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Research paper

Carbon allocation and partitioning in *Populus tremuloides* are modulated by ectomycorrhizal fungi under phosphorus limitation

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The fate of carbon (C) captured by forest trees during photosynthesis is influenced by the supply of other resources. Fixed C may be partitioned among biomolecules within the leaf and/or allocated throughout the tree to growth, storage and maintenance activities. Phosphorus (P) availability often limits tree productivity due to its high biological demand and strong interactions with soil minerals. As ectomycorrhizal (ECM) fungi play critical roles in enhancing phosphate (P_i) acquisition by their hosts, these symbioses will influence the fate of C within trees and forested ecosystems. Using *Populus tremuloides* Michx. (trembling aspen) in symbiosis with *Laccaria bicolor* (Marie) P.D. Orton or *Paxillus involutus* (Batsch) Fr., we assessed C acquisition, allocation and partitioning under P_i limitation, specifically focusing on primary and secondary C compounds. Both ECM fungi moderated the effects of low P on photosynthesis and C partitioning among carbohydrates and secondary metabolites by sustaining P_i uptake and translocation in *P. tremuloides* under P_i limitation. As leaf P declined, reductions in photosynthesis were accompanied by significant shifts in C partitioning from nonstructural carbohydrates (NSCs) to phenolic glycosides and tannins. Carbon partitioning in roots exhibited more complex patterns, with distinct increases in NSCs in nonmycorrhizal (NM) plants under P_i limitation that were not evident in plants colonized by either ECM symbiont. In general, aspen colonized by *L. bicolor* exhibited C partitioning patterns intermediate between those of NM and *P. involutus* aspen. The C cost of symbiosis was pronounced for plants supporting *P. involutus*, where ECM plants exhibited maintenance of photosynthesis yet reduced biomass in comparison with NM and *L. bicolor* aspen under P_i replete conditions. Our results indicate that the ECM symbiosis affects the disposition of C in forest trees in part by altering the acquisition of other limiting resources from soils, but also through ECM species-specific influences on host physiology. This modulation of C partitioning will have broad implications for forest ecosystem C capture, storage and cycling where nutrient resources may be limited.

Keywords: aspen, carbohydrates, *Laccaria bicolor*, *Paxillus involutus*, phenolic glycosides, stress physiology.

Introduction

Forest ecosystem productivity is often limited by mineral nutrient availability (LeBauer and Treseder 2008, St Clair et al. 2008, Hou et al. 2012). Phosphorus (P) is a major mineral nutrient required by forest trees in significant amounts (Elser et al. 2007), yet P availability is limited in soils due to high biological demand and the chemical binding of inorganic phosphate (P_i) and organic phosphorus (P_o) sources to aluminum, iron (Fe) and/or calcium (Ca) ions/compounds prevalent in many natural soils (von Uexküll and Mutert 1995, Batjes 1997).

To overcome P_i limitation, plants have developed a suite of phosphate starvation responses (PSR) that moderate internal P homeostasis, maximize P acquisition from the environment and acclimate plants to low P environments (Vance et al. 1995, Raghothama and Karthikeyan 2005, Plaxton and Tran 2011, Zhang et al. 2014). Increases in the root-to-shoot ratio and changes in root architecture

increase soil exploration and P acquisition (Lynch 2011). Root exudation, increased P_i uptake capacity and metabolic pathway changes increase P acquisition from soils and P-use efficiency within the plant (López-Arredondo et al. 2014).

In addition to PSR systems, plant–mycorrhizal symbioses significantly alter plant–soil interactions and enhance P uptake (Plassard and Dell 2010). The colonization of roots by ectomycorrhizal (ECM) fungi alters root architecture, and hyphal ramification of the soil increases the volume of soil exploited for mineral uptake by the host plant (Hetrick 1991, Schack-Kirchner et al. 2000, Agerer 2001, Hagerberg et al. 2003). Ectomycorrhizal fungal uptake systems often have higher affinity for P_i, increasing P_i acquisition from the soil solution (Cumming 1996, Colpaert et al. 1999, Desai et al. 2014). Increased carbon (C) inputs to the soil in the form of exudates and enzymes may alter the chemistry of the rhizosphere and the subsequent availability of P from a broad spectrum of P-containing compounds for ECM fungi and root uptake (Cumming 1993, Leyval and Berthelin 1993, Wallander 2000, Casarin et al. 2004, Liu et al. 2008, Courty et al. 2010).

Depending on the effectiveness of nutrient limitation responses and mycorrhizal nutrient scavenging, photosynthesis, biochemical pathways and plant tissue composition may change when nutrient resources are limiting (Keski-Saari and Julkunen-Tiitto 2003, Hale et al. 2005, Donaldson et al. 2006, Baum et al. 2009). The production of C-based secondary compounds (CBSCs) in plants depends on the balance between C and nutrient availability (Bryant et al. 1983). In leaves of *Populus alba* × *P. grandidentata*, for example, the concentrations of tannins and salicylates were elevated under low nitrogen (N) (Kleiner et al. 1998). Indeed, many plant species accumulate anthocyanins and other phenolic compounds under nutrient limitation, indicating that CBSCs may also contribute to the acclimation of plants to stressful environments (Dixon and Paiva 1995, Donaldson et al. 2006, Chen et al. 2009).

Although plant responses involving CBSCs under environmental stress are well studied (Koricheva et al. 1998, Gayler et al. 2008, Caretto et al. 2015), considerably less is known about changes in secondary C metabolism in plants in the ECM association. In the *Larix decidua–Suillus tridentinus* symbiosis, higher levels of major secondary C-containing compounds were observed in the root apices of young mycorrhizas (Weiss et al. 1997) and concentrations of CBSCs in the foliage of *Salix* spp. depended on fungal partner and host species (Baum et al. 2009). However, little is known of the interactions between nutrient limitation and ECM fungi on the accumulation of CBSCs in plants.

In the current study, we assessed the influences of *Laccaria bicolor* and *Paxillus involutus* colonization on P_i limitation responses of *Populus tremuloides* (trembling aspen), specifically investigating photosynthesis and partitioning of C between carbohydrates and secondary metabolites. Trembling aspen is widely distributed across North America and fills both early and late successional niches. The root microbiome is diverse, including a wide variety of ectomycorrhizal associates (Kaldorf et al. 2004, Fox et al. 2013, Bonito et al. 2016). *Laccaria bicolor* and *P. involutus*, common ECM symbionts of poplars that have been used extensively in tree physiology research, were used in this study because of this knowledge base as well as their known divergence in affecting P_i acquisition in their hosts (Cumming 1996).

We hypothesized that: (i) photosynthesis would be reduced due to P_i limitation, but increased in ECM plants in response to stimulated C demand in the root system by the fungal partners; (ii) nonmycorrhizal aspen would allocate more C to secondary compounds than to the production of plant biomass under P_i limitation; (iii) changes in photosynthesis, partitioning and growth would be more pronounced in nonmycorrhizal plants than in plants colonized by *L. bicolor* and *P. involutus* due to enhanced P_i acquisition by these symbionts; and (iv) differences between ECM symbionts would reflect fungal-specific changes in P_i acquisition or C demand induced by each symbiont.

Materials and methods

Fungal inoculation and plant culture

The *L. bicolor* (Marie) P.D. Orton fungal strain S238N (Institut National de la Recherche Agronomique, Nancy, France) and *P. involutus* (Batsch) Fr. fungal strain ATCC 200,175 (American Type Culture Collection) were used as symbionts to colonize seedlings of *P. tremuloides* Michx. (trembling aspen, 'aspen') (seed source from the Canadian Natural Resources, National Tree Seed Center, Fredericton NB, Canada). Fungal cultures were maintained on a modification of modified Melin Norkrans (MMN) agar medium (Cumming et al. 2001). For inoculum production, cultures were grown aseptically in liquid MMN medium for 3 weeks at 25 °C in the dark in static culture. Cultures were macerated by blending briefly (three pulses for 3 s each in a common blender) to produce fungal mycelial slurries to serve as inoculum (Molina et al. 1982).

The experimental system for the production of ECM aspen consisted of CP512 treepots (Stuewe and Sons, Corvallis, OR, USA) each containing ~5 l of a mixture (2:1) of sterile acid-washed sand and vermiculite. For establishing mycorrhizal seedlings, aliquots of fungal slurry (equal to ~0.2 g dry weight fungal tissue) were added ~2 cm below the surface of the sand (Desai et al. 2014). For nonmycorrhizal (NM) plants, only MMN liquid medium was added instead of fungal slurry.

Seeds of *P. tremuloides* were planted ~0.5 cm deep in the pots and were kept moist by watering four-times daily with d. H_2O. Seedlings were thinned to one seedling per pot 10 days after germination. Thereafter, seedlings were watered with 160 ml of nutrient solution daily (40 ml per pot every 4 h of the light period). The nutrient solution contained 1.0 mM NO_3, 0.4 mM NH_4, 0.5 mM K, 0.2 mM Ca, 0.1 mM Mg and SO_4, 50.5 μM Cl, 20 μM Fe-EDTA, 20 μM B, 2 μM Mn and Zn, and 0.5 μM Cu, Na, Co and Mo. This watering regime was sufficient

to maintained pots near field capacity and, we assume, the concentrations of nutrients near these basal setpoints. Solution pH was adjusted to 5.6 with 0.1 N NaOH. After an additional 2 weeks, P_i treatments in the form of KH_2PO_4 were added to the baseline nutrient solution to deliver treatment solutions containing 5 μM (P_i limitation) or 100 μM (control). These levels were selected based concentrations where limited (100 μM) and elevated (5 μM) stress responses (antioxidant enzyme and organic acid exudation) were reported by Desai et al. (2014). Eight replicate seedlings per P_i/ECM fungal treatment combination were established. Seedlings were maintained in a climate-controlled greenhouse with supplemental lighting (mixed metal halide sources) providing a 14-h photoperiod and day/night temperatures of 24/19 ± 3 °C. Seedlings were grown for 46 days after the commencement of P_i treatments (days after treatment, DAT).

Photosynthesis and photosystem measurements

Net photosynthesis was measured on 15, 35 and 45 DAT using a Li-COR 6400 portable photosynthesis system with a 6-cm^2 chamber with red/blue LED light sources (Li-COR Biosciences, Lincoln, NE, USA). Photosynthetic measurements were made between 09:00 h and 16:00 h daily on the most fully expanded leaf of each plant (fourth to fifth leaf from the top). Net CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were measured at 380 μmol mol^{-1} CO_2. Measurements were made at saturating light intensity of 1500 μmol m^{-2} s^{-1} and a chamber temperature of 24 °C. A–C_i curves were generated using a sequence of 380, 50, 100, 150, 250, 580, 800, 1000, 1200 and 1500 μmol mol^{-1} CO_2 mole fraction. The maximum rate of electron transport (J_{max}) and the maximum rate of Rubisco-mediated carboxylation (V_{cmax}) were derived from these measurements (Sharkey et al. 2007).

Growth measurements, tissue P concentration and mycorrhizal colonization

Leaf P concentration was measured using four 5-mm leaf discs sampled from the leaves used during photosynthetic measurements, above. Leaf punches were flash frozen in liquid nitrogen and stored at −20 °C. Leaf discs were ashed at 475 °C for 1 h, dissolved in 1 ml 50% HCl, and crucibles rinsed with 1 ml d.H_2O. The resulting 2 ml digests were vortexed until clear. Root P concentration was determined on a sample of root tissue (roots <1 mm) collected at the final harvest. The sample was dried, ground in a cyclonic sample mill and an aliquot digested as above. Tissue P in leaf and root digests was analyzed using the malachite green method (Martin et al. 1999).

At harvest, plants were separated into roots and shoots and roots were washed thoroughly with d.H_2O to remove adhering substrate. Root samples (~1 g) from the center of root systems were stored in sterile distilled water at 4 °C to determine root colonization. The remaining shoot and root tissues were dried at 60 °C for 48 h to determine dry weight. Percent ECM fungal colonization was quantified using the gridline intercept method (Giovannetti and Mosse 1980). Aspen roots were well colonized by each ECM fungus, and ranging from 72% to 77% for *L. bicolor* and from 77% to 82% for *P. involutus*; there was no evidence of short root formation in NM plants.

Tissue C-based compounds

Tissue samples for carbohydrate and CBSC assays were flash frozen in liquid nitrogen and stored at −80 °C. Metabolites were analyzed for three plant tissues: young leaves representing the four youngest leaves from top; mature leaves, which were the remaining fully expanded leaves below young leaves; and fine root tissue representing pooled roots with diameter <1 mm.

For glucose, fructose, sucrose and starch measurements, frozen leaf and root tissues were ground cryogenically using a cryo-bath and ball mill equipped with a cryo-block (2600 Cryo-Station, 2650 Cryo-Block and 2000 Geno Grinder, SPEX SamplePrep, Metuchen, NJ, USA). Frozen powdered leaf and root material (20 mg) was transferred to microcentrifuge tubes to which was added 375 μl of a mixture containing 80% (v/v) ethanol and 10 mM 2-(*N*-morpholino)ethanesulphonic acid (MES), pH 6. The tubes were mixed thoroughly and incubated at 80 °C for 30 min. Extracts were clarified by centrifugation (4500*g*, 10 min), and the supernatant was transferred into a 96-well, deep-well microplate, stored at 4 °C. The pellets were further extracted with 225 μl of 80% (v/v) ethanol, 10 mM MES pH 6, and 375 μl of 50% (v/v) ethanol, 10 mM MES pH 6, and supernatants pooled in the cooled deep-well plate. Leaf starch remaining in the pellets was converted to glucose by incubation with amylase and amyloglucosidase (Sigma-Aldrich, St Louis, MO, USA). The glucose resulting from starch degradation and the glucose, fructose and sucrose in the ethanolic extracts were assayed using a continuous enzymatic substrate assay (Rogers et al. 2004).

For CBSC analyses, plant tissue samples (~1 g) were pulverized in liquid nitrogen using a mortar and pestle and ground in the dark in 10 ml of 80% ice-cold methanol. Ground samples were sonicated for 15 min in an ice bath and centrifuged (1700*g*, 5 min). Sample extracts were stored at −20 °C and the following CBSC analyses were done on each.

Total condensed tannins were measured according to Porter et al. (1985). Methanolic extracts (250 μl) were mixed 1:1 v/v with 70% acetone, 1:3 with *n*-butanol plus 0.1 ml ferric reagent (2% w/v $NH_4Fe(SO_4)_2 \cdot 12\ H_2O$ in 2 M HCl), heated at 100 °C for 1 h, cooled to room temperature, and optical density (OD) taken at 550 nm. Total phenolic glycosides in the methanolic extracts were assessed following Waterhouse (2002). Methanolic extracts (100 μl) were diluted with 400 μl of 80% methanol and 2.5 ml of 10% Folin-Ciocalteu phenol reagent and 2 ml 700 mM Na_2CO_3 were added and OD at 725 nm was determined following a 60-min incubation at room temperature. Total condensed tannins and phenolic glycosides were expressed as gallic acid equivalents per gram of plant material on fresh weight basis.

Specific CBSCs in the extracts were separated by high-performance liquid chromatography (HPLC (Varian, Walnut Creek, CA, USA) using a Luna 5 μm C18 column (250 × 4.60 mm) (Phenomenex 519,880-40, Torrance, CA, USA) and detection at 254 nm. The mobile phase consisted of a gradient of solvent A (0.5% MeOH in phosphoric acid) and solvent B (100% acetonitrile) that increased stepwise over 45 min. The elution steps were (%B) 0:30:50:65:80 each step for 5 min, followed by column cleaning with 100% MeOH for 15 min and re-equilibration for 5 min with 0.5% MeOH in phosphoric acid while maintaining a constant flow rate of 1.0 ml min^{-1} (Mellway et al. 2009). The HPLC standards for secondary metabolites were (+)-catechin, chlorogenic acid, p-OH-cinnamic acid, myricetin, quercetin, kaempferol and procyanidin B2 obtained from Sigma Chemical Company (St Louis, MO, USA). Isorhamnetin, salicin, salicortin and tremulacin standards were kindly provided by Dr Richard Lindroth (University of Wisconsin, Madison, WI, USA).

Statistical analysis

The experiment was established as a randomized two-way factorial design, with two P_i treatments (5 μM and 100 μM P_i) and three ECM fungal treatments (nonmycorrhizal (NM) control, *L. bicolor* (Lb), *P. involutus* (Pax)) with eight replicates of each combination. Samples taken on the same plants at 15, 30 and 45 DAT during the experiment (photosystem function and leaf P concentration, $n = 3$) were analyzed as a repeated measures (DAT) two-way factorial analysis of variance (ANOVA). While DAT was often important in influencing photosynthesis responses, few higher-level interactions containing DAT led to presentation of effects for the P_i × Myc interactions only in the results. The effects of P_i concentration and ECM fungal colonization on plant variables at the end of the experiment (biomass and tissue P, $n = 5$; tissue secondary metabolites, $n = 3$) were analyzed using two-way ANOVA (P_i × Myc). Data were log-transformed as needed to meet the assumptions of ANOVA. Tukey's HSD tests were utilized to identify significant differences among treatment means. Metabolite profiles were evaluated using Pearson correlation coefficients and ANOVA-simultaneous component analysis (ASCA). ASCA is designed to evaluate data matrices containing complex multigroup and multivariate data, such as those generated in metabolomics studies (Smilde et al. 2005). ASCA builds an orthogonal model through which total variation of the dataset is separated into parts corresponding to different factors, and both the covariance between the multiple variables and the design of the experiment are taken into account (Jansen et al. 2005, Smilde et al. 2005). Principal component analyses (PCAs) are then performed on centered data to obtain information on variable contribution to the ASCA component scores. Factor loadings for these components provide insight into the variables most strongly influencing aspen metabolic profiles. Statistical analysis was carried out using SAS JMP 7.0 (SAS Institute, Cary, NC, USA) and the MetStaT protocol in R (Dorscheidt 2013).

Results

Colonization and tissue P concentrations

The concentration of P in aspen leaves was significantly reduced by P_i limitation and this effect was dependent on mycorrhizal status/symbiont ($P < 0.001$ for the P_i × Myc interaction) (Table 1). At 100 μM P_i, the leaf P concentration of aspen colonized by *P. involutus* was 81% and 57% greater than that of NM plants or plants colonized by *L. bicolor*, respectively. At 5 μM P_i, leaf P of aspen colonized by *L. bicolor* and *P. involutus* was 2.8- and 2.1-fold greater than that of NM plants. Comparing symbionts, leaf P concentration of aspen colonized by *L. bicolor* was unaffected by P_i limitation, whereas leaf P of plants colonized by *P. involutus* was reduced by 45% at 5 μM P_i, yet this was as high as that of NM plants grown at 100 μM P_i (Table 1). Root P concentrations in NM aspen were consistently lower than those in roots colonized by either ECM fungus and, as with leaf P concentration, root P was not affected by growth P concentration in *L. bicolor*, but declined in roots colonized by *P. involutus* at the low P_i treatment (Table 1).

Photosystem parameters

Photosynthetic CO_2 uptake (A) was dependent on both P_i and Myc treatments and was related to leaf P concentration (Table 2, Figure 1). A was 63% lower in NM plants grown at 5 than at 100 μM P_i, but was not affected by low P_i in plants colonized by *L. bicolor* or *P. involutus* due to the maintenance of leaf P concentrations under P_i limitation (regression slopes for *L. bicolor* and *P. involutus* in Figure 1 were nonsignificant). Across ECM fungal treatments, stomatal conductance (g_s) was reduced by 27% at low P_i (Table 2). While this effect was consistent across mycorrhizal treatments, plants colonized by *L. bicolor* and *P. involutus* had greater g_s in comparison with NM plants (Table 2). Rates of transpiration (E) of aspen followed those of g_s, where rates at 5 μM P_i were 25% less than that of plants grown at 100 μM and were consistent across all mycorrhizal treatments (Table 2). No

Table 1. Tissue P concentrations of nonmycorrhizal (NM) aspen seedlings and seedlings colonized by *L. bicolor* (Lb) and *P. involutus* (Pax) as affected by P_i treatment. Means ± standard errors followed by the same letter within a column are not statistically different at $P \leq 0.05$ by Tukey's HSD.

P_i	ECM	Leaf P (mg P gdw^{-1})	Root P
5 μM	NM	0.89 ± 0.08c	0.45 ± 0.05c
	Lb	2.45 ± 0.32ab	1.34 ± 0.22ab
	Pax	1.88 ± 0.28b	0.78 ± 0.16bc
100 μM	NM	1.90 ± 0.17b	0.74 ± 0.07bc
	Lb	2.19 ± 0.26b	1.08 ± 0.14ab
	Pax	3.43 ± 0.11a	1.42 ± 0.18a
P_{P_i}[1]		<0.001	0.022
P_{ECM}		<0.001	<0.001
$P_{P_i \times ECM}$		<0.001	0.027

[1]Probability of the P_i, ECM and P_i × ECM treatment effects.

Table 2. Leaf photosynthetic parameters of nonmycorrhizal (NM) aspen seedlings and seedlings colonized by *L. bicolor* (Lb) and *P. involutus* (Pax) as affected by P_i treatment. Means ± standard errors followed by the same letter within a column are not statistically different at $P \leq 0.05$ by Tukey's HSD.

P_i	ECM	A[1]	g_s[2]	E[3]	C_i[4]	V_{cmax}[5]	J_{max}[6]
5 μM	NM	6.1 ± 0.8b	0.137 ± 0.052b	3.14 ± 0.41	189 ± 29	36.9 ± 2.4c	41.3 ± 5.1c
	Lb	13.6 ± 1.4a	0.239 ± 0.052ab	3.07 ± 0.47	212 ± 28	53.5 ± 3.1b	67.0 ± 9.6b
	Pax	15.8 ± 1.5a	0.227 ± 0.046ab	3.56 ± 0.51	242 ± 18	58.1 ± 4.6b	73.9 ± 10.4b
100 μM	NM	13.5 ± 1.0a	0.223 ± 0.054ab	3.51 ± 0.53	224 ± 21	72.8 ± 2.3a	94.5 ± 4.8a
	Lb	17.3 ± 1.3a	0.317 ± 0.049a	4.61 ± 0.27	235 ± 23	56.0 ± 4.6b	69.4 ± 8.9b
	Pax	14.9 ± 1.7a	0.262 ± 0.046ab	4.23 ± 0.26	261 ± 33	63.8 ± 4.4ab	70.0 ± 8.1b
P_{P_i}[7]		0.004	0.011	0.014	0.162	<0.001	<0.001
P_{ECM}		<0.001	0.009	0.322	0.139	0.120	0.626
$P_{P_i \times ECM}$		0.009	0.668	0.346	0.941	<0.001	<0.001
P_{DAT}		0.110	<0.001	0.113	<0.001	0.004	<0.001

[1]Steady-state photosynthesis (μmol CO_2 m^{-2} s^{-1}).
[2]Stomatal conductance (mol m^{-2} s^{-1}).
[3]Tanspiration (mmol m^{-2} s^{-1}).
[4]Leaf intracellular CO_2 (μmol mol^{-1}).
[5]Maximum carboxylation rate of rubisco (μmol CO_2 m^{-2} s^{-1}).
[6]Maximum electron transport rate (μmol electrons m^{-2} s^{-1}).
[7]Probability of the P_i, ECM, P_i × ECM and DAT treatment effects, respectively.

significant changes in intercellular CO_2 concentration (C_i) were observed due to P_i limitation or mycorrhizal colonization (Table 2).

Changes in A were a reflection of altered photochemistry in aspen. The maximum rate of carboxylation of RuBP (V_{cmax}) and light saturated rate of electron transport (J_{max}) both varied with P_i treatment and ECM fungal colonization (Table 2). Nonmycorrhizal aspen exhibited the highest V_{cmax} at 100 μM P_i with a 50% reduction at 5 μM P_i (Table 2) and V_{cmax} declined as leaf P declined (Figure 1). As colonization by with ECM fungi sustained plant leaf P concentrations under P_i limitation, V_{cmax} was unaffected by P_i treatment in ECM aspen (Figure 1). Similarly, NM aspen exhibited the greatest J_{max} at 100 μM P_i and the greatest inhibition at 5 μM P_i (–59%), whereas J_{max} was unaffected by P_i treatment in plants colonized by *L. bicolor* or *P. involutus* (Table 2).

Growth parameters

Plant biomass declined with limiting foliar P concentrations in nonmycorrhizal aspen, but this response was modulated by *L. bicolor* and *P. involutus*, with an abrupt threshold of ~1.5 mg P g^{-1} (Figure 2). Shoot growth of aspen did not differ among mycorrhizal treatments at 100 μM P_i, however reductions in shoot biomass at 5 μM P_i in NM plants (–74%) were substantially greater than reduction in plants colonized by *L. bicolor* (–48%) or *P. involutus* (–45%) (Table 3). Patterns of root allocation under P_i limitation and among mycorrhizal treatments were more complex than shoot responses. At 100 μM P_i, plants colonized by *P. involutus* allocated 55% less C to roots than NM plants and 46% less than plants colonized by *L. bicolor* (Table 3). Root growth was reduced by 91% in NM plants at 5 μM P_i, whereas reductions in plants colonized by *L. bicolor* and *P. involutus* were not statistically different from their corresponding root masses at 100 μM P_i (Table 3).

Tissue C partitioning

Phosphate (P_i) availability and ECM symbionts significantly altered C partitioning in young and mature leaves of aspen (Table 4). Across ECM treatments, young leaves of plants grown with 5 μM P_i exhibited lower leaf starch (–42%), sucrose (–41%), glucose (–30%) and sucrose (–35%) concentrations and higher phenol (+38%) and tannin (+38) concentrations (Table 4). However, the extent of these reductions was differentially modulated by *L. bicolor* and *P. involutus*, with partitioning in aspen colonized by *L. bicolor* exhibiting patterns intermediate between NM aspen and aspen colonized by *P. involutus* (Table 4). Phenolic glycoside and tannin concentrations in young leaves were also higher in NM aspen compared with mycorrhizal aspen grown at 100 μM P_i, suggesting that the ECM associations increase resource availability under this treatment as well as under P_i limitation. Mature leaf nonstructural carbohydrate (NSC, starch + soluble sugars) partitioning was similarly affected by P_i availability, and increases in phenolic glycoside and tannin accumulation were also noted in NM aspen under P_i limitation (Table 4).

Across all treatments, total NSC accumulation was negatively correlated with the accumulation of total CBSCs (phenolic glycosides + tannins) in young leaves ($r = –0.624$, $P = 0.006$), but not in mature leaves ($r = –0.169$, $P = 0.518$). In young leaves, NSC and total CBSC accretion were positively and negatively correlated, respectively, with leaf P concentration (Figure 3). A similar relationship was noted for NSCs and total CBSCs in mature leaves ($r = 0.563$, $P = 0.019$) (data not presented). Both ECM fungi modulated these C partitioning relationships, maintaining higher NSCs and lower total CBSCs in leaves primarily by maintaining leaf P concentrations under P_i limitation (Figure 3, Table 4).

In contrast to leaves, NSC and total CBSC concentrations in roots of aspen were not correlated ($r = 0.145$, $P = 0.566$) and

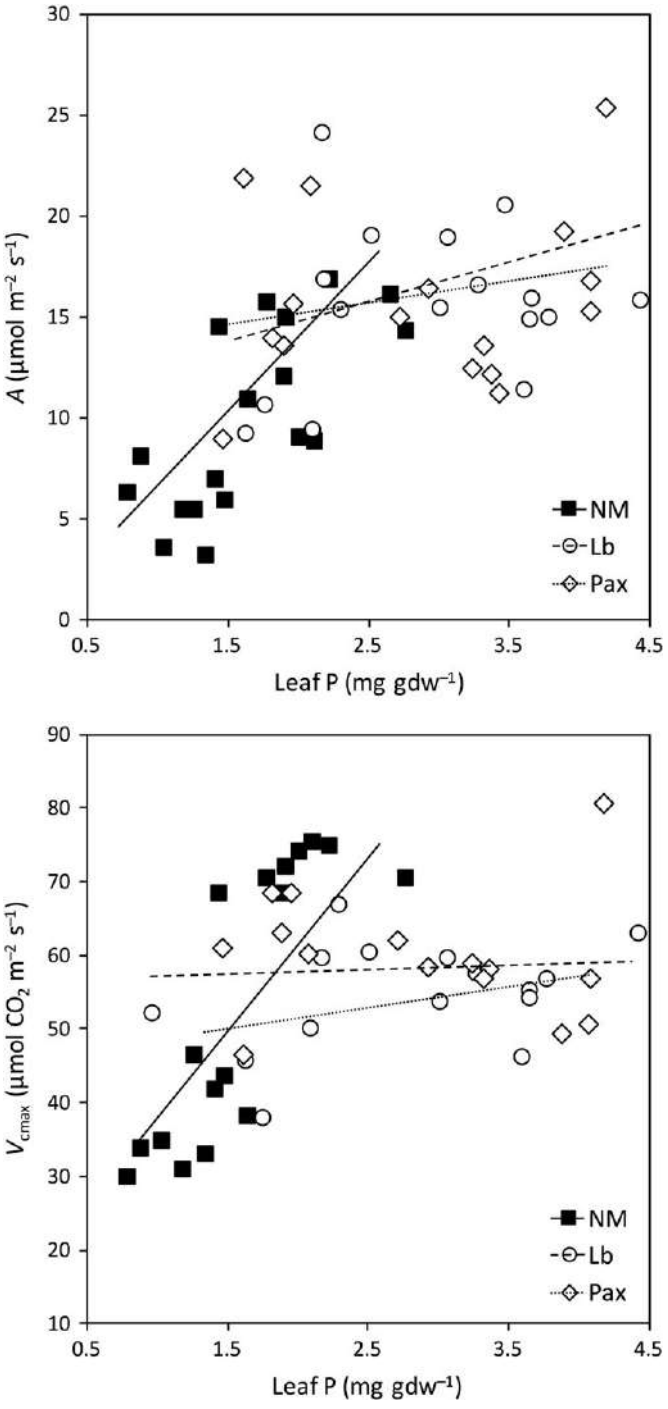

Figure 1. Steady-state photosynthetic CO_2 uptake (A) (top) and the maximum rate of RuBP carboxylation (V_{cmax}) (bottom) in relation to leaf P concentrations for nonmycorrhizal (NM) aspen seedlings and seedlings colonized by *L. bicolor* (Lb) and *P. involutus* (Pax).

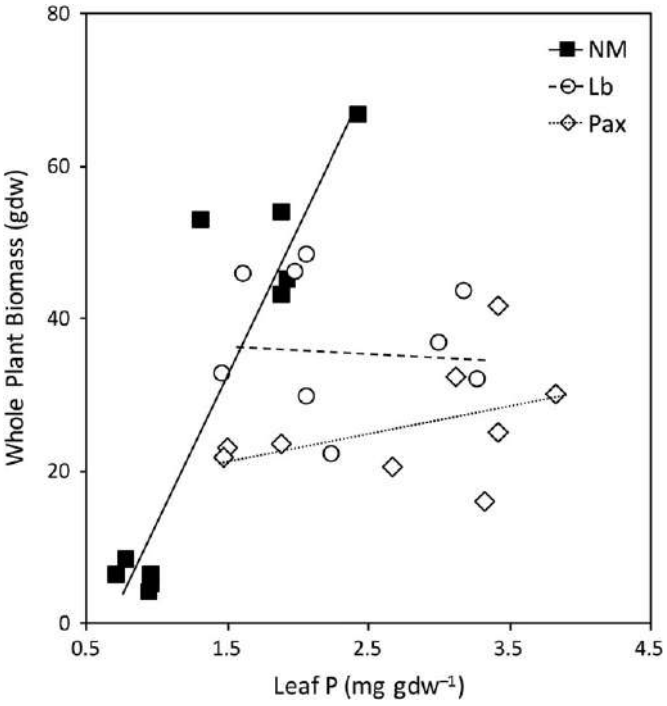

Figure 2. Whole plant biomass in relation to leaf P concentrations for nonmycorrhizal (NM) aspen seedlings and seedlings colonized by *L. bicolor* (Lb) and *P. involutus* (Pax).

there were no relationships with root P concentration ($r = 0.001$ and 0.044 for NSCs and CBSCs, respectively). However, C partitioning in roots was significantly altered by the treatment factors. In NM aspen, P_i limitation increased the accumulation of starch (3.9-fold), sucrose (2.9-fold), glucose (1.5-fold) and fructose (1.7-fold), whereas only sucrose increased in roots colonized *L. bicolor* (+29%) and *P. involutus* (+49%) (Figure 4, Table 4). In addition to these fundamental C shifts due to P_i limitation, there were fundamental shifts in C partitioning in aspen roots resulting from mycorrhizal colonization. Both *L. bicolor* and *P. involutus* increased root starch (3.3- and 5.7-fold, respectfully) and sucrose (2.7- and 2.8-fold), but decreased glucose and fructose concentrations, compared with NM roots (Figure 4, Table 4). In addition, roots of aspen colonized by *P. involutus* exhibited greater concentrations of tannins when P_i was not limiting (Figure 4, Table 4).

Across all treatments, the accumulation of specific CBSCs in young leaves was negatively correlated with leaf P, starch, glucose, fructose and sucrose accumulation and, in general, all positively correlated among themselves and with phenolic glycosides, but less so with tannins (Table 5; see Table S1 available as Supplementary Data at *Tree Physiology* Online). For example, young leaf catechin concentration declined ~8-fold across the range of leaf P concentrations (Figure 5), with leaves of plants colonized by *L. bicolor* and *P. involutus* maintaining catechin concentrations at or well below concentrations of NM plants, especially those grown at 5 μM P_i (Figure 5). Other CBSCs exhibited similar patterns in young leaves (Table 5; see Table S1 available as Supplementary Data at *Tree Physiology* Online). These patterns were also evident in mature leaves, although correlations between CBSCs and NSCs were minimal (Table 5; see Table S1 available as Supplementary Data at *Tree*

Table 3. Biomass of nonmycorrhizal (NM) aspen seedlings and seedlings colonized by *L. bicolor* (Lb) and *P. involutus* (Pax) as affected by P_i treatment. Means ± standard errors followed by the same letter within a column are not statistically different at $P \leq 0.05$ by Tukey's HSD.

P_i	ECM	Shoot (gdw)	Root (gdw)	Root:shoot	Total plant (gdw)
5 μM	NM	1.76 ± 0.28c	4.0 ± 0.91d	2.75 ± 0.91b	5.76 ± 0.71c
	Lb	3.82 ± 0.27b	25.0 ± 2.8bc	6.51 ± 0.47a	28.8 ± 2.9b
	Pax	4.10 ± 0.4b	18.0 ± 0.6c	4.52 ± 0.48ab	22.1 ± 0.7b
100 μM	NM	6.88 ± 0.27a	45.1 ± 4.4a	6.71 ± 0.95a	52.0 ± 4.2a
	Lb	7.34 ± 0.9a	37.1 ± 5.3ab	5.58 ± 0.96ab	46.4 ± 5.4a
	Pax	7.50 ± 0.8a	21.4 ± 4.3c	2.99 ± 0.68b	28.9 ± 4.2b
P_{P_i}[1]		<0.001	<0.001	0.450	<0.001
P_{ECM}		<0.001	<0.001	<0.029	<0.001
$P_{P_i \times ECM}$		<0.001	<0.001	0.003	<0.001

[1]Probability of the P_i, ECM and P_i × ECM treatment effects, respectively.

Table 4. Partitioning of C among nonstructural carbohydrates, total phenolic glycosides, and tannins (mg gfw^{-1}) in young leaves, mature leaves and fine roots of nonmycorrhizal (NM) aspen seedlings and seedlings colonized by *L. bicolor* (Lb) and *P. involutus* (Pax) as affected by P_i treatment. Means ± standard errors followed by the same letter within a column are not statistically different at $P \leq 0.05$ by Tukey's HSD.

P_i	ECM	Starch	Glucose	Fructose	Sucrose	Phenols	Tannins
Young leaf							
5 μM	NM	17.9 ± 0.7d	4.78 ± 0.36d	4.27 ± 0.32c	10.4 ± 0.1d	23.00 ± 1.43a	10.71 ± 0.58a
	Lb	26.0 ± 2.5c	5.95 ± 0.15c	5.08 ± 0.28c	10.6 ± 0.2d	11.06 ± 1.05bc	6.81 ± 0.52ab
	Pax	22.2 ± 1.9c	7.12 ± 0.84bc	6.41 ± 0.16b	13.8 ± 0.1c	8.75 ± 1.37 cd	7.33 ± 1.42ab
100 μM	NM	33.9 ± 0.3b	7.53 ± 0.28b	7.60 ± 0.17b	17.5 ± 0.2b	16.06 ± 1.88ab	9.75 ± 0.94a
	Lb	36.7 ± 2.1ab	7.30 ± 0.38b	7.42 ± 0.15b	17.3 ± 0.1b	8.95 ± 1.47 cd	7.03 ± 2.04ab
	Pax	41.6 ± 1.3a	10.13 ± 0.23a	9.06 ± 0.02a	24.1 ± 0.1a	6.26 ± 0.57d	4.30 ± 0.75b
P_{P_i}[1]		<0.001	<0.001	<0.001	<0.001	0.007	0.136
P_{ECM}		<0.001	<0.001	<0.001	<0.001	<0.001	0.011
$P_{P_i \times ECM}$		0.553	0.164	0.013	0.021	0.842	0.401
Mature leaf							
5 μM	NM	18.7 ± 0.3c	4.06 ± 0.15d	4.23 ± 0.15d	11.2 ± 0.6d	13.45 ± 5.14a	10.56 ± 3.34a
	Lb	19.7 ± 0.7c	5.02 ± 0.45d	4.92 ± 0.45 cd	11.9 ± 0.6d	7.09 ± 1.23b	7.00 ± 3.04ab
	Pax	22.8 ± 0.4c	5.93 ± 0.12c	6.19 ± 0.12bc	14.0 ± 0.1c	6.37 ± 0.88b	6.41 ± 2.41b
100 μM	NM	36.8 ± 1.6b	7.72 ± 0.14b	8.71 ± 0.14ab	16.8 ± 0.8b	7.31 ± 0.24b	6.68 ± 0.15ab
	Lb	36.3 ± 0.5b	7.78 ± 0.23b	7.71 ± 0.23ab	18.1 ± 1.1ab	6.69 ± 0.29b	7.42 ± 0.20ab
	Pax	48.4 ± 2.5a	9.93 ± 0.13a	8.44 ± 0.13a	20.9 ± 0.2a	7.12 ± 0.98b	6.88 ± 2.60b
P_{P_i}[1]		<0.001	<0.001	<0.001	<0.001	0.013	0.313
P_{ECM}		<0.001	<0.001	0.010	<0.001	0.123	0.007
$P_{P_i \times ECM}$		0.491	0.158	0.060	0.071	0.013	0.211
Fine root							
5 μM	NM	7.34 ± 0.47b	6.05 ± 0.22a	6.33 ± 0.10a	7.34 ± 0.21c	2.92 ± 0.33	3.28 ± 0.52ab
	Lb	5.54 ± 0.31c	1.92 ± 0.01c	1.97 ± 0.03c	9.10 ± 0.39b	2.61 ± 0.52	3.01 ± 0.41ab
	Pax	9.54 ± 0.53a	1.19 ± 0.15d	1.14 ± 0.17d	10.64 ± 0.12a	2.27 ± 0.34	2.26 ± 0.27b
100 μM	NM	1.86 ± 0.09d	3.99 ± 0.15b	3.80 ± 0.06b	2.57 ± 0.09d	2.56 ± 0.65	2.20 ± 0.54b
	Lb	6.13 ± 0.08 c	1.73 ± 0.18c	2.30 ± 0.15c	7.05 ± 0.09c	2.86 ± 0.61	2.46 ± 0.51b
	Pax	9.97 ± 0.12a	1.58 ± 0.08 cd	1.34 ± 0.01d	7.10 ± 0.04c	4.21 ± 0.31	6.42 ± 0.70a
P_{P_i}[1]		<0.001	<0.001	<0.001	<0.001	0.242	0.353
P_{ECM}		<0.001	<0.001	<0.010	<0.001	0.635	0.080
$P_{P_i \times ECM}$		<0.001	<0.001	<0.001	<0.001	0.112	0.002

[1]Probability of the P_i, ECM and P_i × ECM treatment effects, respectively.

Physiology Online). In roots, in contrast, specific CBSCs exhibited positive correlations with glucose and fructose concentration, but not with starch, sucrose, phenolic glycosides or tannins (Table 5; see Table S1 available as Supplementary Data at *Tree Physiology* Online).

ANOVA-simultaneous component analysis (ASCA) was applied to the combined NSC and CBSC data. ASCA decomposes this data matrix into effect matrices (e.g., ECM and P_i) and residual matrices to evaluate variation among treatment effects within the multivariate data (Zwanenburg et al. 2011). Similar to

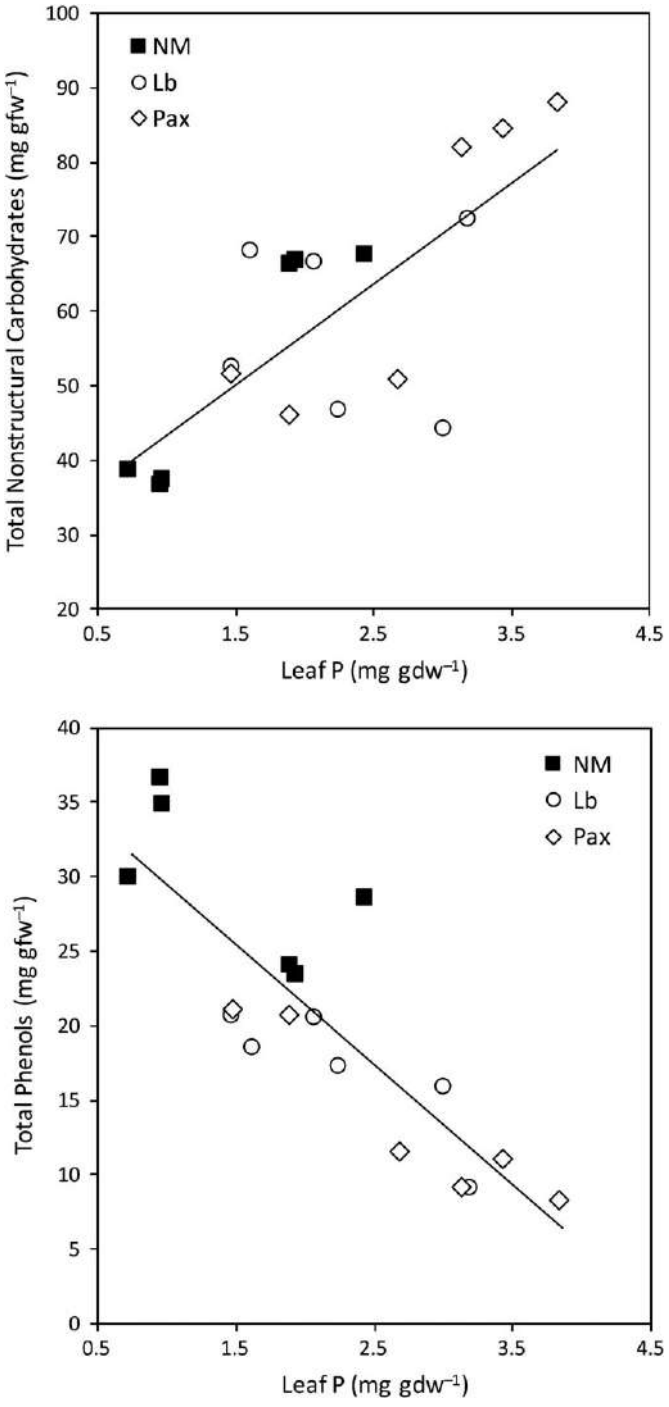

Figure 3. The accumulation of total nonstructural carbohydrates (top) and total phenols (bottom) in relation to leaf P concentrations in young leaves for nonmycorrhizal (NM) aspen seedlings and seedlings colonized by *L. bicolor* (Lb) and *P. involutus* (Pax).

PCA, ASCA provides PCs and loading scores that reflect data dispersion, but these take into account structured treatments.

In young leaves, distinct differences in the fate of C in aspen seedlings were evident among ECM treatments. Nonmycorrhizal aspen and aspen colonized by *L. bicolor* or *P. involutus* fell into three distinct clusters (Figure 6), with 90% of that variation between mycorrhizal treatments accounted for in PC1 that reflected trade-offs between NSCs and CBSCs primarily separating NM from the two ECM fungal treatments (Figure 6). A further separation in C partitioning was evident in PC2, separating *P. involutus* from NM and *L. bicolor* aspen based on soluble sugars and kaempferol. Seedlings colonized by *P. involutus* accumulated between 25% and 41% more sugars than other ECM treatments, whereas kaempferol concentrations in young leaves from NM and *L. bicolor* aspen were twofold greater than those from *P. involutus* seedlings (Table 4). Separation of mature leaves was also evident via ASCA and similar to that of young leaves, with NSCs and CBSCs separating NM and the two ECM treatments on PC1 and NSCs and kaempferol on PC2 (data not presented).

The three ECM treatments were also clearly separated based on root chemistry (Figure 7). In this case, the accumulation of starch and sucrose and reduced monosaccharides, tremulacin and myricetin separated roots colonized by *L. bicolor* or *P. involutus* from NM roots along PC1, while starch and phenolic glycosides, specifically catechin and kaempferol, contributed to separation of the three mycorrhizal treatments along PC2 (Figure 7).

Discussion

Soil nutrient limitation, especially N and P, limits the productivity of many natural and planted forests (Kirkman et al. 2001, Wardle et al. 2004, St Clair et al. 2008, Hou et al. 2012). Under such conditions, trees depend on integrated physiological adjustments as well as symbiotic mycorrhizal fungi to enhance nutrient acquisition and increase nutrient-use efficiency, and together these will influence C capture, allocation and partitioning in trees and the fate of C in forested ecosystems.

Phosphate limitation and ECM alter C fixation and allocation in aspen

Phosphorus limitation often reduces the rate of photosynthesis and plant productivity (Wissuwa et al. 2005, Boyce et al. 2006, Thomas et al. 2006). Under P_i limitation, photosynthetic CO_2 uptake was reduced in NM aspen, but remained unaffected in plants colonized by either symbiont (Table 2). This maintenance of photosynthesis at low P_i was related to the maintenance of leaf P concentrations above a limiting threshold of ~1.5 mg g^{-1} (Figure 1). Reductions in *A* were accompanied by parallel reductions in J_{max} and V_{cmax} (Table 2), indicating that low leaf P concentration in NM aspen constrained the biochemistry of C fixation (Loustau et al. 1999, Bown et al. 2007). In contrast, enhanced P_i acquisition by *L. bicolor* or *P. involutus* at low P_i sustained the reactions of CO_2 fixation at the whole plant and biochemical levels.

Although ECM colonization moderated reductions in plant growth under P_i limitation, the two ECM symbionts differentially influenced host plant growth, C allocation and growth-tissue P concentration relationships (Table 3, Figure 3). The C cost of sustaining *P. involutus* in symbiosis was significant, with aspen

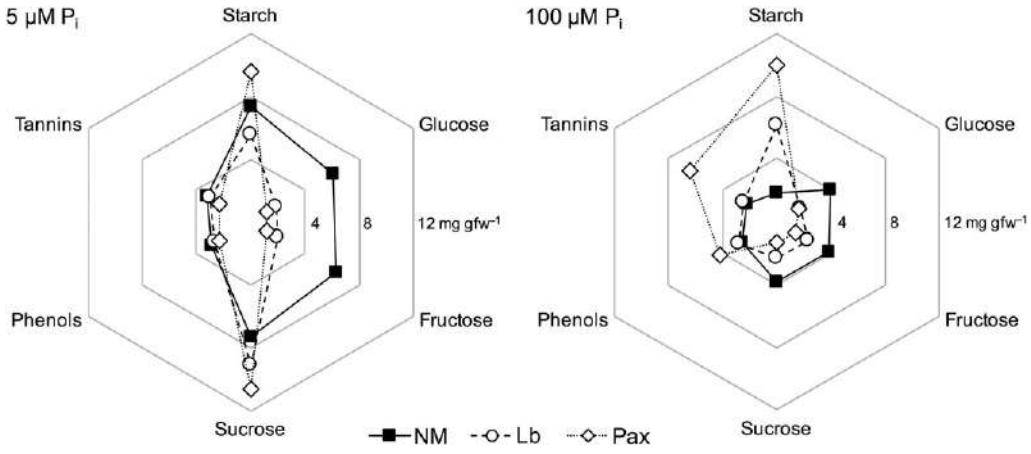

Figure 4. Partitioning of C between starch, sugars, total phenolic glycosides and total tannins in fine roots of nonmycorrhizal (NM) aspen seedlings and seedlings colonized by *L. bicolor* (Lb) and *P. involutus* (Pax) grown at 5 μM (left) and 100 μM (right) P_i.

Table 5. Partitioning of C among phenolic glycosides and flavonoids (mg gfw^{-1}) in young leaves, mature leaves and fine roots of nonmycorrhizal (NM) aspen seedlings and seedlings colonized by *L. bicolor* (Lb) and *P. involutus* (Pax) as affected by P_i treatment. Means ± standard errors followed by the same letter within a column are not statistically different at $P \leq 0.05$ by Tukey's HSD.

P_i	ECM	Salicin	Catechin	Kaempferol	Salicortin	Procyanidin	Tremulacin	Myrcitin
Young leaf								
5 μM	NM	2.84 ± 0.15a	4.35 ± 0.77a	0.251 ± 0.098ab	5.46 ± 2.15a	7.01 ± 1.00a	2.09 ± 0.14a	2.06 ± 0.03a
	Lb	2.47 ± 0.68a	2.08 ± 0.39ab	0.249 ± 0.046a	3.35 ± 0.17a	2.32 ± 0.15bc	0.78 ± 0.01abc	0.44 ± 0.05bc
	Pax	2.04 ± 0.20a	2.44 ± 0.15ab	0.186 ± 0.043ab	3.35 ± 1.23a	2.50 ± 0.35bc	0.83 ± 0.31abc	0.51 ± 0.08abc
100 μM	NM	3.11 ± 0.64a	2.42 ± 0.30ab	0.297 ± 0.066a	5.22 ± 1.15a	4.62 ± 1.30ab	1.35 ± 0.37ab	1.54 ± 0.64ab
	Lb	1.47 ± 0.26ab	1.44 ± 0.44bc	0.204 ± 0.029ab	2.02 ± 0.42a	2.04 ± 0.29bc	0.54 ± 0.07bc	0.26 ± 0.03c
	Pax	0.81 ± 0.16b	0.82 ± 0.14c	0.077 ± 0.016b	1.43 ± 0.31a	1.30 ± 0.25c	0.32 ± 0.06c	0.16 ± 0.02c
P_{P_i}[1]		0.007	<0.001	0.183	0.174	0.016	0.012	0.006
P_{ECM}		0.001	<0.001	0.016	0.073	<0.001	<0.001	<0.001
$P_{P_i \times ECM}$		0.046	0.203	0.114	0.369	0.425	0.662	0.553
Mature leaf								
5 μM	NM	1.41 ± 0.15a	2.79 ± 0.23a	0.080 ± 0.003ab	2.36 ± 0.20a	2.07 ± 0.08a	0.466 ± 0.052a	0.436 ± 0.078a
	Lb	1.18 ± 0.13a	1.45 ± 0.09a	0.078 ± 0.007ab	1.52 ± 0.11b	1.49 ± 0.23ab	0.322 ± 0.036ab	0.290 ± 0.029a
	Pax	1.06 ± 0.14a	3.17 ± 0.86a	0.066 ± 0.018ab	1.76 ± 0.08ab	1.30 ± 0.12b	0.281 ± 0.009b	0.250 ± 0.017a
100 μM	NM	1.47 ± 0.20a	1.94 ± 0.43a	0.122 ± 0.001a	2.26 ± 0.08ab	2.02 ± 0.10ab	0.414 ± 0.028ab	0.299 ± 0.003a
	Lb	1.10 ± 0.03a	2.12 ± 0.40a	0.098 ± 0.018ab	1.90 ± 0.16ab	1.41 ± 0.10ab	0.342 ± 0.031ab	0.281 ± 0.027a
	Pax	1.15 ± 0.12a	1.97 ± 0.47a	0.051 ± 0.008b	2.09 ± 0.24ab	1.41 ± 0.14ab	0.336 ± 0.025ab	0.268 ± 0.026a
P_{P_i}[1]		0.787	0.346	0.339	0.147	0.958	0.659	0.293
P_{ECM}		0.078	0.305	0.008	0.017	0.003	0.006	0.053
$P_{P_i \times ECM}$		0.800	0.128	0.169	0.389	0.814	0.314	0.258
Fine root								
5 μM	NM	0.370 ± 0.089	1.31 ± 0.21	0.300 ± 0.046	0.607 ± 0.122	0.880 ± 0.132a	0.107 ± 0.015a	1.28 ± 0.27a
	Lb	0.130 ± 0.046	0.80 ± 0.10	0.157 ± 0.020	0.343 ± 0.039	0.363 ± 0.030b	0.037 ± 0.007b	0.17 ± 0.023c
	Pax	0.103 ± 0.050	0.96 ± 0.25	0.210 ± 0.055	0.320 ± 0.052	0.347 ± 0.024b	0.027 ± 0.003b	0.23 ± 0.01bc
100 μM	NM	0.143 ± 0.020	0.87 ± 0.07	0.180 ± 0.017	0.337 ± 0.020	0.223 ± 0.026b	0.040 ± 0.005b	0.56 ± 0.24ab
	Lb	0.163 ± 0.012	0.84 ± 0.19	0.197 ± 0.044	0.353 ± 0.047	0.387 ± 0.038b	0.033 ± 0.007b	0.28 ± 0.04bc
	Pax	0.097 ± 0.047	1.21 ± 0.04	0.243 ± 0.034	0.350 ± 0.057	0.410 ± 0.098b	0.040 ± 0.010b	0.29 ± 0.04bc
P_{P_i}[1]		0.604	0.877	0.809	0.233	0.005	0.125	0.7052
P_{ECM}		0.053	0.237	0.263	0.115	0.417	0.004	<0.001
$P_{P_i \times ECM}$		0.249	0.192	0.145	0.085	<0.001	0.009	0.009

[1]Probability of the P_i, ECM and P_i × ECM treatment effects, respectively.

total plant mass being 44% less than NM plants grown at 100 μM P_i, primarily reflecting significantly less C allocation to root biomass (Table 3). Biomass of aspen colonized by *L. bicolor* was intermediate between NM aspen and aspen colonized by *P. involutus* at 100 μM P_i. Although there was evidence of a significant cost to aspen of maintaining the ECM symbioses,

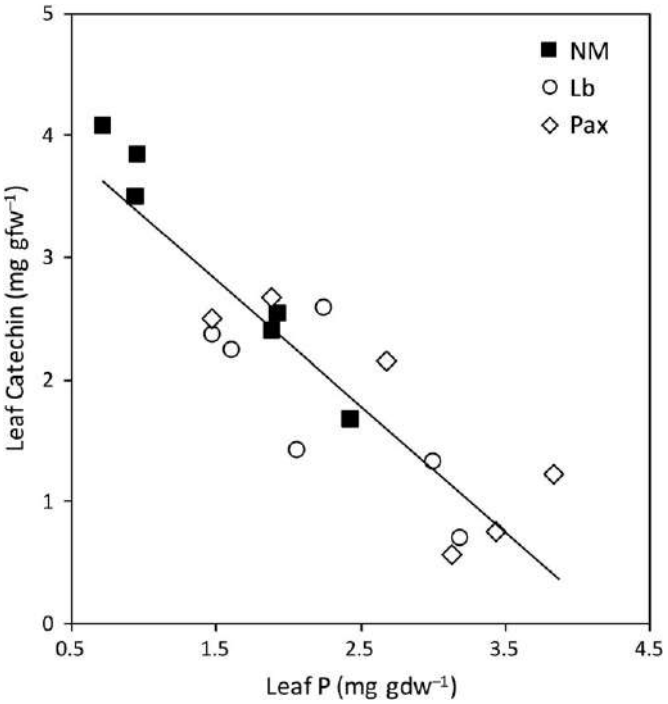

Figure 5. The accumulation of catechin in young leaves in relation to leaf P concentrations for nonmycorrhizal (NM) aspen seedlings and seedlings colonized by *L. bicolor* (Lb) and *P. involutus* (Pax).

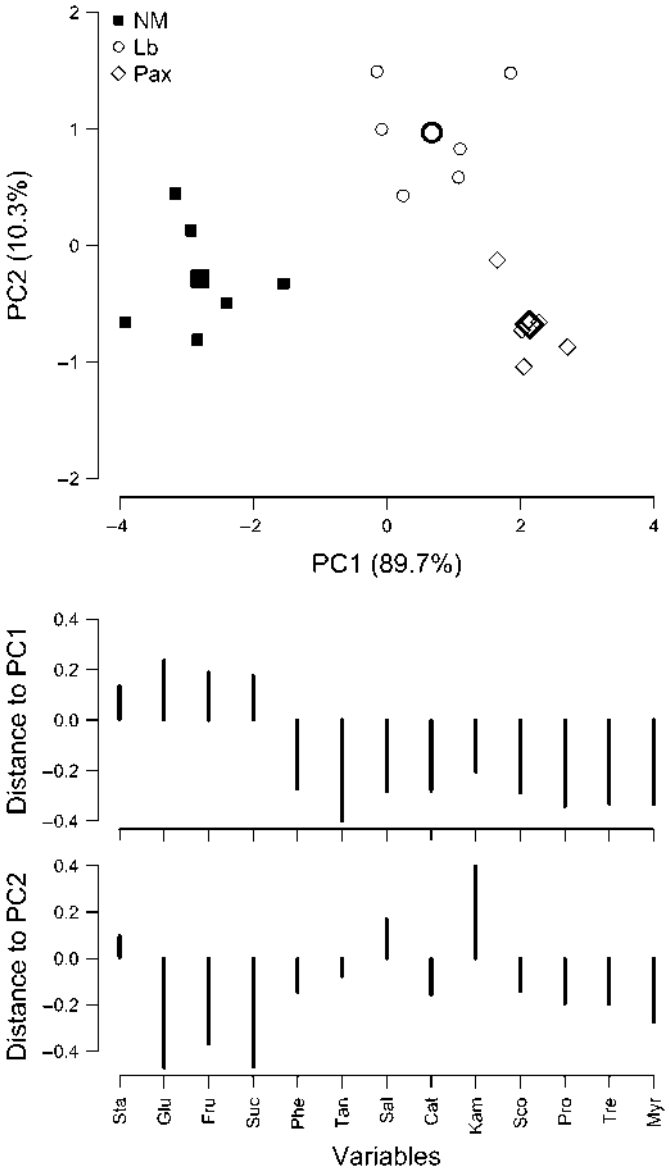

Figure 6. ASCA separation of nonmycorrhizal (NM) aspen seedlings and seedlings colonized by *L. bicolor* (Lb) and *P. involutus* (Pax) based on variation among primary and secondary carbon metabolites in young leaves (top) and the relative influences of each on the principal components (bottom). Bold/larger symbols in upper figure are the multivariate mean responses for each mycorrhizal treatment. Abbreviations at bottom: Sta = starch, Glu = glucose, Fru = fructose, Suc = sucrose, Phe = phenolics, Tan = tannins, Sal = salicin, Cat = catechin, Kae = kaempferol, Sco = salicortin, Pro = procyanidin B2, Tre = tremulacin, Myr = myricetin.

photosynthesis was not stimulated (Table 2), although the highest individual rates were recorded in mycorrhizal plants (Figure 1). Ectomycorrhizal fungi may or may not stimulate CO_2 assimilation when growth is limited die to sink demand (e.g., Conjeaud et al. 1996, Wright et al. 2000, Heinonsalo et al. 2010), but variation among ECM and differences in experimental systems makes generalization difficult.

These changes in allocation belowground may reflect C allocation shifts from roots to the fungal partners. Although we did not assess hyphal biomass in this study, mycorrhizal colonization alters C allocation through the construction of hyphae, fungal respiration, and other fungal metabolic demands (Conjeaud et al. 1996, Choi et al. 2008, Makita et al. 2012). These changes may greatly increase the nutrient capture capacity of the plant and lead to reduced need for root surface area under P replete conditions, yet sustained P_i acquisition under P_i limitation (Lopéz-Bucio et al. 2002).

These differences between *L. bicolor* and *P. involutus* are supported by other reports of ECM-species variation in P acquisition and C allocation/partitioning (Cumming 1996, van Tichelen and Colpaert 2000, Heinonsalo et al. 2010). For example, significant variation in C allocation occurs in ectomycorrhizal *Pinus sylvestris*, with *P. involutus* allocating less C to roots than *L. bicolor*, which maintained a more even balance between root and shoot (Fransson et al. 2007), patterns noted here for aspen. Similarly, *P. involutus* exerted a significant C cost on *Betula pedula* (Wright et al. 2000), aligning with patterns noted for aspen in the current experiment. Thus, the interplay between fungal C-use efficiency, the acquisition of nutrient resources such as P, and plant acclimation to symbiont C demand and P exchange will ultimately influence the growth and C allocation outcomes in the ECM symbiosis.

Phosphate limitation and ECM change tissue chemistry and C partitioning in aspen

Plant C partitioning changes under nutrient stress (Kleiner et al. 1998, Hale et al. 2005, Yin et al. 2009, Kleczewski et al. 2012)

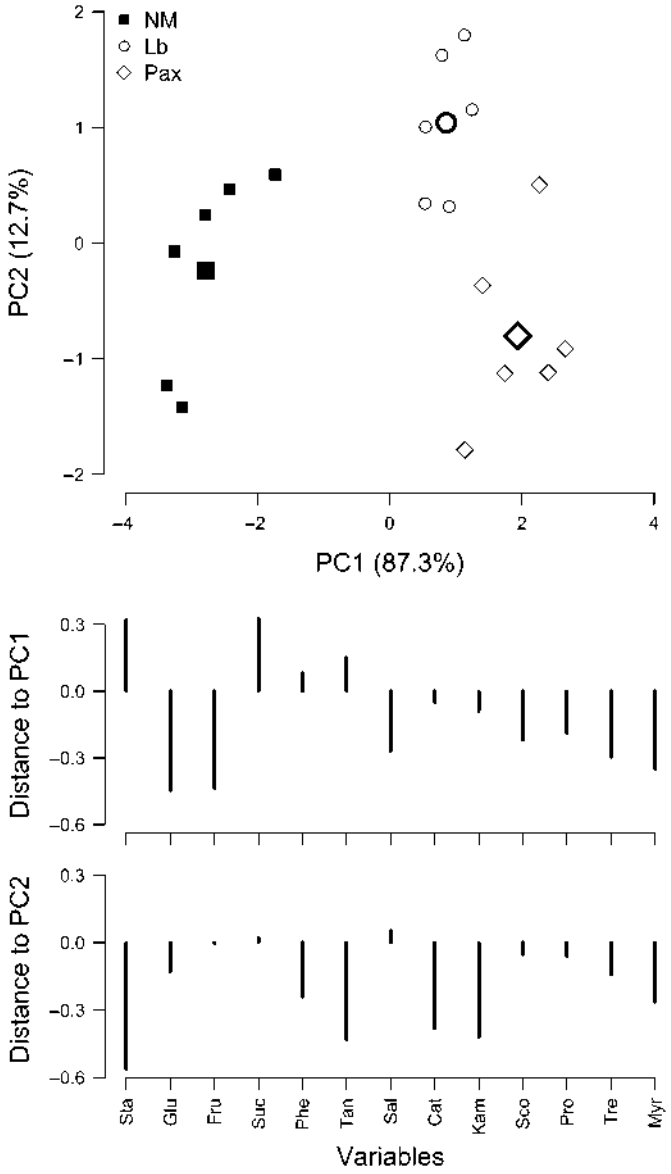

Figure 7. ASCA separation of nonmycorrhizal (NM) aspen seedlings and seedlings colonized by *L. bicolor* (Lb) and *P. involutus* (Pax) based on variation among primary and secondary carbon metabolites in fine roots (top) and the relative influences of each on the principal components (bottom). Bold/larger symbols in upper figure are the multivariate mean responses for each mycorrhizal treatment. Abbreviations as in Figure 6.

and such changes may be further modified during mycorrhizal symbiosis (Baum et al. 2009, Sanchez-Zabala et al. 2013). In the current experiment, NSC concentrations declined in young and mature leaves of aspen under P_i limitation (Figure 3). These changes were mitigated in plants colonized by *P. involutus* while plants colonized by *L. bicolor* exhibited carbohydrate profiles more similar to NM plants. In roots, NSC patterns were more complex, as starch, sucrose and monosaccharides increased in NM aspen under P_i limitation, whereas only sucrose increased in roots colonized by *L. bicolor* or *P. involutus* (Figure 4). Elevated monosaccharide concentrations in NM roots at low P_i may reflect part of the PSR signaling system designed to initiate P-deprivation acclimation pathways (Zhang et al. 2014). Patterns of elevated sucrose and generally lower monosaccharide concentrations in ECM roots may reflect the preferential use of glucose and fructose by the symbionts and the sink stimulation of sucrose transport to ECM roots (Nehls and Hampp 2000, Wright et al. 2000, Nehls 2008).

In the current study, CBSC concentrations were typically higher in NM aspen, and the CBSC profiles of young leaves, mature leaves and roots delineated ECM species-specific influences on aspen metabolism. The accumulation of CBSCs in tissues may reflect partitioning of C to storage under P limitation or physiological acclimation pathways associated with free radical production under nutrient limitation (Hale et al. 2005, Baum et al. 2009, Abdel-lateif et al. 2016) as well as changes in host physiology in support of colonization (Weiss et al. 1997, Jung and Tamai 2012, Sanchez-Zabala et al. 2013). In aspen leaves, the accumulation of catechin and kaempferol were major responses of aspen to P_i-ECM treatments, with these and other CBSCs accumulating under P_i limitation (Figure 6, Table 4). *Paxillus involutus* was more effective at mediating these perturbations to C partitioning in aspen than *L. bicolor*, which may reflect the influence of this symbiont in constraining plant growth compared with NM and *L. bicolor* aspen. In another study on *P. tremuloides*, foliar tannins increased up to 5-fold under N limitation and relative growth rates of plants were negatively correlated with C partitioned to foliar tannins and phenolic glycosides (Donaldson et al. 2006). In *Eucalyptus*, low P_i affected metabolites of the phenylpropanoid pathway, with quinic acid and catechin increasing in leaves as P_i declined from 100 to 5 μM (Warren 2011). Thus, these shifts of C from growth and NSC pools to CBSCs under P_i limitation in aspen reflect fundamental shifts in metabolism resulting from nutrient stress.

In aspen roots, there were also distinct shifts in C partitioning induced by P_i limitation and by colonization by ECM. Notable were lower concentrations of glucose and sucrose under all P_i conditions and shifts to sucrose under P_i limitation in ECM roots, whereas large increases in all NSCs and several CBSCs were induced by low P_i in NM aspen roots (Figure 4). These increases in sugars, especially sucrose, may reflect integrated PSR systems response to P limitation (Lopéz-Arredondo et al. 2014). In roots colonized by *P. involutus*, the large reduction in tannins, which are present in hyphae of this species (Gafur et al. 2004, Jacob et al. 2004), grown at low P_i may reflect diversion of C away from tannins to fungal hyphal growth or other PSR systems, and not a response of the aspen host per se. This is supported by the lack of correlations between phenols or tannins and any of the specific CBSC in roots (see Table S1 available as Supplementary Data at *Tree Physiology* Online).

Variation between ECM for P_i acquisition, C relations and stress responses in aspen

Ectomycorrhizal fungus species vary greatly in their influences on tree root physiology and response to P availability in the

environment (Plassard and Dell 2010, Cumming et al. 2015). There are differences among fungi in their capacity to transport P_i (Cumming 1996, Van Tichelin and Colpaert 2000), produce mineral-dissolving exudates (Casarin et al. 2004, Johansson et al. 2009) and exude enzymes to alter P_o-P_i equilibria in the rhizosphere (Ali et al. 2009, Alvarez et al. 2012). In the current study, *L. bicolor* and *P. involutus* were used as model ECM species. *Laccaria bicolor* is a cosmopolitan species frequently associating with *Populus* in forest ecosystems (Martin et al. 1999, Ostry et al. 2011); *P. involutus* is equally far-ranging (Lamaison and Polese 2008), and both have been used extensively in mycorrhizal research. Our previous worked indicated that these ECM fungi differ in their P starvation responses (Cumming 1996), thus we selected them for use here. The modification of P and C relations of aspen would be expected to vary greatly based on fungal symbiont community, and these two fungi may encompass only a portion of the potential range of aspen C metabolism modification in response to P limitation. Further, although roots of aspen will be, by-and-large, colonized by ECM fungi in nature, we compared NM control aspen responses to aspen colonized by these symbionts to elucidate the capacity of aspen to respond to resource limitation and the fundamental shifts in C and P relations brought about by the two ECM symbionts.

Aspen colonized by *L. bicolor* and *P. involutus* exhibited large differences in C allocation between shoots and roots and, although the responses to P_i limitation were similar, allocation to roots differed between the symbionts. These fundamental differences may reflect differences in C demand by each partner or complex interactions between aspen roots and the two symbionts. Such differences in C allocation modulated by different ECM fungi are often noted, especially for root:shoot ratios (Karst et al. 2008), and may reflect mycorrhizal species-specific C construction and maintenance costs (Colpaert et al. 1996, Nehls and Hampp 2000, Hobbie 2006).

The two ECM fungal symbionts also differed in their influence on aspen C partitioning. Such differences in NSC partitioning may reflect differential demand and use of carbohydrates in roots, whereas CBSC responses reflect differential alleviation of P_i limitation stresses in aspen by *L. bicolor* and *P. involutus*. Taken together with patterns of growth, photosynthesis and P concentrations in tissues, it is evident that aspen colonized by *L. bicolor* responds to P_i limitation in an intermediate manner between NM plants and those colonized by *P. involutus,* and such differences may reflect different ecological niches/roles of these two fungi or unique host–fungal identity interactions.

Conclusions

Aspen responded to P_i limitation with reductions in photosynthesis and growth, reductions in carbohydrates in leaves and increases in roots, and increases in secondary metabolite production that reflected altered C allocation and partitioning. These changes were significantly less or not evident in aspen colonized with *L. bicolor* and *P. involutus* due to the maintenance of P_i acquisition by ECM under P_i limitation. However, aspen associated with these two symbionts exhibited fundamental differences in C allocation and partitioning regardless of growth P_i environment. Aspen colonized by *P. involutus* exhibited less perturbation to growth and tissue NSC and CBSC profiles than plants colonized by *L. bicolor*. The alleviation of these stresses was not related to P acquisition per se, but to slower growth of plants with *P. involutus* reflecting fundamental changes in C–P relations in aspen induced by this ECM fungus. The alleviation of stress responses by *L. bicolor* and *P. involutus* indicates that ECM associations play critical roles in maintaining nutrient homeostasis in aspen. However, differences in C allocation and partitioning among growth and NSC and CBSC pools indicate that ECM symbionts do not uniformly affect host P_i stress responses, and the diversity of the aspen root microbiome and ECM species-specific changes in C metabolism are important for determining the quantity and quality of C cycling in forested ecosystems.

Supplementary Data

Supplementary Data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

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References

Abdel-lateif KS, Maghrabi IA, Eldeab HA (2016) The plant natural products: their antioxidants, free radical scavengers, DNA protection and antimicrobial activities. J Bioprocess Biotech 6:293.

Agerer R (2001) Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11: 107–114.

Ali MA, Louche J, Legname E, Duchemin M, Plassard C (2009) *Pinus pinaster* seedlings and their fungal symbionts show high plasticity in phosphorus acquisition in acidic soils. Tree Physiol 29:1587–1597.

Alvarez M, Huygens D, Díaz LM, Villanueva CA, Heyser W, Boeckx P (2012) The spatial distribution of acid phosphatase activity in ectomycorrhizal tissues depends on soil fertility and morphotype, and relates to host plant phosphorus uptake. Plant Cell Environ 35:126–135.

Batjes NH (1997) A world data set of derived soil properties by FAOU/NESCO soil unit for global modeling. Soil Use Manage 1:9–16.

Baum C, Toljander YK, Eckhardt K-U, Weih M (2009) The significance of host-fungus combinations in ectomycorrhizal symbioses for the chemical quality of willow foliage. Plant Soil 323:213–224.

Bonito G, Hameed K, Ventura R, Krishnan J, Schadt CW, Vilgalys R (2016) Isolating a functionally relevant guild of fungi from the root microbiome of *Populus*. Fungal Ecol 22:35–42.

Bown HE, Watt MS, Clinton PW, Mason EG, Richardson B (2007) Partitioning concurrent influences of nitrogen and phosphorus supply on photosynthetic model parameters of *Pinus radiata*. Tree Physiol 27:335–344.

Boyce RL, Larson JR, Sanford RL Jr (2006) Phosphorus and nitrogen limitations to photosynthesis in Rocky Mountain bristlecone pine (*Pinus aristata*) in Colorado. Tree Physiol 26:1477–1486.

Bryant JP, Chapin FS III, Klein DR (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40:357–368.

Caretto S, Linsalata V, Colella G, Mita G, Lattanzio V (2015) Carbon fluxes between primary metabolism and phenolic pathway in plant tissues under stress. Int J Mol Sci 16:26378–26394.

Casarin V, Plassard C, Hinsinger P, Arvieu JC (2004) Quantification of ectomycorrhizal fungal effects on the bioavailability and mobilization of soil P in the rhizosphere of *Pinus pinaster*. New Phytol 163:177–185.

Chen F, Zhao N, Tschaplinski TJ, Liu CJ (2009) Genomics of secondary metabolism in *Populus*: interactions with biotic and abiotic environments. Crit Rev Plant Sci 28:375–392.

Choi DS, Jin HO, Chung DJ, Sasa K, Koike T (2008) Growth and physiological activity in *Larix kaempferi* seedlings inoculated with ectomycorrhizae as affected by soil acidification. Trees 22:729–735.

Colpaert JV, van Laere A, van Assche JA (1996) Carbon and nitrogen allocation in ectomycorrhizal and non-mycorrhizal *Pinus sylvestris* L. seedlings. Tree Physiol 16:787–793.

Colpaert JN, Katiak VT, Assche VJ, van Laere A (1999) Short-term phosphorus uptake rates in mycorrhizal and non-mycorrhizal roots of intact *Pinus sylvestris* seedlings. New Phytol 143:589–597.

Conjeaud C, Scheromm P, Mousain D (1996) Effects of phosphorus and ectomycorrhiza on maritime pine seedlings (*Pinus pinaster*). New Phytol 133:345–351.

Courty PE, Garbaye J, Franc A (2010) Temporal and functional pattern of secreted enzyme activities in an ectomycorrhizal community. Soil Biol Biochem 42:2022–2025.

Cumming JR (1993) Growth and nutrition of nonmycorrhizal and mycorrhizal pitch pine (*Pinus rigida*) seedlings under phosphorus limitation. Tree Physiol 13:173–187.

Cumming JR (1996) Phosphate-limitation physiology in ectomycorrhizal pitch pine (*Pinus rigida*) seedlings. Tree Physiol 16:977–983.

Cumming JR, Swiger TD, Kurnik BS, Panaccione DG (2001) Organic acid exudation by *Laccaria bicolor* and *Pisolithus tinctorius* exposed to aluminum in vitro. Can J For Res 31:703–710.

Cumming JR, Zawaski C, Desai S, Collart FR (2015) Phosphorus disequilibrium in the tripartite plant ectomycorrhiza-plant growth promoting rhizobacterial association. J Soil Sci Plant Nutr 15:464–485.

Desai S, Naik D, Cumming JR (2014) The influence of phosphorus availability and *Laccaria bicolor* symbiosis on phosphate acquisition, antioxidant enzyme activity, and rhizospheric carbon flux in *Populus tremuloides*. Mycorrhiza 24:369–382.

Dixon RA, Paiva NL (1995) Stress-induced phenylpropanoid metabolism. Plant Cell 7:1085–1097.

Donaldson JR, Kruger EL, Lindroth RL (2006) Competition-and resource-mediated tradeoffs between growth and defensive chemistry in trembling aspen (*Populus tremuloides*). New Phytol 169:561–570.

Dorscheidt T (2013) Package 'MetStaT': Statistical metabolomics tools. https://cran.r-project.org/web/packages/MetStaT/index.html.

Elser JJ, Bracken MES, Cleland EE et al. (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 10:1135–1142.

Fox MD, Tackaberry LE, Drouin P, Bergeron Y, Bradley R, Massicotte HB, HYH Chen (2013) Microbial community structure of soils under four productivity classes of aspen forests in northern British Columbia. Ecoscience 20:264–275.

Fransson PMA, Anderson IC, Alexander IJ (2007) Does carbon partitioning in ectomycorrhizal pine seedlings under elevated CO_2 vary with fungal species? Plant Soil 291:323–333.

Gafur A, Schützendübel A, Langenfeld-Heyser R, Fritz E, Polle A (2004) Compatible and incompetent *Paxillus involutus* isolates for ectomycorrhiza formation in vitro with poplar (*Populus* × *canescens*) differ in H_2O_2 production. Plant Biol 6:91–99.

Gayler S, Grams TEE, Heller W, Treutter D, Priesack E (2008) A dynamical model of environmental effects on allocation to carbon-based secondary compounds in juvenile trees. Ann Bot 101:1089–1098.

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol 84: 489–500.

Hagerberg D, Thelin G, Wallander H (2003) The production of ectomycorrhizal mycelium in forests: relation between forest nutrient status and local mineral sources. Plant Soil 252:279–290.

Hale BK, Herms DA, Hansen RC, Clausen TP, Arnold D (2005) Effects of drought stress and nutrient availability on dry matter allocation, phenolic glycosides, and rapid induced resistance of poplar to two Lymantriid defoliators. J Chem Ecol 31:2601–2620.

Heinonsalo J, Pumpanen J, Rasilo T, Hurme K-R, Ilvesniemi H (2010) Carbon partitioning in ectomycorrhizal Scots pine seedlings. Soil Biol Biochem 42:1614–1623.

Hetrick BAD (1991) Mycorrhizas and root architecture. Experientia 47: 355–362.

Hobbie EA (2006) Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. Ecology 87:563–569.

Hou E, Chen C, McGroddy ME, Wen D (2012) Nutrient limitation on ecosystem productivity and processes of mature and old-growth subtropical forests in China. PLoS One 12:1–12.

Jacob C, Courbot M, Martin F, Bruna A, Chalota M (2004) Transcriptomic responses to cadmium in the ectomycorrhizal fungus *Paxillus involutus*. FEBS Lett 576:423–427.

Jansen JJ, Hoefsloot HCJ, van der Greef J, Timmerman ME, Westerhuis JA, Smilde AK (2005) ASCA: analysis of multivariate data obtained from an experimental design. J Chemometrics 19:469–481.

Johansson EM, Fransson PMA, Finlay RD, van Hees PAW (2009) Quantitative analysis of soluble exudates produced by ectomycorrhizal roots as a response to ambient and elevated CO_2. Soil Biol Biochem 41:1111–1116.

Jung NC, Tamai Y (2012) Anatomical observation of polyphenol changes in epidermal cells during the development of *Quercus acutissima-Scleroderma verrucosum* ectomycorrhizae. Trees 26:301–310.

Kaldorf M, Renker C, Fladung M, Buscot F (2004) Characterization and spatial distribution of ectomycorrhizas colonizing aspen clones released in an experimental field. Mycorrhiza 14:295–306.

Karst J, Marczak L, Jones MD, Turkington R (2008) The mutualism-parasitism continuum in ectomycorrhizas: a quantitative assessment using meta-analysis. Ecology 89:1032–1042.

Keski-Saari S, Julkunen-Tiitto R (2003) Early developmental responses of mountain birch (*Betula pubescens* subsp. *czerepanovii*) seedlings to different concentrations of phosphorus. Tree Physiol 23:1201–1208.

Kirkman K, Mitchell RJ, Helton R, Drew MB (2001) Productivity and species richness across an environmental gradient in a fire-dependent ecosystem. Am J Bot 88:2119–2128.

Kleczewski NM, Herms DA, Bonello P (2012) Nutrient and water availability alter belowground patterns of biomass allocation, carbon partitioning, and ectomycorrhizal abundance in *Betula nigra*. Trees 26: 525–533.

Kleiner KW, Raffa KF, Ellis DD, McCown BH (1998) Effect of nitrogen availability on the growth and phytochemistry of hybrid poplar and the efficacy of the *Bacillus thuringiensis* cry1A(a) d-endotoxin on gypsy moth. Can J For Res 28:1055–1067.

Koricheva J, Larsson S, Haukioja E, Keinanen M (1998) Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of meta-analysis. Oikos 83:212–226.

Lamaison J-L, Polese J-M (2008) The great encyclopedia of mushrooms. HF Ullmann, Königswinter, Germany, p 240.

LeBauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89:371–379.

Leyval C, Berthelin J (1993) Rhizodeposition and net release of soluble organic compounds by pine and beech seedlings inoculated with rhizobacteria and ectomycorrhizal fungi. Biol Fertil Soils 15:259–267.

Liu Q, Grace LJ, Hedley MJ, Loganathan P (2008) Effect of mycorrhizal inoculation on rhizosphere properties, phosphorus uptake and growth of pine seedlings treated with and without a phosphate rock fertilizer. J Plant Nutr 31:137–156.

López-Arredondo DL, Leyva-González MA, González-Morales SI, López-Bucio J, Herrera-Estrella L (2014) Phosphate nutrition: improving low-phosphate tolerance in crops. Annu Rev Plant Biol 65:95–123.

Loépez-Bucio J, Hernández-Abreu E, Sánchez-Calderón L, Nieto-Jacobo MF, Simpson J, Herrera-Estrella L (2002) Phosphate availability alters architecture and causes changes in hormone sensitivity in the Arabidopsis root system. Plant Physiol 129:244–256.

Loustau D, Ben P, Brahim M, Gaudillere J, Dreyer E (1999) Photosynthetic responses to phosphorus nutrition in two-year-old maritime pine seedlings. Tree Physiol 19:707–715.

Lynch JP (2011) Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. Plant Physiol 156:1041–1049.

Makita N, Hirano Y, Yamanaka T, Yoshimura K, Kosugi Y (2012) Ectomycorrhizal-fungal colonization induces physio-morphological changes in *Quercus serrata* leaves and roots. J Plant Nutr Soil Sci 175: 900–906.

Martin M, Barberis E, Celi L (1999) Determination of low concentrations of organic phosphorus in soil solution. Commun Soil Sci Plant Anal 30: 1909–1917.

Mellway RD, Tran LT, Prouse MB, Campbell MM, Constabel CP (2009) The wound-, pathogen-, and ultraviolet B-responsive MY134 gene encodes an R2R3 MYB transcription factor that regulates proanthocyanidin synthesis in poplar. Plant Physiol 150:924–941.

Molina R, Palmer JG, Schenck NC, Paul MN (1982) Isolation, maintenance and pure culture manipulation of ectomycorrhizal fungi. In: Schenck N (ed) Methods and principles of mycorrhizal research. American Phytopathological Society, St Paul, MN, pp 115–129.

Nehls U (2008) Mastering ectomycorrhizal symbiosis: the impact of carbohydrates. J Exp Bot 59:1097–1108.

Nehls U, Hampp RD (2000) Carbon allocation in ectomycorrhizas. Physiol Mol Plant Pathol 57:95–100.

Ostry ME, Anderson NA, O'Brien JG (2011) Field guide to common macrofungi in eastern forests and their ecosystem functions. USDA-Forest Service General Technical Report NRS–79, Newtown Square, PA, 82 pp.

Plassard C, Dell B (2010) Phosphorus nutrition of mycorrhizal trees. Tree Physiol 30:1129–1139.

Plaxton WC, Tran HT (2011) Metabolic adaptations of phosphate-starved plants. Plant Physiol 156:1006–1015.

Porter LJ, Hrstich LN, Chan BG (1985) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25: 223–230.

Raghothama KG, Karthikeyan AS (2005) Phosphate acquisition. Plant Soil 274:37–49.

Rogers A, Allen DJ, Davey PA et al. (2004) Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their life-cycle under free-air carbon dioxide enrichment. Plant Cell Environ 27:449–458.

Sanchez-Zabala J, Majada J, Martín-Rodrigues N, Gonzalez-Murua C, Ortega U, Alonso-Graña M, Arana O, Duñabeitia MK (2013) Physiological aspects underlying the improved outplanting performance of *Pinus pinaster* Ait. seedlings associated with ectomycorrhizal inoculation. Mycorrhiza 23:627–640.

Schack-Kirchner H, Wilpert KV, Hildebrand EF (2000) The spatial distribution of soil hyphae in structured spruce-forest soil. Plant Soil 224: 195–205.

Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaas EL (2007) Fitting photosynthetic carbon dioxide response curves for C3 leaves. Plant Cell Environ 30:1035–1040.

Smilde AK, Jansen JJ, Hoefsloot HCJ, Lamers R-JAN, van der Greef J, Timmerman ME (2005) ANOVA–simultaneous component analysis (ASCA): a new tool for analyzing designed metabolomics data. Bioinformatics 21:3043–3048.

St Clair SB, Sharpe WE, Lynch JP (2008) Key interactions between nutrient limitation and climatic factors in temperate forests: a synthesis of the sugar maple literature. Can J For Res 38:401–414.

Thomas DS, Montagu KD, Conroy JP (2006) Leaf inorganic phosphorus as a potential indicator of phosphorus status, photosynthesis and growth of *Eucalyptus grandis* seedlings. For Ecol Manage 223:267–274.

Vance CP, Uhde-Stone C, Allan DL (1995) Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol 157:423–447.

van Tichelen KK, Colpaert JV (2000) Kinetics of phosphate absorption by mycorrhizal and non-mycorrhizal Scots pine seedlings. Physiol Plant 110:96–103.

von Uexküll HR, Mutert E (1995) Global extent, development and economic impact of acid soils. Plant Soil 171:1–15.

Wallander H (2000) Uptake of P from apatite by *Pinus sylvestris* seedlings colonized by different ectomycorrhizal fungi. Plant Soil 218: 249–256.

Wardle DA, Walker LR, Bardgett RD (2004) Ecosystem properties and forest decline in contrasting long-term chronosequences. Science 305:509–513.

Warren CR (2011) How does P affect photosynthesis and metabolite profiles of *Eucalyptus globulus*? Tree Physiol 31:727–739.

Waterhouse A (2002) Determination of total phenolics. Curr Protoc Food Analyt Chem I1.1.1–I1.1.8.

Weiss M, Mikolajewski S, Peipp H, Schmitt U, Schmidt J, Wray V, Strack D (1997) Tissue-specific and development-dependent accumulation of phenylpropanoids in larch mycorrhizas. Plant Physiol 114:15–27.

Wissuwa M, Gamat G, Ismail AM (2005) Is root growth under phosphorus deficiency affected by source or sink limitations? J Exp Bot 56: 1943–1950.

Wright DP, Scholes JD, Read DJ, Rolfe SA (2000) Changes in carbon allocation and expression of carbon transporter genes in *Betula pendula* Roth. colonized by the ectomycorrhizal fungus *Paxillus involutus* (Batsch) Fr. Plant Cell Environ 23:39–49.

Yin C, Pang X, Chen K (2009) The effects of water, nutrient availability and their interaction on the growth, morphology and physiology of two poplar species. Environ Exp Bot 67:196–203.

Zhang Z, Liao H, Lucas WJ (2014) Molecular mechanisms underlying phosphate sensing, signaling, and adaptation in plants. J Integr Plant Biol 56:192–220.

Zwanenburg G, Hoefsloot HCJ, Westerhuis JA, Jansen JJ, Smilde AK (2011) ANOVA–principal component analysis and ANOVA–simultaneous component analysis: a comparison. J Chemometrics 25: 561–567.