

Potential regulation of ectomycorrhizae by paper birch in response to soil type and fertilization: Linking the growth differentiation balance hypothesis and carbon allocation models

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Abstract

Ectomycorrhizae (EM) are beneficial relationships between tree roots and fungi that increase tree survival and stress tolerance in resource (e.g. nutrient, water) limiting environments. EM abundance is often negatively correlated with increasing nutrient availability (e.g. through fertilization), which may affect long term and health of trees, especially in managed systems where fertilization is commonplace. However, the mechanisms behind this pattern are relatively unknown.

Fertilization impacts metabolic tradeoffs between tree growth and the accumulation of secondary metabolites, which have multiple roles in improving tree health. Over a fertility gradient, secondary metabolism is predicted to respond parabolically, such that rapidly growing trees contain lower concentrations of secondary metabolites and vice versa. We hypothesize that increased nutrient availability affects plant carbon allocation and investment in secondary metabolism in a way that reduces EM colonization. Such information is important because fertilizers are often overused, (ex. urban and nursery settings) which may prove unfavorable to the establishment and survival of trees.

Growth, carbon allocation, carbohydrates, phenolics, lignin, and EM were measured in foliage and roots of fertilized and non-fertilized paper birch (*Betula papyrifera* Marsh) growing in contrasting soil types (nutrient poor subsoil and nutrient rich topsoil). Overall, levels of foliar phenolics and root-to-shoot ratios decreased with increasing fertility. Root phenolics and lignin decreased upon fertilization of subsoil, but increased in fertilized topsoil. EM abundance was strongly negatively correlated with levels of root phenolics and lignin, suggesting that the host may regulate its EM associations by manipulating general defense responses in complex interactions with resource availability.

Introduction

In exchange for increased uptake of limiting nutrients and water, EM fungi (EMF) obtain soluble carbon (mainly sugars and amino acids) from their tree host. EMF are vital for the survival and persistence of trees across environments and are especially valuable to trees in drought conditions¹.

EM often decrease in abundance upon the application of mineral fertilizer. Traditionally this decrease was attributed to direct toxicity of nutrients (e.g. nitrogen) to EMF¹. Recent studies suggest that mycorrhizae may be regulated by differential allocation of carbon to roots, with increased allocation occurring in situations where EM are most beneficial². This model of EMF regulation matches with known patterns of tree growth responses to fertilization whereby more carbon is directed belowground in nutrient-limiting environments and aboveground in nutrient-rich environments³ (Figure 1).

Carbon allocation is also a major player in physiological tradeoffs, particularly between growth (primary metabolism) and the accumulation of secondary metabolites (which are often associated with stress tolerance/defense) under resource constraints. The Growth Differentiation Balance Hypothesis (GDBH) predicts that secondary metabolism will respond parabolically to resource availability⁴ (Figure 2). This tradeoff between growth processes and secondary metabolism has implications for overall tree health, as secondary metabolites function as antioxidants, ultraviolet screens, and as toxins against pests and pathogens.

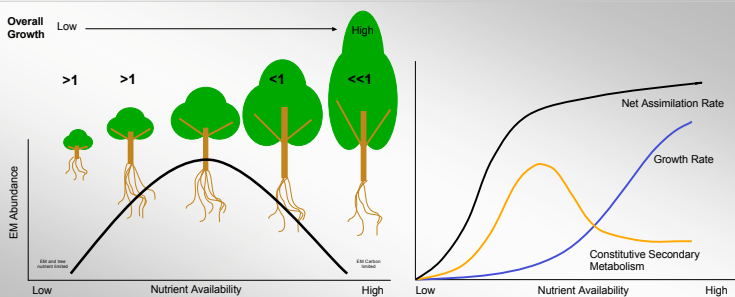


Figure 1. The relationship between EM abundance and carbon partitioning between above and belowground tree growth. EM response curve modified from Treseder and Allen 2002.

Figure 2. Differential responses of Photosynthetic rate, Growth, and Secondary metabolism of a tree over a fertility gradient. Modified from Herms and Mattson 1992.

Our goal was to explore patterns of tree growth and primary and secondary metabolic tradeoffs to determine if and how these patterns interact to model EM abundance and stress tolerance in paper birch. This study aimed to characterize tree responses to changing nutrient availability in two contrasting soil types, nutrient poor subsoil and nutrient rich topsoil, because trees may respond differently depending on initial soil physico-chemical characteristics and nutrient levels. Furthermore, we examined the relationships between the ability of trees to chemically protect their tissues and their need to sustain mycorrhizae to try and model how trees regulate two energetically competing beneficial processes under stressful (drought) and unstressful conditions. (Data on drought effects are not reported in this poster.)

Materials and Methods

- 96 aseptically germinated paper birch seedlings were transferred to 1.9 L pots containing either nutrient poor subsoil or fertile topsoil (Figure 3).
- Pots were moved to a greenhouse set at 24° C, equipped with HID lights (450 umol photons m⁻² sec⁻¹) on a 16:8 photoperiod. Trees were irrigated to capacity daily (250 ml H₂O pot⁻¹)
- Factors in the experiment were soil type, fertilization (yes/no) and drought (yes/no) applied in 2³ factorial combination with 12 biological replicates per treatment combination

Treatments

- Soil type (Figure 3): subsoil from excavation project or field topsoil (both obtained at OARDC)
- Fertilization: 515 mg 30:10:7 NPK and 640 mg 30:0:0 on days 10 and 56
- Drought: Irrigation reduced by 50% weekly between days 121-142 of the experiment

Figure 3. A visual comparison of sub- (left) and topsoil.



Trees were destructively harvested on day 143 and 144. Measurements of above- and belowground components of seedling growth, soluble sugars, starch, soluble phenolics, lignin, and EM colonization percentage were taken and analyzed statistically using the Generalized Linear Model function of SPSS v.15 to determine treatment effects on all measured variables. Means were separated by LSD analysis. Pearson's correlations were calculated to determine variable associations.

Results

Table 1. Results of generalized linear model analysis of treatment effects and one of their interactions on measured variables. For each variable, upper rows show Wald chi-square values, lower rows associated significance level. Bold text indicates significant treatment effects P < 0.05.

Response Variable	Soil	Fertilizer	Drought	S x F
TSM (g)	78.471	35.325	0.222	4.118
TLA (m ²)	87.200	51.311	0.232	9.243
LAR (m ² g ⁻¹)	0.801	<0.001	0.630	0.802
RSR	28.342	7.931	2.819	0.051
NAR (g m ⁻² day ⁻¹)	45.640	3.466	2.000	5.988
RGR (g g ⁻¹ day ⁻¹)	0.801	0.003	0.157	0.014
Foliar Starch (% DM)	3.268	4.048	0.184	0.760
Foliar Phenolics (mg g ⁻¹)	1.523	0.991	0.254	0.061
Root Starch (% DM)	3.956	0.320	0.058	0.416
Root Phenolics (mg g ⁻¹)	0.227	1.383	0.942	0.289
Foliar Lignin (mg g ⁻¹)	0.914	0.076	0.145	0.368
Root Lignin (mg g ⁻¹)	0.993	0.340	<0.001	<0.001
EM Abundance (%)	4.481	4.392	0.185	27.873

Table 2. Means (standard errors) for soil and fertilizer treatment effects on all response variables. Different letters indicate significant differences by LSD analysis (P < 0.05).

Response Variable	Fertilizer	Subsoil	Topsoil	
Mass (g)	7.87 (0.03)	b	1.10 (0.034)	a
TLA (m ²)	9.807 (0.200)	b	12.910 (7.360)	a
LAR (m ² g ⁻¹)	7.040 (5.701)	b	6.160 (5.840)	c
RSR (mg g ⁻¹)	0.819 (0.16)	a	1.79 (0.15)	b
NAR (g m ⁻² day ⁻¹)	11.82 (1.08)	a	11.42 (1.08)	a
RGR (g g ⁻¹ day ⁻¹)	0.810 (0.140)	b	0.880 (0.140)	b
Foliar Starch (% DM)	3.12 (1.08)	a	3.58 (1.03)	a
Foliar Phenolics (mg g ⁻¹)	0.89 (0.04)	a	0.96 (0.04)	a
Foliar Lignin (mg g ⁻¹)	14.71 (1.3)	a	16.21 (2.2)	ab
Root Starch (% DM)	8.87 (0.71)	b	8.01 (0.69)	b
Root Phenolics (mg g ⁻¹)	6.68 (0.6)	b	4.99 (0.5)	a
Root Lignin (mg g ⁻¹)	17.61 (1.6)	b	17.61 (1.6)	b
EM Abundance (%)	63.63 (4.34)	bc	66.37 (4.03)	b

Abbreviations: LAR: leaf area ratio; RSR: root to shoot ratio; NAR: Net assimilation rate; RGR: Relative growth rate; DM: Dry mass

Table 3. Non-parametric pairwise correlations between measured variables. Only correlations for P < 0.10 are shown.

Response Variable	Total Mass	LAR	RSR	RGR	Foliar Sugar	Foliar Starch	Foliar Phenolics	Foliar Lignin	Root Sugar	Root Starch	Root Phenolics	Root Lignin
RSR	Rho: -0.388 Significance: 0.005 N: 79	Rho: -0.647 Significance: <0.001 N: 79										
RGR	Rho: 0.062 Significance: 0.869 N: 79	Rho: -0.134 Significance: 0.869 N: 79	Rho: -0.001 Significance: 0.991 N: 79									
Foliar Starch	Rho: -0.075 Significance: 0.816 N: 79	Rho: -0.148 Significance: 0.616 N: 79	Rho: -0.488 Significance: 0.001 N: 79	Rho: 0.339 Significance: 0.001 N: 79								
Foliar Phenolics	Rho: -0.321 Significance: 0.003 N: 79	Rho: -0.386 Significance: 0.001 N: 79	Rho: 0.494 Significance: 0.001 N: 79	Rho: 0.224 Significance: 0.001 N: 79	Rho: 0.286 Significance: 0.001 N: 79							
Foliar Lignin	Rho: 0.224 Significance: 0.001 N: 79	Rho: -0.386 Significance: 0.001 N: 79	Rho: 0.494 Significance: 0.001 N: 79	Rho: 0.224 Significance: 0.001 N: 79	Rho: 0.286 Significance: 0.001 N: 79	Rho: -0.388 Significance: 0.001 N: 79						
Root Sugar	Rho: 0.484 Significance: 0.001 N: 79	Rho: -0.139 Significance: 0.616 N: 79	Rho: -0.001 Significance: 0.991 N: 79	Rho: 0.339 Significance: 0.001 N: 79	Rho: 0.286 Significance: 0.001 N: 79	Rho: -0.388 Significance: 0.001 N: 79	Rho: -0.206 Significance: 0.001 N: 79					
Root Starch	Rho: -0.397 Significance: 0.001 N: 79	Rho: -0.139 Significance: 0.616 N: 79	Rho: -0.001 Significance: 0.991 N: 79	Rho: 0.339 Significance: 0.001 N: 79	Rho: 0.286 Significance: 0.001 N: 79	Rho: -0.388 Significance: 0.001 N: 79	Rho: -0.206 Significance: 0.001 N: 79	Rho: -0.274 Significance: 0.001 N: 79				
Root Phenolics	Rho: 0.333 Significance: 0.001 N: 79	Rho: -0.386 Significance: 0.001 N: 79	Rho: 0.494 Significance: 0.001 N: 79	Rho: 0.224 Significance: 0.001 N: 79	Rho: 0.286 Significance: 0.001 N: 79	Rho: -0.388 Significance: 0.001 N: 79	Rho: -0.206 Significance: 0.001 N: 79	Rho: -0.274 Significance: 0.001 N: 79	Rho: 0.088 Significance: 0.001 N: 79			
Root Lignin	Rho: 0.389 Significance: 0.001 N: 79	Rho: -0.386 Significance: 0.001 N: 79	Rho: 0.494 Significance: 0.001 N: 79	Rho: 0.224 Significance: 0.001 N: 79	Rho: 0.286 Significance: 0.001 N: 79	Rho: -0.388 Significance: 0.001 N: 79	Rho: -0.206 Significance: 0.001 N: 79	Rho: -0.274 Significance: 0.001 N: 79	Rho: 0.088 Significance: 0.001 N: 79	Rho: 0.003 Significance: 0.991 N: 79		
EM	Rho: -0.200 Significance: 0.001 N: 79	Rho: -0.647 Significance: <0.001 N: 79	Rho: 0.494 Significance: 0.001 N: 79	Rho: 0.224 Significance: 0.001 N: 79	Rho: 0.286 Significance: 0.001 N: 79	Rho: -0.388 Significance: 0.001 N: 79	Rho: -0.206 Significance: 0.001 N: 79	Rho: -0.274 Significance: 0.001 N: 79	Rho: 0.088 Significance: 0.001 N: 79	Rho: 0.003 Significance: 0.991 N: 79	Rho: -0.333 Significance: 0.001 N: 79	

Abbreviations: LAR: leaf area ratio; RSR: root to shoot ratio; RGR: relative growth rate; EM: ectomycorrhizal abundance

Figure 4. Comparison of a fertilized (left) vs. unfertilized control.



Discussion

- Growth responses of paper birch closely followed predictions, with fertilization increasing overall growth, but decreasing root to shoot ratios (Table 1, 2, Figure 4). The majority of measured variables responded as predicted by the GDBH, with increased growth being negatively correlated with the accumulation of phenolics and sugar. However, data indicated a strong relationship between component growth rate (root to shoot ratio) and the specific accumulation of phenolics in these tissues, suggesting that tradeoffs between growth and secondary metabolism may be affected by specific tissue growth rates and overall assessments of tree growth may not be adequate when carrying out tests of the GDBH (Table 3).
- As predicted by existing models, increasing nutrient availability resulted in decreased EM abundance (Table 1, 2). A strong positive correlation between EM abundance and tree root to shoot ratios (Table 3) suggests that increasing resource allocation belowground favors EM colonization rates. In situations where root to shoot ratios decreased, such as with fertilization, strong negative relationships between the accumulation of root soluble secondary metabolites, root lignin and EM abundance were detected (Table 3).
- We conclude that the parabolic response of EM abundance over a fertility gradient may result from decreased allocation to secondary metabolic processes in mycorrhizal roots that are rapidly developing under conditions of low soil fertility. As nutrients become less limiting to tree growth, allocation belowground and root growth slow, resulting in the accumulation of sugars that are used to support secondary metabolism and prevent EM colonization in situations when the needs for EM are minimal.

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