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The role of seedling nutrient status on development of ectomycorrhizal fungal communities in two soil types following surface mining disturbance

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ABSTRACT

Severe disturbances, such as those caused by surface mining, sever connections between ectomycorrhizal (ECM) fungi and their hosts. In this study, we examined the importance of internal plant nutritional status and soil type on the abundance and composition of ECM communities in newly established reclamation areas. Ectomycorrhizal fungi were examined on two aspen (*Populus tremuloides*) seedling stock types that differed in root nutrient concentrations at time of planting. Stock types were planted into two salvaged soil types, an upland forest soil and a lowland peat-mineral soil mix, placed on a reclaimed mining site. Two growing seasons following planting, ECM fungal richness and abundance was low with only four operational taxonomic units identified across the reclamation site. Initial seedling nutrient status affected the total amount of ECM fungi on seedling roots; seedlings with initially high root nutrient reserves (N, P and starch) had more root tips colonized by ECM fungi than seedlings with initially lower nutrient tissue concentrations. Soil type did not affect total amount of ECM colonization; however, the relative abundance of an individual species, *Cenococcum geophilum*, was influenced by soil type. Seedling nutrient reserves, independent of soil nutrition, affects the amount of ECM root colonization, while soil type affects the relative abundance of some ECM fungi colonizing roots.

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1. Introduction

Restoring functional interactions between vegetation and soils is important to create self-sustaining ecosystems following landscape level disturbances (Macdonald et al., 2012). In the boreal forest, trees develop intimate associations with ectomycorrhizal (ECM) fungi, which affect tree survival and growth through their effects on resource uptake. Ectomycorrhizal hyphae extending from colonized roots are physical linkages that functionally connect tree roots to soils, where hosts supply the fungi with photosynthetically-derived sugars and the fungi provide water and soil-derived nutrients to their hosts. Communities of ECM fungi are highly diverse on micro-spatial scales, with multiple species often within centimeters of each other (Bruns, 1995). Though the relationship between mycorrhizal species diversity and host plant productivity is often context-dependent (Jonsson et al., 2001), high mycorrhizal diversity has been demonstrated to increase nutrient uptake and seedling growth (Baxter and Dighton, 2001; Velmala et al., 2014). During severe soil disturbances such as surface mining, vegetation, soils, and parent geological material are stripped to access resource deposits. Following mining, ECM associations must re-establish with planted seedlings. However, depending on the type and severity of disturbance, the diversity of the ECM fungal community is often much lower than in undisturbed areas (Kipfer et al., 2011; Read, 1991). Restoring ECM fungal communities can be a challenge in heavily disturbed soils, which generally have a low ectomycorrhizal inoculum potential (Bois et al., 2005; Hankin et al., 2015).

Seedling establishment is the first step towards re-vegetation on heavily disturbed sites. On these sites, seedlings are often exposed to stress such as poor nutrient availability, drought, or mineral toxicity. Elevated nutrient (nitrogen, phosphorous, and potassium) and non-structural carbon reserves (sugar and starch) in the tissues of planted seedlings can increase seedling growth and nutrient acquisition in stressful conditions such as those found in disturbed areas (Quoreshi and Timmer 2000a; Landhäusser et al., 2012; Schott, 2013). Although the higher reserves in seedlings are often only temporary, the improved growth

Abbreviations: ECM, ectomycorrhizal; FFM, forest floor material; NSC, non-structural carbohydrate; PMM, peat mineral mix.

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performance persists beyond the presence of elevated tissue nutrient levels (Schott, 2013). What underlies this longer-term response is not clear; however, it could be driven by belowground interactions such as those formed between plants and mycorrhizal fungi. The effect of seedling nutrient and carbon reserve status on the establishment of mycorrhizal symbionts in disturbed sites has received little attention. Prior studies have shown that soil nutrient availability influences the outcome between mycorrhizal fungi and their host (Johnson, 1993; Johnson et al., 1992); however, host nutrient and carbon reserve status may also influence the outcome of mycorrhizal interactions (Nylund, 1988). For instance, Quoreshi and Timmer (1998, 2000b) found that nutrient loading (i.e., artificially increasing nutrient reserves) black spruce seedlings (*Picea mariana* [Mill.] BSP.) stimulated mycorrhizal formation during inoculation. Since a balance exists between internal plant carbon and nutrients which governs mycorrhizal symbioses (Johnson, 2010), alterations to the nutrient levels in plant tissues may influence the development of the ECM symbiosis. High amounts of nutrients in the roots, particularly N, can inhibit the development of ECM fungi (Richards and Wilson, 1963). As ECM fungi rely predominantly on their host for carbon, variation in carbon reserves in seedling roots may also influence the abundance of ECM fungi.

In addition to seedling physiology, soil characteristics may also influence the abundance and species composition of ECM fungi occurring at disturbed sites. Soils commonly used in restoration of surface mines in the boreal forests are materials salvaged prior to mining: forest floor material (FFM), which is composed of the litter layer plus a portion of the underlying mineral soil from upland forest sites, and peat, which is salvaged from lowland peatlands and is often mixed with underlying mineral subsoil resulting in a peat-mineral mix (PMM). As can be expected, these two materials differ greatly in soil structure and chemistry, nutrient availability, and their plant and fungal propagule bank, which reflects the plant and fungal communities present at the sites prior to salvage (McMillan et al., 2007; Dimitriu et al., 2010; Schott, 2013). Salvaged soils may differ in the ECM propagules they retain in addition to acting as different habitats suitable for some ECM species, dependent upon fungal physiology, and thus lead to the development of dissimilarly structured ECM fungal communities.

The objective of this study is to characterize the respective influence of host internal plant nutritional status and soil type on the establishment of ECM fungal communities in highly disturbed areas. Specifically, we assessed the influence of *P. tremuloides* seedling nutrient and carbon reserve status on the early development of an ECM fungal community two years after planting and how the early ECM community was influenced by soil type (FFM and PMM). We hypothesized that seedlings with initially lower nutrient reserves would have greater ECM fungal abundance compared to seedlings with higher nutrient reserves because a lower nutrient status ought to lead to a greater necessity to develop and facilitate the establishment of the ECM symbiosis (Johnson et al., 1997). As *P. tremuloides* is an upland tree species, we expected a more abundant ECM community when seedlings were planted in FFM than in PPM.

2. Materials and methods

2.1. Site description

The study is located in the Central Mixedwood subregion of the boreal forest (Natural Regions Committee, 2006). Uplands of this region are typically dominated by white spruce (*Picea glauca* (Moench) Voss) and trembling aspen (*P. tremuloides* Michx.). The low lying areas are wetlands dominated by black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.). The Central Mixedwood subregion endures long, cold winters and short, warm summers. Average daily temperatures range from −18.8 °C in January to 16.8 °C in July (Environment Canada 2013). Average annual rainfall and snow is 342 mm and 156 mm, respectively. The growing season (May–September) of our sample collection (2012) was near average with 350 mm of precipitation and a 12.7 °C average temperature (Environment Canada, 2013).

2.2. Soil salvage and placement

The field site was a reclaimed overburden dump (>100 ha) 26 km north of Fort McMurray, Alberta, Canada. Over six months beginning August 2008 and prior to building the overburden structure, the surface soils were stripped and stockpiled by soil type (forest floor material and peat-mineral mix). Upland forest floor material (FFM) was comprised of a mixture of the top 30 cm and included the organic L, F, H, and the mineral A and a portion of the B soil horizons of a Gray Luvisol occurring under stands of white birch (*Betula papyrifera* Marsh.), balsam poplar (*Populus balsamifera* L.), and/or trembling aspen (*P. tremuloides* Michx.). Peat-mineral mix (PMM) was salvaged from lowland sites where the surface material was stripped to a depth of roughly 30 cm, which included the transition/peat layer and mineral soil beneath it and was monitored to ensure a mix of 60:40 (volume:volume) peat to mineral soil. In 2009, after the soils had been stripped and stockpiled on-site, the dump was filled with overburden material (sodic and/or saline) and the site was sloped with a height to volume ratio of six. In 2010, the construction of the overburden dump finished and 1 m of subsoil (low-sodic soil salvaged from the C-horizon from a depth of 60 cm to 300 cm) was placed across the surface. Capping soil was placed at a depth of 50 cm; placement commenced in August 2010 and was finished in June 2011. The study site was 1.5 ha in size; the salvaged and stockpiled FFM and PMM soil types were placed in alternating 20 m wide and 65 m long strips. A pair of FFM and PMM strips (40 m × 65 m) was considered an experimental block ($n = 5$).

2.3. Tree seedling production and planting

Aspen seedlings were grown commercially from an open-pollinated seed source collected in the Fort McMurray (Alberta, Canada) region. Seedling growing conditions during nursery production are described in more detail in Schott et al. (2013). Briefly, seeds were sown into Styroblock containers (5-12A, 220 ml, Beaver Plastic, Edmonton, Alberta) and grown for a single growing season at Smoky Lake Nursery (Smoky Lake, Alberta, Canada). Once seedlings had reached an average height of 35 cm, they were assigned to two treatments: *high feed* and standard. Standard seedlings were grown under typical seedling production conditions and fertilized with a mixture of macro and chelated micronutrients (78 ppm N, 77 ppm P, 161 ppm K, 46 ppm S) while the high feed seedlings received double the amount of the same fertilizer (i.e., 156 ppm N, 154 ppm P, 322 ppm K, 92 ppm S, including chelated micronutrients). Fertilization continued at these concentrations until early fall after which all fertilization ceased. Dormancy and hardening was induced by leaving seedlings outside and seedlings were lifted and stored frozen (−3 °C) once day temperatures were below freezing. Roots of seedlings were not examined for ectomycorrhizas prior to planting. Aspen seedlings were planted in early spring of 2011 in alternating rows of high feed and standard seedlings within each capping treatment and block. Seedlings were regular spaced at 1.3 m (5917 stems/ha). At the time of planting, high feed seedlings were of similar size as

Table 1
Initial *Populus tremuloides* seedling characteristics by feed type (n=10). Feed types are nutrient loaded (High) and unloaded (Standard). Means are presented ± 1 SE.

	Feed		
	High	Standard	*P*
Seedling height (cm)	43.5 (2.43)	41.0 (2.43)	0.47
Root collar diameter (mm)	4.71 (0.191)	4.92 (0.191)	0.44
Root:Shoot ratio	3.58 (0.162)	3.58 (0.162)	0.98
Shoot total NSC (% dry wt.)	12.3 (0.50)	13.7 (0.55)	0.08
Root total NSC (% dry wt.)	29.9 (0.66)	30.0 (0.66)	0.9
Root sugar (% dry wt.)	16.2 (0.46)	18.8 (0.46)	0.001
Root starch (% dry wt.)	13.7 (0.62)	11.2 (0.62)	0.01
Root nitrogen (%)	2.46 (0.081)	1.41 (0.081)	<0.001
Root phosphorous (ug g^{-1})	3513.0 (67.23)	2697.2 (67.23)	<0.001

standard seedlings; however, high feed seedlings had higher concentrations of root macronutrients (N and P) as well as higher root non-structural starches (Table 1). Seedling size and root:shoot ratios were similar between stock types.

2.4. Mycorrhizal root collection and identification

In 2012, after two growing seasons, single root sections (approximately 30–40 cm long) were collected from five seedlings of each treatment combination in each block (total 100 seedlings). These sections were clearly outside of the initial root plug and would have been roots grown subsequent to planting. Prior to collection, roots were traced to their stem to ensure correct feed type was collected. Root samples were stored at −20 °C until further processing. After thawing, adhering soil and debris was gently removed by lightly rinsing roots with tap water over a 0.4 mm sieve. The root systems were then cut into 1 cm fragments and mixed in water; a number of segments were then randomly selected such that, for each seedling, 150–200 root tips were examined under a dissecting microscope. Root tips were classified as either non-mycorrhizal or mycorrhizal, with mycorrhizal root tips being further classified into unique morphotypes based on presence of hyphae, mantle color and texture, and root tip thickness and shape (Agerer, 1991). Four representative samples of each morphotype per root sample were collected and separately placed into 0.2 μL microcentrifuge tubes for DNA-based identification (see below). The amount of ectomycorrhizal colonization was determined by dividing the number of living mycorrhizal root tips by the total number of living mycorrhizal and nonmycorrhizal root tips found in a subsample. Following examination, root systems were dried at 100° C for one hour to halt enzymatic breakdown of root carbohydrates, followed by at least 48 h of drying at 70° C. Fine roots (<1 mm) were separated from the root system and first ground using a mortar and pestle, followed by grinding for fifteen minutes in a micro-ball mill (MRC International, Holon, Israel) to ensure uniform particle size. The fine roots were then analyzed for total nitrogen (N), total phosphorous (P), and nonstructural carbohydrate (NSC) concentration. Total N was determined by the dumas combustion method using the Costech Model EA 4010 Elemental Analyzer (Costech International Strumatzione, Florence, Italy, 2003). Total P was determined by nitric acid digestion then colourimetry using the SmartChem Discrete Wet Chemistry Analyzer (Westco Scientific Ltd., Brookfield, CT, USA, 2007). Nonstructural carbohydrates were analyzed using a phenol-sulfuric acid assay for total sugar concentrations and enzyme digestion for total starch concentration according to Chow and Landhäusser (2004). The methods for analyzing total N, P, and NSC concentrations in seedlings prior to planting were the same as those described above.

2.5. Molecular confirmation of ectomycorrhizal morphotypes

Genomic DNA from mycorrhizal root tips was extracted using Sigma Extraction Buffer and Neutralization Solution B (Sigma–Aldrich, Gillingham, Dorset, UK) according to the manufacturer's protocol. To confirm morphotype identities, twenty seedlings were selected at random, from which all extractions from each morphotype present were amplified using a nested polymerase chain reaction (PCR) using the fungal-specific nested primer combinations of NSA3/NLC2 and NSI1/NLB4 (Martin and Rygiewicz, 2005). In addition, one extraction for each morphotype on each remaining seedling was also amplified using the same procedure and primer combinations. Reactions using the outer primers consisted of 8 μL of RedTAQ (Sigma, Gillingham, Dorset, UK), 5.4 μL autoclaved MilliQ water, and 0.8 μL each of 10 μM NSA3 and 10 μM NLC2 combined with 1 μL of template DNA in a 16 μL reaction. Amplifications were performed with an initial denaturation at 95 °C for five minutes, followed by 30 cycles of 95 °C for 90 s, 67 °C for 60 s, and 72 °C for 90 s, with a final extension of 72 °C for ten minutes. Reactions using the inner primers consisted of 8 μL of RedTAQ (Sigma, Gillingham, Dorset, UK), 5.4 μL autoclaved MilliQ water, and 0.8 μL each of 10 μM NSI1 and 10 μM NLB4 combined with 1 μL of PCR product from the other reaction in a 16 μL reaction. Amplifications were performed with an initial denaturation at 95 °C for five minutes, followed by 27 cycles of 95 °C for 90 s, 55 °C for 60 s, and 72 °C for 90 s, with a final extension of 72 °C for ten minutes. Success of the reaction was determined by gel electrophoresis using 1% agarose gel and GelRed gel stain (Biotium Inc., Hayward, California, USA) and successful PCR products were purified with ExoSAP-IT (USB, Cleveland, Ohio, USA). Bi-directional sequencing was conducted with BIGDYE v3.1 (Applied Biosystems, Foster City, California, USA) using the NLB4 and NSI1 primers, with 10 μl reactions containing 2 μl autoclaved milli-q water, 1.5 μl 5 × buffer sequencing buffer, 1 μl BIGDYE, 0.5 μl of 10 μM primer, and 5 μl of PCR product. The resulting products were precipitated according to the manufacturer's instructions for EDTA/ethanol. Bi-directional sequences were analyzed on an ABI Prism 3730 Genetic Analyzer (Applied Biosystems, Foster City, California, USA). Sequences were edited with Geneious software (Biomatters, Auckland, New Zealand). Nucleotides were converted to Ns if they had phred scores below 15. The ends of sequences were trimmed using an error probability of 3%. Any sequences with more than 10% Ns remaining or total length less than 300 base pairs were disregarded from further analysis. Sequences were aligned along the ITS regions using a multiple alignment into operational taxonomic units (OTUs). Consensus sequences were queried against Genbank, using nBLAST. Sequences derived from nBLAST were examined for chimeras or other technical errors and erroneous sequences were discarded, according to Nilsson et al. (2012). Sequences with ≥97% sequence similarity were considered to be reasonable approximations of fungal species. Sequences of all ectomycorrhizal fungal species were then submitted to Genbank; accession numbers are listed in Table 2.

2.6. Statistical analyses

All non-normal colonization data was transformed using the arcsine transformation prior to analysis. Initial (2011) characteristics of high and standard feed seedlings were compared using *t*-tests. All 2012 data was analyzed as a randomized block split-plot design, with capping material as the whole-plot factor and feed type as the split-plot factor; block ($n = 5$) and the interactions of block with feed and soil type were set as random factors. Analysis of variance (ANOVA) was used to determine the effects of soil and feed type and their interaction on 2012 seedling growth (height,

Table 2
Operational taxonomic units of ectomycorrhizal fungi identified across two stock types of *Populus tremuloides* seedlings grown in a reclaimed site in northern boreal forest (Alberta, Canada).

Genbank Accession	Query/hit length	Reference	Identities (%)	Score	Closest Genbank match
KM115028	931/738	AY948191	99	1301	*Hebeloma leucosarx*
KR709300	716/902	AF430254	99	1258	*Hebeloma velutipes*
KM115029	812/1058	AY394885	98	1200	*Meliniomyces bicolor*
KM115030	882/877	JQ712012	98	1473	*Thelephora terrestris*

stem diameter) and nutritional characteristics (root starch, root sugar, leaf total nitrogen, leaf total phosphorous, and leaf total potassium). Due to inconsistencies in matching OTUs to ECM morphotypes (see Section 3), relative abundance was examined for one species, *Cenococcum geophilum* Fr., which was easily recognized due to its distinct radiate mantle morphology (LoBuglio, 1999). To determine relative abundance, the total number of colonized tips of *Cenococcum geophilum* was divided by the total number of all colonized tips found on the seedling. ANOVAs were used to test for the effects of soil type, feed type, and their interaction on total ECM colonization and relative colonization of *Cenococcum geophilum*. All statistics were run using SAS 9.2 (SAS Institute Inc., Cary, North Carolina, US).

3. Results

3.1. Seedling growth and nutrient reserve status after two growing seasons in the field

Total seedling height was affected by feed type, ($F_{(1,4)} = 11.6$; $p = 0.03$), but not by soil; high feed seedlings grew taller than standard feed seedlings (Table 2). Similarly, seedling root collar diameter was also greater in high feed than in standard seedlings, ($F_{(1,4)} = 36.12$; $p < 0.004$). Despite having different tissue nutrient concentrations when planted, feed type did not significantly influence either current foliar or root nutrient concentrations (Table 2). Current root non-structural carbohydrate (NSC) concentrations, including sugar, starch, and total NSC, were also unaffected by feed type (Table 2). Soil type did not affect seedling foliar or root nutrient concentrations (Table 2). Total concentrations of fine root NSC was unaffected by soil type; however, seedlings grown in PMM had marginally higher concentrations of fine root sugars, ($F_{(1,4)} = 7.09$; $p = 0.056$), than seedlings grown in FFM, though concentrations of root starch was unaffected by soil type (Table 2).

3.2. Soil and seedling feed type: ectomycorrhizal community composition

A total of four OTUs were assigned from two observed morphotypes: *Hebeloma leucosarx*,*H. velutipes, Thelephora terrestris*, and *Meliniomyces bicolor* (Table 3). Of the 140 root tips analyzed for the presence of fungal DNA, 30% of consensus sequences obtained were discarded due to insufficient sequence quality and 32% were discarded due to non-mycorrhizal species identity. These latter tips were then subtracted from counts of colonization. For the first morphotype, consensus sequences from 24 root tips remained, of which 52% were colonized by *H. leucosarx*, 27% were colonized by *H. velutipes,* and 21% were colonized by *T. terrestris.* For the second morphotype, consensus sequences from 28 root tips remained, of which 82% were colonized by *M. bicolor* and 18% were colonized by *T. terrestris*.

Total colonization of root tips by ECM fungi (including the four OTUs as well as *Cenococcum geophilum*) was affected by seedling feed type, ($F_{(1,4)} = 17.7$; $p = 0.013$), but not by soil type, ($F_{(1,4)} = 0.1$; $p = 0.78$) (Fig. 1). High feed seedlings had a greater percentage of root tips colonized by ECM fungi ($24.6 \pm 3.84\%$ SE) than standard seedlings ($14.9 \pm 3.84\%$ SE). While feed type had a significant effect on colonization, only root collar diameter of the measured current growth and nutritional characteristics (height, diameter, root sugar, root starch, leaf nitrogen, leaf phosphorous, and leaf potassium), was positively correlated with colonization, ($r^2_{(98)} = 0.197$, $p = 0.0496$).

While only feed type affected the total colonization by ECM fungi, relative colonization by *C. geophilum* was affected by soil type ($F_{(1,4)} = 11.57$; $p = 0.027$, Fig. 2). Seedlings grown in FFM had a greater percent of root tips colonized by *C. geophilum* ($5.6 \pm 1.52\%$ SE) than seedlings grown in PMM ($0.64 \pm 0.33\%$). The relative colonization by the other OTUs was unable to be determined due to uncertainty concerning fungal identity, with multiple fungal OTUs being attributed to single morphotypes.

4. Discussion

4.1. Seedling nutrient reserve status and ectomycorrhizal colonization

Seedlings planted with initially higher nutrient reserves had greater colonization by ECM fungi than standard feed seedlings. This finding is contrary to the accepted relationship between mycorrhizal development and nutrient supply: as nutrient availability (predominantly N and P) increases in soils, ECM colonization tends to decrease (Hoeksema et al., 2010; Nilsson

Table 3
Populus tremuloides seedling ($n = 5$) characteristics by treatment. Feed types are nutrient loaded (High) and unloaded (Standard); soil types are forest floor material (FFM) and peat-mineral mix (PMM). Mean are presented ± 1SE. The interaction between soil type and feed type was not significant ($P > 0.05$) for all variables.

	Feed			Soil type		
	High	Standard	*P*	FFM	PMM	*P*
Seedling height (cm)	87.0 (5.74)	77.2 (4.33)	0.027	80.5 (5.52)	83.6 (5.06)	0.41
Root collar diameter (mm)	11.4 (0.63)	9.5 (0.44)	0.004	10.1 (0.54)	10.8 (0.69)	0.45
Root sugar (% dry wt.)	5.1 (0.51)	4.8 (0.35)	0.33	4.6 (0.47)	5.3 (0.38)	0.056
Root starch (% dry wt.)	7.8 (1.29)	9.3 (1.37)	0.12	8.7 (1.17)	8.4 (1.50)	0.54
Root total NSC (% dry wt.)	12.9 (0.65)	14.0 (0.64)	0.25	13.3 (0.57)	13.7 (0.71)	0.48
Root nitrogen (%)	0.70 (0.068)	0.70 (0.049)	0.99	0.63 (0.044)	0.77 (0.064)	0.14
Root phosphorous (%)	0.10 (0.011)	0.10 (0.009)	0.64	0.10 (0.007)	0.10 (0.012)	0.72
Foliar nitrogen (%)	2.0 (0.07)	2.0 (0.09)	0.41	1.90 (0.071)	2.09 (0.078)	0.10
Foliar phosphorous (%)	0.16 (0.012)	0.16 (0.012)	0.98	0.16 (0.010)	0.17 (0.014)	0.22
Foliar potassium (%)	0.81 (0.074)	0.76 (0.042)	0.39	0.78 (0.070)	0.78 (0.049)	0.91

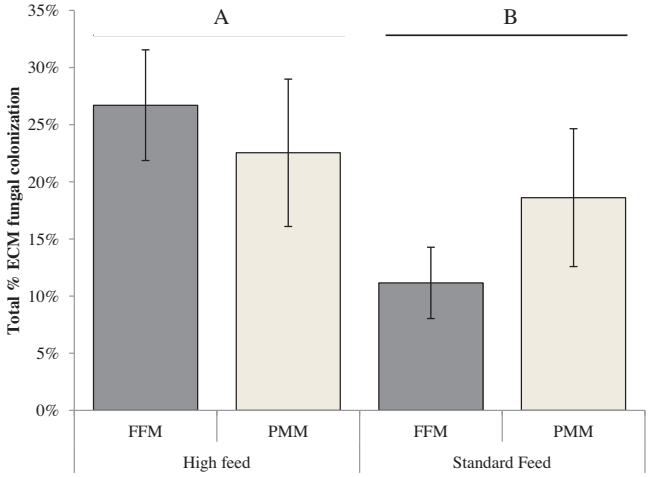

Fig. 1. Total ectomycorrhizal colonization of high and standard feed aspen (*Populus tremuloides*) seedlings growing in two soil types, forest floor material (FFM) and a peat-mineral mix (PMM) ($n = 5$). Significance is denoted on the graph using letters. High feed seedlings had more colonization than standard feed seedlings ($P = 0.013$), neither soil type nor the interaction between the two had a significant effect.

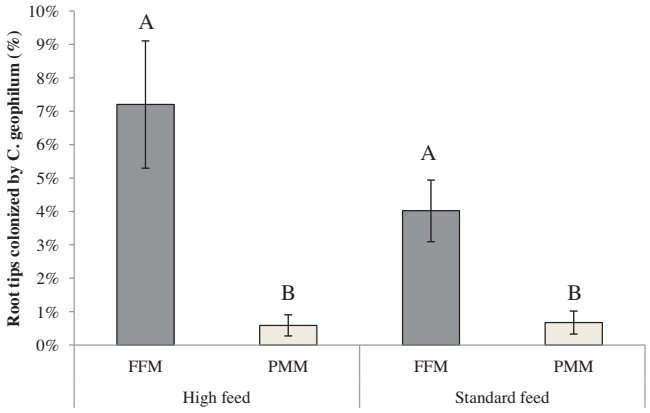

Fig. 2. Relative colonization of *Cenococcum geophilum* on the roots of high and standard feed aspen (*Populus tremuloides*) seedlings planted in two soil types, forest floor material (FFM) and a peat-mineral mix (PMM) ($n = 5$). Colonization by *C. geophilum* was higher in FFM than PPM soils ($P = 0.027$) but was unaffected by seedling feed type. Significance is denoted on the graph using letters.

et al., 2005). However, our results highlight the importance of differentiating between soil nutrient availability and plant nutrient reserves contained in their tissues (Dixon et al., 1981). Contrary to our prediction that seedlings grown in FFM would have higher ECM colonization than those grown in PPM, soil type had no significant influence on total ECM colonization. This may be due to the fact that N and P availability, as measured by plant root simulator probes (Schott, 2013), did not differ between soil types. Specifically, total nitrogen availability (organic nitrogen, ammonia, and ammonium) was 40 ± 11 (SE) $\mu g\ 10\ cm^{-2}\ 91\ days^{-1}$ in the FFM and $41 \pm 23\ \mu g\ 10\ cm^{-2}\ 91\ days^{-1}$ in the PMM; phosphorous availability was $4.1 \pm 1.1\ \mu g\ 10\ cm^{-2}\ 91\ days^{-1}$ in the FFM and $2.2 \pm 0.2\ \mu g\ 10\ cm^{-2}\ 91\ days^{-1}$ in the PMM (Schott, 2013). In the absence of differences in soil nutrient availability, ECM colonization was likely increased by initially elevated plant nutrient reserves, though the underlying mechanisms are unclear. The increased colonization was not related to current plant nutrient reserves: after two growing seasons in the field, there were no differences in seedling nutrient or carbohydrate concentrations between high feed and standard seedlings. Rather than current conditions, we propose that the increased colonization of high feed seedlings was the result of the initial elevated nutrient and starch reserves (Table 1), a legacy which might have allowed high feed seedlings to facilitate colonization by ECM fungi. In order to investigate these mechanisms, we would need data on initial colonization of the seedlings by ECM fungi as well as data on carbon acquisition, root turnover, and nutrient reserves throughout both growing seasons. This research indicates that seedling quality (e.g., nutrient or carbon reserve status) at the time of planting can play a significant role in determining the abundance of mycorrhizal associations present on seedlings, which may prove to be a driver for the continued improved growth performance found in these seedlings.

4.2. Ectomycorrhizal fungal community characteristics

We classified four operational taxonomic units present on seedlings: *Hebeloma leucosarx, H. velutipes, Meliniomyces bicolor,* and *Thelephora terrestris,* as well as a fifth species, identified as *Cenococcum geophilum* due to its distinct radiate mantle morphology. The presence of *C. geophilum*, *H. leucosarx*, *H. velutipes,* and *T. terrestris* at our site is to be expected, as they often colonize roots of trees establishing on recently disturbed sites. For instance, *C. geophilum* has been found on sites recently disturbed by fire (Visser, 1995), clearcut logging (Ingleby et al., 1998), glacial activity (Mühlmann and Peintner, 2008), and in soil following surface mining (Bois et al., 2005). Members of the genus *Hebeloma* have been found on sites recently disturbed by wildfire (Visser, 1995), deforestation (Obase et al., 2007), and surface mining (Hankin et al., 2015). *Thelephora terrestris* has been found on sites after fire (LeDuc et al., 2013), deforestation (Obase et al., 2007), and surface mining (Onwuchekwa et al., 2014). These four species are also commonly found colonizing the roots of trees grown in forest nurseries (Menkis et al., 2005). Little autecological information is available on *M. bicolor*; this study reports the first observed instance of *M. bicolor* occupying a site recently disturbed by mining activities, though it has been observed following fire disturbance (Bent et al., 2011; Sousa et al., 2014).

The abundance of *C. geophilum* was mediated by soil type; *C. geophilum* was more prevalent in FFM than PMM. Fungal physiology may explain some of the observed variation in the relative abundance of *C. geophilum*. The forest floor soil type has a lower water holding capacity than PPM (Schott, 2013); *C. geophilum* is recognized as more drought tolerant, as it has an abundance of melanin, a class of polymer which contributes to the tolerance of drought stress, allowing the fungi to persist where other species may be inhibited (Cripps, 2001; Fernandez and Koide, 2013). Early successional species of ECM fungi such as *C. geophilum* are typically generalists with low host specificity (Dickie et al., 2013), which may explain its greater sensitivity to environmental factors such as water or nutrient availability relative to host characteristics (Dickie, 2007).

Recently disturbed sites typically harbor fewer species of ECM fungi than intact forests (Nara et al., 2003); correspondingly, the number of fungal OTUs found at our site was low. Overall, species richness at the site was less than half of that found in other areas of similar strip mining disturbances (Bauman et al., 2011; Dimitriu et al., 2010). There are several possible explanations underlying the discrepancy. The soil types were stockpiled for two years prior to placement on the site; stockpiling for as little as six months can reduce the ability of fungi to colonize potential hosts due to propagule (both spore and hyphal) death (Persson and Funke, 1988). The age of other comparable mine areas is five to twenty years compared with two years at our site; time since disturbance has been shown to increase ECM fungal abundance and richness

after clearcutting and wild fire (Twieg et al., 2007). A single ECM host was planted at the site which likely recovers fewer ECM fungal species than sites planted with mixed species, as many ECM fungi display host specificity (Ishida et al., 2007). A study examining the fungal community in similar reclamation soils after a single growing season found similarly low rates of colonization, though more species were recovered, possibly due to the fact that cover soils were directly placed onto the reclamation site and not stockpiled and/or the presence of three potential ECM hosts (*P. tremuloides*, *Picea glauca* and *Pinus banksiana*) compared to one at our site (Hankin et al., 2015). Though the overall fungal richness at the site was low, subsequent establishment of vegetation, spore dispersal, and soil development may increase ECM fungal richness in the future (Dickie et al., 2013; Fujiyoshi et al., 2010).

5. Conclusions

We examined the effect of the nutritional status of planted aspen seedlings on the abundance of ECM fungi and its dependence on the soil type in which the seedlings were growing. We hypothesized that fungal abundance, measured by colonization level, would be less for seedlings planted with higher nutrient reserves (high feed), as seedlings would need to invest less into the symbiosis than seedlings with lower nutrient status. Contrary to our predictions, high feed aspen seedlings had greater current total colonization than standard feed seedlings. Total colonization of roots did not differ between soil types, nor was there an interaction between soil and feed type. A total of five species, one identified through distinct morphology and four ECM fungal OTUs, were found recurring across a relative large area (1.3 ha), four of which are commonly found in highly disturbed sites. While total colonization was influenced by only seedling feed type, the relative abundance of *C. geophilim* was influenced by soil type. The results of this study suggest that initial seedling quality (e.g., nutrient or carbon reserve status) can play a significant role in structuring the developing ECM fungal community.

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