The Influence of Soil Fertility on Rhizosphere Effects in Northern Hardwood Forest Soils

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Enhanced levels of microbial biomass and activity in the rhizosphere arise from labile C released from roots but the factors that mediate such “rhizosphere effects” are poorly understood. We hypothesized that the magnitude of rhizosphere effects would be reduced by increases in soil fertility, consistent with plant C allocation theory, which predicts decreased C flux to roots in fertile soils. Replicate plots of sugar maple (Acer saccharum Marshall) and northern red oak (Quercus rubra L.) at the Turkey Hill Plantations, New York, and yellow birch (Betula alleghaniensis Britton) at the Hubbard Brook Experimental Forest, New Hampshire, were fertilized from 2001 to 2003. In fall of 2003, rhizosphere and bulk soils were sampled from the organic horizon of control and fertilized plots and analyzed for microbial biomass and nutrient transformation rates. In general, fertilization reduced microbial biomass and activity in plots of all three species, and the magnitude of such effects was generally greater in rhizosphere than in bulk soil. In red oak soils, fertilization reduced rhizosphere effects on microbial biomass, net N mineralization rates, and phosphatase enzyme activity ($P = 0.026, 0.091, \text{and} 0.061$, respectively). In contrast, fertilization only reduced rhizosphere effects on microbial biomass in sugar maple soils ($P = 0.019$). In yellow birch soils, fertilization decreased rhizosphere effects on microbial biomass ($P = 0.015$). These results suggest that soil fertility may mediate the degree to which roots affect microbial activity in forest soils, possibly due to fertilizer-induced shifts in belowground C supply.

Abbreviations: DWE, dry weight equivalent; HBF, Hubbard Brook Experimental Forest; MB, microbial biomass; MRA, maximum respiratory activity; THP, Turkey Hill Plantations

Since then, there have been numerous reports of enhanced rhizosphere effects for mature trees in forest soils (van Hees et al., 2005). Reports of enhanced microbial activity in the rhizosphere of tree roots date back to the studies of Netti (1955) and Ivarson and Katznelson (1960), who reported order of magnitude differences in the abundance of bacteria between rhizosphere and bulk soil for oak and birch seedlings, respectively. Since then, there have been numerous reports of enhanced rhizosphere activity for tree seedlings in mesocosms (Norton and Firestone, 1991; Bradley and Fyles, 1995; Priha et al., 1999a). Few studies of rhizosphere effects for mature trees in forest soils have been published (Hogberg and Hogberg, 2002; Hernesmaja et al., 2005), however, despite evidence that rhizosphere C fluxes represent a substantial contribution to the C balance of forest soils (Fahey et al., 2005; van Hees et al., 2005).

Because rhizodeposition is the principal factor influencing rhizosphere microbial biomass and activity (Paterson, 2003), factors that regulate rhizosphere C fluxes (e.g., tree species, developmental stage, mycorrhizal status) may also control the magnitude of rhizosphere effects. For example, Cardon et al. (2002) reported reduced rhizosphere microbial biomass when belowground C allocation was decreased in red oak (Quercus rubra L.) seedlings. In silver birch (Betula pendula L.) seedlings, microbial biomass was positively correlated with the number of root tips, suggesting a relationship between C allocation to roots and rhizosphere effects (Priha et al., 1999b). Whether such relationships between belowground C allocation and rhizosphere effects exist in forests is unknown. In a recent study, Phillips and Fahey (2006) reported that the magnitude of rhizosphere effects in...
effects on soil microbes was negatively correlated with soil pH in monospecific stands of several tree species, suggesting a link between rhizosphere C fluxes (and by extension rhizosphere effects) and soil fertility. An informative test of the idea that rhizosphere effects are mediated by belowground C allocation in trees is the response of the rhizosphere effect to increases in soil fertility. If rhizosphere effects are smaller in fertile than infertile soils, then it is likely that rhizosphere effects are linked to belowground C allocation patterns in trees (Giardina et al., 2004). Such a result would be consistent with reports of fertilizer-induced decreases in fine root biomass (Haynes and Gower, 1995; Pregitzer et al., 1995; Nadelhoffer, 2000) and mycorrhizal colonization (Treseder, 2004) in forest soils, and with increased root exudation of seedlings under low-nutrient conditions (van Scholl et al., 2006).

Roots and rhizosphere processes also may increase nutrient availability in forest soils, as suggested by reports of greater N and P availability in the rhizosphere relative to the bulk soil (Phillips and Fahey, 2006). Enhanced N availability is believed to result from the accelerated turnover of rhizosphere microflora owing to starvation and protozoan grazing, and increased decomposition of organic matter due to positive priming effects (Badalucco and Kuikman, 2001). Whether such rhizosphere effects on N availability respond to changes in the fertility of forest soils is unknown, but would be consistent with reports of root-induced increases in net N mineralization in nutrient-poor mineral soils (Bradley and Fyles, 1996; Ehrenfeld et al., 1997). Root-induced stimulation of P availability also has been reported for trees (Haussling and Marschner, 1989; Hernesmaa et al., 2005; Phillips and Fahey, 2006). Although the degree to which soil fertility influences such effects is unknown, some evidence suggests that the magnitude of rhizosphere effects on P availability in forest soils is coupled to belowground C allocation patterns and rhizosphere C fluxes. In forest soils, a large proportion of the P is bound to organic matter through ester bonds, and roots release phosphatase enzymes and low-molecular-weight organic acids to access P (Tate et al., 1991; Hinsinger, 1998). In addition, rhizosphere microbes—including mycorrhizal fungi—release phosphatase enzymes, which may further contribute to enhanced P availability in the rhizosphere (Marschner, 1995; Grierson and Comerford, 2000). Thus, decreases in fine root biomass, mycorrhizal colonization, and rhizosphere microbial biomass in fertile soils might all contribute to decreased rhizosphere effects on P availability (Fox and Comerford, 1992; Clarholm, 1993).

Tree species also differ in the quantity and chemical quality of rhizodeposition (Grayston et al., 1996), and such differences might influence the response of trees to changes in soil fertility. Phillips and Fahey (2006) reported that the magnitude of rhizosphere effects on various microbial variables ranged from 0 to 50% across six tree species planted in a common soil and that, in general, rhizosphere effects on N and P availability were greater for ectomycorrhizal than arbuscular mycorrhizal tree species. The role of mycorrhizal fungi in rhizosphere C flux and rhizosphere effects is poorly understood but it is likely that the fungi play an important role in mediating rhizosphere effects (Hogberg and Hogberg, 2002) and the response of rhizosphere processes to changes in soil fertility (Jones et al., 2004).

The objective of this study was to examine the response of rhizosphere effects to fertilization of three broadleaf deciduous tree species in a mature forest. We hypothesized that fertilization would suppress rhizosphere effects on microbial biomass and N and P cycling. This study is the first, to our knowledge, to examine how fertilization alters rhizosphere effects on nutrient availability in mature forest trees.

**MATERIALS AND METHODS**

**Study Sites and Treatments**

Soil and root samples were collected from two sites: an ~65-yr-old plantation forest at the Turkey Hill Plantations (THP) in Tompkins County, New York (42°27′00″ N, 76°25′00″ W), and an ~85-yr-old northern hardwood forest at the Hubbard Brook Experimental Forest (HBEF) in the White Mountains, New Hampshire (43°56′N, 71°45′ W). Average annual precipitation at the THP is 874 mm, with annual temperatures of −6 and 18°C in January and June, respectively. Monospecific plots (0.4 ha) were established at the THP between 1939 and 1941 by Robert F. Chandler to examine the effects of tree species on soils. Soils at the THP are coarse-loamy, moderately well-drained Typic Dystrochrepts (pH = 4–5) developed from sil-poor till derived from local sandstones and siltstones (Pallant and Riha, 1990). Most soils were under intensive cultivation before planting.

The HBEF receives an average of 1400 mm of precipitation per year, with mean annual temperatures from −9°C in January to 19°C in July. Soils at the HBEF are predominantly well-drained, coarse-loamy, mixed, frigid, acidic Typic Haplorthods (pH = 3.9) developed in shallow glacial till overlying sedimentary and igneous rocks (Likens and Bormann, 1995).

At the THP, paired experimental plots (10 by 10 m) were established in two monospecific stands of northern red oak and two monospecific stands of sugar maple. In all four stands, the basal area of the dominant species was >90% of the total, and they ranged in elevation from 440 to 460 m. At the HBEF, paired experimental plots (12 by 12 m) were established in two stands dominated by yellow birch. In both stands, yellow birch comprised >80% of the total basal area, and the plots ranged in elevation from 480 to 640 m (Hardy et al., 2001). Within each stand, one of the paired plots was fertilized monthly throughout the growing season (May–October) from 2001 to 2003. The water-soluble fertilizer consisted of a macronutrient and micro-nutrient mixture of: N = 167, P = 58, K = 254, Ca = 310, and Mg = 37 kg ha⁻¹ yr⁻¹ with the N primarily as NO₃⁻. The amount of N added represented approximately two times the in situ net N mineralization rates at the HBEF (Grootman et al., 2001) and the THP (R.P. Phillips and W.X. Zhu, unpublished data, 2002). Fertilizer (as a solid) was applied to the soil surface manually by broadcast application.

**Soil and Root Sampling**

Soils and fine roots were sampled in July and August 2003 at the HBEF and in August and September 2003 from the THP. At both sites, four transects were established across each plot at least 2 m from the plot edges. On each transect, five to eight soil samples were collected with a 5-cm-diameter polyvinyl chloride core and composited into a single sample. Samples were collected on the same day from both fertilized and control plots, and no samples were collected in the first week after each fertilizer application. At the THP, soils were collected from the upper 4 cm of the A horizon (the primary rooting zone). At the HBEF, samples were collected from the forest floor (Oa + A horizons) and to a depth of 6 cm on average. Within each plot, soil cores from a single transect were placed into a sorting basket where large aggregates could be broken apart gently, and fine roots (<1 mm) and adhering rhizosphere could be removed with forceps. Rhizosphere soil was operationally defined as soil adhering to roots.
labeled as the change in extractable \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) before and after the incubation. Immediately following filtration, KCl extracts were acidified with several drops of 6 mol L\(^{-1}\) HCl to prevent microbial growth and refrigerated at 4°C. Extractable \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) were determined on a flow-injection autoanalyzer (OI Analytical, College Station, TX).

A modification of the method described in Tabatabai (1994) was used to measure acid phosphatase enzyme activity. Toluene (200 µL), modified universal buffer (4 mL adjusted to pH = 6.5), and buffered \( p \)-nitrophenol phosphate solution (1 mL) were added, respectively. Concentrations of \( p \)-nitrophenol released by phosphatase activity were determined on a tabletop spectrophotometer at 420 nm using \( p \)-nitrophenol standards. To correct for the color of the background matrix, a second control sample that received no \( p \)-nitrophenol phosphate solution was incubated and extracted using the same procedure. Phosphatase enzyme activity was estimated as the amount of \( p \)-nitrophenol released after correcting for controls (mmol \( p \)-nitrophenol kg\(^{-1}\) h\(^{-1}\)).

**Calculations and Statistics**

Fertilizer effects on rhizosphere and bulk soil variables were analyzed with a randomized complete-block design, with block as a fixed factor such that each of the nonspecific stands represented a block. The statistical model was run for each species individually (i.e., tree species were not contrasted) due to large differences in soil properties, climate, and land-use history between the HBEF and THP. Rhizosphere effects—calculated as the percentage difference of a given response variable between paired rhizosphere and bulk soil samples—were analyzed for their statistical difference from zero (i.e., positive or negative rhizosphere effects) and for differences between control and fertilized treatments. Positive rhizosphere effects indicate a greater or lesser pool or flux in rhizosphere soil relative to bulk soil, while no rhizosphere effect (not significantly different from zero) indicates no differences in the pool or flux between rhizosphere and bulk soil. In view of the low number of replicate plots (\( n = 2 \)), rhizosphere effects for individual species were considered statistically different from zero at \( \alpha = 0.1 \) unless noted otherwise. Non-normal data were log-transformed to meet conditions of normality and all data were analyzed in SAS Version 9.1 (SAS Institute, 2003).

**RESULTS**

**Microbial Biomass and Activity**

In general, fertilization significantly decreased microbial biomass and activity in both the rhizosphere and bulk soil fractions of all three species, the only exception being red oak plots, where the suppressive effects of fertilization were present in the rhizosphere only (Table 1). In sugar maple plots, fertilization had stronger effects on MB-C (40% decrease, \( P = 0.0001 \)) and MRA (49% decrease, \( P = 0.0001 \)) than on MB-N (18% decrease, \( P = 0.041 \)). In red oak plots, fertilization decreased MB-C by 22% (\( P = 0.001 \)) and MRA by 48% (\( P = 0.094 \)) in the rhizosphere but had no significant effects on these variables in the bulk soil (\( P = 0.126 \) and 0.547, respectively). Fertilization had no effects on MB-N in either soil fraction of the red oak plots. In yellow birch plots, fertilization suppressed MB-C by 37% (\( P < 0.0001 \)), MB-N by 47% (\( P < 0.0001 \)), and MRA by 32% (\( P = 0.007 \)) in both rhizosphere and bulk soil.
In control plots of all three species, significant rhizosphere effects (i.e., the percentage difference between rhizosphere soil and bulk soil) on microbial biomass were generally observed (Fig. 1). The magnitude of the rhizosphere effect on MB-C and MB-N ranged from 12 to 36% in control plots, and was greatest in sugar maple plots. Positive rhizosphere effects on MRA were generally observed in control plots, but the magnitude of such effects were not significantly different from zero. Fertilization generally suppressed the rhizosphere effect on microbial biomass (i.e., the percentage difference between rhizosphere and bulk soil was smaller) in both sugar maple and red oak plots (Fig. 1). In sugar maple plots, rhizosphere effects on MB-N were significantly lower in fertilized plots than in control plots ($P = 0.026$). Similarly, rhizosphere effects on MB-C in red oak plots were significantly lower in control plots relative to fertilized plots ($P = 0.019$). In contrast to patterns in sugar maple and red oak plots, the magnitude of rhizosphere effects on MB-C and MB-N was greater in fertilized yellow birch plots ($P = 0.015$ and $P = 0.017$, respectively).

**Nutrient Transformations**

Fertilization had varying effects on net N transformations and phosphatase enzyme activity in soils of different species (Table 2). In sugar maple and yellow birch plots, net N mineralization was significantly suppressed by fertilization ($P < 0.0001$ and 0.014, respectively), and the magnitude of the effect was similar between rhizosphere and bulk soils. The response of net nitrification to fertilization differed between these species, however, being significantly suppressed in yellow birch ($P < 0.001$) but not sugar maple ($P = 0.463$). Fertilization effects in red oak soils differed dramatically from those in sugar maple and yellow birch as net N mineralization increased dramatically (182 and 239% in rhizosphere and bulk soil, respectively) and net nitrification was negligible. In yellow birch soils, fertilization decreased phosphatase enzyme activity in rhizosphere ($P = 0.008$) but not in bulk soil ($P = 0.118$). Fertilization had no effects on phosphatase activity in sugar maple or red oak soils.

Positive rhizosphere effects on net N mineralization and phosphatase were detected in the control plots of all three species but to a much lesser degree in fertilized plots (Table 2). In sugar maple and yellow birch plots, net N mineralization was significantly suppressed by fertilization ($P < 0.0001$ and 0.014, respectively), and the magnitude of the effect was similar between rhizosphere and bulk soils. The response of net nitrification to fertilization differed between these species, however, being significantly suppressed in yellow birch ($P < 0.001$) but not sugar maple ($P = 0.463$). Fertilization effects in red oak soils differed dramatically from those in sugar maple and yellow birch as net N mineralization increased dramatically (182 and 239% in rhizosphere and bulk soil, respectively) and net nitrification was negligible. In yellow birch soils, fertilization decreased phosphatase enzyme activity in rhizosphere ($P = 0.008$) but not in bulk soil ($P = 0.118$). Fertilization had no effects on phosphatase activity in sugar maple or red oak soils.
Ecosystem-Level Rhizosphere Effects

The percentage of rhizosphere soil (as a fraction of the total mass of soil) ranged from 15 to 40%, and decreased significantly in response to fertilization in plots of all three species (Table 3). This decrease in the amount of adhering soil may have been due to fertilizer-induced decreases in fine root biomass (Phillips and Fahey, 2007), as fine root biomass and the percentage of rhizosphere soil were positively correlated in plots of sugar maple, red oak, and yellow birch ($r^2 = 0.47, 0.60,$ and 0.47, respectively). Thus, fertilization decreased the ecosystem-level magnitude of rhizosphere effects by decreasing both nutrient transformation rates and the volume of soil defined as rhizosphere. The product of these two variables is defined as the ecosystem rhizosphere effect (RE$_{e}$), which estimates the contribution of rhizosphere transformation rates to the whole soil profile (Phillips and Fahey, 2006). Fertilization decreased RE$_{e}$ on net N mineralization from 12 to 3% in sugar maple soils ($P = 0.049$) and from 18 to 5% in red oak soils ($P = 0.028$). Fertilization did not affect the RE$_{e}$ on net N mineralization in yellow birch soils ($P = 0.644$). Ecosystem rhizosphere effects on phosphatase activity were significantly decreased by fertilization in red oak and yellow birch soils but not in sugar maple soils.

DISCUSSION

Enhanced levels of microbial biomass and activity in the rhizosphere have long been known to result from the release of exudates, secretions, sloughed cells, and other debris from fine roots to soil (Katznelson et al., 1948; Rovira, 1965). However, the mechanisms by which roots affect rhizosphere processes are still poorly understood (Jones et al., 2004), owing to the complexity of the interactions between roots, microbes, and soil factors, and the lack of suitable methods for studying such interactions without physically altering the plant–soil system. This is especially true for forest soils, where the depth of the rooting zone and spatial heterogeneity of soils impose significant constraints on quantifying rhizosphere processes (Smith, 1990).

In this study, we quantified rhizosphere effects as the difference in response variables between rhizosphere and bulk soil, with rhizosphere soil operationally defined as soil adhering to fine roots after gentle shaking sensu Wollum (1994). Although not without significant drawbacks (Jones, 2003), this approach is useful for obtaining a sufficient mass of soil for process-based assays, and is believed to provide a time-integrated estimate of rhizosphere effects (Phillips and Fahey, 2006). It is important to note, however, that rhizosphere “hot spots” where microbial activities and nutrient transformation rates may be an order of magnitude greater than in bulk soil (Badalucco and Kuikman, 2001; Belnap et al., 2003) cannot be detected using this method, and thus failure to detect rhizosphere effects in this study may also result in part from the coarse spatial resolution of the method.

Table 2. Net N mineralization, net nitrification, and phosphatase enzyme activity in rhizosphere and bulk soil of control and fertilized plots. Values are means (and SE) of replicate plots ($n = 2$).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Net N mineralization</th>
<th>Net N nitrification</th>
<th>Phosphatase enzyme activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rhizosphere (mg N kg$^{-1}$ OM d$^{-1}$)</td>
<td>Bulk soil (mg N kg$^{-1}$ OM d$^{-1}$)</td>
<td>Rhizosphere (mmol kg$^{-1}$ OM h$^{-1}$)</td>
</tr>
<tr>
<td>Sugar maple</td>
<td>Control: 11.7 (3.2) a</td>
<td>10.9 (0.9) b</td>
<td>10.6 (1.9) a</td>
</tr>
<tr>
<td>Fertilized: 13.8 (2.4) a</td>
<td>11.2 (0.1) a</td>
<td>-8.7 (2.4) b</td>
<td>-8.5 (0.4) b</td>
</tr>
<tr>
<td>Red oak</td>
<td>Control: 15.0 (3.7) a</td>
<td>12.3 (3.1) a</td>
<td>4.4 (3.0) a</td>
</tr>
<tr>
<td>Fertilized: 11.4 (2.0) b</td>
<td>9.3 (1.0) b</td>
<td>1.2 (0.9) b</td>
<td>0.9 (0.9) b</td>
</tr>
<tr>
<td>Yellow birch</td>
<td>Control: 11.7 (3.7) a</td>
<td>12.3 (3.1) a</td>
<td>4.4 (3.0) a</td>
</tr>
<tr>
<td>Fertilized: 11.4 (2.0) b</td>
<td>9.3 (1.0) b</td>
<td>1.2 (0.9) b</td>
<td>0.9 (0.9) b</td>
</tr>
</tbody>
</table>

† Different letters denote significant differences between control and fertilized plots within a given soil fraction (rhizosphere and bulk soil) and tree species ($\alpha = 0.10$).

Species effects on microbial activity and nutrient availability have been reported for sugar maple, red oak, and yellow birch (Finzi et al., 1998; Templer et al., 2003; Lovett et al., 2004), but species-level comparisons in this study should be done with caution, given the differences in climate, soils, and

Fig. 2. Rhizosphere effects on net N mineralization rate (net N min.) and phosphatase enzyme activity in control and fertilized plots of sugar maple, red oak, and yellow birch. Rhizosphere effects are presented as means (and one standard error) for each treatment ($n = 2$). Means greater than zero (positive rhizosphere effects) are noted by the symbols $† = P < 0.1$, $*= P < 0.05$, $** = P < 0.01$, and $*** = P < 0.001$. Different letters indicate significant differences between control and fertilized plots for each tree species for a given variable ($\alpha = 0.05$).
Belowground Carbon Supply and Rhizosphere Effects

Forests of the northeastern United States are generally thought to be nutrient limited (Aber and Melillo, 1991), and they are therefore an appropriate setting for testing the hypothesis that rhizosphere effects on microbial activity are decreased by increased soil fertility. Forest fertilization studies can be difficult to interpret, however, because myriad factors may influence the magnitude and direction of ecosystem responses. Factors such as stand age, the timing and quantity of fertilizer application, the chemical composition of fertilizer (e.g., N or N–P–K), and the duration of fertilization can all influence ecosystem responses to changes in nutrient availability (Tietema et al., 1998; Jelts et al., 2004; Magill et al., 2004). In addition, fertilization can have direct effects on soil processes independent of root or rhizosphere activity via formation of recalcitrant soil C (Neff et al., 2002) and N suppression of phenol oxidase activity, a lignin-degrading enzyme produced by white-rot fungi (Fog, 1988). In this study, fertilization had direct negative effects on microbial biomass in plots of all three species, consistent with previous forest fertilization studies using N (Saiya-Cork et al., 2002; Frey et al., 2004; Waldrop et al., 2004) and N plus other macronutrients (Fahey et al., 1998; Fisk and Fahey, 2001).

By quantifying differences between paired rhizosphere and bulk soil samples, we were able to separate the direct suppressive effects of fertilization on bulk soil microbial processes (Tables 1 and 2) from the indirect effects via decreased rhizosphere activity (Fig. 1 and 2). In addition, we quantified changes in the mass of rhizosphere and bulk soil fractions in control and fertilized plots to estimate the potential ecosystem contribution of these rhizosphere processes (Table 3).

Because soil microbial biomass is considered a temporally integrated measure of labile C (Zak et al., 1993), we attribute differences in rhizosphere and bulk soil microbial biomass to differences in the amount of root-derived C in each soil fraction. In both sugar maple and red oak soils, fertilization decreased rhizosphere effects on microbial biomass, suggesting decreased belowground C allocation and decreased C flux from roots to soil microbes. Additional support for this interpretation comes from fertilizer-induced decreases in the colonization of sugar maple and red oak roots by mycorrhizal fungi (Phillips and Fahey, 2007), as mycorrhizal fungi may comprise a significant proportion of the rhizosphere microbial biomass in forest soils (Hogberg and Hogberg, 2002). An important question is whether such patterns arose from decreased fine root biomass or decreased C flux per unit fine root. In fertilized sugar maple plots, fine root biomass decreased relative to controls, suggesting that the decreased rhizosphere effects may have resulted, in part, from fewer fine roots. In contrast, fine root biomass in fertilized red oak plots did not differ from control plots, suggesting that the reduced rhizosphere effects may have resulted from decreased rhizosphere C flux. This interpretation would be consistent with the findings of Zak et al. (1993) and Cardon et al. (2002), who reported that changes in the rhizosphere microbial biomass resulted from changes in rhizosphere C flux in big-tooth aspen (Populus grandidentata Michx.) and red oak seedlings, respectively.

Fertilization increased rhizosphere effects on microbial biomass in yellow birch soils. A possible explanation for this response is that such effects resulted from fertilizer-induced increases in rhizosphere C flux consistent with the findings of Uesman et al. (2000), who reported increased root exudation in black locust (Robinia pseudoacacia L.) seedlings fertilized with N. However, this explanation seems unlikely given the small but nonsignificant decreases in mycorrhizal colonization of birch roots in fertilized plots (Phillips and Fahey, 2007). Alternatively, the increased rhizosphere effects in birch may have resulted from the stimulatory effects of nutrients on the rhizosphere microbes. Increased rhizosphere effects have been reported for other Betula species growing in fertile soils (Bradley and Fyles, 1996), and may be indicative of an N-limited rather than C-limited rhizosphere microbial biomass (Cheng et al., 1996; Priha and Smolander, 2003). At the HBEF, rhizosphere C fluxes in yellow birch are appreciable in magnitude (Phillips and Fahey, 2005) and consist primarily of C-rich, N-poor compounds (Smith, 1976), suggesting that N availability may be relatively low in the rhizosphere. This is also supported by the small (8%) enhancement of net N mineralization in the rhizosphere relative to the bulk soil (Fig. 1) in yellow birch control soils. However, we cannot rule out that other factors such as N-induced shifts in the rhizosphere microbial community (Frey et al., 2004; Waldrop et al., 2004) contributed to the increased rhizosphere effects in birch soils.

Table 3. Rhizosphere effects (RE) on net N mineralization and phosphatase enzyme activity in control and fertilized plots. Values are means of replicate plots (n = 2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RS</th>
<th>Net N mineralization</th>
<th>Phosphatase</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>RE</td>
<td>RE e†</td>
<td>RE e‡</td>
</tr>
<tr>
<td>Sugar maple</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>28 a‡</td>
<td>43 12 a ‡</td>
<td>28 8 a</td>
</tr>
<tr>
<td>Fertilized</td>
<td>24 b</td>
<td>12 3 b</td>
<td>23 5 a</td>
</tr>
<tr>
<td>Red oak</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>30 a</td>
<td>61 18 a</td>
<td>26 8 a</td>
</tr>
<tr>
<td>Fertilized</td>
<td>25 b</td>
<td>21 5 b</td>
<td>5 1 b</td>
</tr>
<tr>
<td>Yellow birch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>25 a</td>
<td>26 7 a</td>
<td>22 6 a</td>
</tr>
<tr>
<td>Fertilized</td>
<td>23 b</td>
<td>26 6 a</td>
<td>-9 -2 b</td>
</tr>
</tbody>
</table>

† Ecosystem rhizosphere effects (REe) are calculated as the product of rhizosphere effects (percentage difference between rhizosphere and bulk soil) and the percentage of rhizosphere soil (RS) in each plot, and represent the contribution of rhizosphere transformation rates to the whole soil profile.

‡ Different letters indicate significant differences between control and fertilized plots for each tree species for a given variable (α = 0.05).
Rhizosphere Effects on Nutrient Transformations

In control plots of all three species, net N mineralization rates were greater in rhizosphere than bulk soil. Root-induced stimulation of N mineralization has been reported for seedlings (Norton and Firestone, 1996; Ehrenfeld et al., 1997; Prittha et al., 1999a), but rarely for mature trees in forest soils (Phillips and Fahey, 2006). In general, N availability in the rhizosphere is controlled by the quantity and chemical quality of C from rhizodeposits and soil organic matter, the physiological activity and turnover of the microbial biomass, plant uptake rates, and soil fertility (Badalucco and Kuikman, 2001). Although the enhanced rhizosphere microbial biomass in control plots of these soils (Fig. 1) might be expected to immobilize N (Norton and Firestone, 1996), accelerated N turnover in the rhizosphere may have resulted from increased faunal grazing (Setala and Huhta, 1991). In addition, accelerated rhizosphere N cycling may have resulted from positive priming effects, which occur when microbial utilization of N-poor rhizodeposits results in enhanced soil organic matter decomposition due to microbial release of exoenzymes to access N (Paterson, 2003).

In red oak soils, fertilization reduced rhizosphere effects on net N mineralization and the percentage of rhizosphere soil, and thus decreased the ecosystem-level rhizosphere effects from 18% in control plots to 5% in fertilized plots. One interpretation of these results is that the fertilizer-induced decreases in rhizosphere effects resulted from reduced priming effects. Reductions in priming effects could have occurred if increases in soil fertility resulted in greater microbial utilization of rhizodeposits rather than organic matter, consistent with the preferential substrate utilization hypothesis (Cheng, 1999). Soil organic matter in red oak soils is generally considered of low chemical quality as a microbial substrate (Finzi et al., 1998; Lovett et al., 2004), and thus a large proportion of the heterotrophic activity in soil probably results from microbial respiration of rhizodeposits (Kelting et al., 1998). Reduced priming effects in oak soils might be expected given the tight coupling between rhizosphere C flux and the soil microbial biomass reported for red oak seedlings (Cardon et al., 2002). The effects of soil fertility on priming effects have been controversial (Cheng and Kuzyakov, 2005), however, with reports of greater priming effects in both infertile soils (Cardon, 1996; Kuzyakov, 2002) and fertile soils (Hungate et al., 1997; Cheng, 1999). Moreover, it would be premature to conclude that fertilization reduced priming effects in red oak without direct evidence of fertilizer effects on decomposition in the rhizosphere.

Rhizosphere effects on net N mineralization did not respond to fertilization in yellow birch soils. Interpretation of net N mineralization is complicated by the fact that it represents the balance between two simultaneous processes, gross N mineralization and N immobilization, both of which may be better indicators of microbial activity in forest soils (Hart et al., 1994; Fisk and Fahey, 2001). In a separate forest fertilization experiment in northern hardwood forests, Fisk and Fahey (2001) reported that fertilizer-induced decreases in net N mineralization rates in bulk soil were primarily the result of increased immobilization of \( \text{NO}_3^- \). We did not measure gross rates in this study, but it is plausible that gross N mineralization was reduced in fertilized birch soils but that no changes in net N mineralization were detected due to increased N immobilization by the rhizosphere microbial biomass (Norton and Firestone, 1996). More research is needed to elucidate whether there are differences between rhizosphere and bulk soil gross N transformation rates in forest soils.

Fertilization suppressed soil microbial activity as indicated by reduced rhizosphere effects on phosphatase activity in red oak and yellow birch (Fig. 2). Although both roots and mycorrhizal fungi produce phosphatase enzymes (Dodd et al., 1987; Hinsinger, 1998), the similar response of the two ectomycorrhizal tree species to fertilization suggests that the mycorrhizal fungi may be mediating rhizosphere effects on enzyme activity in the soil. Grierson and Comerford (2000) reported that ~26% of the total phosphatase activity in the root system of loblolly pine (\textit{Pinus taeda} L.) resulted from mycorrhizal tips and unsuberized roots, and that phosphatase activity in bulk soil was minimal. Similarly, Fox and Comerford (1992) reported rhizosphere phosphatase activity in ectomycorrhizal slash pine (\textit{Pinus elliottii} Engelm.) was two to five times greater than in bulk soil. Fertilized red oak had 18% lower mycorrhizal colonization than control plots (Phillips and Fahey, 2007) and such reductions probably contributed to the large reduction (21%) in rhizosphere effects on phosphatase activity. In contrast, fertilized yellow birch had reduced rhizosphere effects on phosphatase activity, but mycorrhizal colonization was not significantly reduced (Phillips and Fahey, 2007). Perhaps birch roots rather than mycorrhizal tips were the primary source of rhizosphere phosphatase enzymes as noted for Norway spruce (Finishing and Claassen, 1996). It is also possible, however, that the reduced rhizosphere effects on phosphatase activity in birch resulted from shifts in fungal community composition (Fransson et al., 2004). Similarly, fertilized-induced shifts in mycorrhizal community composition could also have contributed to this pattern, as suggested by the significant reduction in one of the two birch ectomycorrhizal morphotypes (Phillips and Fahey, 2007).

In sugar maple plots, rhizosphere effects on phosphatase activity were unaffected by fertilization. This result was surprising given that fertilized roots had significantly lower mycorrhizal colonization than control roots, and a large percentage of rhizosphere C flux from sugar maple roots is probably mediated by arbuscular mycorrhizal hyphae (Phillips and Fahey, 2005). One possible explanation is that release of phosphatase enzymes to the rhizosphere may be less quantitatively important for P nutrition in arbuscular mycorrhizal trees than hyphal acquisition of inorganic P (Hinsinger, 1998) and thus less sensitive to changes in soil fertility. A second possible explanation is that fertilization may have stimulated and depressed different factors that control rhizosphere effects, thereby resulting in no net change; that is, if decreased C inputs from mycorrhizal hyphae in fertilized plots were offset by increased exudation from actively growing maple fine roots (Smith, 1976) or fertilizer-induced increases in glomalin (J. Aber, personal communication, 2001). A third possibility is that the fertilizer inputs were sufficient to satisfy the N but not P demands of sugar maple, thereby resulting in N-induced increases in phosphatase activity (Saiya-Cork et al., 2002). In one of two control sugar maple plots, N/P ratios in green foliage were 15.8 (data not shown), which closely approaches the Redfield value of 16, suggesting possible P limitation of sugar maple growth. Such low P availability in these soils may result, in part, from the high density of non-indigenous earthworms (Suarez et al., 2004).
CONCLUSIONS

We quantified the degree to which rhizosphere effects were influenced by changes in soil fertility to examine the potential role of roots in mediating microbial activity and nutrient availability in nutrient-poor forest soils. We hypothesized that rhizosphere effects on soil microbes and nutrient availability would be diminished under conditions of high soil fertility. Our results suggest that for some species on some sites (e.g., red oak at the THP), the rhizosphere effect on microbial activity and nutrient availability may be reduced by increases in soil fertility, and that such effects may be coupled to changes in belowground C supply. To the extent that the fertilizer-induced changes in belowground processes reflect belowground dynamics in northern hardwood forest soils, we suggest that roots probably play an important role in influencing nutrient availability in nutrient-poor forest soils through rhizosphere effects. Future studies that establish links between belowground C allocation and rhizosphere effects will advance our understanding of how plant–microbial interactions influence ecosystem processes.

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