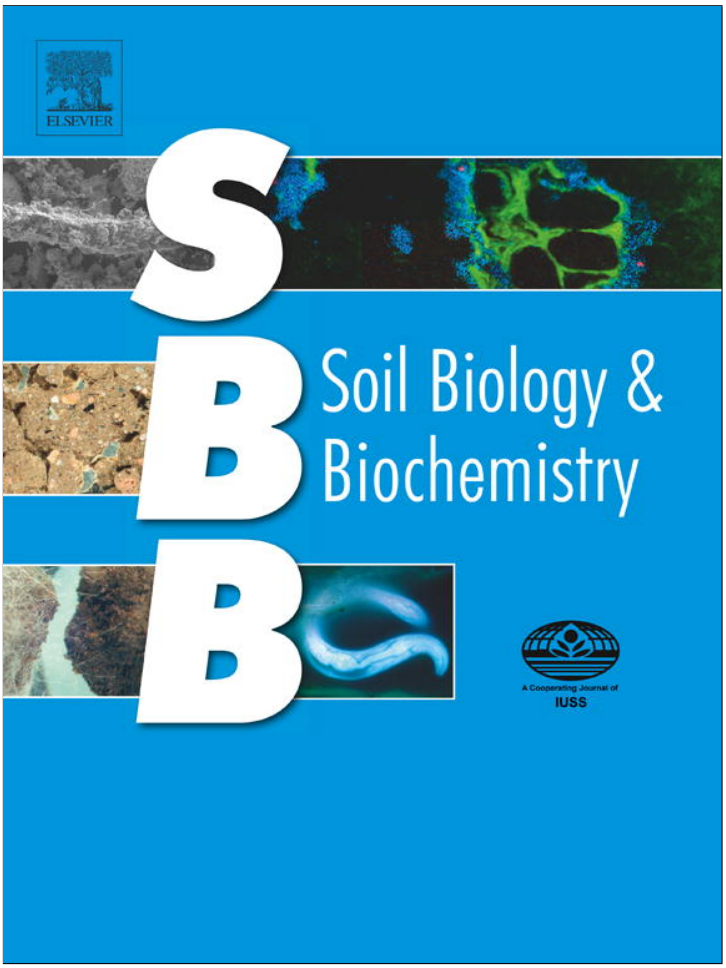

Soil Biology & Biochemistry 63 (2013) 61–72

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Linkages between the forest floor microbial community and resource heterogeneity within mature lodgepole pine forests

Anne C.S. McIntosh*, S. Ellen Macdonald, Sylvie A. Quideau

Department of Renewable Resources, GSB 751, University of Alberta, Edmonton, AB T6G 2H1, Canada

ARTICLE INFO

Article history:
Received 28 November 2012
Received in revised form
21 February 2013
Accepted 25 March 2013
Available online 8 April 2013

Keywords:
Community-level physiological profiling (CLPP)
Phospholipid fatty acid analysis (PLFA)
Pinus contorta
Above-ground below-ground interactions
Ecosystem properties and processes
Multiple carbon-source substrate-induced respiration (MSIR)

ABSTRACT

Below-ground microbial communities are influenced by a wide variety of above and below-ground abiotic and biotic factors. Thus, variation in properties, such as vegetation composition and cover, litter cover, soil pH, and nutrient availability likely influence their structure and composition. Examining microbial community variation within forests dominated by just a single tree species provides insights into the factors, other than dominant tree species, that influence the structure and function of below-ground microbial communities at the within-stand scale. In this study we examined fine scale (within-stand) patterns in microbial communities within mature lodgepole pine forests, and their relationships with abiotic and biotic factors. Specifically, the existence of fine-scale microbial structure (using phospholipid fatty acids – PLFAs) and function (using respiration of multiple carbon substrates – MSIR) community types; which PLFAs and carbon substrates were indicators of these, and which above- and below-ground properties and processes were related to these community types. At 108 sampling points in 12 0.48-ha plots abundance of understory plants, canopy tree size and cover, downed wood biomass, forest floor thickness, litter cover, pH, decomposition rate, available soil nutrients, microbial PLFAs and MSIR were assessed. Cluster and indicator species analysis revealed four fine-scale structural (PLFA) and four functional (MSIR) microbial community types. The biotic and abiotic variables measured had low explanatory power for describing the MSIR communities but high explanatory power for describing the PLFA communities. The main factors contributing to the separation of the structural (PLFA) microbial community types were understory plant species. Our findings suggest that the spatial partitioning of understory plant species and their rhizosphere resources (e.g., root exudates, nutrients) creates heterogeneity that influences the patterns of variation in below-ground microbial communities, in particular the microbial structure.

1. Introduction

Below-ground microbial communities may be influenced by a wide variety of biotic and abiotic factors. Vegetation composition regulates litter inputs (i.e., quality and quantity of litter) and plant root exudate composition, and thus can influence below-ground biota and processes affected by these (e.g., Zak et al., 2003). Both the canopy trees and the understory vegetation must be considered when examining the influence of vegetation on soil microbial communities in forests. Plant species diversity affects resource heterogeneity for soil microbial communities through effects on diversity of litter quality and quantity and root exudates. Thus, higher plant diversity may result in greater diversity in soil microbial communities (Hooper et al., 2000). Variation in tree species composition has been shown to affect structural and functional microbial community composition, at both the within-stand (Saetre and Baath, 2000) and among-stand scales (e.g., Grayston and Prescott, 2005).

Abiotic environmental resources/factors including microclimate, soil pH, texture, nutrient status (e.g., C:N ratio), and moisture can also influence the structural and functional composition of microbial communities (e.g., Birkhofer et al., 2012). Indeed, some studies have suggested that factors such as seasonal patterns in moisture may be more important drivers of soil microbial community structure and function than is vegetation (e.g., Swallow et al., 2009).

The factors influencing below-ground microbial community are expected to differ across a gradient of spatial scales (Ettema and

* Corresponding author. Tel.: +1 780 492 9121; fax: +1 780 492 4323.
E-mail addresses: amcintos@ulberta.ca (A.C.S. McIntosh), ellen.macdonald@ualberta.ca (S.E. Macdonald), sylvie.quideau@ualberta.ca (S.A. Quideau).

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http://dx.doi.org/10.1016/j.soilbio.2013.03.028

Wardle, 2002). At the micro-scale (mm to cm), changes in roots, organic particles, soil structure, etc. may influence the microbial community, although little research has been done at this scale (Leckie, 2005). At a within-stand scale (a few m; hereafter 'fine scale'), changes in factors such as plant community composition may affect the soil microbial community. For example, Pennanen et al. (1999) identified structural microbial communities that existed at scales of <3–4 m within boreal coniferous forests and these were correlated with proximity to tree and understory plants. At the landscape scale, differences in forest type and topography are likely to influence the structure and function of the forest floor microbial community (e.g., Brockett et al., 2012). Thus, the spatial scale at which microbial communities are studied will be important in understanding the influential factors as well as the linkages between soil and vegetation communities (Ettema and Wardle, 2002).

Understory plant communities have been identified at the fine-scale within forest stands dominated by a single canopy species, and these plant communities were related to below-ground factors including several below-ground microbial PLFAs (McIntosh, 2012). However, we do not know if forest floor microbial communities also vary at the fine scale in these forests, nor which abiotic and biotic factors may contribute to such patterns. If below-ground communities are primarily driven by overstory species composition, then single canopy species forests will not show a pattern of variation in fine-scale microbial communities. However, if below-ground microbial communities are related to understory communities or to abiotic factors that do vary at this scale then they will show fine-scale patterning in their composition. Thus, examining patterns of variation in microbial communities within forests dominated by a single tree species can provide insights into the biotic and abiotic factors that influence the structure and function of below-ground microbial communities at the fine scale.

Lodgepole pine (*Pinus contorta* Douglas ex Loud. var. *latifolia* Engelm.) is often the single canopy species in forests across its' wide climatic and geographical range in North America. Thus, these forests provide a valuable study ecosystem for examining fine-scale patterns in below-ground microbial communities in single canopy species forests and their relationships with resource heterogeneity within them. We addressed three key research questions regarding the relationships between forest floor microbial community structural (as measured by PLFA) and functional (as measured by MSIR) composition and overstory, understory, and below-ground attributes in lodgepole pine forests of Alberta: i) Are there fine-scale structural or functional microbial communities within mature lodgepole pine forests? ii) If so, what structural and functional variables are indicators of these microbial communities? and iii) Which above- and below-ground factors contribute to the separation of these microbial communities? We hypothesized that fine-scale microbial communities would occur, but that with just one dominant overstory species, separation of the microbial communities would be primarily influenced by above- and below-ground variation in abiotic and biotic factors associated with fine scale heterogeneity in the understory plant communities.

2. Materials and methods

2.1. Study area

The study area was located in the Upper Foothills natural subregion of Alberta (Natural Regions Committee 2006) in lodgepole pine forests in west-central Alberta (~53.2 °N; 116.8 °W). The regional natural disturbance regime is one of relatively frequent stand-initiating wildfire (Beckingham et al., 1996). The climate is temperate continental with mean daily maximum air temperatures during the growing season ranging from 16.2 °C in May, to 20.6 °C in August. Mean monthly precipitation during the growing season is as follows: 57.9 mm (May), 106.7 mm (June), 106.2 mm (July), and 82.2 mm (August), with a mean annual precipitation of 562.4 mm (30 year climate normal 1971–2000). The study stands were approximately 110–120 years old (mature) and were located on brunisolic gray luvisolic soils (SCWG, 1998). The study area was classified as ecosite UF e1.1 – Pl/green alder/feather moss (Beckingham et al., 1996). The overstory included only lodgepole pine; there were a very few white (*Picea glauca* (Moench) Voss) and black (*Picea mariana* (Mill.)) spruce, trembling aspen (*Populus tremuloides* Michx.) and balsam fir (*Abies balsamea* (L.) Mill) in the lower canopy and almost no seedlings or saplings. The understory was dominated by feather mosses, including *Pleurozium schreberi* (Brid.) Mitt., *Ptilium crista-castrensis* (Hedw.) De Not. and *Hylocomium splendens* (Hedw.) Schimp. and the hair cap moss *Polytrichum commune* Hedw. Common forbs included *Cornus canadensis* L. and *Linnaea borealis* L., common small shrubs included *Rosa acicularis* Lindl. and *Vaccinium myrtilloides* Michx.; *Alnus crispa* (Aiton) Pursh was the dominant tall shrub and the common graminoid was *Calamagrostis montanensis* (Michx.) Beauv.

Three study units (i.e., blocks in a statistical sense) ranging in size from 4.8 to 8.8 ha were sampled during the growing season of 2008 (Table 1). The study units were relatively flat topographically, were similar to one another and covered by fairly homogeneous mature lodgepole pine forest representative of the dominant forest cover type in the region. Within each study unit four 60-m × 80-m (0.48 ha) plots were established. Each plot was surrounded by a minimum of 20-m (~one tree height) of similar composition pine forest in order to minimize edge effects. Plots were placed as close together as possible with the constraint of ensuring uniform overstory stand conditions within each plot. Within each plot nine systematically-located nested sample points served as the centre-points for sampling the overstory, downed wood, understory and below-ground variables (n = 3 blocks*4 plots*9 sample points = 108 sampling points). These sample points were located 20–30 m apart from one another to reduce spatial auto-correlation.

2.2. Data collection

The overstory plant community was sampled in 8-m fixed-radius (0.02 ha) circular subplots in which live/dead status, species and diameter at 1.3 m height (dbh) were measured for all trees (dbh ≥ 5 cm and ht > 1.3 m). These data were used to calculate basal area and density separately for live and dead stems.

Table 1
Summary of site characteristics of lodgepole pine (*Pinus contorta*) study units in Upper Foothills of Alberta. Given are the locations and mean values for each of the three study units; the minimum and maximum values across subplots within each study unit are in parentheses.

Study unit	Latitude/longitude	Basal area (m^2 ha^{-1})	Density (trees ha^{-1})	Dbh (cm)	Canopy cover (%)
1	53.2248/116.8094	39.6 (26.7–56.2)	1420 (950–1900)	18.3 (5–34.7)	63.9 (56.2–86.9)
2	53.24129/116.8288	37.3 (21.6–55.1)	978 (550–1350)	21.5 (6.6–43.3)	59.2 (51.4–70.7)
3	53.22647/116.8212	40.3 (27.1–54.0)	1182 (450–1850)	20.1 (8.0–38.3)	62.1 (54.9–77.4)

To estimate canopy cover, hemispherical photographs were taken in mid-July at 1.4 m height at each sample point using a digital Nikon Coolpix 4500 with FC-E8 fisheye lens. We calculated gap fraction of images using SLIM (Spot Light Intercept Model v. 3.01) and subtracted gap fraction from 100 to estimate canopy cover (see McIntosh, 2012 for details).

The downed woody material (DWM) was measured along 8-m transects at each sampling point using the line intersect method (Brown et al., 1982). Biomass (Mg ha^{-1}) estimates for each of six diameter size classes (0–0.5 cm, 0.5–1.0 cm, 1–3 cm, 3–5 cm, 5–7 cm and >7 cm) were calculated using the equation and coefficients for Central Alberta Foothills lodgepole pine stands (Delisle and Woodard, 1988; Nalder et al., 1997; see McIntosh, 2012 for further details). Percent cover of DWM was estimated during assessment of understory communities (see below).

The understory plant community (i.e., forest floor mosses, forest floor lichens, forbs, graminoids and shrubs) was sampled within 1-m × 1-m quadrats located at each sample point. Percent cover (0–100) of each species/taxa was estimated. Cover estimates were also recorded for litter, tree/snag boles, downed woody material (diameter ≥ 3 cm), exposed mineral soil and rock. Understory species richness and diversity (i.e., Shannon Index, Magurran, 1988) were calculated per quadrat. Basal area of tall shrubs and saplings (i.e., >1.3 m ht and <5 cm dbh, e.g., *A. crispa*) was determined in 4-m radius circular subplots centred at each of the sampling points. The thickness of the forest floor (excluding the recent litter fall, or L layer, but including both Fibric and Humic layers – i.e., F/H, mm) was measured in each of the four corners of the nine understory vegetation quadrats within each plot.

Plant Root Simulator (PRS) probe ion exchange membranes (Western Ag Innovations, Inc., Saskatoon, SK, Canada) were used to measure soil nutrient availability. The anion exchange PRS™-probes simultaneously adsorbed all nutrient anions, including NO_3^-, PO_4^{3-} and SO_4^{2-}. Cation exchange PRS™-probes simultaneously adsorbed nutrient cations such as B^+, NH_4^+, K^+, Ca^{2+} and Mg^{2+}. A chelating pre-treatment of the anion PRS™-probe also permitted the adsorption of micronutrient metals such as Cu^{2+}, Fe^{2+}, Mn^{2+} and Zn^{2+}. One pair (1 cation and 1 anion) of probes was installed vertically at each of the four corners of the understory quadrats. The top of the ion exchange membrane was placed at the interface between the forest floor and mineral soil. Probes were installed for the duration of the growing season (mid-June–mid-September 2008), and this was not sufficient time for saturation of probe membranes to occur. After probes were removed at the end of the growing season, they were cleaned with deionized water and shipped to Western Ag for analysis; the four probe pairs from individual quadrats were pooled prior to elution and analysis. We did not analyze the data for several elements because their calculated nutrient supply rates were predominately below the minimum detection limits (Cd, Cu, NO_3^- and Pb).

Decomposition rate was measured using nylon mesh bags (1.5 mm mesh size) with four 90-mm diameter Whatman cellulose filter papers buried at the forest floor-mineral soil interface at each sample point for the growing season. Filter papers were oven dried for 1 day (pre-burial) and 3 days (post-burial) at 70 °C and weighed before and after burial. Decomposition rate was calculated as % weight loss.

Forest floor samples were collected to measure pH, microbial multiple carbon source substrate-induced respiration (MSIR) and phospholipid fatty acid analysis (PLFA) of the below-ground microbial community, as described below. We collected forest floor samples (i.e., the entire thickness of the combined F and H layers) from each of the four corners of the nine understory quadrats per plot using aseptic techniques and combined them to form a single homogeneous sample (~50 g) per quadrat. These were then divided into portions to be used for pH, MSIR, and PLFA. Samples for MSIR and pH were sieved (4 mm) and kept refrigerated (4 °C) in plastic bags prior to analysis. PLFA samples were stored at −86 °C and then freeze-dried prior to PLFA extraction.

Forest floor pH was measured potentiometrically in a saturated paste in equilibrium with a soil suspension of a 1:4 soil:liquid mixture. We used 0.01 M $CaCl_2$ in place of water following the instructions for measuring pH of field-moist organic samples as described in Kalra and Maynard (1991).

MicroResp™ was used to measure the community-level physiological profile (MSIR) for each forest floor sample (Campbell et al., 2003; Chapman et al., 2007). We prepared detection agar plates containing a gel-based bicarbonate buffer with indicator dye that responded to the pH change within the gel resulting from carbon dioxide evolved from the soil (Cameron, 2008); plates were stored in a closed desiccation chamber in the dark in between analysis. Each MSIR respiration substrate was prepared as 30 mg of substrate per gram of water (Cameron, 2008); a separate set of substrates was prepared for each of the three study units because of observed differences in soil moisture content among them (see McIntosh, 2012 for details). Fifteen substrates commonly used in carbon MSIR analysis and thought to be associated with plant root exudates (e.g., Garland and Mills, 1991; Stevenson et al., 2004) were used: five amino acids (L-alanine, L-arginine, glutamine, L-lysine, γ aminobutyric acid), six carbohydrates (n-acetyl glucosamine, L-(+)-arabinose, D-(+)-galactose, glucose, mannose, trehalose), four carboxylic acids (citric acid, L-malic acid, oxalic acid, 3,4-dihydroxybenzoic acid) and water as a control to measure basal respiration.

Field-moist sieved forest floor samples were incubated in a dark chamber at 25 °C for ~24 h prior to analysis and then added to the 96-well microtiter deep well plates after 30 μl of each substrate was dispensed (three replicate substrate wells per sample, two forest floor samples per deep well plate). The deep well plate was then hermetically sealed with a gasket, face-to-face, with the detection plate and incubated in the dark at 25 °C for six hours. The colour change in the detection plate was then read on a standard laboratory microplate reader (detection plate read before and after 6 h of incubation, absorbance = 570 nm) and respiration rates were calculated (μg CO_2–C g^{-1} h^{-1}). A maximum of 16 samples could be analyzed in a day, so samples were randomly selected each day to reduce bias associated with differences in time since collection and all analyses were completed within two weeks of when the samples were collected. One sample had five carbon substrate respiration rates below basal respiration and was excluded from analysis. To standardize for differences in the total forest floor respiration among samples, respiration was expressed as the respiration response for an individual substrate (p_i) as a proportion of total respiration rates from all 15 substrates for a given forest floor sample (Degens et al., 2000). Catabolic evenness was calculated using the Simpson-Yule index ($1/\sum p_i^2$, Magurran, 1988).

Microbial phospholipid fatty acid (PLFA) analysis produces a lipid profile of microbial communities. We transferred 0.30 g of each freeze dried forest floor sample to a muffled test tube and then analyzed each of them for PLFAs by extraction with a single-phase chloroform mixture, lipid fractionation on a solid-phase-extraction Si column and then subjected them to a mild methanolysis using a modified Bligh and Dyer extraction (Bligh and Dyer, 1959; Frostegård et al., 1991; White and Ringelberg, 1998). The resulting fatty acid methyl esters were then analyzed (see McIntosh, 2012 for details). The MIDI peak identification software (MIDI, Inc., Newark, DE) was used to identify individual fatty acids. Fatty acids were designated X:YωZ following standard labelling conventions. PLFAs present in 5% or less of the samples were

excluded from analysis. PLFAs for 16:1ω9c and 16:1ω11c were combined and 18:2ω6,9c and a18:0 were combined for analysis as they could not be distinguished. We excluded two samples with <85% peak matching from analysis. There were a total of 53 PLFAs included in the final analysis and these were also summed to provide a measure of total PLFA biomass (nmol g^{-1} forest floor). PLFAs that have been previously identified as associated with soil microorganisms were combined into PLFA biomarker groups as follows: fungal PLFAs (18:1ω9c, 20:1ω9c and 18:3ω6c; Myers et al., 2001; Hamman et al., 2007); arbuscular mycorrhizae (16:1ω5c; Frostegård and Bååth, 1996; Olsson, 1999); bacterial PLFAs (10:0 3OH, 12:0, 12:0 2OH, 12:0 3OH, 14:0, i14:0, 15:0, a15:0, i15:0, i16:0, i17:0, a17:0, 17:0, 17:0cyclo, 18:1ω5c, 18:1ω7c; Bååth et al., 1992; Frostegård and Bååth, 1996; Myers et al., 2001; Hassett and Zak, 2005) and actinomycetes (10-methyl branched fatty acids; 10me16:0, 10me17:0 and 10me18:0; Kroppenstedt, 1985; Brennan, 1988). We also calculated the ratio of fungal to bacterial PLFAs. Aside from the measurement of total biomass of PLFAs and the biomass for biomarker groups, all measured PLFAs were expressed on a mol% basis to standardize for differences in the total amount of forest floor PLFAs among samples.

The microbial metabolic quotient for each forest floor sample was calculated as the soil basal respiration (i.e., SIR with water as the substrate) divided by the microbial biomass (i.e., the total PLFA biomass) (qCO_2 – Anderson and Domsch, 1978).

2.3. Statistical analyses

Multivariate outlier analysis of the PLFA and MSIR datasets were conducted in PC-ORD and subplots with >2.5 standard deviations mean distance were excluded from all statistical analyses; results below are for 100 PLFA subplots and 99 MSIR subplots.

Hierarchical, agglomerative clustering was used to determine if the i) 53 PLFAs and ii) 15 MSIR carbon substrates grouped into discrete structural (PLFA) and functional (MSIR) microbial community types. We used a flexible beta linkage method with $\beta = -0.25$ and Sørenson's distance measure and indicator species analysis (ISA; Dufrêne and Legendre, 1997) was used to prune the dendrogram, with individual PLFAs and carbon substrates treated as the 'species'. The final cut-off for number of community types was based on the lowest average *P*-value of PLFA or carbon substrate 'indicator species' (McCune and Grace, 2002), that also had a minimum mean indicator value of 25. Once the final community types had been identified, ISA was used to identify which PLFAs or carbon substrates were contributing to the separation of the microbial community types, using Monte Carlo permutations ($n = 5000$).

Unconstrained ordination (nonmetric multidimensional scaling; NMS) was used to examine the multivariate patterns in the i) structural (PLFA – mol%) and ii) functional (MSIR – proportional respiration) below-ground microbial communities (McCune and Grace, 2002). We used the Sørenson distance measure and completed 100 runs with real data and 100 Monte Carlo randomized runs, starting with a five-dimensional solution and stepping down to a one-dimensional solution. The number of dimensions of the final solution was based on evaluation of the scree plot and the reduction in stress with step-down in dimensionality of the preliminary runs (McCune and Grace, 2002). Stability of the solution (stability criterion = 0.00005) was assessed by plotting stress versus iteration. After checking the optimal number of dimensions and best solution from the preliminary runs, we ran a final NMS with the number of dimensions determined from the preliminary runs, using the starting configuration that worked best in our preliminary runs. We then calculated the Pearson correlation coefficients of the PLFAs, the MSIRs and for variables in our secondary ('environmental') matrix with the axes of their respective ordinations. Correlations of the proportional respiration of carbon substrates with axes of the PLFA ordination and mol percent for each PLFA with axes of the MSIR ordination were determined to evaluate potential associations between the structure and function of the microbial community along with the other environmental factors that we measured. Cluster analysis, ISA and NMS were conducted using PC-ORD (Version 5 MjM Software Design, Gleneden Beach, OR).

Constrained ordination (distance-based redundancy analysis; db-RDA) was used to evaluate the relationships between i) structural (PLFA mol percent) and ii) functional (MSIR proportional respiration) microbial community composition and the above- and below-ground variables we measured (see Legendre and Anderson, 1999; Lepš and Šmilauer, 2003; ter Braak and Šmilauer, 2009). We used the Bray–Curtis distance measure and excluded negative eigenvalues in our PCoA. Environmental variables (same variables as for the NMS ordinations but excluding the microbial variables) were tested for inclusion in the db-RDA with forward step-wise selection based on 499 Monte Carlo permutations, blocked by block ($\alpha = 0.05$). A final db-RDA including only the significant variables was run testing the significance of the first and all canonical axes. Canoco for Windows Version 4.56 (ter Braak and Šmilauer, 2009) was used to run the db-RDAs.

For the resource and environmental variables that were significant in the db-RDA mean values were compared among the: i) structural; and ii) functional microbial community types using ANOVAs with 'community type' as a fixed effect and 'study unit' and 'plot within study unit' as random effects. The residuals were assessed for the assumptions of normality and homogeneity of variance; response variables were transformed when necessary. Significant main effects were explored further using post-hoc linear contrasts to compare among the microbial community types using Bonferroni-adjusted *P*-values (family-wise $\alpha = 0.05$). These analyses were run using SAS (Proc Mixed, SAS Institute Inc., Version 9.2 (32-bit), Cary, NC, USA: SAS Institute Inc., 2008).

3. Results

Within the lodgepole pine stands there were four PLFA microbial community types identified by cluster analysis of data from the 100 subplots (Fig. 1A); each of these had several significant PLFA 'indicator species' (Table 2A). There was good separation among these four PLFA community types in the NMS ordination (3-dimensional solution final stress = 11.7 after 62 iterations) with 90.6% of the variation explained (Fig. 2A and B). Several PLFAs were strongly ($R^2 > 0.5$) correlated with the ordination axes, but the only 'environmental' variables strongly ($R^2 > 0.25$) correlated with the NMS axes were the PLFA biomarker groups (non-mycorrhizal fungi, mycorrhizal fungi, and bacteria functional groups, and the ratio of fungi to bacteria) (Fig. 2A and B). PLFA community type 1 loaded towards the upper portions of all three axes and was positively associated with the mol% of 18:2ω6,9c/a18:0, fungi:bacteria ratio, and the biomass of non-mycorrhizal fungi (Fig. 2A and B). The second community type loaded towards the lower ends of axes 1 and 2 and middle of axis 3, was positively associated with PLFAs 17:0, 18:0, 18:1ω5c, 20:2ω6,9c, and 20:4ω6,9,12,15c, and was negatively associated with non-mycorrhizal fungi biomass (Fig. 2A and B). Community type 3 loaded towards the lower ends of axes 1 and 3 and the upper end of axis 2 and was positively associated with PLFAs 16:1ω11c, 16:1ω5c, i17:0, and 18:1ω7c, and with mycorrhizal fungi biomass (Fig. 2A and B). The fourth community type loaded towards the lower end of axis 1, upper end of axis 2, and middle of axis 3 without strong associations with individual

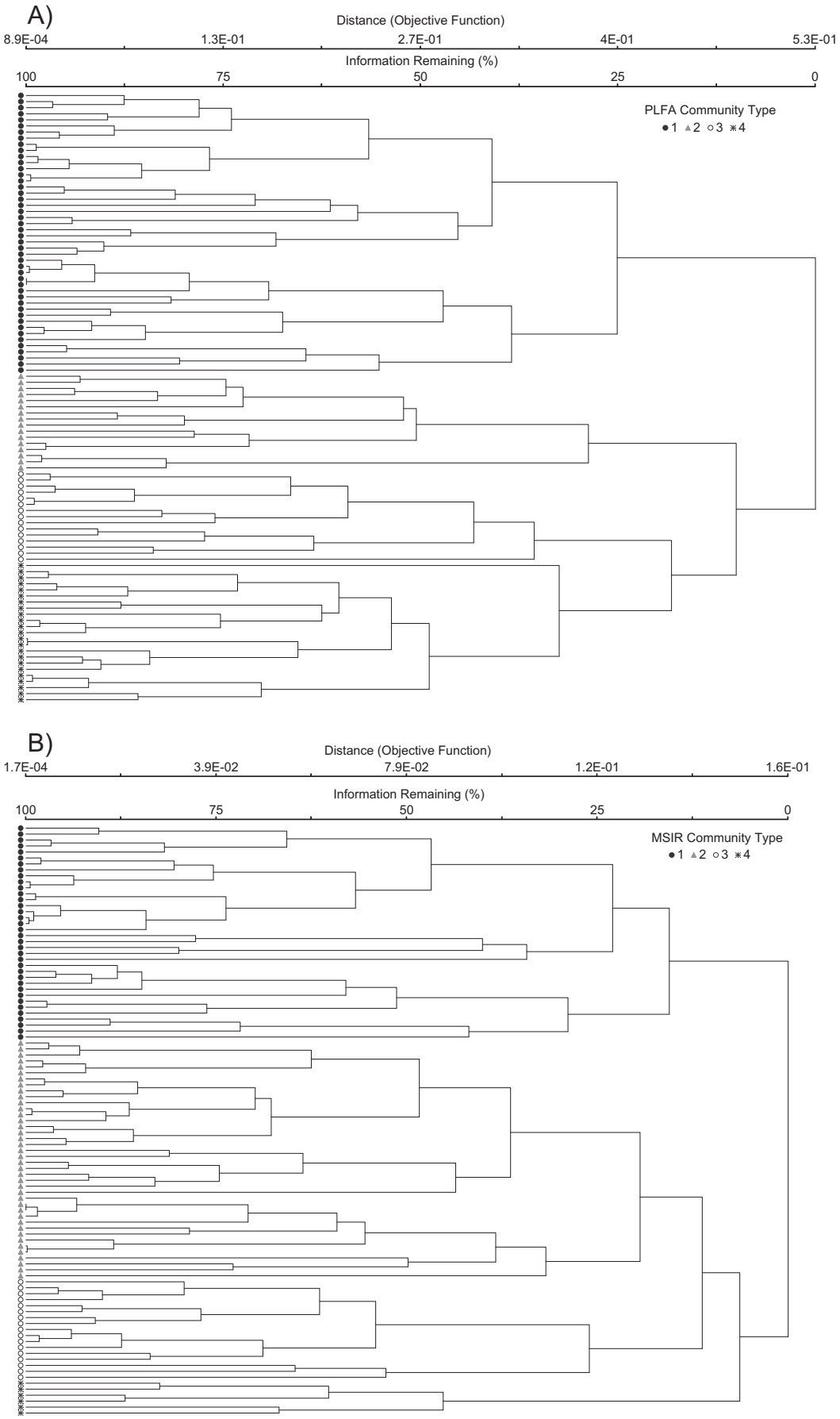

Fig. 1. Dendrogram of hierarchical agglomerative cluster analysis of the microbial A) PLFAs and B) MSIRs showing the four community types for each. Cluster analysis used a flexible beta linkage method with $\beta = -0.25$ and Sørenson's distance measure. Chaining = 1.6% for PLFA and 2.4% for MSIR microbial communities.

Table 2
Results of indicator species analysis examining A) PLFA and B) MSIR microbial community types. Given are the A) PLFAs and B) MSIRs that had an indicator value >25 and that were significant at $\alpha = 0.05$ (Dufrêne and Legendre, 1997), listed in order by descending indicator value within the community type for which they were an indicator. *N* is the sample size for each community type. Mean A) PLFA mol% or B) MSIR proportional respiration (±SE) for each of the indicators for the microbial community types are also provided. The mean A) PLFA or B) carbon substrate values for the microbial community they were an indicator for are highlighted in bold.

Community type	*N*	Variable	Indicator value	*P*	Mean (±S.E.) for community type			
					Type 1	Type 2	Type 3	Type 4
A) PLFA community type								
1	46	17:1ω8c	36.3	0.001	**1.08 (0.08)**	0.74 (0.17)	0.60 (0.15)	0.03 (0.03)
		18:2ω6,9c/a18:0	33	0.0002	**13.93 (0.34)**	10.12 (0.44)	8.42 (0.46)	9.75 (0.35)
		20:1ω9c	32.9	0.007	**0.83 (0.05)**	0	0.62 (0.10)	0.86 (0.08)
		15:0	27.7	0.0002	**1.03**	0.90 (0.02)	0.82 (0.02)	0.97 (0.02)
		16:0	27.2	0.0002	**15.56 (0.17)**	13.30 (0.22)	13.81 (0.25)	14.63 (0.20)
		18:1ω9c	26.6	0.0008	**13.68 (0.16)**	12.85 (0.31)	11.88 (0.31)	12.98 (0.14)
2	16	20:2ω6,9c	94.2	0.0002	0.06 (0.03)	**3.06 (0.33)**	0.13 (0.10)	0
		20:4ω6,9,12,15c	41.8	0.0002	1.57 (0.07)	**3.19 (0.33)**	1.52 (0.08)	1.36 (0.07)
		17:0	39.3	0.0002	0.74 (0.02)	**1.31 (0.11)**	0.63 (0.03)	0.65 (0.04)
		18:0	31.9	0.0002	2.71 (0.04)	**3.77 (0.18)**	2.62 (0.06)	2.73 (0.09)
3	15	20:1ω7c	52.1	0.0002	0.02 (0.01)	0 (−)	**0.15 (0.02)**	0.06 (0.02)
		16:1,2OH	37.6	0.001	0.16 (0.02)	0.17 (0.05)	**0.39 (0.05)**	0.25 (0.03)
		a17:1ω9c	37.4	0.0006	0.12 (0.03)	0.07 (0.05)	**0.50 (0.11)**	0.11 (0.04)
		i15:1	35.5	0.0002	0.38 (0.02)	0.39 (0.03)	**0.73 (0.08)**	0.42 (0.04)
		i17:0	31.7	0.0002	0.63 (0.01)	0.69 (0.03)	**0.94 (0.02)**	0.69 (0.01)
		18:1ω5c	30.8	0.001	0.89 (0.06)	1.33 (0.06)	**1.55 (0.06)**	1.26 (0.08)
		16:1ω11c	30.7	0.0002	0.78 (0.03)	0.92 (0.04)	**1.15 (0.04)**	0.90 (0.05)
		i14:0	30.5	0.0004	0.31 (0.01)	0.33 (0.01)	**0.42 (0.03)**	0.32 (0.02)
		16:1ω5c	30.5	0.0002	0.89 (0.06)	1.33 (0.12)	**1.55 (0.08)**	1.26 (0.09)
		10me18:0	30.2	0.01	0.61 (0.03)	0.55 (0.05)	**0.79 (0.08)**	0.66 (0.05)
		11me18:1ω7c	29.8	0.0002	0.70 (0.02)	0.76 (0.04)	**0.94 (0.04)**	0.75 (0.03)
		18:1ω7c	29.6	0.0002	8.18 (0.21)	10.71 (0.24)	**11.74 (0.19)**	8.97 (0.23)
		a15:0	28.5	0.0002	2.25 (0.05)	2.23 (0.07)	**2.74 (0.06)**	2.37 (0.07)
		i16:1	28.3	0.003	0.74 (0.02)	0.70 (0.03)	**0.88 (0.03)**	0.78 (0.04)
		10me16:0	28.2	0.002	3.51 (0.09)	3.16 (0.13)	**4.18 (0.15)**	3.97 (0.14)
		17:0c	28.1	0.0002	1.89 (0.04)	1.83 (0.07)	**2.21 (0.07)**	1.92 (0.05)
		19:0c	27.5	0.02	6.01 (0.17)	5.67 (0.21)	**6.78 (0.45)**	6.24 (0.19)
		i15:0	27.1	0.003	5.35 (0.12)	4.95 (0.16)	**5.75 (0.18)**	5.12 (0.10)
		a17:0	27	0.003	1.00 (0.02)	0.95 (0.01)	**1.10 (0.04)**	1.02 (0.02)
		16:0,2OH	25.9	0.001	0.01 (0.01)	0 (−)	**0.05 (0.02)**	0.01 (0.01)
4	23	17:1ω7c	49.6	0.0002	0.44 (0.11)	0.71 (0.21)	0.82 (0.22)	**1.93 (0.11)**
		15:1ω8c	38.6	0.0002	1.41 (0.09)	1.34 (0.11)	1.33 (0.17)	**2.56 (0.25)**
		20:0	33.2	0.0002	2.52 (0.08)	2.45 (0.16)	2.44 (0.11)	**3.69 (0.18)**
		14:0	27.7	0.01	1.51 (0.05)	1.47 (0.05)	1.51 (0.05)	**1.72 (0.05)**
B) MSIR community type								
1	36	Oxalic acid	27.8	0.01	**0.086 (0.002)**	0.074 (0.001)	0.083 (0.002)	0.067 (0.003)
		Glutamic acid	26.1	0.04	**0.076 (0.001)**	0.072 (0.001)	0.071 (0.002)	0.071 (0.001)
2	40	L-alanine	27.0	0.02	0.056 (0.011)	**0.066 (0.001)**	0.062 (0.001)	0.059 (0.004)
3	17	Citric acid	28.6	0.001	0.065 (0.002)	0.067 (0.001)	**0.078 (0.002)**	0.062 (0.003)
4	6	Malic acid	30.2	0.0002	0.088 (0.002)	0.088 (0.002)	0.099 (0.002)	**0.119 (0.003)**
		Glucose	26.8	0.008	0.078 (0.001)	0.076 (0.001)	0.074 (0.001)	**0.084 (0.003)**

PLFAs, but negative associations with total bacteria and non-mycorrhizal fungi biomass (Fig. 2A and B).

The constrained ordination further illustrated separation among the four PLFA community types and showed which environmental variables were associated with these (Fig. 3). The first four db-RDA axes, which were all significant, accounted for 88.3% of the PLFA-environment relationship (Table 3A). The four PLFA community types separated along the first two db-RDA axes (Fig. 3). The relative locations of subplots of the different PLFA community types were consistent with the indicator species PLFA analysis, although not all indicator PLFAs were highly correlated ($r > 0.4$) with the ordination axes (Fig. 3A). The PLFA locations in ordination space (on the db-RDA bi-plot) reflected their correlation with the environmental gradients (Fig. 3). One overstory variable (canopy cover), seven understory plant species, and one below-ground variable (available Al) were significantly correlated with the PLFA community composition and collectively explained 22.2% of the variation in the PLFA data (Table 3A, Fig. 3B). Most strongly correlated with axis 1 were the understory cover of *C. montanensis* (negative), *Chamerion angustifolium* (positive), *Lycopodium annotinum* (positive), *P. schreberi* (negative), and *P. commune* (positive) and availability of Al (negative); understory cover of *R. acicularis* was most highly correlated with axis 2 (negative); canopy cover (positive) and understory cover of *C. canadensis* (negative) had the strongest correlation with axis 3 (Table 3A).

Differences in mean values for the environmental variables among the four PLFA community types were consistent with the relationships found in the db-RDA (Table 4A). Community type 1 had the highest canopy cover, which was significantly higher than type 2 and it also had significantly lower cover of *L. annotinum* compared with types 2 and 3. Understory cover of *C. canadensis* was significantly higher in type 2 than in types 1 and 4. For community type 3, understory cover of *C. angustifolium* was significantly higher than for type 1, cover of *P. schreberi* was significantly lower compared with types 1 and 4, and cover of *P. commune* was significantly higher than for types 1 and 2. There were no significant differences in available soil Al, and cover of *C. montanensis* or *R. acicularis* among any of the four PLFA community types (Table 4A).

There were four MSIR microbial community types identified by cluster analysis of data from the 99 subplots (Fig. 1B) and each of these had one or more significant carbon substrate 'indicator

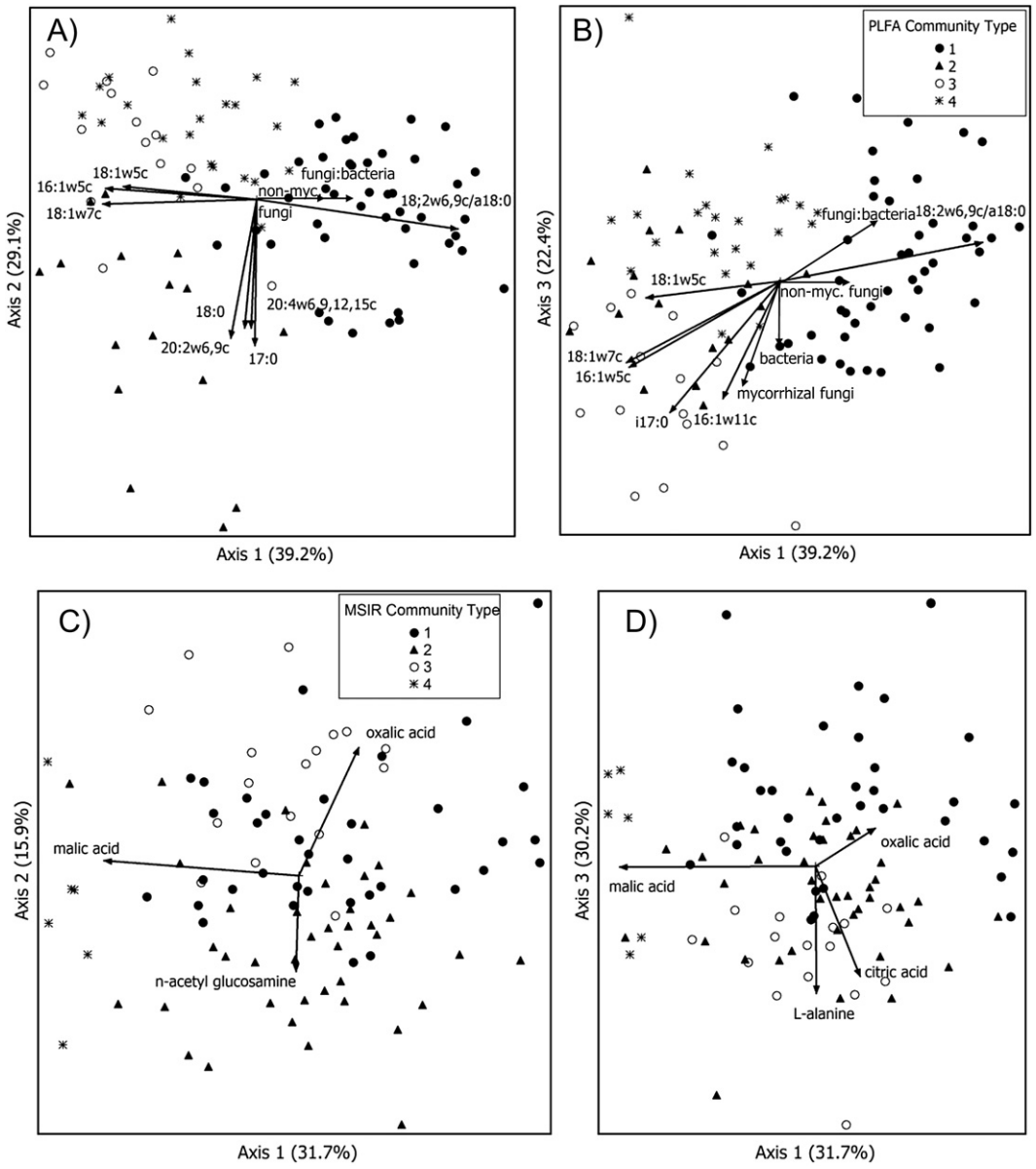

Fig. 2. Results of nonmetric multidimensional scaling (NMS) ordination of A & B) PLFA and C & D) MSIR community composition. The final ordinations were 3-D solutions, so two plots are presented for each ordination. Each symbol in the plots is a subplot, which is coded by PLFA (panels A and B) or MSIR community type (panels C and D) (see Fig. 1 and Table 2). The amount of variation explained by each axis is included in parentheses. The angles and lengths of the vectors for the individual PLFAs, carbon substrates, and environmental variables (including PLFA biomarker groups) overlain on the ordination vectors indicate direction and strength of associations of them with the ordination axes. The cut-offs for display were: for PLFAs, $R^2 > 0.5$ (to improve readability because so many individual PLFAs were highly correlated and obscured interpretation); for carbon substrates and environmental variables, $R^2 > 0.25$. No environmental variables had an $R^2 > 0.25$ with the axes of the carbon substrates ordination.

species' (Table 2B). The unconstrained ordination illustrated the separation among the four MSIR community types; the NMS 3-dimensional solution (final stress = 18.1 after 29 iterations) explained 77.9% of the variation in the dataset (Fig. 2C and D). Several of the MSIR carbon substrates had strong correlations (with $R^2 > 0.25$) with the NMS ordination axes; however, none of the 'environmental' variables were strongly correlated with the ordination axes (Fig. 2C and D). MSIR community type 1 loaded towards the middle of axis 2 and upper end of axis 3 and was positively associated with oxalic acid (Fig. 2C and D). The second community type loaded towards the lower ends of axes 2 and 3 and was positively associated with n-acetyl glucosamine, citric acid, and L-alanine (Fig. 2C and D). Community type 3 loaded towards the lower ends of axis 1 and 2 and upper end of axis 2 and was negatively associated with oxalic acid (Fig. 2C and D). The fourth community type loaded towards the very lower end of axis 1, lower end of axis 2, and middle of axis 3 and was positively associated with malic acid respiration (Fig. 2C and D).

The constrained ordination suggested some separation among the four MSIR community types, although the separation was less distinct than with the NMS (Fig. 4). The first three db-RDA axes, which were all significant, accounted for 100% of the MSIR-environment relations (Table 3B). The four MSIR community types only partially separated along the first two db-RDA axes: community type 1 was centred in the middle of axis 1 and towards the middle and lower half of axis 2, community type 2 was spread across axes 1 and 2 and overlapped with the other types, types 3 and 4 were loaded towards the upper end of axis 1 with some

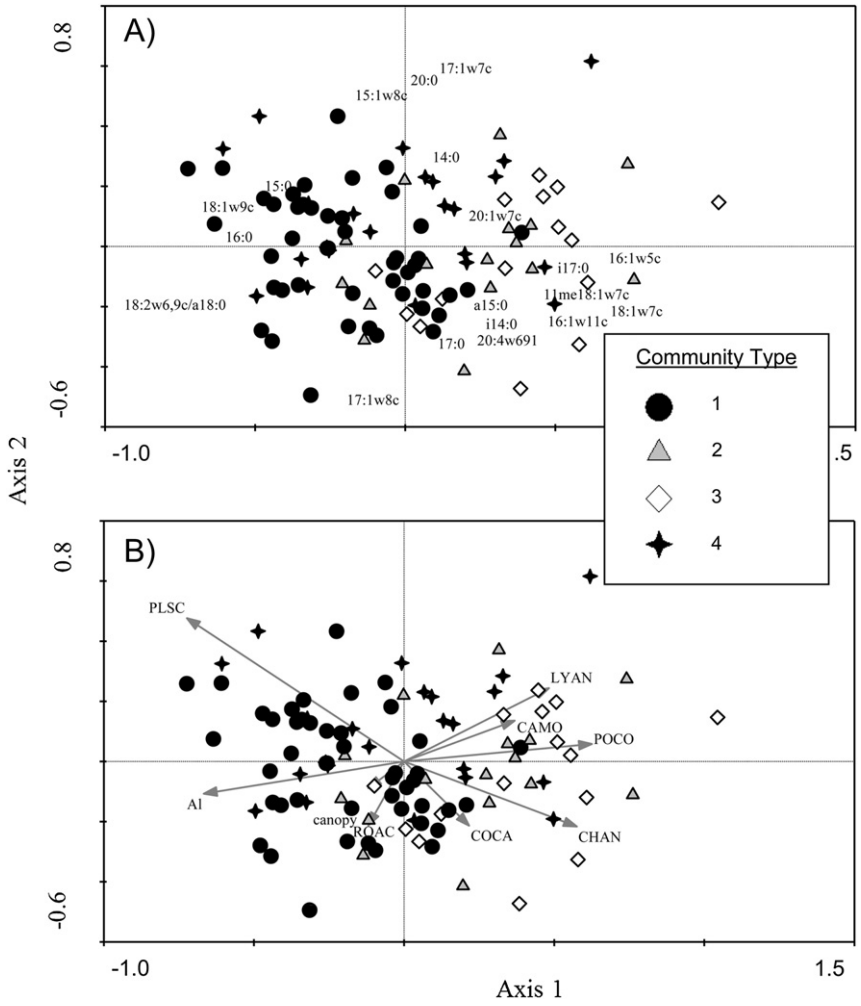

Fig. 3. Results of distance-based redundancy analysis of PLFA microbial community composition delineated by the four PLFA community types identified by hierarchical cluster analysis: A) PLFA names indicate the locations of PLFAs which had a Pearson correlation coefficient >0.4 with either of the first two axes, and B) the direction and length of the vector for environmental variables (description in lowercase letters, see Table 4 for details) reflects the direction and strength of correlation for variables that had a Pearson correlation coefficient >0.4 with either of the first two axes. Each symbol is a subplot, which is coded by PLFA community type (see Fig. 2A). The environmental and species scores were scaled up 1.8 and 3.7 times, respectively, to those of sample scores to improve readability.

overlap with types 1 and 2 (Fig. 4). The MSIR locations in ordination space relative to the subplots of the different MSIR community types (on the db-RDA bi-plot) were mostly consistent with the MSIR indicator species analysis (Fig. 4). However, glutamic acid and glucose were not highly correlated ($r > 0.4$) with the ordination axes, and carbohydrate trehalose, which was not a significant indicator, was (Fig. 4B). Several environmental variables (understory cover of *Vaccinium vitis-idaea*, litter cover, and biomass of DWM size class 4) were significantly correlated with the three axes but collectively these explained only 6.7% of the variation in the MSIR data (Table 3B, Fig. 4B). The understory cover of *V. vitis-idaea* (positive) and litter cover (negative) were most strongly correlated with axis 1, whereas DWM of Class 4 was most strongly correlated (positive) with axis 3; none of the variables were strongly correlated with axis 2 (Table 3B).

The only differences in mean values for the significant environmental variables among the four MSIR community types were for cover of litter and *V. vitis-idaea* (Table 4B). Community type 2 had significantly higher litter cover compared with type 3, with intermediate levels in types 1 and 4. The cover of *V. vitis-idaea* was significantly higher in MSIR community type 4 compared with types 1 and 2, with intermediate levels in type 3.

4. Discussion

Our results show that, even in forest stands with a single species homogeneous overstory, soil microbial communities are organized into distinct, fine-scale community types. We identified four fine-scale structural (PLFA) and four fine-scale functional (MSIR) microbial community types. As we hypothesized, the majority of the environmental variables associated with these below-ground microbial communities were understory plant species. There are two potential explanations for the observed linkages between the microbial community and the understory plant community: i) both communities are responding to some shared environmental factor; or ii) spatial partitioning of plant species and their associated litter and rhizosphere resources (e.g., root exudates, nutrients) creates heterogeneity, which in turn influences the patterns of variation in below-ground microbial communities. We consider these further below.

In these pine forests, the only important overstory variable associated with below-ground community composition was percent canopy cover. Although our sample points included only a moderate range of variation in canopy cover, the PFLA microbial community types were associated with variation in canopy cover.

Table 3
Results of distance-based redundancy analyses (dbRDAs) for the A) PLFA and B) MSIR microbial community types. The trace value (sum of all the canonical eigenvalues) and the eigenvalues of the first four axes are presented, along with the species–environment correlations, and the cumulative percentage of the variance explained for PLFAs/MSIRs and PLFA/MSIR-environment. Inter-set correlations (Pearson) of significant above- and below-ground variables from the db-RDA step-wise forward selections are listed (see Table 4 for description of variables), ordered by their correlations (from high to low) with the first axis. The inter-set correlation values for the axis where the correlation was strongest are highlighted in bold.

	Axis 1	Axis 2	Axis 3	Axis 4
A) PLFA community				
Trace: 0.234				
Eigenvalues[a]	0.125	0.042	0.028	0.011
Species–environment correlations	0.730	0.569	0.542	0.544
Cumulative percentage variance				
PLFA variance explained (%)	13.4	18.0	21.0	22.2
PLFA-environment correlation variance (%)	53.4	71.6	83.6	88.3
Inter-set correlations				
PLSC	−**0.42**	0.37	0.02	0.21
Al	−**0.37**	−0.08	0.08	0.29
CHAN	**0.37**	−0.19	0.15	0.22
POCO	**0.36**	0.04	0.23	0.03
LYAN	**0.28**	0.19	−0.09	−0.11
CAMO	**0.21**	0.10	0.00	0.14
COCA	0.13	−0.17	−**0.30**	0.18
Canopy cover	−0.07	−0.07	**0.37**	−0.10
ROAC	−0.07	−**0.16**	0.11	−0.04
B) MSIR community				
Trace: 0.065				n/a
Eigenvalues[a]	0.042	0.015	0.008	n/a
Species–environment correlations	0.602	0.464	0.398	n/a
Cumulative percentage variance				
MSIR variance explained (%)	4.3	5.9	6.7	n/a
MSIR-environment correlation variance (%)	64.6	87.9	100.0	n/a
Inter-set correlations				
Litter cover	−**0.39**	0.35	0.05	n/a
VAVI	**0.39**	0.16	0.27	
DWM – Class 4	−0.26	−0.24	**0.30**	

[a] Axis 1 and all combined axes were significant at $P = 0.02$.

These findings suggest that the cover of trees is an important regulator of the structure of below-ground microbial communities, even in a forest with only one overstory species. Interestingly, canopy cover was not a significant indicator of the understory plant community types identified by McIntosh (2012) (although dbh was). Thus, any influences of the overstory cover on soil microbial communities are not a result of indirect influences on understory plant community composition, and instead appear more likely to be related to the influence of canopy cover on variation in microclimatic conditions such as soil moisture and soil temperature (microclimatic factors for which we do not have data at the scale of this analysis).

Seven understory plant species were associated with structural (PLFA) microbial community composition within the stands (*C. montanensis*, *C. angustifolium*, *C. canadensis*, *L. annotinum*, *P. schreberi*, *P. commune*, and *R. acicularis*). Interestingly, all of these understory species were indicators of understory plant community types within these forests too (McIntosh, 2012). The importance of these species in development of the microbial community types was further supported by the fact that they all (except *C. montanensis* and *R. acicularis*) showed significant differences in cover among structural microbial community types. However, it is unclear whether this is due to both the plant and microbial communities responding to some shared environmental factor(s), or whether it results from variation in the litter and rhizosphere resources (e.g., root exudates, nutrients) among plant community types. *C. angustifolium*, *C. montanensis*, *C. canadensis,* and *L. annotinum* are all generalists in terms of soil moisture and nutrient levels (Haeussler et al., 1990; Matthews, 1993; Gucker, 2012). However, the moss species *P. schreberi* may indicate more acidic conditions (Anderson et al., 1995). Further, we observed large pockets of *P. commune* in very wet depressions in these forests, thus it could indicate higher soil moisture conditions. The auto-ecological properties of *P. schreberi* and *P. commune* suggest they may be associated with structural microbial community types that are affected by soil pH and moisture. Overall, our results suggest that variation in below-ground microbial communities may be largely driven by the existence of fine-scale variability in understory plant composition within these homogeneous forests and the associated heterogeneity in litter and/or root exudates.

The overlap among the four fine-scale structural microbial community types we identified suggests that differences among the community types were in relative abundance (mol%) of PLFAs, rather than any individual PLFAs being unique to a particular community. This suggests that microbes, as indicated by their PLFA profile, have fairly broad environmental tolerances but that the fine-scale heterogeneity was sufficient to result in detectable changes in their relative abundance, and thus development of distinct structural microbial community types.

Table 4
The mean values (±SE) for each of the four A) PLFA and B) MSIR community types of the above- and below-ground variables that were significant in the distance-based redundancy analyses (db-RDAs) described inTable 3. Different lower case letters (a, b) after mean values indicate significant differences for individual variables among A) PLFA or B) MSIR community types.

>Variable	Code	Units	Mean (±S.E.)			
			Type 1	Type 2	Type 3	Type 4
A) PLFA community						
Pleurozium schreberi[b]	PLSC	%	29.4 (4.0)b	14.1 (4.4)ab	1.9 (0.6)a	30.8 (5.4)b
Aluminum	Al	μg −10 cm^2 − burial $length^{-1}$	74.1 (2.3)	64.8 (3.8)	64.0 (2.9)	65.4 (3.3)
Chamerion angustifolium[a]	CHAN	%	2.5 (0.8)a	8.3 (2.7)b	14.9 (3.6)b	6.5 (1.5)ab
Polytrichum commune[a]	POCO	%	2.0 (0.9)a	3.0 (1.4)a	13.1 (5.3)b	5.5 (2.1)ab
Lycopodium annotinum[a]	LYAN	%	0.3 (0.1)a	7.5 (3.2)b	9.3 (3.1)b	6.7 (3.2)ab
Calamagrostis montanensis	CAMO	%	5.2 (1.4)	6.9 (4.7)	13.0 (4.1)	5.4 (2.4)
Cornus canadensis[a]	COCA	%	10.9 (1.2)a	19.1 (4.0)b	9.8 (1.8)ab	9.3 (1.3)a
Canopy cover	Canopy	%	63.9 (0.8)b	58.5 (1.2)a	62.5 (2.3)ab	59.8 (0.6)ab
Rosa acicularis	ROAC	%	4.9 (0.8)	2.9 (0.9)	6.3 (1.1)	4.3 (1.1)
B) MSIR community						
Litter cover[b]	Litter	%	49 (3)ab	57 (3)b	41 (5)a	34 (4)ab
Vaccinium vitis-idaea	VAVI	%	2.5 (0.7)a	3.1 (1.1)a	5.2 (2.3)ab	14 (7.1)b
Downed woody material – size class 4	DWM – Class 4	Mg ha^{-1}	1.9 (0.4)	1.7 (0.4)	2.2 (0.7)	2.0 (0.7)

[a] Square root-transformed for analysis.
[b] Log-transformed for analysis.

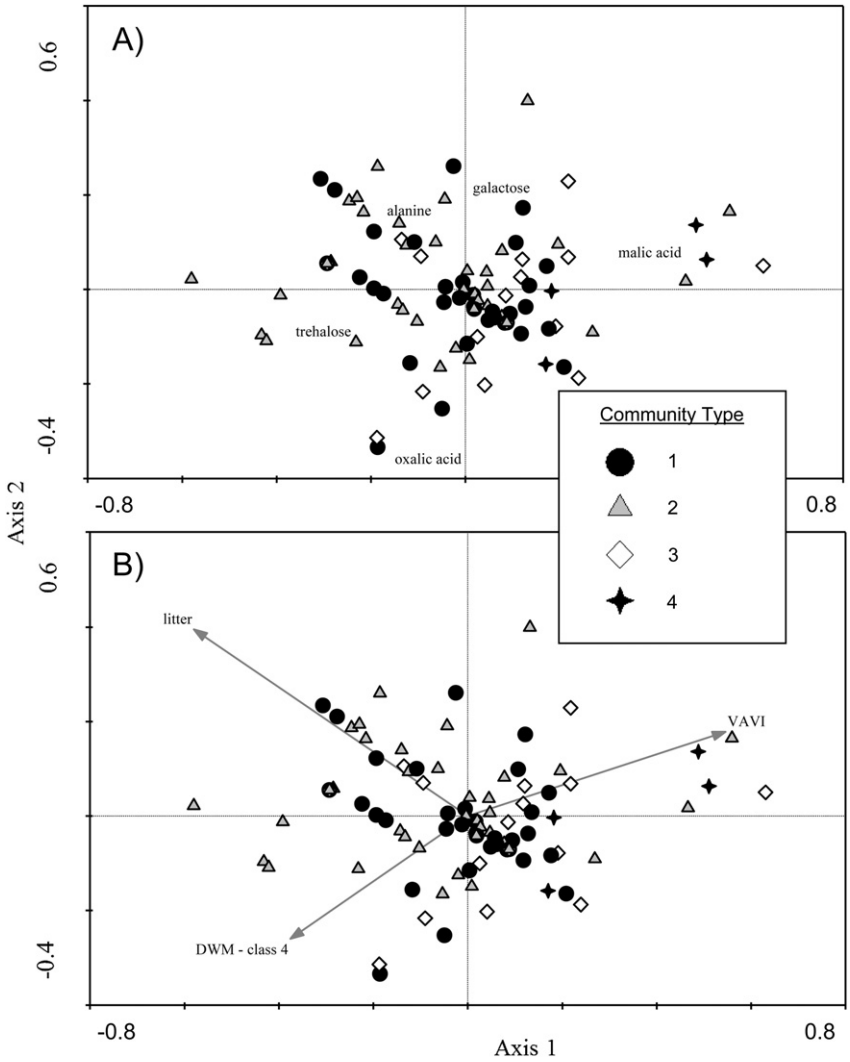

Fig. 4. Results of distance-based redundancy analysis of MSIR microbial community composition delineated by the four MSIR community types identified by hierarchical cluster analysis: A) carbon substrate names indicate the locations of carbon substrates which had a Pearson correlation coefficient >0.4 with either of the first two axes, and B) the direction and length of the vector for environmental variables (description in lowercase letters, see Table 4 for details) reflects the direction and strength of correlation for variables that had a Pearson correlation coefficient >0.4 for either of the first two axes. Each symbol is a subplot, which is coded by MSIR community type (see Fig. 2B). The environmental and species scores were scaled up 1.5 and 4.4 times, respectively, to those of sample scores to improve readability.

There are several possible explanations for the weak separation among the four functional (MSIR) microbial communities. Both diurnal and seasonal variability in temperature and moisture conditions likely influence the functioning of the below-ground microbial community. However, our MSIR samples represented only a single moisture and temperature combination (those during the incubation). MSIR is dependent on microbes being active under the conditions in which they are measured (Preston-Mafham et al., 2002). Thus, our MSIR results may reflect the influence of a subset of dominant microbes that grew well in the lab conditions, rather than the lack of *in situ* patterns in functional microbial communities. To detect patterns reflective of field conditions it may be more appropriate to conduct MSIR incubations under conditions that are more representative of the *in situ* situation (e.g., incubate at lower temperatures). Alternatively, there may be some separation of functional microbial communities that exists at a different scale than we measured; for example, at a scale of mm to cm (Kirk et al., 2004). Finally, there is a greater likelihood of redundancy in the functional composition of microbes compared with the structural composition, and thus only large difference in functional composition may be detected using this approach (Kirk et al., 2004). Other studies have, like us, found stronger discrimination among the structure than the function of microbial communities (e.g., Swallow et al., 2009) or have found differences in both the structure and function of microbial communities, but across different forest types (e.g., Brockett et al., 2012).

The environmental variables we measured explained very little of the variation (<7% of the variability in the dataset) in the functional microbial community types; only cover of litter and *V. vitis-idaea* were significantly different among community types. Litter plays an important role in biogeochemical cycling within forests, as microbial community function depends on both the quantity and quality of litter. Higher cover of litter will provide more available substrate for decomposition processes to occur; the rate of decomposition of this litter will, in turn, be influenced by its quality and the structure and function of the microbial community that decomposes it. While *V. vitis-idaea* has been shown to be an indicator species for conifer patches in boreal mixedwood forests (Chávez and Macdonald, 2010), it is a habitat generalist that is not an indicator for particular environmental factors. Thus its influence on the microbial function is likely to be related to its litter and root

exudates rather than an association with particular environmental conditions.

The low explanatory power of the measured abiotic and biotic factors for the functional microbial types suggests either that there is not much separation because of redundancy in function, or that factors that we did not measure have more influence on the functional microbial communities. One such potential variable is soil moisture, which has been shown to be linked to the functioning of microbial communities (Brockett et al., 2012). We expect that forest floor moisture will vary both spatially within the forest stand and temporally across the growing season as a function of the relative influences of snowmelt, precipitation, antecedent soil moisture conditions, and evaporative demand (Schume et al., 2003). However, quantifying temporal and spatial variation in soil moisture at this fine scale was beyond the scope of this study. A paired study by Piña (2012) quantified soil moisture at multiple depths and demonstrated the greatest variation in soil moisture conditions in the middle of the growing season when evaporative demand plays the critical role in regulating soil moisture. Therefore, an alternative option to continuous sampling throughout the growing season could be to sample the microbial community in the middle of the growing season when fine scale variation in forest floor moisture is likely to be greatest. Future studies could also measure additional soil attributes such as soil temperature, and soil organic carbon and nitrogen, in order to evaluate their influence on the functioning of microbial communities.

Overall, this study provided new insights into the relationships of below-ground structural and functional microbial communities and micro-habitat resource partitioning at the within-stand fine scale in forests with a single overstory species. Our findings demonstrate the importance of spatial heterogeneity in the understory plant community in structuring below-ground microbial PLFA communities. However, spatial heterogeneity of above- and below-ground factors appears to have less influence on functional microbial communities, at least for the variables and spatial scale that we measured in this study. While we were unable to explicitly test the mechanisms that may be driving the relationships between the above and below-ground communities, it is evident that understory and below-ground communities operate with very close linkages to one another.

Acknowledgements

This project was supported by funding from the Forest Resource Improvement Association of Alberta and Foothills Research Institute awarded to E.M. and Co Principal Investigator Uldis Silins and Natural Sciences and Engineering Research Council of Canada – Canada Graduate Scholarship and an Izaak Walton Killam Memorial Scholarship awarded to A.M.. The authors thank Pablo Piña, Peter Presant, Emily Turton, Macdonald and Quideau lab members for assistance with field and lab work. We thank the two anonymous reviewers for their valuable comments on the manuscript.

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