incubated at 23 °C for 4 h. We chose 4 h as an incubation time because previous work has shown proteolytic enzyme activity to be linear over this period (Berthrong and Finzi 2006; Rothstein 2009). Enzyme activity in all the initial and incubated subsamples was terminated through the addition of 3 ml of a trichloroacetic acid solution. Proteolytic rates for each soil were calculated from the difference between amino acid concentrations in the incubated and initial subsamples of each soil assayed using the OPAME method described above (Jones et al. 2002).

For every soil fraction at each sample date, we also assayed the potential activities of the chitinolytic

enzyme, *n*-acetyl-glucosaminidase (NAG), acid phosphatase and the ligninolytic enzymes, phenol oxidase and peroxidase. All the assays were run using a 1 g subsample of each soil homogenized in a pH 5.0 sodium acetate buffer at 23 °C. NAG and acid phosphatase activities were determined using a fluorometric microplate assay, while phenol oxidase and peroxidase activities were determined using a colorimetric microplate assay (Saiya-Cork et al. 2002).

Carbon and nitrogen mineralization

For the mineral soil rhizosphere and bulk fractions for the August sample date only, we measured the rate of C mineralization (Talbot and Finzi 2008). Five grams of each sample were placed in 480-ml Mason jars fitted with rubber septa, aerated, and sealed. An initial CO_2 concentration was measured using an infrared gas analyzer (EGM-4; PP Systems) immediately after each jar was sealed by sampling the headspace of each Mason jar with a 10-ml syringe. The jar remained sealed and then the concentration of CO_2 was then measured again after incubating at 23 °C for 1 and 2 h. Carbon mineralization was calculated as the rate of increase (i.e., the slope of the relationship between CO_2 concentration and time) in the concentration of CO_2 over the incubation period. For all samples the increase in CO_2 concentration was linear over the incubation period (average $R^2 = 0.99$).

For the mineral soil rhizosphere and bulk fractions for the June and August sample date only, we measured the rate of net N mineralization and nitrification. Rates of net N mineralization and net nitrification were measured by quantifying the change in the 2 M KCl extractable pool sizes of NH_4^+ and NO_3^- after a 28 day soil incubation in the lab (Finzi et al. 1998b).

Statistics

We used repeated measures ANOVA to test for significant differences in exudation rates as well as root biomass and surface area for each tree species and every sample date. For simplicity, we presented the average of the root surface area data across sampling dates when there were no significant date effects or interactions. We used two-way repeated measures ANOVA to test for significant differences in amino acid pools, enzyme activity, and C and N mineralization between the bulk soil and rhizosphere soil for each tree species and every sample date. When the interaction between species and soil fraction was significant, we analyzed the simple main effects for comparisons between adjusted least square means of the bulk and rhizosphere soil for a given species and sample date at the $p < 0.05$ level. We used linear regression to test the significance of the relationship between enzyme activity in the rhizosphere and bulk soil with the rate of root exudation and C mineralization. To account for correlation between enzyme activities, we also calculated principle components (PC) scores across all five enzymes and examined whether the PC's were significantly related to C mineralization or exudation. On those PC's that had significant relationships, we examine the loadings on the PC to determine which enzyme was driving the relationships. Data were analyzed using the MIXED, PRINCOMP, and GLM procedures in SAS (version 9; SAS Institute, Cary, N·C.). Residuals were assessed for normality and homogeneity of variance and log-transformations were used to meet model assumptions when needed.

We used meta-analysis techniques to synthesize the rhizosphere effect on the activity of the five enzymes we assayed for each tree species. Meta-analysis is commonly used to analyze data from multiple independent studies (e.g., Rustad et al. 2001; Knorr et al. 2005; Treseder 2008), but it can also be used to analyze and synthesize treatment effects on soil enzyme activity within one experiment (e.g., Saiya-Cork et al. 2002). We performed a weighted meta-analysis using Meta-Win 2.1 (Rosenberg et al. 2000). One assumption of meta-analysis is that all observations are independent. To ensure independence when analyzing data from multiple sample dates, previous analyses have used the average of the response variable across all the sample dates to calculate effect sizes (Rustad et al. 2001; Saiya-Cork et al. 2002). Thus, for each enzyme, we calculated an average across all three sampling dates for the rhizosphere and bulk soil of each species. We then calculated the rhizosphere effect size on activity by calculating the natural log of the response ratio ($ln(RR)$) and the variance about this metric. Response ratios were calculated as the mean value for each enzyme activity assayed in the rhizosphere soil divided by the mean value of activity in the bulk soil for a given species. To test hypothesis (2), we then calculated the grand mean

and the bias corrected bootstrapped 95 % CI's for the rhizosphere effect on total enzyme activity for each tree species. Grand means of *ln*(*RR*) above zero indicated an increase in activity and below zero indicated a decrease in activity in the rhizosphere compared to the bulk soil. Finally, we used categorical random effects models to test for differences in the rhizosphere effect on enzyme activity between tree species.

Results

Root exudation

The tree roots of all four species exuded a measurable quantity of C ranging from 2.6 to 6.9 μg C cm $FRSA^{-2}$ day^{-1} at the root system scale and from 0.36 to 1.10 g C m^{-2} day^{-1} at the plot scale (Table 1). At the root system scale, there were no significant differences in measured rates of exudation between species or sampling date (Table 1). Further, when scaled to the plot level, exudation rates did not differ despite significant differences in fine root biomass and surface area between species (Table 1).

Amino acid concentrations

In hemlock and beech soil, the concentration of amino acids was significantly greater in rhizosphere compared to bulk soil in May and August (Fig. 1b; Table S1). In June, rhizosphere amino acid concentrations were significantly higher in hemlock. The only other significant increase in rhizosphere amino acid concentrations was observed in maple soil in August. Across all sampling dates, the concentration of amino acids was 54 % higher in rhizosphere relative to bulk soils for both hemlock and beech, 23 % higher in sugar maple rhizospheres and there was no difference observed for ash.

Extracellular enzyme activity

Extracellular enzyme activities were strongly stimulated in the rhizospheres of beech, while rhizosphere effects were fewer in number for hemlock and sugar maple and almost absent in ash. For beech and hemlock, proteolytic enzyme activity was significantly greater in the rhizosphere than the bulk soil for every sample date except for the hemlock soils in June (Fig. 1a; Table S1). The only other significant increase in proteolytic enzyme activity was observed in maple soil in May (Fig. 1a; Table S1).

N-Acetyl-glucosaminidase activity was significantly higher in rhizosphere soil of beech on every sample date (Fig. 1c; Table S1). The only other significant rhizosphere effect on NAG activity was observed in maple soils in August (Fig. 1c; Table S1).

Acid phosphatase activity was significantly greater in the rhizosphere than the bulk soil of beech in June and August (Fig. 1d; Table S1). There were no significant rhizosphere effects observed for the other species.

Phenol oxidase activity was significantly greater in the rhizospheres of hemlock in May and June, and in beech in May and August (Fig. 1e; Table S1). In August, both hemlock and beech soils had a

Table 1 Fine root characteristics and exudation rates for each tree species

Species	Fine root biomass (g m^{-2})	Mass specific FRSA (cm^2 g^{-1})	Exudation sampling date	Measured exudation rate (μg C cm $FRSA^{-2}$ day^{-1})	Measured exudation rate (mg C g $root^{-1}$ day^{-1})	Plot level exudation rate (g C m^{-2} day^{-1})
Maple	456 (69)^{ab}	474 (35)^{a}	June	6.89 (1.74)	2.40 (0.72)	1.10 (0.36)
			August	2.61 (1.42)	1.26 (0.74)	0.58 (0.33)
Ash	367 (42)^{bc}	337 (16)^{b}	June	4.46 (1.61)	1.59 (0.67)	0.58 (0.25)
			August	2.97 (0.99)	1.00 (0.38)	0.36 (0.13)
Hemlock	504 (53)^{a}	230 (31)^{c}	June	4.41 (1.70)	0.69 (0.22)	0.35 (0.11)
			August	4.94 (2.37)	1.26 (0.50)	0.88 (0.32)
Beech	347 (11)^{c}	332 (16)^{b}	June	5.96 (1.09)	2.27 (0.36)	0.75 (0.15)
			August	3.20 (1.07)	1.16 (0.47)	0.40 (0.15)

Note Different lowercase letters indicate significant differences between species for a given measurement

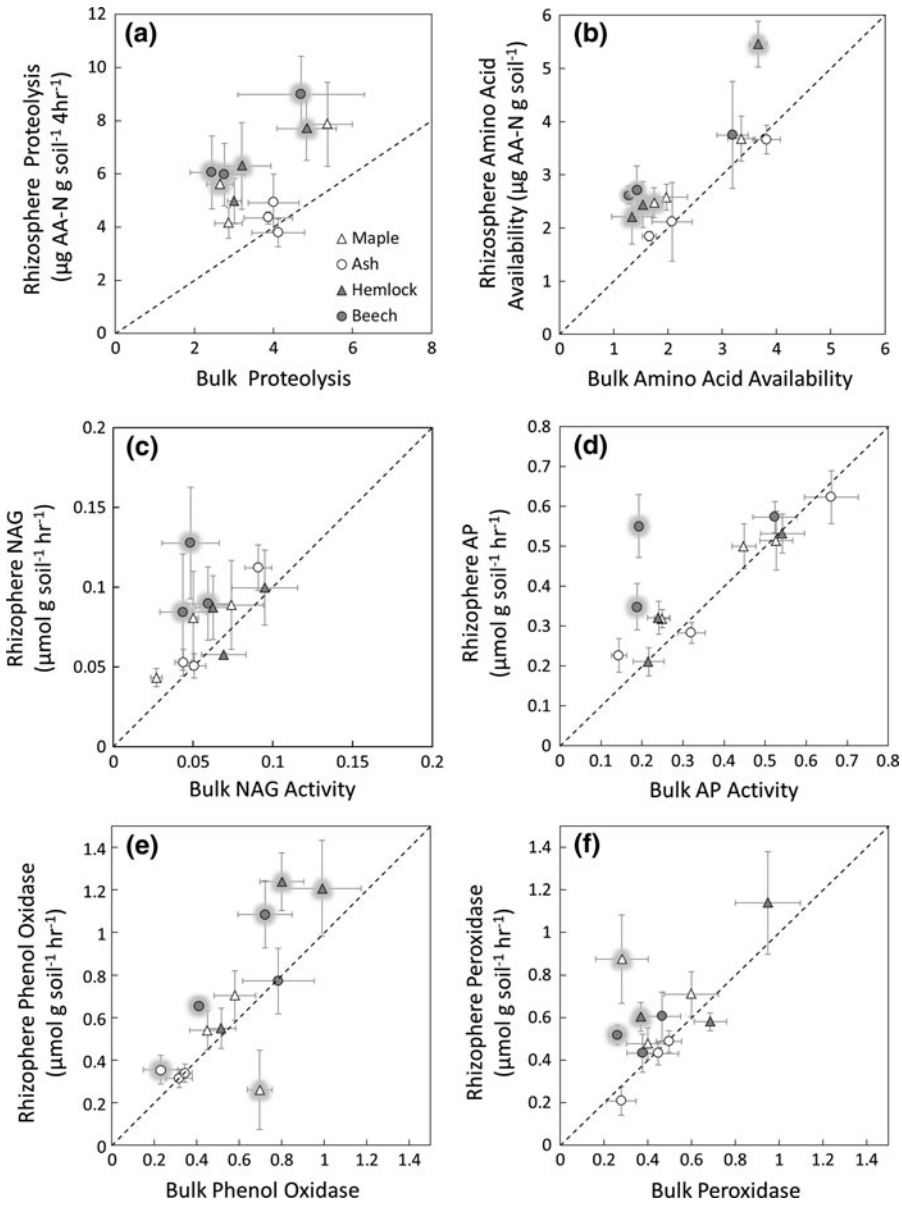

Fig. 1 Plots of mean ± 1 SE **a** rates of proteolysis, **b** extractable amino acid concentrations, and the activities of **c** NAG, **d** acid phosphatase, **e** phenol oxidase, and **f** peroxidase in the bulk soil versus the rhizosphere for all species and sampling dates. Values above the *dashed 1:1 line* indicate a larger effect in rhizosphere compared to bulk soil. *Gray borders* around symbols indicate significant differences ($p < 0.05$) between the bulk and rhizosphere soil for a given species and sampling date. Numerical data are reported in Table S1

significant rhizosphere effect on peroxidase activity (Fig. 1f; Table S1). Maple soil had a positive rhizosphere effect on phenol oxidase and a negative rhizosphere effect on peroxidase in June (Fig. 1e, f; Table S1). For ash soil, there was a rhizosphere effect on phenol oxidase activity in August (Fig. 1f; Table S1).

Using meta-analysis to synthesize across all the sample dates and enzymes, we found that beech had the largest rhizosphere effect on total enzyme activity (Fig. 2). Hemlock and sugar maple had similar rhizosphere effects, although there was greater variability in the rhizosphere effect in maple soils (Fig. 2). Comparing across species, the rhizosphere effect in beech was significantly greater than in ash, where there was no significant rhizosphere effect (Fig. 2).

C and N mineralization

Rates of C mineralization were significantly greater in the rhizosphere than in the bulk soil of maple, hemlock, and beech in August (Fig. 3f). The largest

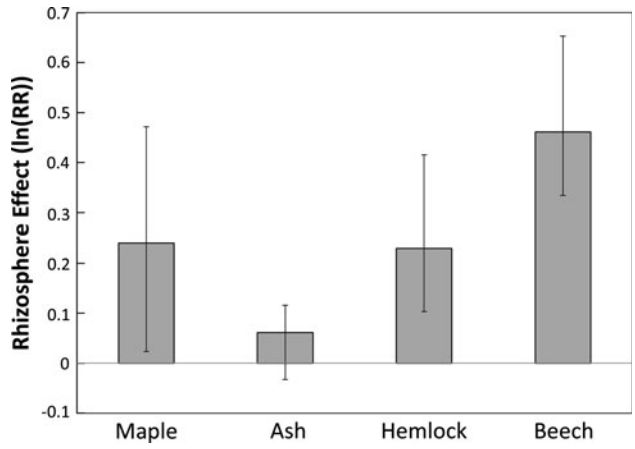

Fig. 2 Grand means of the rhizosphere effect, *ln*(*RR*) with 95 % CI's, on total enzyme activity for the soils of all four tree species

rhizosphere effect on C mineralization was observed in beech soil and the smallest in hemlock soil, with rates 82 and 31 % greater in the rhizosphere than the bulk soil, respectively. There was no difference between fractions in ash soils (Fig. 3f).

The rate of C mineralization in the bulk and rhizosphere soil was positively correlated to the mean activity of proteolytic, NAG, phenol oxidase and peroxidase enzymes in these soil fractions (Fig. 3a–d). There was no significant relationship between rates of C mineralization and acid phosphatase activity (Fig. 3e). In the principle components analysis, the first PC explained 60 % of the variation in C mineralization ($p < 0.001$; Fig. S1). However, it was difficult to distinguish which enzyme was driving this relationship because they had similar loadings on the first PC (Table S2).

Net N mineralization was significantly greater in the rhizosphere than in the bulk soils of maple in June and August, and in beech in August (Fig. 4a, b). Maple soils had the highest rates and the largest rhizosphere effects on net N mineralization and nitrification (Fig. 4). Further, over half of the mineralized N in the rhizosphere of maple soils in June was nitrified (Fig. 4c). In hemlock soil, there were no differences in net mineralization between soil fractions, and in general net immobilization of N. The bulk soil in ash tended to have greater rates of N mineralization than the rhizosphere soil, but the difference was not significant (Fig. 4a, b).

Discussion

Root inputs of C appear to support increased microbial and enzymatic activity in rhizospheres of every species except ash (Table 1; Fig. 3f). The rate of microbial respiration was higher in the rhizosphere compared to bulk soil and was highly correlated with the activity of four enzymes responsible for depolymerizing N from SOM (Fig. 3a–d). The magnitude of the rhizosphere effects was not clearly distinct between mycorrhizal associations, but the form of N produced differed between ECM and AM rhizospheres (Figs. 1b, 2, 4). Root C inputs primarily enhanced inorganic N availability in maple rhizospheres, whereas they enhanced amino acid availability in hemlock and beech rhizospheres (Figs. 1b, 4). These results suggest an interaction between mycorrhizal association and SOM chemistry on the form of N produced and taken up by roots in the rhizosphere.

Across species, the exudation of C by roots at the plot level ranged from 0.35 to 1.10 g C m^{-2} day^{-1} (Table 1). Our measurements are comparable to previously reported values (Table 2) and suggest that the exudation of C by fine roots into the rhizosphere represents a small but important transfer of photosynthate-C to rhizosphere microbes. Most of the data in Table 2 were derived from culture studies and it is unclear whether exudation in the laboratory is similar to patterns and types of exudates in the field suggesting a critical need for future rhizosphere research.

Contrary to hypothesis (1), there were no significant differences in rates of root exudation between species or sampling dates (Table 1). Our inability to resolve species and seasonal differences in exudation rates is probably the result of infrequent sampling and high inherent spatiotemporal variability in exudate fluxes (Phillips et al. 2008). Previous studies that documented differences in exudation rates between ECM

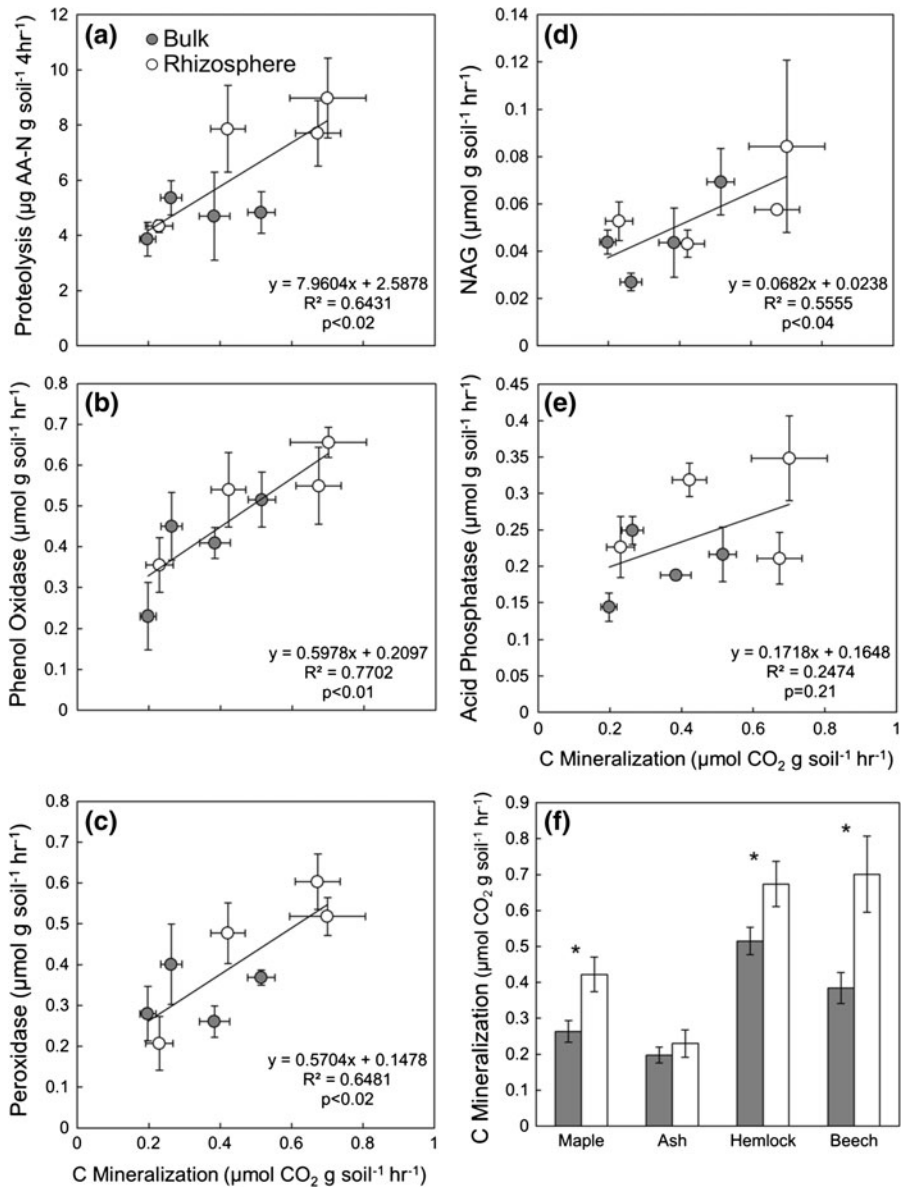

Fig. 3 Relationship between the rate of C mineralization with **a** proteolysis, **b** phenol oxidase, **c** peroxidase, **d** NAG, and **e** acid phosphatase activities in August of 2010. Values are means ± 1 SE for the bulk soil (*filled symbols*) and rhizosphere soil (*open symbols*) for all four tree species. In **f**, the values represent the rate of C mineralization (mean ± 1 SE) in the rhizosphere and bulk soil for each species in August of 2010. *Asterisks* indicate significant differences ($p < 0.05$) in the C mineralization rate between the bulk (*gray background*) and rhizosphere soil (*white background*) for a given species

and AM tree roots were conducted in controlled environments (i.e., greenhouse or soil mesocosms) or with samples drawn over multiple years in the field (Smith 1976; Phillips and Fahey 2005; Table 2). Based on the available data we could not support hypothesis (1), but also note that more extensive field sampling may be able to resolve species level differences in root exudation should they exist.

There was a gradient in the magnitude of rhizosphere effects between tree species that could not be easily characterized by mycorrhizal association or SOM chemistry (Fig. 2). However, the low and high end members of this gradient, ash and beech, were predicted by hypothesis (2). In ash, there were equivalent concentrations of amino acids, rates of C and N mineralization and proteolytic enzyme activity in the AM influenced rhizosphere and bulk soil (Figs. 1, 2, 3f). In beech, the rate of C mineralization, amino acid concentration and extracellular enzyme activity in ECM influenced rhizosphere soil far exceeded the rate measured in bulk soil on almost every sample date (Figs. 1, 3f). Despite this difference in rhizosphere effects, ash soils had among the highest and beech soils had among the lowest rates of

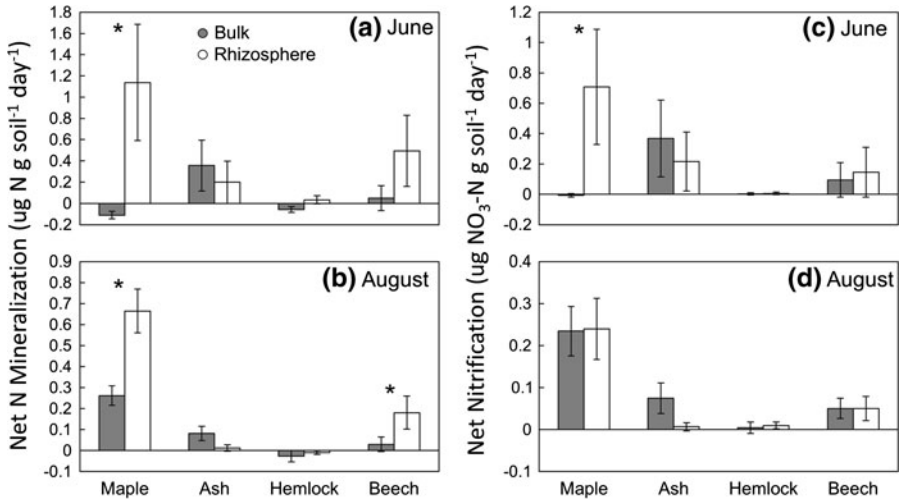

Fig. 4 Rates of net N mineralization (mean ± 1 SE) in the bulk soil (*gray background*) and rhizosphere soil (*white background*) in **a** June and **b** August of 2010 for each species. Rates of net nitrification in bulk soil and rhizosphere soil in **c** June and **d** August of 2010. *Asterisks* indicate significant differences ($p < 0.05$) in rates between the bulk and rhizosphere soil for a given species and sample date

Table 2 Measurements of root exudation rates by trees in the greenhouse and the field

Study	Tree species	Measurement type	Daily flux (mg C g root^{-1} day^{-1})	Estimated yearly flux (g C m^{-2} year^{-1})
Smith (1976)	*Acer saccharum; Fagus grandifolia; Betula alleghaniensis*	Field; mature trees		0.25–2.05
Uselman et al. (2000)	*Robinia psuedoacacia*	Greenhouse; seedlings	0.15–0.52	
Aitkenhead-Peterson and Kalbitz (2005)	*Picea abies*	Greenhouse; seedlings	0.55–1.20	
Fransson and Johansson (2010)	*Pinus sylvestris*	Greenhouse; seedlings	1.0–12.4	
Phillips and Fahey (2005)	*Acer saccharum; Betula alleghaniensis*	Mesocosms; saplings		55–104
Phillips et al. (2008)	*Pinus taeda*	Field; mature trees	Mean = 0.1	9.4
Phillips et al. (2011)	*Pinus taeda*	Field; mature trees	0.31–0.36	23.2–24.8
This study	*Acer saccharum, Fraxinus americana, Tsuga canadensis, Fagus grandifolia*	Field; mature trees	0.35–1.10	

Note For some studies it was not possible to derive a daily or yearly flux of root C

proteolytic enzyme activity and concentrations of amino acids in the bulk soil (Fig. 1a, b, Table S1). These results suggest that ash and beech differ in their nutrient mobilization strategies (Chapman et al. 2006). Beech appears to localize decomposition in the rhizosphere and tightly couple C allocation to rhizosphere microbes with nutrient return, whereas nutrient mobilization in ash is more diffuse and primarily a bulk soil process driven by free-living microbes (Phillips and Fahey 2006; Brzostek and Finzi 2011).

Sugar maple and hemlock had equivalent rhizosphere effects on total enzyme activity, but differed in the form of N produced (Figs. 1b, 2, 4). Throughout temperate forests, sugar maple soils are characterized by high rates of nitrification and nitrate leaching (e.g., Finzi et al. 1998b; Lovett et al. 2004). In the sugar maple soils studied here, AM root inputs enhanced proteolytic enzyme activity and fueled greater rates of the downstream processes, N mineralization and nitrification (Figs. 1a, 4). Thus, it appears that the rapid inorganic N cycling rates in sugar maple soils are, in part, a direct result of AM root inputs to labile SOM. By contrast, hemlock soils are typically characterized by slow rates of N mineralization and

nitrification (e.g., Finzi and Canham 1998; Lovett et al. 2004). In these soils enhanced proteolytic activity in the rhizosphere did not lead to faster rates of inorganic N cycling, suggesting that a significant portion of the amino acids that are produced are taken up by roots and rhizosphere microbes to meet N demand.

Differences in the chemistry of exudates and SOM (e.g., labile in AM soils, more recalcitrant in ECM soils, Finzi et al. 1998a, b) likely resulted in the gradient of rhizosphere responses between ECM and AM trees. In the present study it is not possible to distinguish whether and how SOM or exudate chemistry affected these outcomes, making this an important avenue for future research. Importantly, differences between AM and ECM plots in the forms of N that were depolymerized and mineralized from SOM mirror differences in the forms of N acquired from the soil in the field, with amino acid uptake dominating N uptake in ECM soils and inorganic-N uptake dominating in AM soils (Gallet-Budynek et al. 2009).

We chose our sampling dates to capture different soil moisture and temperature regimes as well important phenological dates in the C balance of the forest (Urbanski et al. 2007). We expected that rhizosphere effects would vary with the photosynthetic activity of the canopy (Hogberg et al. 2010), but there was no emergent temporal pattern in rhizosphere effects across sampling dates. We acknowledge that the limited number of soil sampling dates may be masking important seasonal variation in rhizosphere effects. There were trends in bulk soil enzyme activity across sampling dates. As soil moisture decreased and temperature increased over the growing season, we found that the activity of every enzyme decreased in the bulk soil, except proteolytic enzymes (Table S1). These trends suggest that for most enzymes low soil moisture limits activity by slowing the diffusion of enzymes and substrates (Allison et al. 2010; Brzostek et al. 2012).

We found strong, positive correlations between extracellular enzyme activity and rates of C mineralization in rhizosphere and bulk soil across all tree species (Fig. 3a–d). In the principle components analysis, we found that all five enzymes were equally weighted in their ability to predict C mineralization. These relationships suggest a tight coupling between the aggregate activity of multiple enzyme classes and soil-C efflux, resulting from growth and maintenance respiration in addition to the C-cost of enzyme production (Schimel and Weintraub 2003).

This study contributes to the growing body of evidence demonstrating that plant roots enhance SOM decomposition and soil-N cycling through the allocation of C to the rhizosphere (e.g., Phillips and Fahey 2006; Weintraub et al. 2007; Koranda et al. 2011; Drake et al. 2011). Rhizosphere effects on SOM decomposition were not easily categorized by mycorrhizal association. However, there were clear differences in the form of N produced with amino acid production dominating in ECM rhizospheres and inorganic-N production dominating in AM rhizospheres. This work suggests that climatic and environmental factors affecting belowground C allocation have the potential to impact both the form and amount of N to support primary production in ECM and AM stands.

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