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Root surface phosphatase activities and uptake of ^{32}P-labelled inositol phosphate in field-collected gray birch and red maple roots

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Abstract This study examined select, naturally-occurring tree mycorrhizae for differences related to efficiency of organic phosphorus hydrolysis in forest soils. We investigated the activity of several phosphatases and root respiration in field-collected ectomycorrhizae of American beech and gray birch and VAM of red maple. Root materials were collected in the early and late growing season from a common soil type. American beech occurred in a late-successional stand, whereas gray birch and red maple grew in a mid-successional stand. All of the root types examined had phosphatase activities with *p*-nitrophenyl phosphate, bis-*p*-nitrophenyl phosphate and phytic acid and thus the potential to mineralize monoester and diester forms of organic phosphorus. Rates of hydrolysis at pH 5.0 were greatest with *p*-nitrophenyl phosphate. Although enzyme activity varied with season and ectomycorrhizal morphotype, VAM roots of red maple consistently had the lowest enzyme activities on a length and dry weight basis. Comparison of ^{32}P uptake from inositol phosphate by gray birch and red maple roots suggested that phosphomonoesterase activity was linked to P uptake from this source. Differences between species in oxygen consumption rates were less pronounced than those observed for enzymatic activities, suggesting similar short-term energy demands by the root types examined. The quantitative differences observed between plants growing on a common soil potentially relate to differences in host demand or reflect differences in basic morphology and/or physiology of associated mycobionts. Further study is necessary to understand the importance of these enzymes in the functional ecology of mycorrhizal fungi.

Key words Acid phosphatase · Ectomycorrhizae · Phytase · Phosphorus uptake · Root respiration · Vesicular-arbuscular mycorrhizae

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Introduction

The ability of ectomycorrhizal fungi to enhance growth and acquisition of macronutrients by tree seedlings under controlled conditions is well established (Harley and Smith 1983). Comparisons of ectomycorrhizal (ECM) and nonmycorrhizal seedlings revealed greater total uptake of phosphorus and higher concentrations of macronutrients in the former when grown on nutrient deficient forest soils (Hatch 1937; Bowen 1973). Unfortunately, less is known about the functioning of mycorrhizae for larger trees growing under natural conditions (Fahey 1992).

Mycorrhizae are a vital but incompletely defined component of ecosystem nutrient cycles (Coleman et al. 1992). The distribution of mycorrhizal types and/or mycobiont species may have marked biogeochemical consequences (Entry et al. 1991). Read (1984) developed a conceptual model relating nitrogen-use patterns of different mycorrhizal types to climax vegetation, soil properties and climatic gradients. This approach depicted changes in mycorrhizal function over relatively large differences in elevation. Fahey (1992) proposed a model combining energy and nutrient relationships; in this model, efficiency of utilization influences the outcome of competitive interactions among mycorrhizae. Efficiency is based on fine root morphology, physiology and longevity. Such an approach is applicable at varying degrees of resolution, however, very little comparative data address root function in mature trees.

One goal of this present study was to examine different mycorrhizal types for potential physiological differences related to efficiency of nutrient acquisition. We

examined mycorrhizae of gray birch (*Betula populifolia* Marsh.), red maple (*Acer rubrum* L.) and American beech (*Fagus grandifolia* L.). These are important tree species in seral events occurring on abandoned agricultural sites of northern New York. Mycorrhizal type and host nutrient demand were considered to be the major factors influencing our observations. The study was designed to examine species growing in proximity on a common soil type. Sampling aimed to minimize potential effects of spatial and temporal separation on comparisons of mycorrhizal types. Physiological aspects examined included root oxygen consumption, root surface phosphatase activities and phosphorus uptake from an organic phosphorus source.

Materials and methods

Site description

Root collections were made in a wooded area of the Clarkson University campus (elevation 146 m, 44°40′ N, 75°00′ W) in Potsdam, NY. The cold temperate climate of Potsdam is characterized by long cold winters and short warm summers. Over the period 1980–1990 average annual rainfall for the Saint Lawrence Valley region in New York was 889–1016 mm with mean annual temperatures of 6–8 °C (NOAA 1990). The climax vegetation in the area is Northern hardwood forest (Vankat 1979); our work was carried out in two stands representing mid- and late-stages of a successional sequence.

The canopy at the mid-successional site was dominated by red maple (*A. rubrum* L.) and mature to senescent gray birch (*B. populifolia* Marsh.) trees. Mature quaking aspen (*Populus tremuloides* Michx.) and young white pine (*Pinus strobus* L.) were present but scattered in the stand. This stand, partially enclosed by a stone wall, was at one time cleared for pasture. Ground cover at the site consisted of scattered mosses, ferns, graminoids and spring ephemerals. The late-successional stand was dominated by American beech (*F. grandifolia* L.). Also present were scattered individuals of hemlock (*Tsuga canadensis* L.) Carr. and yellow birch (*Betula alleghaniensis* Britton). Large *F. grandifolia* trees were older than 170 years. Ground cover consisted primarily of scattered ferns and spring ephemeral angiosperms. Both sites were located on ground moraine characterized by moderately drained spodosols. At the mid-successional site the <2 mm fraction of mineral soil had a pH (soil to water, 1:2.5) of 4.76. Loss on ignition (LOI, 3 h at 450 °C) of the oven-dried soil was 4.3%. A pH of 4.24 and LOI of 9.7% were recorded for soil at the late-successional site.

Root sampling

Mycorrhizal roots for various experiments were collected in June and October over the period 1987–1990. The sample dates represente the early and late growing seasons of extreme upstate New York. The large numbers of roots required were obtained by randomly excavating surface horizons at distances of 1–2 m from tree bases on each site. With ECM hosts, root tips were collected by severing higher-order woody roots supporting large numbers of the seasonally dominant morphotypes. Samples were placed in plastic bags containing moist humus from the plot. Beaded roots of red maple were collected and handled in a similar fashion.

In the laboratory processing was initiated immediately by separating fine roots from soil and organic matter under a stream of cold, running water; samples were placed on window screen under continuous washing and roots removed with fine forceps. All intact roots were placed in 0.5 mM $CaCl_2$ at 5–10 °C until further processing.

Final separation of roots from soil materials was carried out under a stereomicroscope. Only those root tips judged to be viable based on color and turgescence (Harvey et al. 1976) were used in experiments. In cases where dark-colored or black mycorrhizae were present, we sectioned random subsamples to determine whether an intact light-colored cortex was present. ECM roots were separated according to morphotype, which were distinguished by color, size, type of branching, surface features, emanating hyphae and rhizomorphs (Agerer 1988).

For root respiration and phosphorus uptake studies, the finest feeder roots of VAM or ECM were left attached to segments of suberized, long roots. The segments of long roots (4–6 cm in length) with large numbers of fine lateral roots obtained resembled the class A roots pictured by Barnard and Jorgensen (1977) in their studies of *Pinus taeda* root respiration. Only fine feeder roots were used in enzyme assays. These were excised at the point of attachment to fine woody roots and subjected to further cleaning with forceps and running water to remove as much adhering organic material as possible without damaging root hairs, attached mycelium, mantle or rhizomorphs.

To minimize root excision effects, all samples were collected and processed in the early morning and experiments were conducted that afternoon.

Phosphatase activity measures

Surface acid phosphomonoesterase (EC 3.1.3.2) activities of roots were measured by a modification of the *p*-nitrophenyl phosphate (*p*NPP) technique of Bartlett and Lewis (1973). Excised roots were added to vials containing 2.0 ml of 50 mM acetate buffer (pH 5.0). A sufficient number of roots was added to provide similar total root lengths in analytical replicates. An attempt was made to use similar root lengths of the different ECM morphotypes and species, but because of varying abundance this was not always possible. The reaction was started by adding 0.5 ml *p*NPP, previously adjusted to the assay pH, to give a final substrate concentration of 10 mM. Reaction vials were incubated at 22–24 °C on an orbital shaker; controls contained buffer and substrate without roots. At the end of the incubation period, 0.5 ml of reaction mix was removed, added to 4.5 ml of 0.5 M NaOH and absorbance measured at 410 nm. The amount of *p*-nitrophenol (*p*NP) released was determined from a standard curve obtained with an alkaline solution of *p*NP (Antibus et al. 1981). Roots were rinsed thoroughly with distilled water prior to root length and dry mass measurements.

Root surface phosphodiesterase activities (E.C. 3.1.4.1) were quantified using the artificial substrate bis-(*p*-nitrophenyl) phosphate (B*p*NPP) as described previously by Hayano (1987) and Ho (1987). The buffer, pH, substrate concentration and volumes used were the same as described above for *p*NPPase assays. Lower activities towards B*p*NPP favored the use of 3-, 6- and 12-h incubations for this assay.

Phytase activities were assayed by the procedure described for *p*NPase except that phytic acid (inositol hexaphosphate) served as substrate. Phytic acid in 50 mM acetate buffer (pH 5.0) was added to vials containing roots in buffer to give a final concentration of 1 mM phosphorus. The release of inorganic phosphate was measured with malachite green at 650 nm according to the method of Penney (1976). Lower rates of hydrolysis of phytic acid relative to *p*NPP dictated use of 6- or 12-h incubations for phytase assays. As roots and hyphae were intact and would be expected to absorb phosphate during experiments, our data for phytase activities represent net rates of inorganic P (Pi) release from an organic P (Po) source.

Root respiration measures

Root oxygen consumption was measured manometrically with a Gilson Differential Respirometer. Sufficient root material was available to study respiration in two gray birch ECM morpho-

types, one American beech ECM morphotype and red maple VAM roots. Roots of each type were randomly allocated to four replicate respiration flasks. Each 15-ml flask contained 130–350 mg fresh wt. (23–41 mg oven dry wt.) of root material in 2.0 ml of 0.5 mM $CaCl_2$. Filter paper wicks saturated with 10% NaOH were used to absorb released CO_2. Flasks with samples were equilibrated for 1 h at 20 °C followed by measurements at hourly intervals for 4 h. The total time between root collection and the termination of respiratory measures was 8–10 h. Oxygen consumption rates (Q_{O_2}) were calculated as described by Umbreit et al. (1964) on a root length and dry weight basis.

^{32}P uptake measures

The time course of ^{32}P uptake from inositol polyphosphate (IPP) and its relationship with phosphatase activity was investigated for one gray birch ECM morphotype and red maple VAM roots. Acid phosphatase was measured at pH 5.0 with *p*NPP as substrate as described above.

A total of 60 root segments was selected for each of the species examined. Five segments were allocated to each of three replicates. Replicates were bundled in cheesecloth bags and suspended in flasks containing 50 ml of ^{32}P-labelled phytic acid solution at 22–24 °C. Separate flasks were used for each root type and harvest. Mixing and aeration were accomplished by bubbling with cotton-filtered compressed air. The uptake solution contained 1 mM phytic acid (6 mM P) adjusted to pH 5.0 with approximately 0.005 mCi/l of ^{32}P-inositol-1,4,5-triphosphate (IPP) (110 Ci $mmol^{-1}$ at positions 4,5; New England Nuclear, Boston, Mass.).

After incubation in labeled phytic acid, each bag of roots was washed thoroughly in distilled water and then three times for 5 min with 20 ml of 1 mM KH_2PO_4. Roots were removed, washed again and dried overnight at 50 °C. After obtaining dry weights, roots were transferred to vials containing 6 ml of scintillation cocktail. Uptake of ^{32}P was assayed by liquid scintillation counting with internal standards. Roots harvested at the time of label addition were used to determine the magnitude of physical adsorption of labeled P. No attempt was made to measure root lengths, and uptake rates were calculated using dry weight data with appropriate corrections for background counts, isotope decay and physical adsorption.

Root characterization and data analysis

Roots were rinsed thoroughly with distilled water prior to obtaining root length and dry weight measures. Root lengths were determined with an image analysis system (Jandel Scientific, Sausalito, Calif.). Entire root samples were spread flat in petri dishes so that no overlap occurred among segments and perimeters were traced and calculated by the image analysis system; root lengths were calculated by halving the perimeter values. Root dry weights were obtained after drying roots at 5 °C for 24 h. Field-collected roots of red maple were examined for the presence of mycorrhizal fungi by staining subsamples with chlorazol black E (Brundrett et al. 1984). Data were analyzed using SPSS/PC$^+$ software (Norusis 1988). Differences among enzyme activities and respiration rates for root types were compared by analysis of variance followed by Tukey's Honestly Significant Difference Test ($\alpha = 0.05$) (Zar 1984).

Results

Root types

Several ECM morphotypes were collected and utilized in experiments with gray birch; the two most frequently used are referred to as smooth and white-tomentose morphotypes. The smooth ECM morphotype was greyish-yellow to greyish-brown and formed irregular-pinnately branched systems of indeterminant growth. The unramified ends were frequently bent and mycorrhizal clusters were often densely packed. Although these appeared smooth at low magnification, inspection at high magnification revealed the presence of abundant, short hyphae with clamp connections. The mantle surface was pseudoparenchymatous. The white-tomentose morphotype was characterized by abundant white hyphae over a darker background. Systems were irregular-pinnately branched but were more open and had thinner branches than the smooth morphotype. Unramified tips were usually bent. Systems were often woolly with attached tangles of white rhizomorphs and surface hyphae. The mantle surface consisted of interwoven hyphae; clamp connections were not observed. One dominant ECM morphotype was collected and utilized in enzyme and respiration studies with American beech. This ECM was characterized by a smooth mantle and a pyramidal branching pattern. Based on the presence of occasional, large-diameter hyphae in the mantle and overall appearance, we feel the mycobiont was probably a *Lactarius* species. However, frequent fruitbody collections at the site yielded primarily *Russula* sp. We found beaded, white- to tan-colored red maple roots to be heavily colonized with hyphae typical of VAM. Hyphal coils and vesicles were frequently observed in the root cortex; arbuscules were infrequent.

Phosphatase activities

All root types examined had surface acid phosphomonoesterase activities as indicated by the hydrolysis of *p*NPP; enzyme activities were linear under our assay conditions for up to 6 h (Fig. 1a). As found in an earlier study (Antibus and Linkins 1992), *p*NPPase activities varied seasonally but the amplitude was small compared with differences among different root types. Gray birch ECM morphotypes examined in June 1987 and 1988 demonstrated significantly higher *p*NPPase activities than VAM roots of red maple (Figs. 1a, b).

In accordance with one of our objectives, we carried out intensive sampling of roots for enzyme and respiratory measurements in August 1988. The range of values for *p*NPPase activity observed for ECMs was quite similar to that found for other field-collected ECMs (Alexander and Hardy 1981; Doumas et al. 1986). On a dry weight basis, gray birch ECMs demonstrated significantly higher *p*NPPase activities than the single ECM morphotype of American beech and red maple VAM roots (Figs 2a). These patterns were still evident when data were expressed on a root length basis (Fig. 2b). However, the length contributed by wefts of external mycelium lowered specific activity values calculated for gray birch tomentose-white ECM.

All roots examined had surface acid phosphodiesterase activities as indicated by the hydrolysis of B*p*NPP

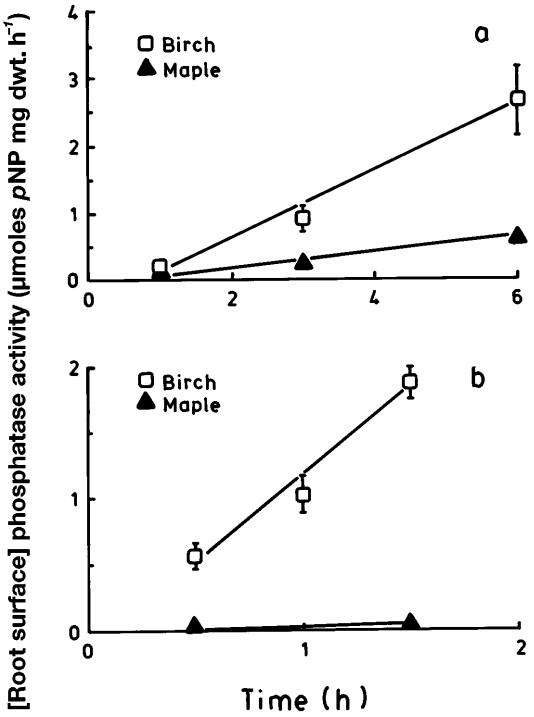


Fig. 1a, b Time course of surface phosphatase activity at pH 5.0 for gray birch ectomycorrhizae (□) and red maple vesicular-arbuscular mycorrhizae (▲). *Vertical bars* represent standard deviations. In cases where no bar is visible, the estimate is smaller than the size of the symbol. **a** Roots were collected and assayed in June 1987. **b** Roots were collected and assayed in June 1988

(Fig. 3). At the same substrate concentrations, B*p*NPPase activities were at least an order of magnitude lower than *p*NPPase activities. All ECM roots examined showed significantly higher B*p*NPPase activities than red maple VAM roots on a dry weight basis. Again, calculation of activity on a length basis greatly reduced B*p*NPPase estimates for the gray birch tomentose-white ECM (Fig. 3b).

All roots examined had phytase activities as indicated by the net release of Pi from phytic acid (Fig. 4). The smooth ECM morphotypes of gray birch and American beech showed significantly higher net Pi releases than the tomentose-white ECM or red maple VAM roots on both a dry weight and length basis (Figs. 4a, b). Specific phytase activity, as for the other enzymes, was lower when expressed on a length than on a dry weight basis.

Root respiration

Rates of oxygen consumption were constant for the first 4 h of respiration runs then gradually declined. Values presented in Figure 5 are based on these initial 4 h. Our data for Q_{O_2} on a dry weight basis are in close agreement with values reported for class A ECM of *Pinus taeda* (Barnard and Jorgensen 1977). On a dry weight basis, the tomentose-white ECM showed significantly higher Q_{O_2} values than the other three root types examined (Fig. 5a). As observed for the enzyme data, Q_{O_2} data expressed on a length basis were lower for this morphotype than the other ECM morphotypes and were similar to VAM roots (Fig. 5b).

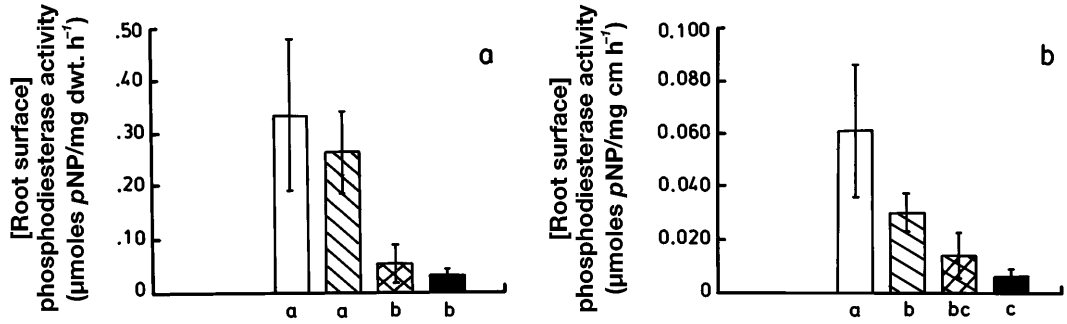
^{32}P uptake

The uptake of ^{32}P from inositol polyphosphate by field-collected roots of both gray birch and red maple was linear over a 12-h incubation period (Fig. 6a). The higher uptake rates of smooth gray birch ECM corresponded with differences observed in *p*NPPase activities compared with red maple VAM (Fig. 6b).

Discussion

Whereas structural differences can be used to characterize various types of mycorrhizae, parallel physiological divergence amongst types has remained difficult to demonstrate (Fahey 1992; Tinker et al. 1992). Both the fungi and the hosts involved in the VAM and ECM roots we examined are taxonomically distinct and likely

Fig. 2a, b Root surface phosphomonoesterase activities of different mycorrhizal types expressed **a** per unit root dry weight and **b** per unit root length. All samples were collected on the same day and measured at pH 5.0. Root types are smooth (□) or tomentose-white (▨) birch ectomycorrhizae, smooth beech (▩) ectomycorrhizae, and red maple (■) VAM. *Bars* with different letters indicate significant differences between samples at $P<0.05$



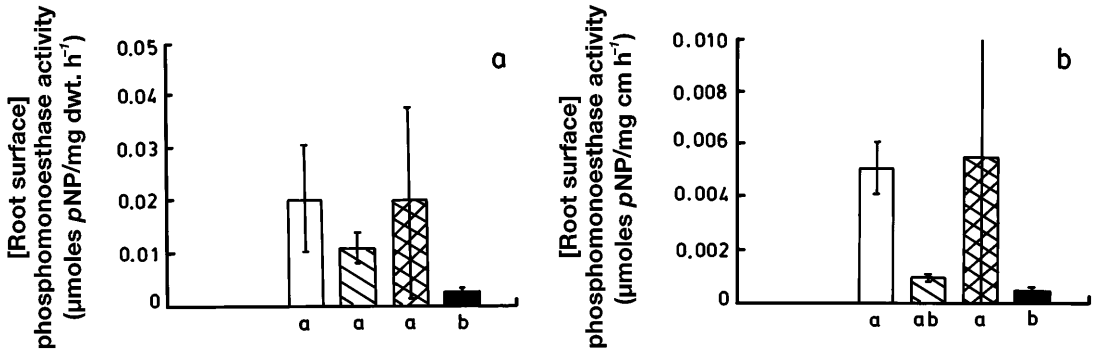


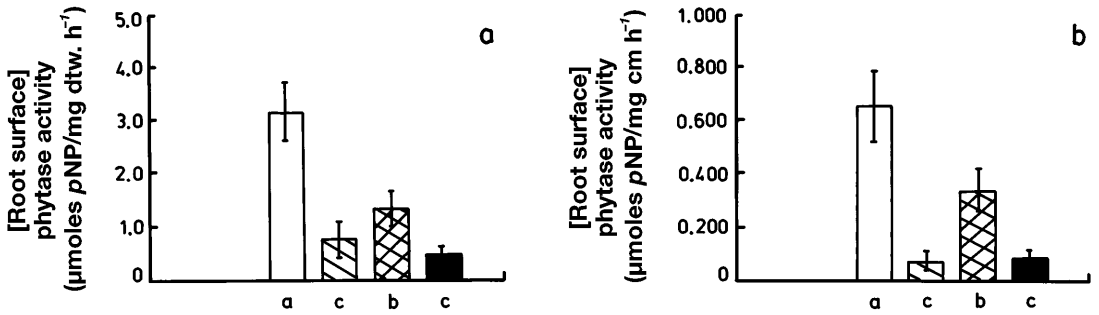
Fig. 3a, b Root surface phosphodiesterase activities of different mycorrhizal types expressed **a** per unit root dry weight and **b** per unit root length. All samples were collected on the same day and measured at pH 5.0. Root types are smooth (□) or tomentose-white (▨) birch ectomycorrhizae, smooth beech (▩) ectomycorrhizae, and red maple (■) VAM. *Bars* with different letters indicate significant differences between samples at $P<0.05$

encompass a range of physiological capabilities. One goal of the present study was to gather information on specific functional attributes of these mycorrhizae of mature trees.

We used root oxygen consumption as a measure of root respiration and carbon utilization rates. ECM formation can clearly alter respiration and carbon allocation in pine seedlings (Rygiewicz and Andersen 1994); however, relatively little comparative information is available for different types of mycorrhizae. In addition, interpretation depends on the scale and manner in which respiration data are expressed (Harley and Smith 1983). Our data show that differences apparent on a weight basis may not be clear on a length basis; the length data incorporate external hyphae, which add little to weight but much to oxygen consumption and surface area. These data highlight the need for precise methods of measuring fungal weight in internal and external root compartments to obtain reliable estimates of carbon costs and efficiency of root symbioses (Fahey 1992; Tinker et al. 1992). Ergosterol analysis currently represents the most sensitive measure of live hyphal biomass requiring a carbon subsidy (Antibus and Sinsabaugh 1993).

We measured acid phosphatases of the various root types as an estimate of Po mineralization potential (Haussling and Marschner 1989). All root types examined in our study showed activity towards several substrates including *p*NPP, B*p*NPP and phytic acid. With regard to ectomycorrhizal phosphatase activities, our results confirm as well as extend prior studies. In resynthesis experiments, ECM colonization may or may not enhance *p*NPPase activity relative to host roots (Antibus et al. 1981; MacFall et al. 1991). The results depended largely on genotypic differences in phosphatase production characteristics of the fungi (Doumas et al. 1986; Ho 1987). However, host P demand, P status and soil P status may regulate phenotypic expression of activity (Alexander and Hardy 1981; Dighton 1983). Our findings agree with previous work demonstrating differences among ECM morphotypes on a common soil and host (Antibus and Linkins 1992). Thus differences observed among gray birch ECM on a common soil are linked to mycobiont attributes, i.e. genotypic differences in enzyme production, mantle surface area and associated flora among morphotypes. We do not know to what extent these properties reflect microspatial differences in litter quality or random colonization events. The question of how resource quality might influence distribution of mycorrhizal symbionts has been considered by Read (1991). It is interesting in this regard to note that the single beech morphotype examined had lower *p*NPPase activities than birch but produced similar or higher levels of B*p*NPPase. Because we lack in-

Fig. 4a, b Root surface phytase activities of different mycorrhizal types expressed **a** per unit root dry weight and **b** per unit root length. All samples were collected on the same day and measured at pH 5.0. Root types are smooth (□) or tomentose-white (▨) birch ectomycorrhizae, smooth beech (▩) ectomycorrhizae, and red maple (■) VAM. *Bars* with different letters indicate significant differences between samples at $P<0.05$



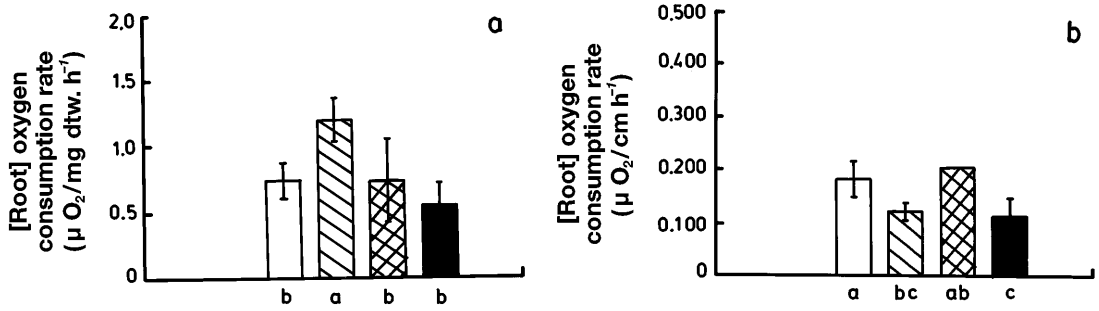


Fig. 5a, b Root oxygen consumption rates of different mycorrhizal types expressed **a** per unit root dry weight and **b** per unit root length. All samples were collected on the same day and measured at 25 °C. Root types are smooth (□) or tomentose-white (▨) birch ectomycorrhizae, smooth beech (▩) ectomycorrhizae, and red maple (■) VAM. *Bars* with different letters indicate significant differences between samples at $P<0.05$

formation on Po forms in the litter types, the question of whether the enzyme differences reflect resource quality remains unresolved. B*p*NPPase activities were generally low compared to *p*NPPase but indicate that these field-collected ECM, like cultures of *Pisolithus tinctorius* (Ho 1987), act as a source of phosphodiesterase activity in forest soils, hence enhancing mineralization of this soil Po component.

Phytase activity has been previously demonstrated in ECM roots (Bartlett and Lewis 1973; Mousain and Salsac 1986) and ECM fungi (Theodorou 1971). Phytase activities were always lower than *p*NPPase but not to the same extent among all morphotypes. Such differences in comparative Pi release from *p*NPP and phytic acid have been observed with cultures of ECM fungi (Antibus et al. 1992; McElhinney and Mitchell 1993), and two possible explanations have been forwarded. Fungi may differ in the rate at which Pi released enzymatically is absorbed (Dighton 1983; Hilger and Krause 1989), or we may be observing differences in the relative contribution of phytase to the total phosphatase activity. Either of these explanations would influence the ability of fungi or roots to compete for inositol-linked P in soils.

The results over several seasons confirm significant differences in *p*NPPase activities between several birch ECM morphotypes and red maple VAM but such differences do not hold for all enzymes assayed. Some birch morphotypes which showed higher *p*NPPase activities than maple VAM did not show concomitant differences in B*p*NPPase or phytase with maple roots. The beech ECM studied did not display enhanced *p*NPPase activity relative to red maple VAM but did show higher B*p*NPPase and phytase activities. Our birch findings broadly agree with those of Dighton and Coleman (1992) on *Rhododendron maximum* where *p*NPPase activities were always significantly higher for ECM than for VAM or lattice roots.

The higher activities of birch ECM than maple VAM may be explained in terms of genotypic differences in enzyme production, hyphal surface area available for enzyme production or regulation of enzyme activity by soil or host P. We feel that the activity measured for maple VAM largely represents root activity as few external hyphae were observed. Ergosterol-based estimates of fungal biomass suggest much higher hyphal to root mass for birch than maple (Antibus and Sinsabaugh 1993). Because birch and maple roots often occurred intermingled in the same soil layer, it is unlikely that differences in enzyme expression relate to external P. Additionally, Dighton and Coleman (1992) found consistent ECM-VAM differences between *R. maximum* root types despite a range of soil P availabilities linked to elevation. As these authors examined mycorrhizae from a common host, they minimized possible confounding effects of host P demand. In our study, we cannot discount possible differences in host P demand as a factor affecting enzyme production.

Correlations between Pi uptake and root surface *p*NPPase, as well as joint regulation by external P levels

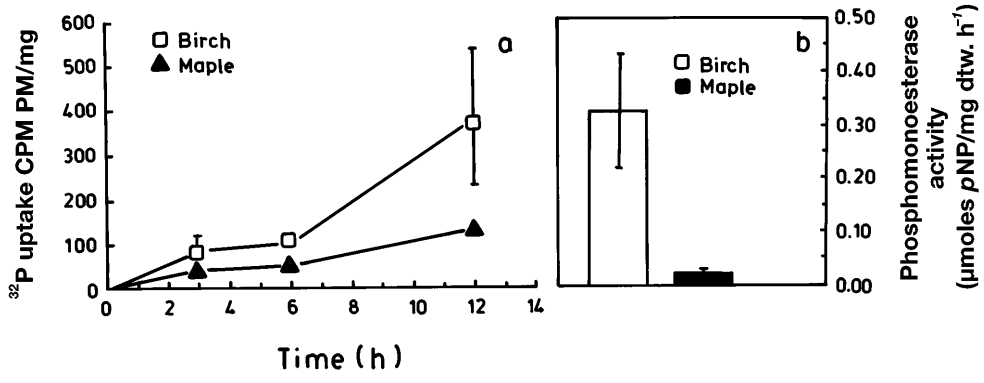


Fig. 6a, b ^{32}P uptake from inositol polyphosphate (**a**) and phosphomonoesterase activities (**b**) of smooth birch (□) ectomycorrhizae and red maple (▲) VAM at pH 5.0

have been established for plant roots (Lee 1988) and ECM fungi (Dighton et al. 1993). In *R. maximum,* Pi influx and *p*NPPase were positively related (Dighton and Coleman 1992), and differences in uptake among mycorrhizal types were consistent with enzyme activity across the sampled elevations. Less is known about the relationship between *p*NPPase and Pi uptake from Po sources. Phosphatase activity correlated well with uptake of Pi from inositol polyphosphate in ECM fungi grown under P-limiting conditions (Antibus et al. 1992). Our data suggest that *p*NPPase differences between birch ECM and maple VAM are likewise related to differences in Pi uptake from inositol polyphosphate. Lower rates of P uptake from inositol phosphate indicate that the lower rates of phytase (measured as Pi released) in maple roots are the result of lower enzyme activity and not higher maple Pi uptake. A quantitative functional difference is apparent at least for birch and maple, with the difference being seasonally consistent for trees on a common site. As little external hyphae were present on red maple roots, our observations do not address the debate over the potential of such VAM hyphae to use organic P sources. Very little is known about the capacity for and regulation of phosphatase production by external hyphae of VAM fungi (Allen 1991). Jayachandran et al. (1992) showed that VAM can significantly increase access to a variety of Po sources. They suggest that qualitative differences in the use of Po exist between VAM and nonVAM *Andropogon gerardii*. Only further research with a wider array of substrates will reveal whether Po use is qualitatively different in maple VAM roots and birch ECM. Our work does not address certain aspects of an intact soil-root system where rhizosphere and soil P fixation effects act to influence uptake (Martin and Cartwright 1971). Still, we believe our findings highlight ecologically important differences in potential to use soluble organic P sources in the root types examined.

The possibility that taxonomically distinct mycobionts provide access to different soil nutrient pools has long intrigued researchers, and has been the topic of recent discussions (Dighton 1991; Read 1991). Clearly, differences exist in enzymatic capacities to mineralize organic N among ECM species and between ECM and ericoid fungi. For nitrogen, the patterns of mycobiont enzyme production appear to parallel the distribution of soil nitrogen forms. Phosphorus represents the other plant macronutrient potentially made available by biochemical mineralization, however, the forms and distribution of soil organic P have been less studied (Harrison 1987) and few generalizations are possible about organic P utilization by mycorrhizae. The ability to release various kinds of enzymes is clearly an important aspect of the functional ecology of fungi and one we have begun to address here. ECM fungi when grown in pure culture produce an array of enzymes (Hutchinson 1990) and some invest significant amounts of carbon in the release of these enzymes (R. Antibus unpublished data). Sinsabaugh et al. (1993) have suggested that decomposer communities maximize efficiency of carbon utilization by investing in enzymes that mobilize nutrients in accordance with supply and demand. In this way, enzymes serve as emergent system properties. Likewise, enzyme activities represent emergent properties of mycorrhizae and components of Fahey's root efficiency model. Latham (1992) has shown that, although plants require a small number of resources, the forms used and the associated costs can influence the competitive balance among tree seedlings. In this way, mycorrhizae could be a factor in the maintenance of stand diversity. We cannot yet determine the extent to which the costs and benefits of investment in a mycobiont or enzymes may act to affect competitive outcomes among trees.

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