# Site properties have a stronger influence than fire severity on ectomycorrhizal fungi and associated N-cycling bacteria in regenerating post-beetle-killed lodgepole pine forests

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# Abstract

Following a pine beetle epidemic in British Columbia, Canada, we investigated the effect of fire severity on rhizosphere soil chemistry and ectomycorrhizal fungi (ECM) and associated denitrifying and nitrogen (N)-fixing bacteria in the root systems of regenerating lodgepole pine seedlings at two site types (wet and dry) and three fire severities (low, moderate, and high). The site type was found to have a much larger impact on all measurements than fire severity. Wet and dry sites differed significantly for almost all soil properties measured, with higher values identified from wet types, except for pH and percent sand that were greater on dry sites. Fire severity caused few changes in soil chemical status. Generally, bacterial communities differed little, whereas ECM morphotype analysis revealed ectomycorrhizal diversity was lower on dry sites, with a corresponding division in community structure between wet and dry sites. Molecular profiling of the fungal ITS region confirmed these results, with a clear difference in community structure seen between wet and dry sites. The ability of ECM fungi to colonize seedlings growing in both wet and dry soils may positively contribute to subsequent regeneration. We conclude that despite consecutive landscape disturbances (mountain pine beetle infestation followed by wildfire), the "signature" of moisture on chemistry and ECM community structure remained pronounced.



## Electronic supplementary material

The online version of this article (doi: 10.1007/s12223-014-0374-7 ) contains supplementary material, which is available to authorized users.


# Introduction

Forest soils are subject to natural disturbances such as wildfire and insect infestations, which kill or stress trees. In recent years, the magnitude of mountain pine beetle (*Dendroctonus ponderosae* Hopkins) infestation of lodgepole pine (*Pinus contorta* var. *latifolia*) forests has reached epidemic proportions in British Columbia (BC), Canada, causing substantial social, economic, and ecological impacts. The mountain pine beetle (MPB) outbreak peaked in 2004 with 140 million square meters of forest attacked

in that year. The current BC Provincial-Level Mountain Pine Beetle model (Walton, 2012) predicts a 58 % loss of provincial pine forest by 2021. The infestation is spreading eastward into the Canadian boreal forest, threatening major losses in lodgepole pine forests and the prospect of losses of other pine species (e.g., jack pine, *Pinus banksiana*) susceptible to MPB attack (Natural Resources Canada 2013). Due to unprecedented scale of this outbreak, its impacts on the future regeneration of managed and unmanaged MPB-killed stands are unknown (Burton 2006; Axelson et al. 2009; Burton 2010). In addition, climate change is both expanding the range of the MPB (Carroll et al. 2006; Mikkelson et al. 2013) and resulting in drier conditions (Hamann and Wang 2006). Stands of dead wood, killed by MPB, and dry conditions create an ideal environment for forest wildfires (Turner et al. 1999).

The effect of MPB attack on forest stands, and optimal mitigation strategies, were highlighted in recent studies (Kaufmann et al. 2008; Burton 2010; Mikkelson et al. 2013). However, the impact of MPB attack on soil chemical and biological properties is not well understood. Tree death due to insect attack has the potential to influence entire forest ecosystems. Beetle attacks have been shown to increase nitrogen (N) concentrations in organic soil horizons in several types of forest (Clow et al. 2011; Griffin et al. 2011; Kana et al. 2012; Keville et al. 2013; Rhoades et al. 2013), although these increases do not seem to result in long-term changes to soil biogeochemistry (Keville et al. 2013; Rhoades et al. 2013). The death of trees also affects the future regeneration potential of the forest through influences on mycorrhizal networks and rhizosphere processes (Simard 2009; Šturšová et al. 2014). Soil microbes play crucial roles in forest nutrient cycling, carrying out key processes in the carbon, sulfur, and nitrogen cycles. In addition to microbes that inhabit bulk soil, ectomycorrhizal (ECM) associations are vital to tree growth in lodgepole pine forests, providing pine hosts with essential nutrients, such as nitrogen (N) and phosphorus (P), in return for a ready source of available carbon (C) to the fungal partner (Simard and Durall 2004). As trees die, these ECM fungi lose their C source and can be diminished or eliminated, and, as a consequence, the availability of N and P in the soil may decrease (Treu et al. 2014). A reduction in ECM fungi may also result in a decrease in associated “mycorrhizal helper bacteria” (Frey-Klett et al. 2007), many of which perform important nutrient cycling roles. For example, N-fixing bacterial genes have been found in association with ECM fungi (Burke et

al. 2006; Izumi et al. 2006). Two key N-cycling processes performed by bacteria include nitrogen-fixation, which can be assayed using the gene for nitrogenase reductase (*nifH*) (Zehr et al. 2003), and denitrification, which can be assayed by targeting the gene encoding dinitrogenase reductase (*nosZ*) (Throbäck et al. 2004).

Wildfire disturbance can alter both aboveground forest properties (removal of trees and other plants) and belowground soil chemistry, microbial communities, and nutrient cycling (Yeager et al. 2005; Certini 2005; Dooley and Treseder 2011; Mataix-Solera et al. 2011; Switzer et al. 2012). Fire has been demonstrated to alter the community structure of both soil fungi (Cairney and Bastias 2007) and ECM fungi (Stendell et al. 1999; Mah et al. 2001; Dahlberg et al. 2001; Smith et al. 2005; Gundale et al. 2005; Rincón and Pueyo 2010; Kennedy and Egger 2010; Buscardo et al. 2012; Barker et al. 2013). In addition, disturbance by MPB has been documented to increase fire severity (Turner et al. 1999; Page et al. 2012), with MPB-attacked (red) stands experiencing higher severities of crown fire than might otherwise be expected (S. Taylor, CFS Forestry Officer, pers. comm.). In light of the increase in wildfire incidence following MPB infestation, it is critical to understand the impact of these disturbances on the biological component of forest soils, and the resulting impact upon natural seedling regeneration.

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In recent years, molecular advances have allowed ecologists using techniques such as length-heterogeneity polymerase chain reaction (LH-PCR) and terminal restriction fragment length polymorphism (T-RFLP) to quickly profile microbial populations in the environment (Hirsch et al. 2010). These “fingerprinting” techniques provide a powerful way to gain information about the richness of the targeted gene (number of genotypes), while the fragment peak height gives some indication of the relative abundance of each genotype within a soil sample. In the case of ECM, analysis of community structure by traditional morphotyping can be complemented by the use of a molecular profiling approach such as LH-PCR.

In this study, we hypothesized that wildfire would affect the ability of the soil to support tree regeneration in MPB-attacked lodgepole pine forests by (1) altering the nutrient status of the soil directly via physical effects and

(2) reducing ECM fungi inoculum both directly (through fungi in the soil being consumed by fire) and indirectly (through death of any living trees remaining), which in turn could result in further decreases in available soil nutrients. We further hypothesized that losses in ECM fungi would have a negative impact upon the soil's potential for supporting seedling regeneration, and that the more severe the fire, the greater the impact would be on soil nutrient status, ECM fungal diversity and structure, and associated N-cycling bacteria. In order to test our hypotheses, we studied rhizosphere soil and root systems of lodgepole pine seedlings regenerating in two soil types (as delineated by moisture), and three fire severity classes.

# Materials and methods

## Study area and site description

The study area was located near Kenny Dam (124° 54′ 27″ W, 53° 36′ 34″ N) in central BC, where the devastation from MPB was severe. The MPB attack occurred over many years but was considered to reach its height in 1999–2000 (Kathy Lewis (UNBC), personal communication), with evidence of attack still present in 2004/2005. The area was not devoid of under storey plants or trees following attack. Some smaller lodgepole pine had escaped the beetle attack, and a variety of other mycorrhizal host species were present in the under storey as well as over storey. These occurred randomly across sites and included, but were not restricted to *Alnus crispa* spp. *sinuata* Regel, *Arctostaphylos uva-ursi* L., *Populus tremuloides* Michx., and *Pyrola asarifolia* Michx. Some areas of MPB-killed stands in the study area were disturbed by a large forest fire (~10,000 ha) in June 2004. The study area was dominated by Brunisolic soil order in dry sites and Luvisolic soil order in wet sites developed on glaciolacustrine deposits (Dawson 1989). Selection of study sites in MPB-killed stands (>80 % attack, determined by surveying trees for the presence of MPB galleries) was completed in late April–May 2005. Level of MPB attack was determined in unburned stands by a visual estimate and the presence of red crowns. In burned stands using fixed radius plots in combination with MPB-enhanced aerial photography, visual identification of beetle galleries under the bark and pitch-tubes was necessary to verify MPB attack. A time domain reflectometer (TDR, Campbell Scientific) with a 12-cm probe for volumetric water content (%) was used to randomly sample target stands during field reconnaissance. The range in site

moisture content across the study area was classified as dry (<20 %) and wet (20–45 %). To accommodate the wet and dry sites, severity conditions were visually aggregated into three post-fire severity classes: high, moderate, and low. The distinction was based on the amount of crown and subsequent cone consumption. A relative measure of duff consumption by depth between sites was also used in defining the classes. Classifications were as follows: high—forest floor and tree crown were completely burned; moderate—forest floor was completely burned and crown was burned but still recognizable; and low—forest floor was partially burned and crown was burned but still recognizable. The two site moisture classes (dry and wet), each with three fire severity classes (high, moderate, and low), were replicated three times for a total of 18 disturbance plots of 1 × 1 $m^2$ each (see Scholefield 2007 for full description of plot selection). Unburned, MPB-attacked lodgepole pine plots were identified on the same soil types as burned plots. Lodgepole pine seed (100 wild seeds (BC 25110) per plot) was sown directly into all plots in May 2005. Forceps were used to insert individual seeds 5 mm beneath the soil at distances of 50 cm apart. Due to competing vegetation, lodgepole pine seedlings were unable to establish in unburned plots; therefore, data for unburned plots are included for soil analysis, but not for root-associated microbe analyses (ECM and N-cycling bacteria).

## Soil sampling and analyses

In each plot, soil was collected in August 2006 from three locations at a depth of 0–12 cm; equal volumes of these soils were combined to form one composite sample (approximately 1 kg) per replicate plot. Soil samples were kept at 4 °C during transport to the laboratory; each sample was then air-dried and passed through a 2-mm sieve prior to analysis. Soils were analyzed (BC Ministry of Forests, Lands and Natural Resource Operations, Research Branch Lab, Victoria, BC) for selected soil properties (total C and N;- pH (water and $CaCl_2$); $SO4_2^-$; conductivity; exchangeable Al, Ca, Fe, K, Mg, Mn, and Na; CEC; available $NH_4$-N; $NO_3$-N; mineralizable $NH_4$-N; and particle size) following procedures in Kalra and Maynard (1991).

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## Seedling harvest and ECM characterization

Harvesting of lodgepole pine seedlings for ECM assessment occurred in

August 2006, and consisted of seedlings grown from unimproved wild seed, sown in May 2005. Depending on date of germination, seedlings were approximately two growing seasons old. All seedlings were randomly selected, harvested with soil surrounding roots, bagged, and kept cool. Seedlings were stored at 4 °C until assessed. A total of 103 seedlings were harvested, 49 from dry sites and 54 from wet sites, representing all three fire severity classes. Seedling roots were gently cleaned of soil and root tips were assessed using standard microscopy techniques (Robertson et al. 2006). Ectomycorrhizal tips were initially described using a dissecting microscope and classified according to standard features such as colour, lustre, tip dimension and shape, branching pattern, and presence or absence of rhizomorphs (mycelial strands) (Agerer 1987–2008; Ingleby et al. 1990). Root tip squash mounts were subsequently examined using bright field microscopy, and descriptions of mantle features, emanating hyphae, rhizomorphs, and other characteristics were used to further categorize the different ECM morphotypes. When possible, ECMs were identified to a species, genus, or family based on their morphological features; when identification was not possible, a descriptive name was assigned. Uncolonized or lightly colonized tips that lacked identifiable, well-developed mantles were classified as one group. Identification to family, genus, or species was made based on similarities in features to published descriptions (Agerer 1987–2008; Ingleby et al. 1990). Only roots that appeared turgid with intact meristems were examined. For each seedling, all tips were assessed and the percent abundance for each morphotype was calculated from this total. A total of 18,696 root tips were characterized according to their fungal-root associations (ECM morphotypes). ECM abundances were calculated by dividing the total number of root tips of each particular morphotype found on all seedlings in a plot by the total number of mycorrhizal root tips counted in that plot. Following ECM assessment, root samples (entire seedling root systems) were frozen at −20 °C for subsequent molecular analysis.

## Molecular characterization of ECM and N-cycling communities

The Ultraclean Soil DNA kit (MoBio, Carlsbad, CA, USA) was used to extract DNA from all whole seedling root systems, following the manufacturer's recommended alternative protocol for increased yield. Before extraction, samples were frozen at −80 °C for 30 min, thawed for

30 min, frozen at −80 °C for 30 min, and thawed again for 30 min to fracture cells. Fungal communities were profiled by length-heterogeneity PCR (LH-PCR) using primers ITS3 and NLB4, while nitrogen-cycling bacterial communities were profiled by T-RFLP of *nifH* and *nosZ* genes, as previously described (Kennedy and Egger 2010).

## Statistical analysis

Data from all soil samples (soil chemistry) and root tips (ECM morphotype, LH-PCR fungal genotypes, and N-cycling bacterial genotypes) analyzed in each plot were averaged in order to create a composite plot profile, resulting in $n = 3$ (plots) for each disturbance. Richness and evenness of morphotypes and genotypes were determined using indices described by Blackwood et al. (2007): richness (S′) and Smith and Wilson evenness ($E_{var}$). All single-variable (soil chemistry, richness/evenness, and morphotype abundances) data were analyzed using Statistica version 6.1. First, data were tested for normality using the Shapiro-Wilk *W* test. If normal, factorial ANOVA (moisture (2 levels), fire (4 levels for soil chemistry, 3 levels for all other datasets), and their interaction) was performed, with the significance level set at $P < 0.05$. Post hoc Tukey HSD tests were performed to determine significant differences between means in disturbances showing significance. Some non-normal results could not be made normal by transformation and so were analyzed using nonparametric Kruskal–Wallis ANOVA. Correlations between soil chemical variables and richness/evenness/morphotype abundances were determined using linear regression.

Multivariate statistical analysis was performed using PC-ORD 5.12. First, LH-PCR (fungal) and T-RFLP (bacterial) profiles from individual seedlings in a plot were averaged together using the program RiboSort (Scallan et al. 2008) and the statistical software R. The relative abundance of genotypes in each composite sample was calculated after relativizing the fluorescent signal strength of each fragment peak to the total peak area of the sample (Osborne et al. 2006). Community structure was assessed graphically with nonmetric multidimensional scaling (NMS) (McCune et al. 2002). This was calculated on the basis of a Sørensen (Bray–Curtis) distance measure with 100 runs with real and randomized data (compared using Monte Carlo simulations) and a maximum of 250 iterations to assess stability. Pearson and Kendall correlations with soil chemistry variables

were assessed, and correlations with values higher than 0.150 were overlaid as vectors on the NMS plot. Cluster analysis on ECM morphotype data was performed using two-way hierarchical cluster analysis with flexible beta linkage and Sørensen (Bray–Curtis) distance measure.

# Results

The majority of soil properties (16 out of 22) were significantly influenced by moisture, with total C and N, $SO_4$-S, conductivity, mineral $NH_4$-N, silt and clay content, and exchangeable Al, Ca, Fe, Mg, and Na, as well as CEC values, higher in wet compared to dry soils. Soil pH (water and $CaCl_2$) and sand content values were higher in dry sites (Table 1). Fire severity (ranging from unburned to high) did not have a significant impact on any soil properties.

**Table 1**

Means (SE) and ANOVA results comparing soil chemical properties by site type (dry, AQ4

| Soil properties | Dry | | | | We | | |
|---|---|---|---|---|---|---|---|
| | Un-burned | Low | Moderate | High | Un-burned | Low | |
| Total C (%) | 1.15 (0.41) | 0.55 (0.06) | 0.70 (0.07) | 0.57 (0.08) | 1.24 (0.11) | 1.58 (0.30) | ( |
| Total N (%) | 0.05 (0.014) | 0.03 (0.001) | 0.04 (0.003) | 0.03 (0.002) | 0.06 (0.001) | 0.07 (0.009) | ( |
| C:N ratio | 22.00 (1.88) | 19.6 (1.11) | 19.9 (1.45) | 19.9 (1.47) | 20.2 (1.41) | 23.8 (1.47) | ( |
| pH (water) | 5.79abcd (0.12) | 5.89b (0.09) | 5.95c (0.06) | 6.01d (0.14) | 5.80abcd (0.05) | 5.52ab (0.07) | ( |
| pH ($CaCl_2$) | 4.97 (0.16) | 4.96 (0.10) | 4.92 (0.08) | 4.98 (0.06) | 4.96 (0.06) | 4.71 (0.07) | ( |
| $SO_4$-S (μg/g) | 0.46a (0.22) | 1.71 (0.42) | 1.4 (0.29) | 1.17 (0.02) | 1.41 (0.94) | 1.69 (0.06) | ( |
| Conductivity (dS/m) | 0.03 (0.003) | 0.02 (0.0002) | 0.02 (0.0001) | 0.01 (0.003) | 0.04 (0.001) | 0.04 (0.002) | ( |
| Exch. Al ($cmol_c$/kg) | 0.16 (0.07) | 0.18 (0.05) | 0.19 (0.05) | 0.16 (0.05) | 0.14 (0.05) | 0.38 (0.11) | ( |
| Exch. Ca ($cmol_c$/kg) | 2.53 (0.69) | 1.24 (0.08) | 0.94 (0.16) | 1.11 (0.05) | 5.08 (0.11) | 4.82 (0.23) | ( |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Exch. Fe ($cmol_c/kg$) | <0.001 | <0.001 | <0.001 | <0.001 | 0.01 (0.004) | 0.03 (0.014) | ( |
| Exch. K ($cmol_c/kg$) | 0.31 (0.05) | 0.22 (0.02) | 0.20 (0.02) | 0.18 (0.04) | 0.29 (0.05) | 0.31 (0.02) | ( |
| Exch. Mg ($cmol_c/kg$) | 0.38 (0.06) | 0.27 (0.03) | 0.20 (0.02) | 0.22 (0.03) | 1.38 (0.22) | 1.15 (0.08) | ( |
| Exch. Mn ($cmol_c/kg$) | 0.09 (0.05) | 0.03 (0.01) | 0.04 (0.01) | 0.04 (0.02) | 0.05 (0.01) | 0.08 (0.02) | ( |
| Exch. Na ($cmol_c/kg$) | 0.01 (0.004) | 0.01 (0.001) | 0.01 (0.003) | 0.01 (0.001) | 0.06 (0.018) | 0.04 (0.011) | ( |
| CEC ($cmol_c/kg$) | 3.47 (0.90) | 1.95 (0.08) | 1.59 (0.14) | 1.73 (0.12) | 7.02 (0.17) | 6.82 (0.40) | ( |
| Avail. P (μg/g) | 132.1 (16.4) | 83.1 (3.64) | 112.6 (4.13) | 86.6 (5.74) | 135.6 (82.9) | 125.4 (12.00) | ( |
| Avail. $NH_4$ (μg/g) | 4.04 (1.63) | 1.19 (0.39) | 2.93 (1.88) | 3.73 (0.55) | 6.27 (1.58) | 5.43 (2.17) | ( |
| Avail. $NO_3$ (μg/g) | <0.001 | 0.10 (0.06) | 0.04 (0.02) | 0.01 (0.01) | 0.07 (0.03) | 0.03 (0.02) | ( |
| Mineral $NH_4$ (μg/g) | 8.12 (1.79) | 4.55 (1.60) | 5.38 (3.94) | 5.60 (1.93) | 22.37 (2.29) | 20.81 (3.87) | ( |
| Sand (%) | 88.1 (3.81) | 84.0 (3.61) | 92.0 (0.42) | 91.1 (0.73) | 42.6 (1.88) | 54.7 (3.86) | ( |
| Silt (%) | 6.8 (3.09) | 12.7 (3.19) | 5.5 (0.42) | 5.5 (0.42) | 44.6 (2.6) | 34.2 (2.47) | ( |
| Clay (%) | 5.1 (0.75) | 3.4 (0.42) | 2.5 (0.03) | 3.4 (0.85) | 12.8 (0.75) | 11.1 (1.58) | ( |
| *n* = 3. Means within the same row followed by the same letter are not significantly | | | | | | | |
| *NS* not significant, *NE* not estimated (interaction could not be estimated using non | | | | | | | |

Ectomycorrhizal morphotype assessment identified a total of 29 unique morphotypes, 25 from dry, and 27 from wet disturbance sites (Table 2). Seedlings had from two to six ECM morphotypes, while plots had from five to 10 each. Table 2 shows mean plot values for percent ECM abundance of each morphotype as affected by disturbance. Four ECM morphotypes showed a significant moisture preference: three to wet sites (Russulaceae 2, *MRA*, and *Piloderma*) and one to dry sites (Russulaceae *Lact*-like1). Most seedlings (approximately 90 %) had some level of tips

considered uncolonized or lightly colonized (i.e., with too little fungal mantle for characterization); no significant difference was identified between moisture or fire severity disturbances for this uncolonized/lightly colonized group ($P > 0.05$, data not shown). Figure 1 shows a two-way cluster analysis of ECM morphotype data, showing some broad clustering of morphotypes according to moisture. Most dry sites clustered together at the top of the diagram, indicating that they had similar morphotype profiles, while wet sites exhibited similar clustering at the bottom.

**Table 2**

Means (SE) and ANOVA results comparing percentage abundance for ECM morph and fire severity (low, moderate, high)

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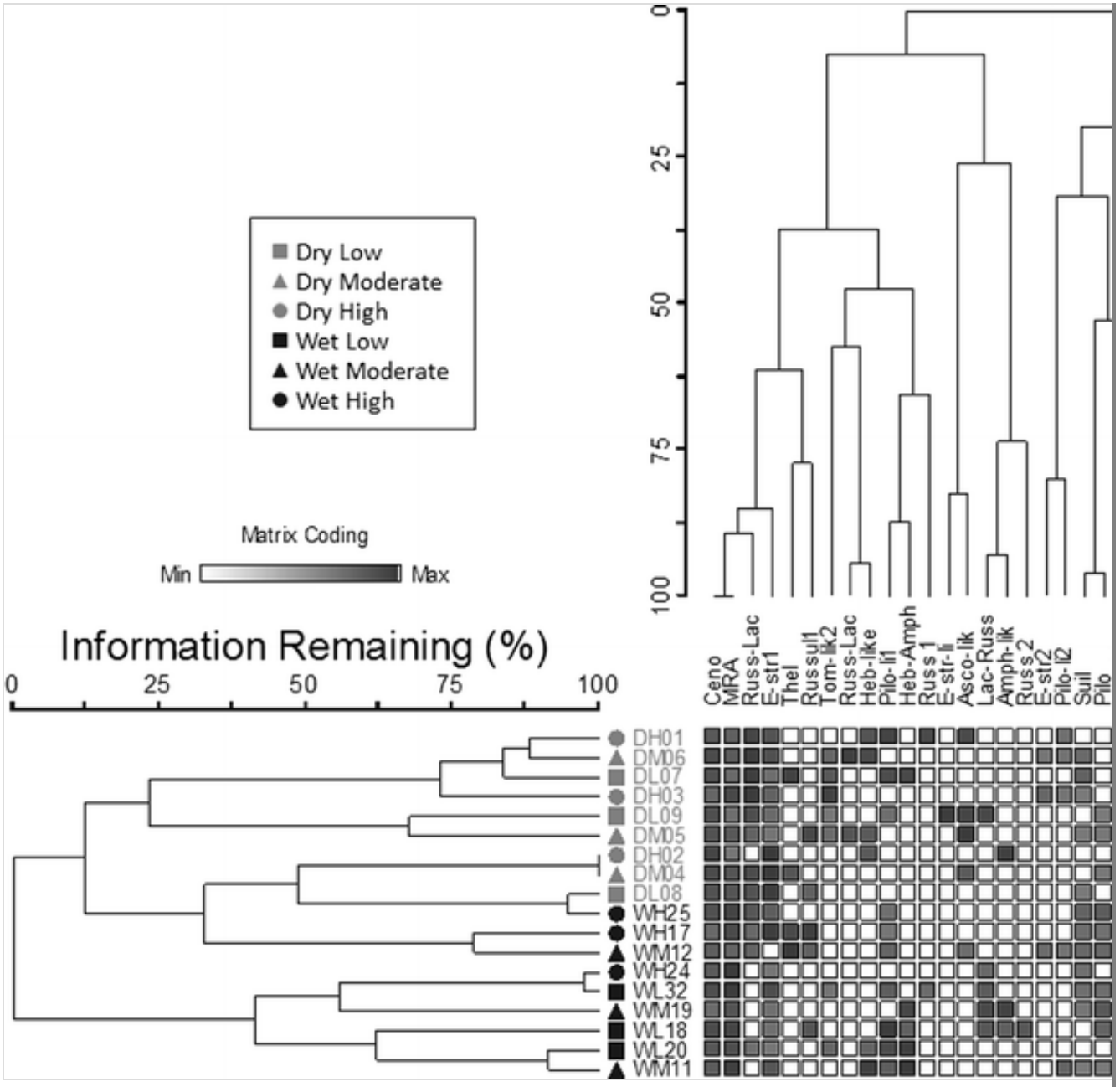
| Description/fungal species | Dry | | | Wet | | |
|---|---|---|---|---|---|---|
| | Low | Moderate | High | Low | Moderate | Hig |
| *Cenococcum* | 10.3 (0.53) | 12.8 (1.66) | 9.4 (4.74) | 11.3 (2.04) | 3.7 (0.95) | 6.1 (2.23 |
| *MRA* | 4.1 (2.25)a | 7.0 (0.76)a | 6.9 (4.78)a | 19.7 (5.20)b | 11.5 (5.67)b | 22.7 (5.87 |
| Russulaceae Lact-like1 | 15.4 (5.01)a | 14.1 (6.48)a | 17.5 (9.86)a | 0.3 (0.29)b | 1.1 (1.10)b | 3.4 (2.31 |
| E-strain1 | 11.4 (9.47) | 18.9 (13.72) | 21.9 (15.62) | 4.6 (3.97) | 3.0 (2.52) | 10.6 (8.16 |
| *Thelephora* | 7.0 (7.00) | 2.8 (2.76) | 0 | 0 | 16.0 (16.02) | 5.1 (5.07 |
| Russulaceae1 | 1.3 (1.34) | 5.5 (5.48) | 0 | 2.8 (2.78) | 1.4 (1.35) | 8.1 (8.14 |
| *Tomentella*2 | 2.2 (2.09) | 1.6 (0.83) | 6.5 (6.48) | 1.0 (0.81) | 0 | 0 |
| Russulaceae Lact-like2 | 0 | 10.3 (6.53) | 0 | 0 | 0 | 0 |
| *Hebeloma-like* | 0 | 7.1 (4.33) | 4.9 (2.58) | 1.0 (0.98) | 7.4 (7.44) | 0 |
| *Piloderma*-like1 | 4.7 (3.97) | 0 | 6.4 (6.41) | 16.3 (7.51) | 2.5 (1.31) | 0.5 (0.34 |
| | 5.5 | | | 9.9 | 17.3 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Heb-Amph-like | (5.50) | 0 | 0 | (7.91) | (9.90) | 0 |
| *Russula*1 | 0 | 0 | 5.3 (5.34) | 0.7 (0.65) | 0 | 0 |
| E-strain-like | 10.3 (10.31) | 0 | 0 | 0 | 0 | 0 |
| Asco-like | 6.2 (6.22) | 17.1 (14.50) | 5.3 (5.34) | 0 | 0.6 (0.58) | 0 |
| *Lactarius*-Russul-like | 5.6 (5.56) | 0 | 0 | 4.3 (2.28) | 5.4 (5.43) | 0.9 (0.86 |
| *Amphinema*-like | 0 | 0 | 7.4 (7.41) | 1.4 (1.43) | 6.5 (6.50) | 0 |
| *Russula*2 | 0 | 0 | 0 | 2.5 (2.51) | 0 | 0 |
| E-strain2 | 0 | 0.1 (0.12) | 0.9 (0.91) | 0 | 0.6 (0.63) | 0 |
| *Piloderma*-like2 | 0 | 0.2 (0.23) | 0.6 (0.38) | 0 | 1.2 (0.92) | 0 |
| *Suillus-Rhizopogon* | 1.4 (1.30) | 0.8 (0.66) | 0.1 (0.06) | 0.4 (0.40) | 1.1 (0.81) | 2.1 (0.75 |
| *Piloderma* | 0.03 (0.03)a | 0.2 (0.09)a | 0a | 1.5 (0.88)b | 3.1 (1.85)b | 2.0 (1.62 |
| Wh rhizo (+)clamps | 0 | 0 | 0 | 0 | 0 | 0.5 (0.48 |
| Lt Br Curling | 0 | 0 | 0 | 3.0 (2.97) | 0 | 0 |
| Russulaceae2 | 0a | 0a | 0a | 3.2 (3.22)b | 1.4 (1.43)b | 4.5 (3.12 |
| *Tomentella*1 | 7.3 (6.94) | 0 | 0.7 (0.69) | 6.2 (5.86) | 0 | 6.8 (6.84 |
| *Lactarius* | 5.5 (5.55) | 0 | 0 | 3.6 (3.62) | 6.4 (6.39) | 0 |
| Wh rhizo (-)clamps | 0.1 (0.07) | 1.6 (1.63) | 0.2 (0.19) | 2.1 (2.05) | 0.4 (0.22) | 2.3 (1.95 |
| *Lactarius*-like | 1.4 (1.40) | 0 | 0 | 0 | 0 | 14.9 (7.73 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Amphinema* | 0 | 0 | 5.8 (5.85) | 4.3 (4.33) | 9.3 (9.32) | 9.6 (6.23 |
| Total morphotypes | *18* | *15* | *16* | *21* | *20* | *16* |
| $n = 3$. Means within the same row followed by the same letter are not significantly HSD test, $P < 0.05$). Soil chemistry factors exhibiting significant ($P < 0.05$) correl abundances are listed | | | | | | |
| *NS* not significant, (+) positive correlation, (−) negative correlation | | | | | | |

**Fig. 1**

Two-way cluster analysis of fungal community structure, as assessed by similarities in ECM morphotype percentage abundance. Morphotype names are abbreviated in the *vertical dendrogram*, with sites listed in *horizontal dendrogram*




Significant differences were not found for richness or evenness values of ECM (fungal morphotypes (richness 8.3–11.3; evenness 0.27–0.40) or LH-PCR genotypes (richness 7.7–13.3; evenness 0.62–0.72) or denitrifying (richness 6.3–11.3; evenness 0.41–0.53) or N-fixing (richness 15.3–20.7; evenness 0.59–0.74) bacteria with respect to moisture or fire severity (all $P > 0.05$; data not shown). However, ECM morphotype richness was consistently, but not significantly, higher from the wet (all fire severities) compared to dry sites. Significant correlations were observed between ECM morphotype richness and soil $SO_4$ (positive, $P = 0.01$, $r^2 = 0.34$), and between ECM morphotype evenness and C:N ratios (positive, $P = 0.01$, $r^2 = 0.34$). There was also a significant correlation between denitrifier richness and available $NO_3$ (positive, $P = 0.03$, $r^2 = 0.26$).

Nonmetric multidimensional scaling ordination of fungal ECM morphotype profiles exhibited clustering according to moisture (Fig. 2a), with wet sites clustering on the left side of the plot, while dry sites clustered to the right. Overlay of the plot with soil chemical properties indicated pH, total C and N, and mineral $NH_4$ were significantly correlated with ECM morphotype profiles. Axis 1 was most highly correlated with pH ($r^2 = 0.524$), while axis 2 was most highly correlated with mineral $NH_4$ ($r^2 = 0.292$). Similar clustering according to moisture was evident in the NMS ordination plot of LH-PCR fungal genotypes (Fig. 2b). As in the ECM morphotype plot, there were significant correlations between LH-PCR genotype profiles and pH, total C and N, and mineral $NH_4$. The impact of moisture on fungal community structure was confirmed by PERMANOVA, which showed both ECM morphotype and LH-PCR genotype profiles of wet sites differed significantly (Table 3) from dry sites. In contrast to the strong effect of moisture, NMS plots showed no clustering due to fire severity, and PERMANOVA analysis found no significant differences between fungal communities in soils from different fire severities. Denitrifying (Supplemental Fig. 1) and N-fixing (Supplemental Fig. 2) bacterial genotypes did not exhibit any clustering due to moisture or fire severity. However, there were significant correlations between N-fixing genotype profiles and C:N and pH.

**Fig. 2**

NMS plots of fungal community structure. $r^2$ values represent the variance explained by each axis. *Line vectors* show correlations of profiles with soil chemistry data. **a** NMS plot of fungal ECM morphotype profiles. Stress = 23.9, instability = 0.00001. **b** NMS plot of fungal LH-PCR profiles. Stress = 23.0, instability = 0.01037. Site type (dry, wet); fire severity (low, moderate, high)

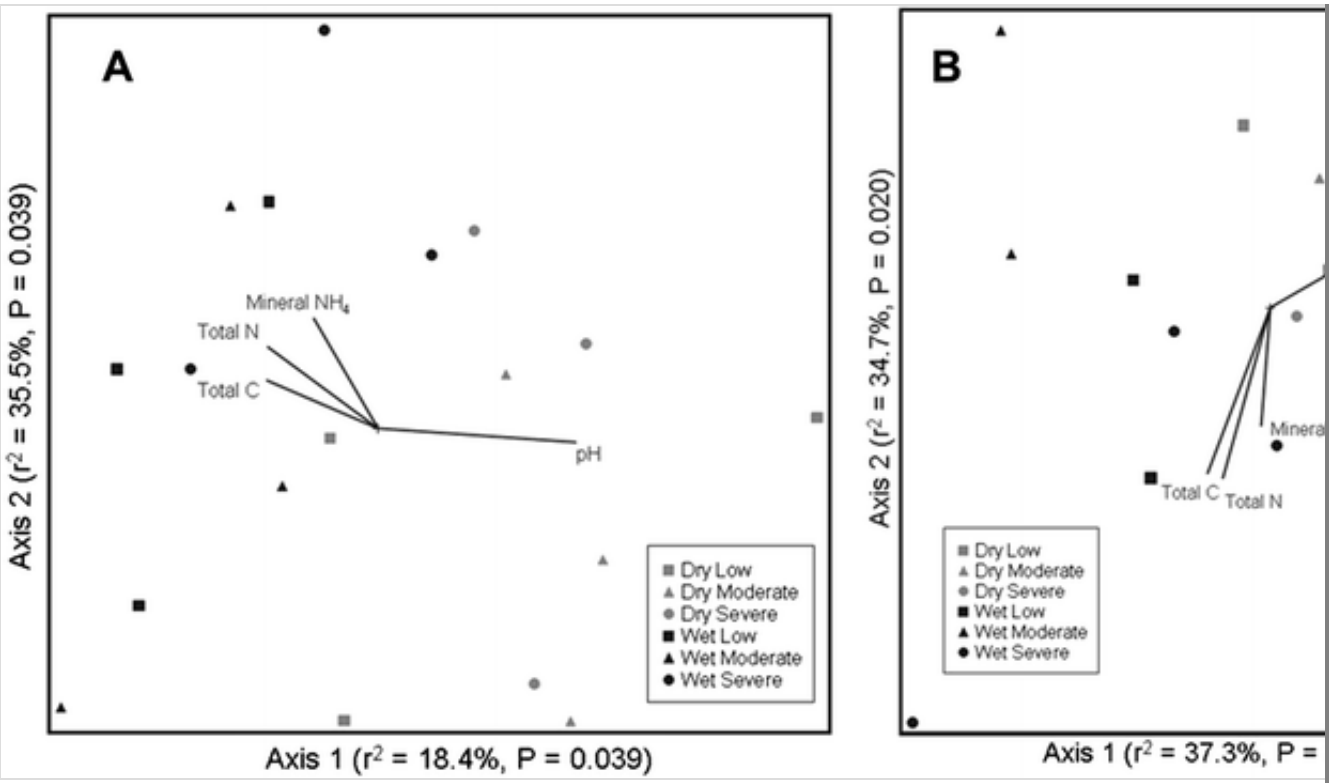



**Table 3**

PERMANOVA results for fungal community structure (as assessed by ECM morphotypes and LH-PCR profiles), denitrifying bacteria community structure (as assessed by *nosZ* T-RFLP profiles), and N-fixing bacterial community structure (as assessed by *nifH* T-RFLP profiles)

| | **Moisture** | **Fire severity** | **Moisture × fire** |
|---|---|---|---|
| Fungi (ECM morphotypes) | 0.0009 | NS | NS |
| Fungi (LH-PCR) | 0.0002 | NS | NS |
| Nitrifying bacteria (*nosZ*) | NS | NS | NS |
| N-fixing bacteria (*nifH*) | NS | NS | NS |
| *P* values are listed by disturbance (moisture, fire severity) | | | |
| *NS* not significant ($P > 0.05$) | | | |

# Discussion

## Wet and dry sites had distinct soil chemistries

Following examination of the full suite of soil chemistry data, it became evident that wet and dry sites were different in almost every aspect of soil chemistry, not just moisture, and as expected, represented two discrete soil

types. Interestingly, despite consecutive disturbances (mountain pine beetle infestation and wildfire), moisture remained the most important factor influencing soil chemistry. The lower pH of wet sites was likely due to increased moisture leading to increased biological activity, which in turn can lead to production of organic acids (Prescott et al. 2000). Similarly, greater microbial biomass in wet sites may also have resulted in higher levels of mineralizable ammonium. This was supported by higher levels of available nitrate and ammonium in wet soils than dry soils, although these were not significant. The sandy nature of dry soil sites could result in increased leaching on these sites, leading to lower levels of nitrate. Although nitrate levels were higher in wet soils, they were still considered low compared to typical levels (Ryan and Waring 1992; Cullings and New 2003; Douglas et al. 2005). This may be due to the higher fungal biomass in wet soils capturing nitrate (Martin et al. 2000).

## Soil type strongly influenced ECM community structure

Our molecular and morphological results showed a clear distinction between ECM fungal communities from wet versus dry sites. The use of both approaches confirmed the trends seen in the data, as each approach has different advantages and limitations. Morphotyping has been extensively used to describe ECM communities, but is time-consuming and requires advanced expertise; in addition, it is possible that one morphotype can have more than one genetic identity. Molecular fingerprinting techniques, such as LH-PCR, are rapid and high-throughput and may be able to resolve species that morphotyping cannot; however, caution must be used in analyzing profiling data due to possible interspecies ITS heterogeneity and PCR amplification bias (Dickie and FitzJohn 2007). In this study, both approaches revealed the same overall trends in ECM community structure, thus confirming one another's results. Complementarity of ECM morphotype and fingerprinting data was also noted by Burke et al. (2005), who found that genotypes detected with TRFLP accounted for 93 % of their colonized root tips.

The cluster analysis revealed the influence of soil type on individual morphotypes. Although some morphotypes occurred in both site types, there were four morphotypes that were restricted to, or found in greater abundance in, either wet or dry sites. The distribution of ECM morphotypes between soil types indicated three broad groups:

"ubiquitous/cosmopolitan," "wet-preferring," and "dry-tolerating." Ubiquitous morphotypes, including *Cenococcum* and E-strain 1, were found in almost all sites. These ascomycetes have been previously described from a wide diversity of habitats and are known to associate with a broad range of coniferous hosts (e.g. Mah et al. 2001; Douglas et al. 2005; Robertson et al. 2006; DeBellis et al. 2006). In particular, the E-strain morphotype has been described as a dominant member of post-fire ECM communities (reviewed in Jones et al. 2003). Morphotypes such as *MRA*, *Piloderma*, and Russulaceae 2 were preferentially found in wet sites and were largely absent from dry sites, while dry-tolerating types, such as Russulaceae-Lact-like1 and Asco-like, were present in both wet and dry sites. These differences in distribution likely represent individual species adaptations to soil moisture (or chemical changes associated with soil moisture) that allow for survival (or, in some instances, for ECM fungi to thrive) in diverse habitats. A fourth group of morphotypes exhibited a patchy, pattern-less distribution, representing a small component of the root community.

Our findings are supported by other studies which have noted significant effects of soil conditions on forest ECM communities. Soil moisture and temperature levels were found to impact ECM community structure to a larger extent than soil nutrient status on lodgepole pine roots (Cullings and New 2003). Field studies of ECM associated with black spruce (Robertson et al. 2006) and subalpine fir (Kranabetter et al. 2009) also found variations in ECM community structure due to differences in moisture. A study of ECM communities from pinyon pine trees growing in different soils noted that soil type was linked to fungal community composition (Gehring et al. 1998). A recent study (Karst et al. 2011) found that Douglas-fir seedlings from low-moisture soils had higher levels of ectomycorrhizal colonization than those from medium and high moisture soils.

## Fire severity had limited impact on soil chemistry or ECM communities

In comparison to the significant influence of soil type, our study found fire severity had a relatively small impact on soil chemistry and root-associated microbial communities. These results contrast with other studies that have found dramatic changes in soil properties due to fire. In a review of the effects of fire on soil aggregation (a measure of soil resilience), Mataix-

Solera et al. (2011) found that aggregation depended upon fire severity and soil type, with high-severity fires having the greatest impact. Certini (2005) conducted a review and found that fire had severe negative effects on forest soil properties, including removal of organic matter, loss of soil nutrients, decreased microbial biomass, and changes in the composition of soil microbiota. A recent meta-analysis on microbial responses to fire found that fire decreased microbial biomass by an average of 33 % and fungal biomass by an average of 47 % (Dooley and Treseder 2011).

Several studies have looked at the impact of fire on forest ECM communities in particular (see Cairney and Bastias (2007) for review). Varied responses of ECM communities to fire have been described, with some studies finding post-fire decreases in ECM biomass/diversity on Scots pine (Dahlberg et al. 2001) and ponderosa pine stands (Stendell et al. 1999; Smith et al. 2005) in Sweden, California, and Oregon, respectively. Other studies have found fire altered ECM community structure in Douglas-fir (Kennedy and Egger 2010), ponderosa pine (Gundale et al. 2005), and spruce (Mah et al. 2001) forests, without necessarily causing large changes in diversity or biomass, while a study on Scots pine forests in Sweden did not find any consistent change in ECM richness or composition following wildfire (Jonsson et al. 1999). These varying responses of ECM to fire may indicate that local conditions (such as moisture in our study) can strongly influence the response of ECM to wildfire.

Previous studies that have included sites burned at different severities suggest the impact of fire severity on ECM communities is difficult to estimate. An assessment of maritime pine (*Pinus pinaster*) in Portugal indicated that soil chemistry and ECM communities in areas burned at different fire return intervals were more similar to each other than to unburned controls (Buscardo et al. 2012). Similarly, Rincón and Pueyo (2010) examined fire severity on ECM communities of a maritime pine forest in Spain and found that fire severity did not affect ECM richness or diversity, while Barker et al. (2013) found the rate of ECM colonization of Douglas-fir seedlings in interior BC forests was unaffected by wildfire severity. In contrast, an earlier study on Scots pine forests in Sweden found ECM abundance and diversity decreased with increased fire severity (Dahlberg et al. 2001). Their study also emphasized the impact of burning on forest floor soil properties, noting that even low-severity burns killed

mycorrhizal fungi in the organic layer of the soil. Other studies focused on the mineral layer of soil have noted that this layer acts as reservoir of resistant propagules of ECM, such as spores and sclerotia, which can rapidly colonize seedling roots post-fire (Stendell et al. 1999; Grogan et al. 2000; Bruns et al. 2002; Rincón and Pueyo 2010; Barker et al. 2013). This suggests that all fire severities in our study may have been hot enough to kill mycorrhizal fungi in the organic layer, allowing colonization of regenerating seedlings from the mineral layer, and resulting in an evenness of effect across fire intensities with respect to both chemical and microbial impacts.

## N-cycling bacteria were unaffected by soil type or fire severity

In contrast to the large impact of moisture on fungal communities, there was no significant impact of moisture (or fire severity) on two types of N-cycling bacterial communities. This concurs with results for burned Douglas-fir pine forests in southern British Columbia, where fire affected ECM community structure, but not the structure of denitrifying or N-fixing bacterial communities (Kennedy and Egger 2010). Similarly, Štursová et al. (2014) found that insect infestation affected forest soil fungal communities more than bacterial communities. The few studies that have been conducted specifically on root-associated N-cycling communities in forests have noted low diversity of nitrogen-fixing bacteria (Burke et al. 2006; Izumi et al. 2006), which is consistent with our results. However, the inability of lodgepole pine to establish on unburned control plots may have prevented our ability to identify an effect of fire severity (at any intensity) on N-cycling bacterial communities. It is also possible that the differences between the fire severities tested were too small to be detected in community analyses that were used. This is supported by the results of Yeager et al. (2005), which showed few significant differences between nitrogen-fixing and ammonia-oxidizing bacterial profiles from moderately and severely-burned mixed conifer forest soils. We did note significant correlations between pH and C:N ratio and N-fixing communities, which was consistent with previous reports that changes in C:N ratio (Deslippe et al. 2005; Coelho et al. 2008) and pH (Hayden et al. 2010) can influence soil *nifH* profiles.

A lack of response to soil type and fire severity by N-cycling bacteria may also indicate greater resilience in bacterial compared to fungal

communities. A review of soil microbe responses to fire found that fire reduced fungal biomass more than overall microbial biomass (Dooley and Treseder 2011). Similarly, fungal communities (especially mycorrhizal fungi) were shown to be more sensitive than bacterial communities to tree harvesting disturbance (Hartmann et al. 2012).

# Conclusions

Our most striking finding was that soil type influenced ECM community much more than fire severity. Similarly, results showed that significant differences in soil chemistry parameters were due to soil type rather than to fire severity, leading us to conclude that local soil chemical variables were controlling ECM community structure. In particular, moisture appeared to be the most important influence on both soil chemistry and ECM composition, with the "signature" of moisture remaining pronounced even after consecutive landscape disturbances. Neither a major insect outbreak nor wildfire could eliminate this signature.

In summary, our results indicate that local soil conditions had a greater impact than fire severity on ECM communities on the roots of regenerating seedlings in post-beetle forests. This has implications for forest management in that it may be more important to consider soil chemistry and local soil moisture regime than fire history of sites selected for "restoration" (Burton 2006) through planting of seedlings.

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# Electronic supplementary material

Below is the link to the electronic supplementary material.

**Supplemental Fig. 1**

(GIF 77 kb)

High resolution image (TIFF 27 kb)

**Supplemental Fig. 2**

(GIF 79 kb)

High resolution image (TIFF 30 kb)

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