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# Phosphorus source alters host plant response to ectomycorrhizal diversity

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**Abstract** We examined the influence of phosphorus source and availability on host plant (*Pinus rigida*) response to ectomycorrhizal diversity under contrasting P conditions. An ectomycorrhizal richness gradient was established with equimolar P supplied as either inorganic phosphate or organic inositol hexaphosphate. We measured growth and N and P uptake of individual *P. rigida* seedlings inoculated with one, two, or four species of ectomycorrhizal fungi simultaneously and without mycorrhizas in axenic culture. Whereas colonization of *P. rigida* by individual species of ectomycorrhizal fungi decreased with increasing fungal richness, colonization of all species combined increased. Plant biomass and N content increased across the ectomycorrhizal richness gradient in the organic but not the inorganic P treatment. Plants grown under organic P conditions had higher N concentration than those grown under inorganic P conditions, but there was no effect of richness. Phosphorus content of plants grown in the organic P treatment increased with increasing ectomycorrhizal richness, but there was no response in the inorganic P treatment. Phosphorus concentration was higher in plants grown at the four-species richness level in the organic P treatment, but there was no effect of diversity under inorganic P conditions. Overall, few ectomycorrhizal composition effects were found on plant growth or nutrient status. Phosphatase activities of individual ectomycorrhizal fungi differed under organic P conditions, but there was no difference in total root system phosphatase expression between the inorganic or organic P treatments or across richness levels. Our results provide evidence that plant response to ectomycorrhizal diversity is dependent on the source and availability of P.

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## Introduction

The extent to which plant productivity is influenced by biodiversity is a question that has generated much recent interest. Although most studies of this relationship have focused on aboveground plant diversity (Tilman et al. 1996; Naeem et al. 1996; Hooper and Vitousek 1997), belowground biodiversity has also been reported to play a role in influencing plant productivity (van der Heijden et al. 1998; Baxter and Dighton 2001; Jonsson et al. 2001). In particular, belowground mutualisms are important biotic components of ecosystems, their functions ranging from nutrient cycling and retention to plant community succession (Wall and Moore 1999). In natural ecosystems, ectomycorrhizal fungi are important belowground mutualists whose presence and diversity can influence plant productivity. For example, Baxter and Dighton (2001) and Jonsson et al. (2001) demonstrated that ectomycorrhizal diversity can influence plant productivity. Although ectomycorrhizal fungi are key components of terrestrial ecosystems (Smith and Read 1997), the relationship between ectomycorrhizal fungal diversity and plant productivity is not well understood.

As a group, ectomycorrhizal fungi benefit their hosts by enhancing nutrient acquisition (Finlay et al. 1989; Abuzinadah and Read 1989), drought tolerance (Parke et al. 1983), and pathogen resistance (Duchesne et al. 1989). However, individual tree species form ectomycorrhizal associations with a diverse community of fungal species in nature, each of which may differ in key physiological attributes. At the root scale, multiple ectomycorrhizal fungal species can coexist within a few centimeters on a single length of root (Gibson and Deacon 1988). Thus, individual trees may host numerous and different species of ectomycorrhizal fungi. In oak-pine forest stands in southwestern Virginia, 138 ectomycorrhizal fungal species were identified, with 45 of these undescribed (Palmer et al. 1993).

Similarly, as many as 100 different ectomycorrhizal fungal species may coexist in a single stand of Douglas fir forest (Trappe and Molina, unpublished results, cited in Allen et al. 1995). On a global scale, the diversity of ectomycorrhizal fungi is also high, with an estimated 5,000–6,000 species worldwide (Molina et al. 1992).

Ectomycorrhizal fungi differ in their physiological capacities to acquire and transfer nutrients to a range of plant hosts (Abuzinadah and Read 1989; Dighton et al. 1993; Bending and Read 1995). However, the significance of this functional diversity is unclear. One possibility is that ectomycorrhizal diversity enhances host access to a variety of nutrient sources. The differential ability of ectomycorrhizal fungi to access organic sources of nutrients is of particular interest, as these nutrient sources would otherwise be less or not available to the plant host. Because a large fraction of the phosphorus in most temperate forest soils occurs in organic forms (Harrison 1987), such as inositol phosphates, nucleic acids and phospholipids, host access to organic P sources may depend on its association with a range of ectomycorrhizal fungi that produce extracellular enzymes capable of acquiring phosphorus in organic form (Dighton 1991).

Despite their diversity and differential physiological functioning, the role of ectomycorrhizal diversity and community composition in determining plant productivity has only recently been investigated. In a study by Jonsson et al. (2001), *Betula pendula* had greater biomass production when inoculated with eight ectomycorrhizal species compared to plants inoculated with single species under low fertility conditions. Likewise, Baxter and Dighton (2001) showed that greater ectomycorrhizal diversity, rather than colonization or composition, increased mycorrhizal root biomass of *Betula populifolia* seedlings at the expense of shoot biomass and increased plant P uptake. These recent studies suggest that ectomycorrhizal diversity can influence plant productivity, but that host identity and soil conditions may influence host responses.

We tested the hypothesis that phosphorus source (inorganic vs organic) and availability alter the response of host plants to ectomycorrhizal diversity. We hypothesized that host response to diversity would be greater under organic than under inorganic phosphorus conditions due to greater reliance of the host on its mycorrhizal associates for access to limiting P. To test this hypothesis, we compared tree seedlings exposed to an experimental ectomycorrhizal richness gradient (cf. Baxter and Dighton 2001) under contrasting inorganic and organic P availability conditions. The ectomycorrhizal richness gradient consisted of nonmycorrhizal or mycorrhizal *Pinus rigida* colonized simultaneously with one, two, or four species of ectomycorrhizal fungi.

## Materials and methods

### Mycorrhizal inoculum

We used six species of ectomycorrhizal fungi in our seedling inoculations (Table 1). Fungal species differed in their growth rates and foraging strategies and were easily distinguished from one another based on color and morphology. Differences among the fungi in their growth and foraging traits suggested that our experimental "communities" would exhibit relative abundance patterns resembling those of natural ectomycorrhizal communities (i.e., dominance by one or several species). Mycorrhizal cultures were maintained on MMN agar at 25°C in the dark. Fungal inoculum was grown in peat/vermiculite (1:8, v/v) moistened with 700 ml MMN/l vermiculite. Inoculum was grown at 25°C in the dark until the medium was fully colonized. Although ectomycorrhizal fungi differed in their relative growth rates, complete colonization of the growth medium was typically achieved in 10–14 weeks.

### Seedling establishment

*Pinus rigida* Miller seedlings were established from seed collected from mature cones in Lebanon State Forest, NJ. Seeds were extracted from the serotinous cones of *Pinus rigida* by heating at 70°C for 15–20 min. Seeds were then surface sterilized for 30 min in 30% w/v hydrogen peroxide (Mason et al. 1983), rinsed with sterile water, and germinated on sterile water agar in Petri dishes at 20°C under continuous fluorescent light for 2 weeks. Seedlings were transferred under sterile conditions within 2 weeks of germination to notched 100×15-mm diameter Petri dishes containing sterile peat/vermiculite (1:8, v/v) and moistened with double-strength low-carbon MMN medium (0.1% w/v glucose), containing either 2 mg P as orthophosphate ($KH_2PO_4$) or 2 mg P as inositol hexaphosphate (dipotassium salt) per Petri dish culture; pH of the MMN medium in peat/vermiculite was 5.2. Plants were watered as needed, but no additional nutrients were added during the experiment. Petri dishes were sealed with Parafilm to reduce contamination and desiccation. Seedlings were maintained in a growth chamber at a day/night regime of 16 h, 22°C/8 h, 18°C at a photon flux density of 150 μmol $m^{-2}$ $s^{-1}$ until inoculation.

**Table 1** Code designation, original host, and state of origin of the six ectomycorrhizal isolates used in this study

| Code | Species | Isolate | Host | Origin |
|---|---|---|---|---|
| AF | *Amanita flavorubescens* Atkinson | AF96-01 | *Pinus rigida* | NJ |
| AL | *Amanita longipes* Bas | AL99-01 | *Pinus rigida* | NJ |
| CG | *Cenococcum geophilum* Fr. | Cege A-145 | *Pseudotsuga mensiezii* | OR |
| LL | *Laccaria laccata* (Scop. ex Fr.) Berk. | LL98-01 | *Pinus rigida* | NJ |
| PB | *Piloderma bicolor* (Peck) Jülich | VT-2331 | *Picea rubens* | VA |
| PT | *Pisolithus tinctorius* (Pers.) Coker et Couch | VT-3303 | *Pinus strobus* | VA |

## Mycorrhizal diversity experiment

To examine whether plant response to ectomycorrhizal diversity was influenced by P source, *Pinus rigida* seedlings were inoculated with a gradient of ectomycorrhizal richness from none to four species under contrasting P availability conditions. Nonmycorrhizal controls consisted of eight replicate seedlings that were inoculated with a previously sterilized inoculum. For the one-species treatment, each of the six fungal species used was inoculated onto eight replicate seedlings. Inoculation of each mycorrhizal species alone allowed us to address the potential for "sampling effect," in which diversity responses may be confounded by the influence of dominant species in the mixture (Wardle 1999). To construct the two- and four-species richness treatments, six pair-wise and six four-wise combinations of the selected ectomycorrhizal fungi were determined at random with no species used twice in the same combination. Table 2 lists the pair-wise and four-wise species combinations used. Each species combination was replicated eight times. Thus, each set of pair-wise and four-wise treatment replicates was unique in species composition but had the same richness. We successfully employed this approach in a prior experiment to distinguish the effect of ectomycorrhizal species richness from community composition (Baxter and Dighton 2001). To compare the effect of phosphorus source on host responses to diversity, half of the seedling replicates received phosphorus as orthophosphate and half as inositol hexaphosphate. This design resulted in 152 experimental seedlings.

Seedling inoculations were accomplished by adding a total of ~5-g fungal inoculum under sterile conditions to each seedling–Petri dish culture. To control for potential differences in inoculum amount and placement across the richness treatments (including nonmycorrhizal controls), each seedling was inoculated by placing ca. 1.25-g inoculum at each of four equidistant points from the origin of the seedling root system (Fig. 1). In the pair-wise and four-wise richness treatments, placement of inoculum was randomized to reduce bias from potential negative interactions among the fungal species initially and to control for possible inoculum positioning effects. After inoculation, seedlings were returned to the growth chamber in a completely randomized design and periodically moistened with filter-sterilized deionized water.

**Table 2** Codes and keys to the two- and four-species combinations of ectomycorrhizal fungi used to inoculate *P. rigida* seedlings

| Code | Pair-wise[a] | Code | Four-wise[a] |
|---|---|---|---|
| A | PT+CG | G | LL+AL+CG+AF |
| B | PT+PB | H | PT+LL+CG+PB |
| C | AL+CG | I | PT+LL+CG+AF |
| D | LL+PB | J | PT+AL+CG+PB |
| E | LL+AF | K | PT+LL+AL+PB |
| F | LL+AL | L | LL+CG+AF+PB |

[a]Species abbreviations are given in Table 1

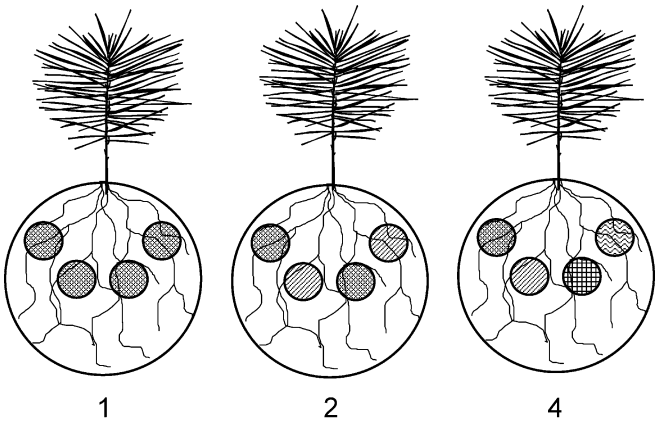


**Fig. 1** Diagram showing the approximate placement of ectomycorrhizal inocula in the one-, two-, and four-species diversity treatments. *Circles* with different fill patterns indicate inocula from distinct fungal species. Total inoculum volume (total circle area per treatment) was the same across the treatments. *1* One-species treatment, *2* two-species treatment, *4* four-species treatment

Seedlings were harvested after 16 weeks. Plant root systems were separated from the shoots, gently cleaned of the growth medium, and placed in tap water. Mycorrhizal colonization was determined by quantifying the total number of ectomycorrhizal root tips of each fungal species observed on a seedling root system and expressed on a root length basis. Root length was determined using the grid-intercept method of Tennant (1975). Biomass of shoots and mycorrhizal roots was then determined on plant material dried for 48 h at 70°C and weighed. For determination of N and P status, shoots and mycorrhizal roots were ground in a Wiley Mill at 40 mesh and digested in concentrated sulfuric acid/hydrogen peroxide (Allen 1989). Plant tissue N was determined using an $NH_4^+$-sensitive probe (Orion Research Inc., Boston, MA, USA). Tissue P was determined by the ascorbic acid method (Hach Company, Loveland, CO, USA).

Root surface acid phosphomonoesterase (phosphatase) activities were determined on individual ectomycorrhizal root tips for each fungal species in both P treatments and at the one- and two-species mycorrhizal richness levels. Phosphatase assays were conducted using a method described by Antibus et al. (1997), modified for root tips. Six to ten excised root tips colonized by each fungal species were collected from three replicates of each P treatment at the two diversity levels. Excised root tips were added to 2 ml of 50 mM acetate buffer, pH 5.0, to which was added 0.5 ml of 50 mM *p*-nitrophenyl phosphate (*p*NPP), pH 5.0. Assays, plus controls without roots, were incubated at 22–24°C for 1 h. Release of *p*-nitrophenol phosphate (*p*NP) was measured by adding 0.5 ml of reaction mix to 4.5 ml of 0.5 M NaOH and reading absorbance at 410 nm. Release of product was calculated from a standard curve using 0.5 ml *p*NP (10, 20, 50, 100, 200, and 500 μmol $ml^{-1}$) in 4.5 ml NaOH. Excised root tips were then dried at 70°C and weighed. Phosphatase activity was expressed as μmol *p*NP $mg^{-1}$ root $h^{-1}$. Total mycorrhizal root phosphatase activity was calculated by extrapolating the phosphatase activities of the individual fungal species to the entire ectomycor-

rhizal root system. This was done by summing the product of the per mass individual root tip activity (μmol *p*NP $mg^{-1}$ root tip) and the total mass (mg) of root tips for each fungal species present on the root system.

### Data analysis

To test whether phosphorus availability affected host response to mycorrhizal diversity or total phosphatase expression, we used a mixed-model nested factorial design. In the model, phosphorus source and mycorrhizal richness were fixed effects, and mycorrhizal composition was nested within richness. We used Tukey's studentized range (HSD) test to distinguish among means. Variables that did not meet the criterion of homogeneity of variances were transformed prior to statistical analyses. All statistical analyses were conducted using Statistical Analysis Software (SAS Institute Inc. 1990).

## Results

### Mycorrhizal colonization

Mycorrhizal fungi successfully established on 88% of plants in the single species treatment under inorganic P conditions and 83% of plants in the organic P conditions. Unsuccessful colonization in the inorganic P treatment was due to failed colonization by *Amanita longipes*, whereas in the organic P treatment, several species failed to colonize in one or two instances. Of plants inoculated with two mycorrhizal species, 50 and 54% of plants in the inorganic and organic P treatment, respectively, were colonized by both species at the end of the experiment. In the four-species richness treatment, a few plants were colonized by all four mycorrhizal species. In the inorganic P treatment, 42% of plants were colonized by three species and 8% colonized by all four species. This dropped to 21% of plants colonized by three species in the organic P treatment, with no plants colonized by all four fungal species.

Colonization density of fungi differed when mycorrhizal species occurred alone in both the inorganic and organic P treatments, though observed differences occurred primarily in the inorganic P treatment (Table 3). In the inorganic P treatment, *Pisolithus tinctorius* showed the highest colonization densities of the six fungal species, followed by *Amanita flavorubescens* and *Cenococcum geophilum*. Although there were differences in mean colonization density among mycorrhizal species occurring alone in the organic P treatment, the only significant difference was between *Pisolithus tinctorius* and *A. longipes*. However, mycorrhizal colonization did not differ significantly for individual fungal species between the inorganic and organic P treatments. We observed no cross-contamination within the experiment or contamination by mycorrhizal fungi or microfungi from outside sources.

In the multiple fungal species treatments, a significant competitive reduction in colonization of some fungal species occurred (Table 3). Reduced colonization density of individual fungi in the presence of competing species occurred to a greater degree in the inorganic than in the organic P treatment. Five of the six fungal species in the inorganic P treatment showed significantly lower colonization rates in the presence of competing fungal species, whereas in the organic P treatment, only two species showed a competitive reduction. Although *A. longipes* generally exhibited overall lower colonization densities than the other fungal species, its colonization rate was significantly higher in the four-species vs the two-species diversity level in the inorganic P treatment; we observed no such pattern in the organic P treatment for any other species.

Overall, fungal colonization density did not differ significantly between the phosphorus treatments or with respect to mycorrhizal richness (Table 4). However, colonization rates of individual fungal species and combinations within each richness level differed across the richness gradient, as indicated by a significant nesting effect in the overall analysis. We also observed no overall differences in fungal colonization with respect to mycorrhizal richness within inorganic and organic phosphorus treatments. However, there was a significant difference in fungal colonization

**Table 3** Colonization density (EM root tips $m^{-1}$ root) (±1 SE) of each ectomycorrhizal species occurring alone and with one or three other species under contrasting inorganic and organic P conditions

| Treatment | Fungal species† | Richness treatment* | | |
|---|---|---|---|---|
| | | 1 | 2 | 4 |
| Inorganic P | *P. tinctorius* | $357.7^{1a}$ (44.5) | $197.1^{2}$ (17.7) | $170.7^{2}$ (22.5) |
| | *A. flavorubescens* | $213.5^{1b}$ (29.9) | $125.6^{12}$ (89.9) | $22.1^{2}$ (11.2) |
| | *C. geophilum* | $134.3^{1bc}$ (11.1) | $62.8^{12}$ (24.4) | $16.3^{2}$ (3.6) |
| | *L. laccata* | $91.0^{1bcd}$ (22.7) | $31.0^{1}$ (10.6) | $6.7^{2}$ (3.7) |
| | *P. bicolor* | $87.5^{1cd}$ (33.6) | $4.9^{2}$ (3.5) | $3.5^{2}$ (1.7) |
| | *A. longipes* | $0.4^{12d}$ (0.4) | $3.6^{2}$ (1.5) | $8.0^{1}$ (3.6) |
| Organic P | *P. tinctorius* | $244.0^{1a}$ (56.1) | $157.5^{1}$ (50.7) | $263.4^{1}$ (44.5) |
| | *A. flavorubescens* | $141.5^{1a}$ (82.6) | $200.2^{1}$ (58.3) | $51.6^{1}$ (26.9) |
| | *C. geophilum* | $168.1^{1a}$ (78.7) | $137.6^{1}$ (39.9) | $14.1^{2}$ (6.9) |
| | *L. laccata* | $46.7^{1a}$ (20.6) | $46.1^{1}$ (10.0) | $23.2^{1}$ (8.1) |
| | *P. bicolor* | $154.7^{1a}$ (63.0) | $48.4^{1}$ (15.8) | $10.0^{2}$ (8.1) |
| | *A. longipes* | $2.4^{1b}$ (1.3) | $2.9^{1}$ (2.9) | $0.0^{1}$ (0.0) |

*Colonization densities of fungal species within rows having the same superscript numbers are not significantly different at $P<0.05$

†Colonization densities of fungal species within *P* treatments and within the 1 species richness column having the same superscript letters do not differ significantly at $P<0.05$

**Table 4** Effect of phosphorus source (P) and ectomycorrhizal richness (R) on overall and within treatment mycorrhizal colonization density (EM tips $m^{-1}$ root) as indicated by *F* values[a] derived from ANOVA[b]

| Colonization density | P | R | P×R interaction | C(R)[b] | P×C(R) interaction |
|---|---|---|---|---|---|
| Overall | 0.31 | 0.93 | 2.38 | 5.88* | 0.87 |
| Within P treatment | | | | | |
| Inorganic P treatment | – | 0.04 | – | 5.69* | – |
| Organic P treatment | – | 2.71 | – | 1.86 | – |

[a]Degrees of freedom for *F* are $F_{(1,15)}$ for P, $F_{(2,15)}$ for R, $F_{(2,15)}$ for P×R, $F_{(15,106)}$ for overall C(R), $F_{(15,54)}$ for inorganic and organic treatments C(R), and $F_{(15,106)}$ for P×C(R)
[b]Overall ANOVA is a two-way mixed model nested factorial design, where P and R are fixed factors and mycorrhizal composition (C) is nested within R. Within P treatment ANOVAs are one-way mixed-model nested designs
*$F$ value significantly different from zero at $P<0.001$

density with respect to mycorrhizal composition; the effect of composition was observed only in the in-organic P treatment.

## Plant response to mycorrhizal richness

Phosphorus source had a significant effect on the growth of *Pinus rigida* seedlings and on their response to ecto-mycorrhizal richness. The response of total plant biomass to mycorrhizal richness differed between inorganic and organic P conditions, as indicated by a significant phosphorus × richness interaction (Table 5). Total plant biomass was significantly lower in the organic than the inorganic P treatment, but only under organic P conditions was there a significant plant growth response to increasing mycorrhizal richness (Fig. 2). Under organic P conditions, total plant biomass increased from the nonmycorrhizal treatment up to the two- and four-species richness levels. No difference in plant biomass was observed between the two- and the four-species richness treatments. The increase in total plant biomass with increasing mycorrhizal diversity in the organic P

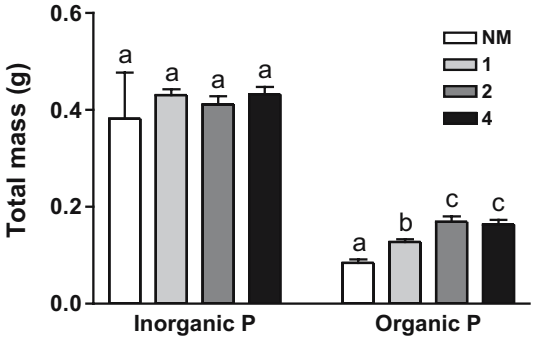


**Fig. 2** Total biomass (g dry mass) of *P. rigida* grown in either inorganic or organic P conditions colonized with one (*1*), two (*2*), or four (*4*) species of mycorrhizal fungi or nonmycorrhizal (*NM*) (mean± SE). Significant differences ($P<0.05$) among diversity levels within phosphorus treatments are indicated by *different letters*

treatment was due to a significant increase in shoot but not root growth across the diversity gradient (data not shown).

Plant nutrient status also showed significant differences between the phosphorus treatments and with respect to ecto-mycorrhizal richness. The response of plant N content and N concentration to mycorrhizal richness differed with phosphorus treatment, as indicated by the significant phosphorus × richness interactions (Table 5). Whole plant N content in the organic P treatment was significantly lower than in the inorganic P treatment. However, the organic treatment showed a significant increase in N content with increasing mycorrhizal richness (Fig. 3a), whereas there was no response to richness in the inorganic P treatment. Plants grown in organic P had significantly higher N concentrations than those grown in inorganic P (Fig. 3b), but there was no overall richness effect (Table 5). A significant phosphorus × richness interaction indicated that plant N concentration in the two phosphorus treatments differed in their response to ectomycorrhizal richness. Under inorganic P conditions, there was a small increase in N concentration in the two-species vs the one-species mycorrhizal treatment, with no other differences. In the organic P treatment, plant N concentration was significantly higher in mycorrhizal than nonmycorrhizal controls; however, no differences were observed among mycorrhizal plants.

Whole plant P content and P concentration differed significantly between phosphorus treatments and with respect

**Table 5** Effect of phosphorus source (P) and ectomycorrhizal richness (R) on total plant biomass (g dry mass) and total N and P status as indicated by *F* values[a] derived from ANOVA[b]

| Response variable | P | R | P×R interaction | C(R)[b] | P×C(R) interaction |
|---|---|---|---|---|---|
| Total biomass | 1,104*** | 4.29* | 7.35** | 0.65 | 0.40 |
| N concentration (% of dry mass) | 452*** | 2.88 | 5.34* | 1.94 | 0.84 |
| N content (mg) | 100*** | 17.9*** | 8.09** | 1.55 | 1.19 |
| P concentration (% of dry mass) | 28.4*** | 12.5*** | 2.98 | 0.64 | 1.39 |
| P content (mg) | 328*** | 15.2*** | 1.40 | 1.18 | 2.36** |

[a]Degrees of freedom for *F* are $F_{(1,15)}$ for P, $F_{(3,15)}$ for R, $F_{(3,15)}$ for P×R, $F_{(15,108)}$ for C(R), and $F_{(15,108)}$ for P×C(R)
[b]ANOVA is a mixed-model nested factorial design, where P and R are fixed factors and mycorrhizal composition (C) is nested within R
*$F$ value significantly different from zero at $P<0.05$
**$F$ value significantly different from zero at $P<0.01$
***$F$ value significantly different from zero at $P<0.001$

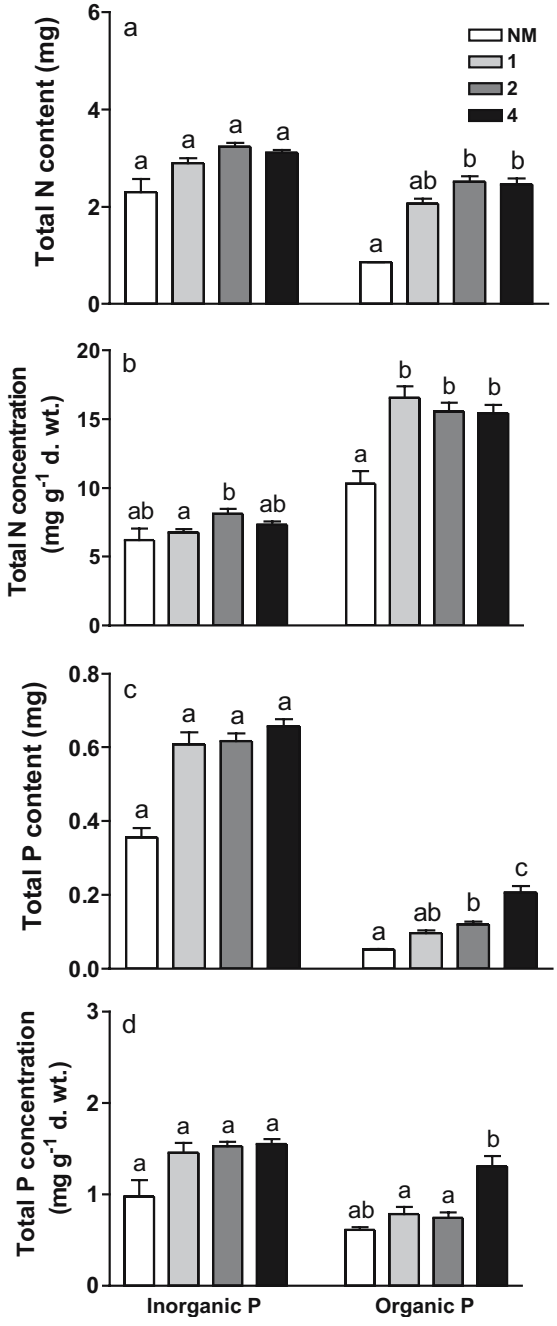


**Fig. 3** Total N and P uptake by *P. rigida* grown in either inorganic or organic P conditions colonized with one (*1*), two (*2*), or four (*4*) species of mycorrhizal fungi or nonmycorrhizal (*NM*) (mean±SE). **a** Total N content (mg); **b** total N concentration (mg $g^{-1}$ dry wt.); **c** total P content (mg); **d** total P concentration (mg $g^{-1}$ dry wt.). Significant differences ($P<0.05$) among diversity levels within phosphorus treatments are indicated by *different letters*

to mycorrhizal richness. Plant P content was significantly higher in the inorganic than in the organic P treatment, and there was a significant overall effect of richness (Table 5). The significant overall richness effect was due to a significant increase in plant P content with increasing mycorrhizal richness up to four species in the organic P treatment (Fig 3c). There were no significant differences in plant P content with respect to richness treatment in the inorganic P treatment. Plant P concentration was significantly higher in the inorganic than in the organic P treatment and exhibited a significant overall richness effect (Table 5). Within the inorganic P treatment, there were no significant differences in P concentration across the richness gradient (Fig. 3d). However, there was a significant increase in plant P concentration between the one- and two-species richness treatments vs the four-species treatment.

## Plant response to mycorrhizal composition

We found no differences in whole plant biomass among individual mycorrhizal species or among distinct mycorrhizal species combinations in the two- and four-wise richness treatments in either the inorganic or the organic P treatment (data not shown). However, in the inorganic P treatment, plants colonized by *Piloderma bicolor* had significantly higher ($P<0.05$) shoot biomass than those colonized by *Pisolithus tinctorius*. No other significant differences in shoot or root biomass among plants colonized by different mycorrhizal species or among distinct mycorrhizal combinations were observed.

Differences in plant nutrient uptake with respect to ectomycorrhizal composition were limited to differences between mycorrhizal and nonmycorrhizal plants. However, in one case in the inorganic P treatment, plants colonized by *Piloderma bicolor* alone had significantly ($P<0.05$) higher N content (i.e., total N uptake) than plants colonized by either *Pisolithus tinctorius* or *A. longipes* (Fig. 4a). Otherwise, we observed no significant differences in N or P uptake among plants colonized by different mycorrhizal species (i.e., single species treatments) or among plants colonized by distinct species compositions (i.e., two- and four-species treatments) (Figs. 4, 5).

There were a few differences in N uptake (i.e., content) between mycorrhizal and nonmycorrhizal plants under inorganic P conditions across the mycorrhizal richness gradient (Fig. 4a–c). At the one-species richness level, plants colonized by *Piloderma bicolor* (PB) had significantly higher N content than nonmycorrhizal controls, whereas at the two-species level, plants colonized by combination B (PT and PB) and combination C (AL and CG) had significantly higher N content than nonmycorrhizal controls. We found no significant differences in N content between mycorrhizal and nonmycorrhizal plants at the four-species richness level. Under organic P conditions, increases in plant N content in mycorrhizal vs nonmycorrhizal plants became more pronounced at higher mycorrhizal richness (Fig. 4d–f). At the one-species richness level, plants colonized by *Laccaria laccata* (LL) had significantly higher N content than nonmycorrhizal controls. Plants colonized by mycorrhizal combination B (PT and PB) and E (LL and AF) at the two-species richness level also had significantly higher N content than nonmycorrhizal controls. This pattern was most pronounced at the four-species richness level, where plants colonized by four different mycorrhizal combinations (G–J) had significantly higher plant N content

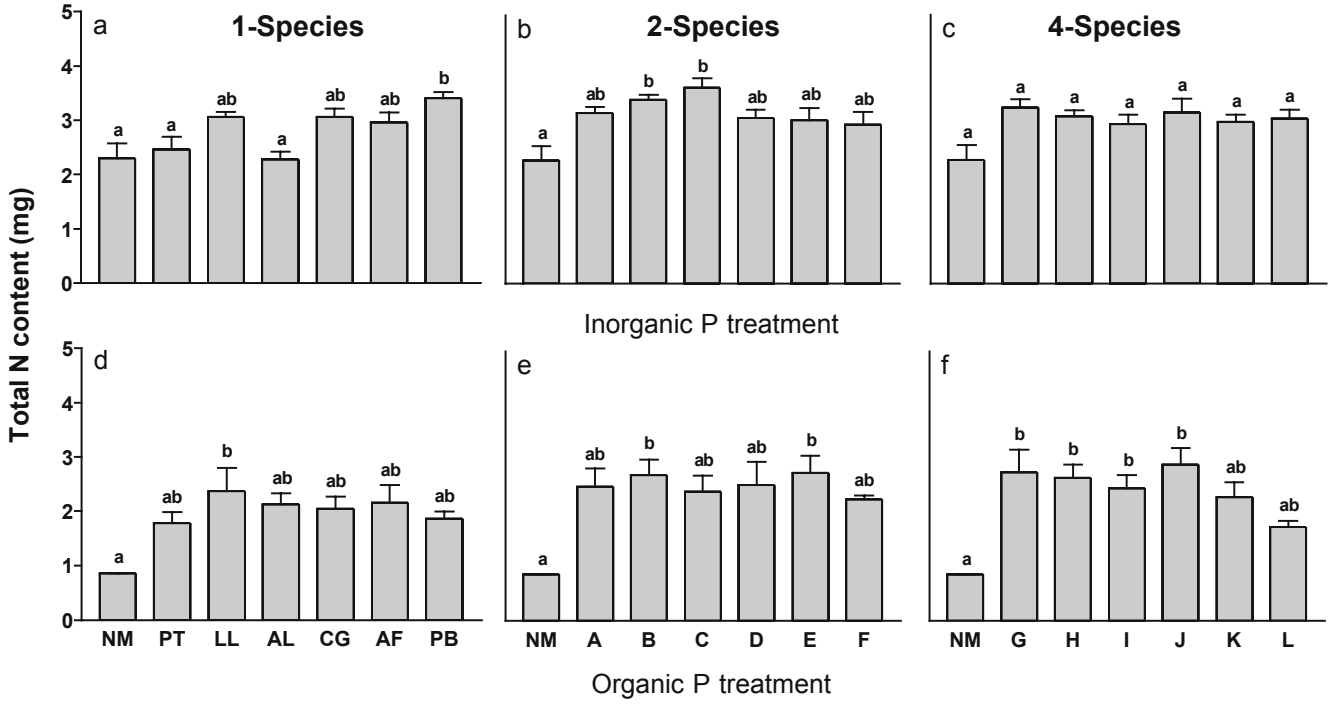


**Fig. 4** Nitrogen content (mg N) of *P. rigida* for each mycorrhizal species combination in the three diversity treatments under either inorganic or organic P conditions (mean±SE). **a**–**c** Inorganic P treatment; **d**–**f** organic P treatment. Significant differences ($P<0.05$) among species combinations are indicated by *different letters*

than nonmycorrhizal controls (Fig. 4f). Thus, where differences in plant N content did occur between mycorrhizal and nonmycorrhizal controls, they were observed under organic P conditions at higher levels of mycorrhizal richness.

Differences in P uptake (i.e., content) between mycorrhizal and nonmycorrhizal plants across the mycorrhizal richness gradient for the inorganic and organic P treatments occurred almost exclusively under inorganic P conditions

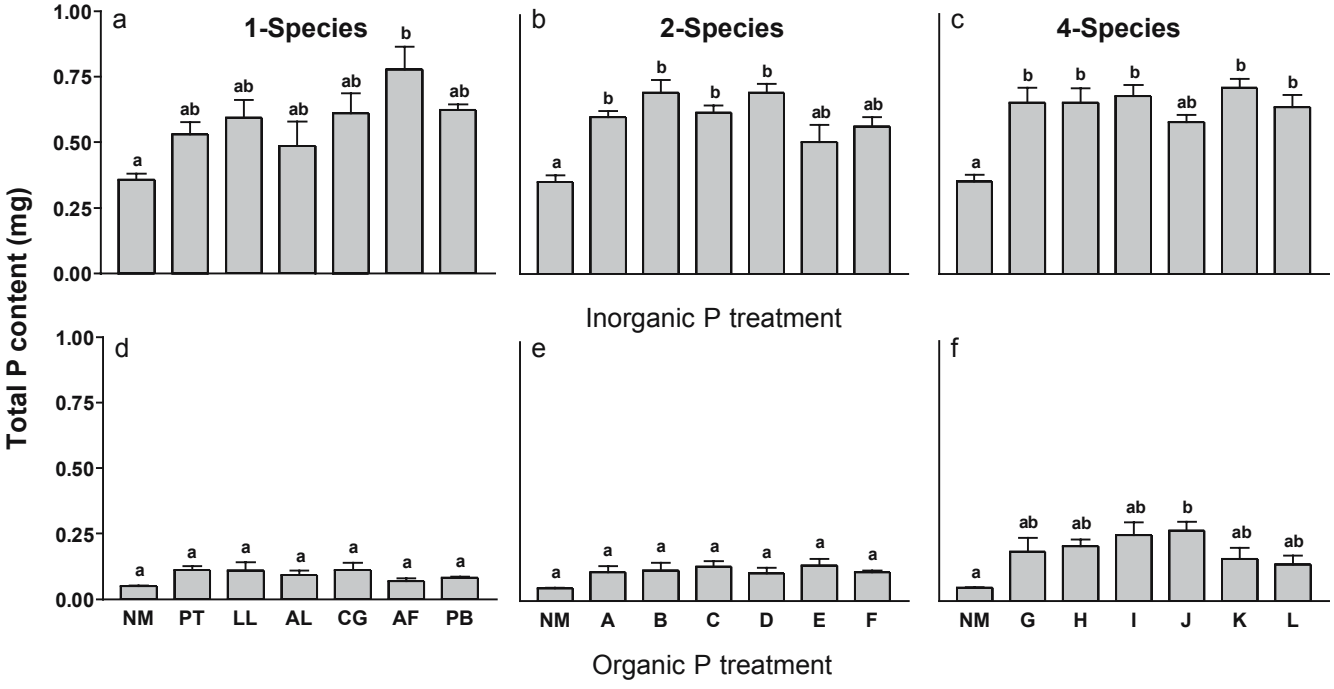


**Fig. 5** Phosphorus content (mg P) of *P. rigida* for each mycorrhizal species combination in the three diversity treatments under either inorganic or organic P conditions (mean±SE). **a**–**c** Inorganic P treatment; **d**–**f** organic P treatment. Significant differences ($P<0.05$) among species combinations are indicated by *different letters*

**Table 6** Mean (±1 SE) surface acid phosphatase activities (μmol *p*NP $mg^{-1}$ $h^{-1}$) of individual root tips of ectomycorrhizal fungi collected from the inorganic (IP) and organic (OP) P treatments at the one- and two-species mycorrhizal richness levels

| Fungal species | One species | | | Two species | | |
|---|---|---|---|---|---|---|
| | IP† | OP | *F* value‡ | IP | OP | *F* value |
| *P. tinctorius* | $0.07^{a}$ (0.01) | $0.21^{ab}$ (0.05) | 8.50* | $0.09^{a}$ (0.02) | $0.15^{a}$ (0.03) | 2.16 |
| *L. laccata* | $0.18^{a}$ (0.05) | $0.17^{a}$ (0.04) | 0.05 | $0.18^{a}$ (0.03) | $0.28^{a}$ (0.05) | 2.68 |
| *A. longipes* | $0.72^{b}$ (0.07) | $0.55^{c}$ (0.10) | 5.39 | $1.35^{b}$ (0.19) | $1.08^{b}$ (0.73) | 0.34 |
| *C. geophilum* | $0.04^{a}$ (0.006) | $0.07^{a}$ (0.01) | 4.89 | $0.05^{a}$ (0.02) | $0.10^{a}$ (0.02) | 2.53 |
| *A. flavorubescens* | $0.27^{a}$ (0.09) | $0.24^{abc}$ (0.06) | 0.09 | $0.18^{a}$ (0.04) | $0.15^{a}$ (0.06) | 0.31 |
| *P. bicolor* | $0.12^{a}$ (0.03) | $0.49^{bc}$ (0.08) | 17.76* | $0.07^{a}$ (0.007) | $0.33^{a}$ (0.06) | 12.13* |

†Phosphatase activities of individual fungal species within *P* treatment columns followed by the same superscript letters do not differ significantly at ($P<0.05$)
‡*F* values are for the comparison of phosphatase activities between IP and OP treatments within richness levels for each fungal species
*means are significantly different at $P<0.05$

(Fig. 5a–c). In the inorganic P treatment, at the one-species richness level, plants colonized by *A. flavorubescens* had significantly higher P content than nonmycorrhizal controls. At the two- and four-species richness levels under inorganic P conditions, combinations A, B, C, and D and combinations G, H, I, K, and L, respectively, had significantly higher plant P content than nonmycorrhizal controls. Only at the four-species richness level in the organic P treatment did we observe a mycorrhizal vs nonmycorrhizal effect on plant P content (Fig. 5d–f); this effect occurred for combination J, which had significantly higher plant P content than nonmycorrhizal controls. Consequently, differences in plant P content between distinct mycorrhizal compositions and nonmycorrhizal controls occurred to a greater degree in the inorganic than in the organic P treatment.

### Phosphatase activities

Differences in phosphatase activities among fungal species occurred in each of the P treatments and at both the one- and two-species mycorrhizal richness levels, but the differences were most pronounced under organic P conditions at the one-species richness level (Table 6). Regardless of treatment, *A. longipes* consistently showed the highest phosphatase activity. Only in the organic P treatment at the one-species level did we observe differences in phosphatase activities among any other species. The differences in this treatment combination were due to significant increases in phosphatase activity of *Pisolithus tinctorius* and *Piloderma bicolor* over that of the inorganic P treatment (Table 6, see *F* values). At the two-species richness level, *Piloderma bicolor* also showed a significant increase in phosphatase activity under organic vs inorganic P conditions. We also observed significantly higher phosphatase activity of *A. longipes* under inorganic P conditions when it was paired with another species than when it occurred alone. No other differences in phosphatase activity were observed between the one- and two-species richness levels. Total phosphatase expression by ectomycorrhizal roots did not differ between phosphorus treatments ($F=0.65$; $P=0.433$) or with respect to ectomycorrhizal richness within either the inorganic ($F=0.20$; $P=0.819$) or organic P treatment ($F=2.29$; $P=0.136$).

## Discussion

Our study provides support for the hypothesis that phosphorus source and availability can alter host plant response to ectomycorrhizal diversity. That growth and nutrient uptake of *Pinus rigida* seedlings exposed to an experimental ectomycorrhizal richness gradient was greater under organic than under inorganic P conditions suggests that ectomycorrhizal diversity can enhance plant access to organically bound nutrients and increase plant productivity under nutrient-limiting conditions. This is consistent with a study by Jonsson et al. (2001) in which growth of *B. pendula* was greater when inoculated with eight ectomycorrhizal species compared to plants inoculated with single species under low fertility (nitrogen) conditions. Our results also suggest that ectomycorrhizal composition was not an important factor in determining host responses regardless of P source. We observed similar results in a previous study of the effects of ectomycorrhizal diversity on plant productivity in *B. populifolia* seedlings (Baxter and Dighton 2001). In addition, our study indicates that differential phosphatase expression by individual fungi under contrasting P conditions could not explain the context-dependent diversity responses we observed.

We used a simplified plant–mycorrhizal system to examine the effect of organic phosphorus on host plant response to ectomycorrhizal diversity. Although this highly controlled approach placed limits our ability to generalize, it allowed us to minimize environmental variability and isolate the effects of phosphorus source and mycorrhizal diversity on plant performance. Because we randomly assigned species to replicate ectomycorrhizal "communities" of varying diversity and included single species mycorrhizal treatments for comparison, we can also explain host responses by changes in mycorrhizal richness and composition rather than sampling effect (Wardle 1999).

Differences in colonization rates among fungal species in the inorganic but not the organic P treatment may have been

due to competition for phosphorus between the plant and its associated fungi. Phosphorus that was readily available to both plant roots and fungi may have resulted in more intense competition among ectomycorrhizal species in the inorganic P treatment. Increased carbon allocation to roots under these competitive conditions could have reduced any benefit to the host of ectomycorrhizal diversity. This is consistent with a study by Baxter and Dighton (2001) in which shoot growth of *B. populifolia* seedlings under similar inorganic nutrient conditions decreased at higher levels of ectomycorrhizal richness. In a study of ectomycorrhizal pine, Rygiewicz and Anderson (1994) observed a shift in carbon allocation to fine roots and fungal hyphae, with a concomitant increase in host root and fungal respiration. Whether C allocation to roots increased at higher mycorrhizal richness under inorganic P conditions remains unclear. By comparison, limited phosphorus availability in the organic P treatment may have reduced competition between plant roots and mycorrhizal fungi and slowed their growth rates. Indeed, we observed that mycorrhizal growth rates were significantly lower in organic vs inorganic P conditions in Petri dish culture (Baxter and Kaszas, unpublished results).

Lower fungal colonization rates by individual fungi at higher treatment levels of ectomycorrhizal richness under inorganic P conditions were likely due to competitive reduction (and possibly competitive exclusion) of some fungal species. Competitive reduction in colonization at higher species richness has been observed in previous studies of host response to ectomycorrhizal diversity (Jonsson et al. 2001; Baxter and Dighton 2001) and suggests that under inorganic P conditions, there was considerable niche overlap among competing fungi. Fewer instances of competitive reduction in the organic P treatment suggest that under these conditions, fungal species experienced less intense competition. This may be due to differences in the ability of some fungal species (e.g., *Piloderma bicolor*) to access the organic P source we used in this study. Indeed, the fungal species in this study differed markedly in their phosphatase activities and hence in their potential to mineralize organically bound P. This is consistent with other studies reporting differential phosphatase expression and organic P mineralization potential among ectomycorrhizal species (Kroehler et al. 1988; Antibus et al. 1992). Indeed, Conn and Dighton (2000) provided preliminary evidence associating differences in phosphatase expression by ectomycorrhizal fungi and mycorrhizal community development on leaf litter patches of different resource qualities. Yet, although species-specific differences in phosphatase expression suggest that some ectomycorrhizal fungi are better competitors for organic P than are others, the relationship between phosphatase expression and access to organic P in soils remains tentative (Tibbett 2002).

That plant growth was lower under organic than inorganic P conditions suggests that P was limiting to plant growth in the organic P treatment. Because conifers tend to accumulate nitrogen under P-limiting conditions, high shoot N concentrations (8.5–24.6 mg $g^{-1}$ dry wt.) and N/P ratios (14–98) of *Pinus rigida* grown in the organic P treatment further indicate that phosphorus was the growth-limiting factor under these conditions (Ingestad and Kähr 1985). Despite lower production by plants grown under limiting organic P conditions, these plants showed a positive growth response to increasing ectomycorrhizal richness. Thus, this study supports the ability of ectomycorrhizal fungi to enhance host access to P from organic sources.

Enhanced growth and nutrient uptake of mycorrhizal vs nonmycorrhizal plants under organic P conditions suggest that ectomycorrhizal fungi facilitated host uptake of organic phosphorus. This is consistent with a study by Perez-Moreno and Read (2000) in which biomass and P concentration increased in mycorrhizal seedlings grown with supplemented litter vs those without litter. Likewise, ectomycorrhizal fungi have been shown to increase plant uptake of P via access to patchy organic nutrient sources (Tibbett and Sanders 2002). Yet, some studies have not observed uptake or transfer of P to the host from organic sources (e.g., Colpaert et al. 1997), perhaps due to different conditions or species used. Ectomycorrhizal fungi are known to release nonspecific surface acid phosphatases capable of hydrolyzing a variety of organic phosphorus compounds (Dighton 1991). Differences in phosphatase activity among our fungi and increased phosphatase expression under organic P conditions for some fungal species suggest that physiological differences in enzyme expression among the fungal species may play a role in the response of *Pinus rigida* to our ectomycorrhizal gradient under organic P conditions. This has important implications for the functional role of ectomycorrhizal diversity and community structure in relation to plant productivity.

Little variation in plant response to compositional shifts in our experimental ectomycorrhizal communities under either P treatment suggests that fungal composition was not an important factor for plant growth and nutrient uptake under these conditions. Similar results were observed in a previous study under inorganic P conditions in which *B. populifolia* exposed to an ectomycorrhizal richness gradient showed little response to individual species or species combinations (Baxter and Dighton 2001). Given differences in surface acid phosphatase production among the ectomycorrhizal species in this study, it was surprising that no compositional effects were observed under organic P conditions. While this could be explained by the similar overall production of phosphatase across richness treatments, it may also suggest that the phosphatase assay—which degrades a relatively unstable and artificial compound (i.e., *p*NPP)—was not a reliable indicator of P acquisition from the stable organic P compound inositol hexaphosphate (Tibbett 2002). Whether the host can influence the total phosphatase production of its mycorrhizal root system by regulating ectomycorrhizal composition or colonization is an intriguing possibility that should be explored in future studies.

The relative role of resource partitioning vs niche overlap may be an important factor in determining the magnitude of diversity-functioning relationships in ecological systems in general (Lawton and Brown 1994; Hooper and Vitousek 1997). Because soils are heterogeneous mixtures of a wide range of nutrient sources and ectomycorrhizal species differ

in their physiological abilities to access many organically bound nutrients, the balance between resource partitioning and niche overlap may also determine the strength of diversity-functioning relationships in plant–mycorrhizal associations (Jonsson et al. 2001). Given greater host reliance on its mycorrhizal symbionts under organic P conditions, increased functional diversity at higher levels of ectomycorrhizal richness may have played a role in enhancing plant performance in this study. Although nutrient conditions in our plant–mycorrhizal system were homogeneous, differences in the physiological capacity of individual fungal species to access organically bound P may have enhanced the positive diversity response we observed in the organic P treatment.

Together, our results provide evidence for a context-dependent relationship between ectomycorrhizal diversity and host plant performance. Context-dependent differences in host responses to ectomycorrhizal richness suggest that the ability of ectomycorrhizal fungi to enhance plant nutrient acquisition—and thereby productivity—depends on an interaction between the functional attributes of the ectomycorrhizal assemblage and the source and host availability of soil nutrients. Given the importance and heterogeneous nature of organic nutrient stores as potential sources of essential nutrients for plants in forest soils (Farley and Fitter 1999), the degree to which plants can effectively access these organic nutrient pools will have an important impact on forest productivity. In this study, we showed that the benefit to plants of ectomycorrhizal diversity is dependent on the availability of organic phosphorus to the host through a diverse fungal community. Thus, ectomycorrhizal diversity may operate as an important determinant of forest productivity in soils relatively low in mineral nutrients but high in organic matter content. The greatest host benefit of ectomycorrhizal diversity should therefore exist in cool temperate and boreal forest ecosystems, where ectomycorrhizal fungi are dominant (Read 1991). If ectomycorrhizal diversity is influenced by soil resource heterogeneity, as has been suggested (e.g., Bruns 1995; Conn and Dighton 2000), this highlights the importance of soil nutrient heterogeneity in determining host reliance on mycorrhizal diversity in natural ecosystems. Hence, the benefit to the plant host of increased ectomycorrhizal diversity appears to derive from an integration of host nutrient acquisition capacity and soil substrate heterogeneity. Based on our study, determining the nature of the relationship between ectomycorrhizal diversity and plant productivity will require a mechanistic understanding of the role of physiological differences among ectomycorrhizal fungi and how these differences relate to host feedbacks and soil nutrient heterogeneity.

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