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## Root controls on soil microbial community structure in forest soils

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**Abstract** We assessed microbial community composition as a function of altered above- and belowground inputs to soil in forest ecosystems of Oregon, Pennsylvania, and Hungary as part of a larger Detritus Input and Removal Treatment (DIRT) experiment. DIRT plots, which include root trenching, aboveground litter exclusion, and doubling of litter inputs, have been established in forested ecosystems in the US and Europe that vary with respect to dominant tree species, soil C content, N deposition rate, and soil type. This study used phospholipid fatty-acid (PLFA) analysis to examine changes in the soil microbial community size and composition in the mineral soil (0–10 cm) as a result of the DIRT treatments. At all sites, the PLFA profiles from the plots without roots were significantly different from all other treatments. PLFA analysis showed that the rootless plots generally contained larger quantities of actinomycete biomarkers and lower amounts of fungal biomarkers. At one of the sites in an old-growth coniferous forest, seasonal changes in PLFA profiles were also examined. Seasonal differences in soil microbial community composition were greater than treatment differences. Throughout the year, treatments without roots continued to have a different microbial community composition than the treatments with roots, although the specific PLFA biomarkers responsible for these differences varied by season. These data provide direct evidence that root C inputs exert a large control on microbial community composition in the three forested ecosystems studied.

**Keywords** Decomposition · Fungi · Phospholipid fatty acids (PLFA) · Seasonal dynamics · Soil organic matter (SOM)

### Introduction

Soils are the largest terrestrial sink for organic carbon (C), storing approximately twice as much C as the atmosphere (Schimel 1995). Carbon storage in soil is mediated by microbes that use plant primary production from above- and belowground litter and soil organic matter (SOM) as their sources of C. In turn, the size and composition of the soil microbial community is controlled through complex interactions with plants (Zak et al. 2000; Bohlen et al. 2002; Butler et al. 2004), and is a function of net primary production, plant C allocation, rhizosphere activity, and litter substrate quality (Smith and Paul 1990; Fisk and Fahey 2001; Myers et al. 2001).

Litter and root exclusion in forested ecosystems has been shown to decrease heterotrophic soil CO<sub>2</sub> efflux (Bowden et al. 1993; Boone et al. 1998; Fisk and Fahey 2001; Rey et al. 2002; Lavigne et al. 2004; Li et al. 2004; Sulzman et al. 2005), although these changes do not appear to alter microbial biomass in the mineral soil (Fisk and Fahey 2001; Siira-Pietikainen et al. 2001, 2003; Nadelhoffer et al. 2004). Root exclusion has been shown to decrease fungal biomass and alter bacterial community structure in organic, but not mineral, horizons of forest soils (Siira-Pietikainen et al. 2001, 2003; Subke et al. 2004); however, changes in the microbial community composition of mineral soils of grasslands have been found with increasing depth, and were attributed to changes in the quantity of root inputs within the profile (Fierer et al. 2003). Spears et al. (2003) found that the addition of coarse woody debris in an old-growth coniferous forest did not alter microbial biomass in the mineral soil. Similarly, the addition of aboveground litter did not affect bacterial or fungal biomass in the mineral soil of a deciduous site in Massachusetts (Nadelhoffer et al. 2004), although an

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increase in fungal biomass (Nadelhoffer et al. 2004) and microbial C (Subke et al. 2004) has been found in the organic layer.

The Detritus Input and Removal Treatment (DIRT) experiment is an inter-site study examining feedbacks between plants, microbes, and SOM through long-term manipulation of both above- and belowground litter inputs to forested ecosystems (Nadelhoffer et al. 2004). The DIRT treatments include root trenching, aboveground litter exclusion, and the doubling of aboveground litter inputs. The DIRT network currently consists of five sites in forested ecosystems that vary with respect to background soil C content, N deposition, tree species, and soil type. Research at the Harvard Forest (Bowden et al. 1993), Bousson Forest (R.D. Bowden, 2004, personal communication), and H.J. Andrews (Sulzman et al. 2005) DIRT sites have shown that these long-term manipulations alter soil CO<sub>2</sub> efflux. Additionally, research at Harvard Forest (Nadelhoffer et al. 2004) and the H.J. Andrews (Lajtha et al. 2005) has also shown differences in dissolved organic C and dissolved organic nitrogen fluxes in response to the DIRT manipulations, while mineral soil C and N concentrations have remained unchanged (Keirstead 2004; Nadelhoffer et al. 2004).

It has been hypothesized that the quantity and chemical composition of litter plays an important role in determining microbial community structure in forest ecosystems (Leckie 2005). The DIRT treatments provide a unique opportunity to examine this theory in a variety of forested ecosystems with the same experimental treatments. The objective of this study was to examine soil microbial biomass and community composition using phospholipid fatty-acid (PLFA) analysis at three of the established DIRT sites. Given the reported changes in soil CO<sub>2</sub> efflux, as well as nutrient input into the various DIRT treatments, we hypothesized that the DIRT treatments would lead to changes in the composition of the microbial community. We expected the largest change to be the loss of fungal biomass from root-trenched plots, with smaller changes in bacterial communities in both the below- and aboveground input manipulations. We also expected the magnitude of these differences to increase with time since the plots have been installed. At one of the three sites, seasonal dynamics in the microbial community were also examined.

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## Materials and methods

### Site descriptions

The three sites in this study are part of the long-term inter-site DIRT project designed to assess how rates and sources of plant inputs control accumulation and dynamics of SOM and nutrients in forest soils (Nadelhoffer et al. 2004). Plant litter inputs have been manipulated at the DIRT plots in the H.J. Andrews

Experimental Forest (AND) in Oregon (44°15'N, 122°10'W, 531 m elevation) since 1997. Mean annual temperature (MAT) at H.J. Andrews headquarters is 8.7°C (1973–2002) and mean annual precipitation (MAP) over the same period is 2,370 mm, which occurs mostly as rain. In general, over 70% of the precipitation occurs during a “wet season” between November and March. Nitrogen deposition to this area is 1.6 kg N ha<sup>-1</sup> y<sup>-1</sup> (Vanderbilt et al. 2003). The DIRT site was established in an undisturbed old-growth Douglas-fir, *Pseudotsuga menziesii* (Mirbel) Franco, western hemlock, *Tsuga heterophylla* (Raf.) Sarg., stand. Other important tree species at the site include western red cedar (*Thuja plicata* Donn ex D. Don) and vine maple (*Acer circinatum* Pursh). Soils are derived from volcanic parent materials and have been classified as coarse loamy mixed mesic Typic Hapludands (Dixon 2003). Daily averages for soil temperature (Campbell Scientific model 107 temperature probe) and volumetric water content (Campbell Scientific CS615) at 10 cm were downloaded from the PRIMET meteorological station located at the H.J. Andrews Experimental Forest (AND LTER 2005).

The DIRT sites at Bousson Experimental Forest (BOU) in Pennsylvania (41°35.99'N, 80°2.53'W, 381 m) were established in 1991, where MAT is 8.3 °C and MAP is 1,050 mm. Atmospheric N deposition at BOU is high at ~13 kg N ha<sup>-1</sup> y<sup>-1</sup> (Holland et al. 2004). The site is in an 80-year-old mixed deciduous forest dominated by black cherry (*Prunus serotina* Ehrh.) and sugar maple (*Acer saccharum* Marsh). Soils are coarse loamy mixed superactive mesic Oxyaquic Fragiudalfs (Cambridge series).

The newest DIRT site included in this study was established in 2000 at Síkfökút Forest (SIK) in Eger, Hungary (47°90'N, 20°46'E, 330 m). This is the driest and warmest site, with a MAT of 10 °C and a MAP of 550 mm. This site also has high N deposition, with an annual rate of ~15 kg N ha<sup>-1</sup> y<sup>-1</sup> (Holland et al. 2004). The plots are located in a mature temperate deciduous oak forest dominated by durmast oak, *Quercus petraea* (Mattuschka) Liebl., and European turkey oak (*Quercus cerris* L.). The soils are brown forest soils with Cambisol features. The most similar US Taxonomic soil order is Alfisols.

### Experimental manipulations

There are six litter input/exclusion treatments at AND and SIK, and five treatments at BOU. At all three sites there are three replicates per treatment. Plots vary in size from site to site dependent on site characteristics. Individual plots at BOU are 3 m×3 m, SIK has 10 m×10 m plots, and AND has 10 m×15 m plots because of the high spatial heterogeneity of this site. On no litter (NL) and no input (NI) plots, litter is excluded with 1-mm-mesh screens. Aboveground litter that is swept off NL plots is added to double litter (DL) plots. Root growth is

prevented in the no roots (NR) plots by trenching to 1 m followed by insertion of impenetrable barriers. The NI treatment combines the NR and NL treatments. Wood inputs are doubled on double wood plots (DW; AND and SIK only) by the addition of chipped whole logs. Control (CO) plots with no manipulation are found at all sites.

### PLFA analysis and extraction

Seven to ten soil cores (2.5 cm diameter, 0–10 cm depth) were collected from each plot using stratified random sampling. The cores were combined to yield one composite sample per plot. Soils were passed through a 2-mm sieve and stored at 4 °C until PLFA extraction within 3 days of sampling. BOU was sampled in May 2003 and SIK was sampled in October 2003. AND was sampled in July 2003, November 2003, and April 2004. Gravimetric water content (GWC) of all soils samples was determined by drying samples at 104 °C for 24 h.

Phospholipid fatty acids were extracted from 2 g of soil using the modified Bligh and Dyer method (Bligh and Dyer 1959; White and Ringelberg 1998). Briefly, soils were suspended in a 2:1:0.8 solution of methanol, chloroform, and phosphate buffer. The soil was then filtered and the chloroform phase separated. Phospholipids were separated from glycolipids and neutral lipids using solid phase extraction columns. Phospholipids were saponified and methylated to fatty-acid methyl esters (FAME). FAMES were run on an Agilent 6890 gas chromatograph (GC) equipped with a flame ionization detector and an Ultra-2 column. Peaks were identified by comparing retention times with known standards. Concentration of each PLFA was obtained by comparing peak areas with a 13:0 standard curve.

Standard nomenclature was used to describe PLFAs. The number before the colon refers to the total number of C atoms and the number following the colon refers to the number of double bonds. The location of any double bonds in the fatty acid molecule is indicated by the number after the 'ω'. The notations: "Me," "OH," "cy," "i," and "a" refer to methyl, hydroxy, cyclopropane groups, and iso- and anteiso-branched fatty acids, respectively.

Total extractable PLFAs were used as an indicator of living biomass. This method has been found to be proportional to other microbial biomass measures (Fritze et al. 2000; Fierer et al. 2003). The total bacterial biomass was calculated by summing the i15:0, a15:0, i16:0, 16:1ω9, 16:1ω5, 17:1ω9, i17:0, a17:0, cy17:0, 18:1ω7, and cy19:0 PLFAs, which are mostly of bacterial origin (Hill et al. 2000; Blume et al. 2002). Specific PLFA markers were used to quantify the relative abundance of various taxonomic groups in a manner similar to Fierer et al. (2003). Gram-positive bacteria were identified by summing i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0; gram-negative bacteria were identified by summing cy17:0, 16:1ω9c, 17:1ω9c, 18:1ω7c, and cy19:0. The

18:2ω6c PLFA was used as a marker for fungi. Actinomycetes were identified using the 10Me16:0, 10Me17:0, and 10Me18:0 PLFAs; 20:4ω6 and 20:2ω6 were used as biomarkers for protozoa.

### Statistical analysis

Phospholipid fatty acid relative abundances (mol %) for all 40 PLFAs identified were used to examine community differences among treatments. Microbial community structure was examined by non-metric multidimensional scaling (NMS; Kruskal 1964; Mather 1976) using the PC-ORD software package (McCune and Mefford 1999). The medium setting of 'autopilot' mode in PC-ORD was used, which attempts to find a stable solution from a random starting position (McCune and Mefford 1999). This method uses a maximum of 200 iterations in 15 runs with real data. The final dimensionality is determined by comparing the final stress values among the best solution for each axis. Significance of axes is assessed by comparing observed final stress with the final stress in 30 runs of randomized data using a Monte Carlo test (McCune and Grace 2002). Final ordinations were rotated to maximize environmental variables represented and separation of treatments on axes 1 and 2. Summed abundances for taxonomic biomarkers were used as overlays in a joint plot to visualize correlations between the ordination and the relative abundance of specific taxonomic groups. In a joint plot the length and direction of each vector is related to correlations with the original PLFA data (McCune and Grace 2002).

Statistical differences among PLFA profiles was assessed using multi-response permutation procedures (MRPP; Mielke 1984; Mielke and Berry 2001) in PC-ORD. MRPP is a nonparametric procedure similar to MANOVA for testing the hypothesis of no difference between two or more pre-existing groups (McCune and Grace 2002). MRPP yields a *P* value to evaluate how likely it is that an observed difference is due to chance, as well as the chance-corrected within-group agreement (*A*), which describes within-group homogeneity compared to random expectation (McCune and Grace 2002). An *A* value equal to 1 is found when all items within a group are identical; when heterogeneity within groups equals expectation by chance *A* = 0. In instances where there were no significant differences among plots with roots (*P* > 0.1) these treatments were combined and compared with the rootless treatments in a two-way analysis.

The statistical significance of summed taxonomic group differences between treatment and season at AND, as well as their interaction, were analyzed with repeated measures analysis of variance (ANOVA) using PROC MIXED (SAS version 8.1, SAS Institute, Inc, Cary, NC, USA). In instances where the interaction between treatment and season was not significant (*P* > 0.05), the interaction was removed and the analysis

was repeated using data from all three sampling dates. When ANOVA resulted in a  $P$  value  $< 0.05$ , pre-planned comparisons among the six treatments were made using orthogonal contrasts. At BOU and SIK, where data were from only one sampling date, the same comparisons were made with ANOVA using the Tukey-Kramer procedure to correct for family-wise error rates (Splus version 6.1, Insightful Corp., Seattle, WA, USA).

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## Results

### Soil moisture content at the time of sampling

There was moderate evidence of a difference in soil water content among treatments at BOU (ANOVA;  $P=0.05$ ), although none of the multiple comparisons among treatments were significant ( $P>0.1$ ; Table 1). At SIK, there was a difference in soil water content (ANOVA;  $P<0.001$ ), with the rootless (NR and NI) treatments being wetter than the CO, DL, and DW treatments. At AND, the NI and NR plots tended to be wetter than the other treatments in July, with virtually no difference among treatments in either November or April. There was a significant difference in soil water content by the sampling date, with the July sampling being drier than either of the other two dates.

### Microbial biomass and community structure

Microbial biomass, as determined by total extractable PLFA, was relatively unaffected by treatment at all three sites (Table 1). The only significant differences in biomass between treatments were found at SIK (ANOVA;  $P=0.05$ ). At that site there was evidence that the DW plots had a larger biomass than the NR plots ( $P<0.05$ ) and suggestive evidence that the DW plots had a larger biomass than the NI plots ( $P<0.1$ ). At AND, there were no treatment effects on microbial biomass, although there were seasonal trends, with low microbial biomass in November and high microbial biomass in April (Table 1;  $P<0.0001$ ).

A NMS of the BOU PLFA data shows that the treatments without roots had different microbial communities than all the other treatments (Fig. 1). There was no evidence of a difference in the PLFA profiles among any of the three treatments (CO, DL, and NL) with roots (MRPP;  $P=0.49$ ). When these treatments were grouped together and compared with the two rootless treatments, we found convincing evidence of a difference in PLFA profiles between the root and rootless treatments (MRPP;  $P=0.002$ ,  $A=0.14$ ). Removing the fungal biomarker from the analysis had little effect on the final NMS solution (data not shown) and actually increased the observed treatment effects (MRPP;  $P=0.002$ ,  $A=0.17$ ), indicating that differences in the bacterial community were driving the treatment differences. An examination of taxonomic markers from the

joint plot suggested that the rootless plots tended to have higher concentrations of actinomycete biomarkers and a lower concentration of gram-negative biomarkers (Fig. 1), although these differences were not statistically different (Table 1).

A NMS of the PLFA profile at SIK showed a similar trend: the plots without roots appeared to have a different PLFA profile than the plots with roots (Fig. 2). Again there was no difference in PLFAs among the treatments with roots (MRPP;  $P=0.34$ ). When these treatments were compared with the rootless treatments, there was moderate evidence of a difference in PLFA profiles (MRPP;  $P=0.03$ ,  $A=0.05$ ). However, if the 18:2 $\omega$ 6,9 PLFA was removed from the analysis there was no evidence of a treatment difference among bacterial communities (MRPP;  $P=0.73$ ). Rootless plots exhibited a significantly lower relative abundance of fungi, a lower fungal:bacterial ratio, and fewer protozoa than the treatments with roots at this site (Table 1).

At AND, the PLFA profiles varied by both treatment and season (Fig. 3). There was no evidence of a difference in PLFA profiles among any of the plots with roots for any sampling date (MRPP;  $P>0.25$ ). In the April and July data there was significant evidence that the rootless plots had a different PLFA profile than all other plots (MRPP;  $P<0.0001$ ,  $A=0.15$  and  $0.13$ , respectively). In the November data there was moderate evidence that the rootless plots were different from all other plots (MRPP;  $P=0.04$ ,  $A=0.05$ ). If the fungal biomarker was removed, the bacterial PLFA profiles of the rootless plots were still significantly different from all other plots in April and July (MRPP;  $P<0.05$ ,  $A=0.03$  and  $0.06$ , respectively), although the differences were much reduced. In November, there was no evidence of a treatment effect on the bacterial communities (MRPP;  $P=0.41$ ). The rootless plots had a lower abundance of fungi regardless of season (ANOVA;  $P<0.05$ ), although the portions of the bacterial community driving the treatment differences varied by sampling date. The rootless plots generally had a larger abundance of actinomycetes and gram-positive bacteria (Fig. 3), although these differences were never significant (Table 1). The rootless plots also had a larger abundance of gram-negative bacteria during April and July, with no difference in November.

### Seasonal changes in microbial community at AND

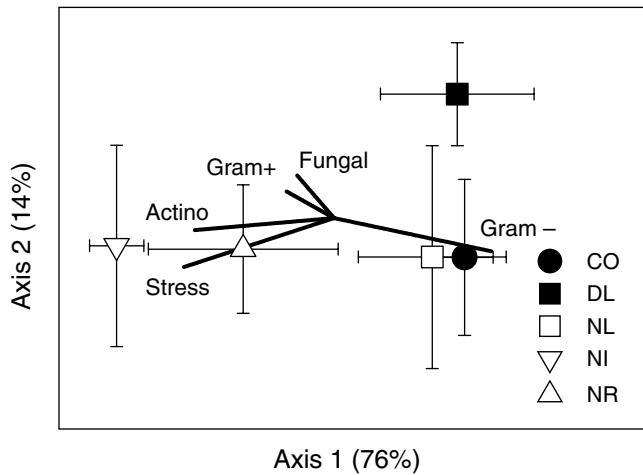
At the three sampling dates at AND, the soils were at different water contents, being much wetter in April and November than in July. However, there were no significant differences in water content among the different litter treatments (Table 1). At a meteorological station located 4 km from the DIRT plots, the average volumetric water content at 10 cm depth in the 14 days preceding sampling was almost threefold higher before the April and November sampling dates than before the July sampling. In addition, the average soil temperature

**Table 1** Treatment, site, and seasonal differences in soil moisture content, microbial biomass, and taxonomic biomarkers

Treatment	Water content (g H <sub>2</sub> O g <sup>-1</sup> soil)	Microbial biomass (nmol PLFA g <sup>-1</sup> soil)	Gram-positive bacteria (mol %)	Gram-negative bacteria (mol %)	Actinomycetes (mol %)	Protozoa (mol %)	Fungi (mol %)	Fungal:bacterial ratio	cy17:0/cy19:0 precursors ratio
<b>AND</b>									
<b>April</b>									
DW	0.48 (0.02)	90.5 (22.5)	13.1 (0.6)	23.0 (0.5)	4.8 (0.3)	0.65 (0.11)	7.9 (0.5) <sup>a</sup>	0.16 (0.01) <sup>a</sup>	0.66 (0.05)
DL	0.47 (0.01)	92.5 (21.1)	13.7 (0.1)	23.7 (1.3)	5.5 (0.7)	0.53 (0.13)	7.9 (0.9) <sup>a</sup>	0.16 (0.02) <sup>a</sup>	0.62 (0.04)
CO	0.49 (0.01)	92.0 (11.1)	15.0 (1.0)	22.6 (0.4)	5.8 (0.2)	0.61 (0.13)	7.9 (0.9) <sup>a</sup>	0.16 (0.02) <sup>a</sup>	0.58 (0.03)
NL	0.47 (0.02)	73.2 (18.1)	13.9 (0.4)	23.2 (1.5)	5.3 (0.2)	0.64 (0.04)	6.7 (1.4) <sup>a</sup>	0.14 (0.03) <sup>a</sup>	0.59 (0.04)
NR	0.51 (0.02)	86.7 (21.1)	15.3 (0.9)	24.7 (0.5)	5.5 (0.6)	0.70 (0.16)	2.7 (0.4) <sup>b</sup>	0.05 (0.01) <sup>b</sup>	0.69 (0.03)
NI	0.51 (0.03)	78.0 (13.0)	16.9 (1.2)	23.5 (0.5)	5.9 (0.4)	0.66 (0.10)	2.6 (0.2) <sup>b</sup>	0.05 (0.01) <sup>b</sup>	0.68 (0.02)
<b>July</b>									
DW	0.30 (0.02)	67.0 (5.2)	12.5 (1.7)	21.0 (1.6)	6.1 (0.3)	0.58 (0.7)	7.4 (1.1) <sup>a</sup>	0.16 (0.03) <sup>a</sup>	0.69 (0.07)
DL	0.31 (0.01)	42.9 (3.0)	12.6 (0.3)	21.7 (1.8)	5.9 (0.2)	0.90 (0.18)	7.1 (0.7) <sup>a</sup>	0.15 (0.02) <sup>a</sup>	0.77 (0.02)
CO	0.32 (0.01)	39.4 (6.6)	13.5 (0.6)	20.7 (0.8)	6.3 (0.2)	1.49 (0.22)	6.3 (0.5) <sup>a</sup>	0.14 (0.01) <sup>a</sup>	0.75 (0.07)
NL	0.34 (0.01)	44.8 (2.5)	13.8 (1.4)	21.9 (0.9)	6.0 (0.1)	0.78 (0.15)	6.8 (1.3) <sup>a</sup>	0.14 (0.03) <sup>a</sup>	0.68 (0.07)
NR	0.41 (0.02)	44.9 (2.0)	14.0 (0.9)	21.8 (0.6)	6.3 (0.9)	1.05 (0.34)	2.8 (0.1) <sup>b</sup>	0.06 (0.001) <sup>b</sup>	0.91 (0.07)
NI	0.40 (0.02)	52.2 (1.9)	14.3 (0.1)	21.8 (0.3)	6.6 (0.4)	0.80 (0.14)	2.7 (0.2) <sup>b</sup>	0.05 (0.005) <sup>b</sup>	0.88 (0.02)
<b>November</b>									
DW	0.43 (0.04)	37.5 (3.9)	14.3 (0.4)	20.9 (1.8)	5.8 (0.3)	1.1 (0.2)	6.7 (0.4) <sup>a</sup>	0.14 (0.01) <sup>a</sup>	0.82 (0.09)
DL	0.46 (0.06)	23.3 (4.9)	15.4 (1.4)	18.2 (1.3)	6.9 (0.9)	2.1 (0.3)	4.5 (0.1) <sup>a</sup>	0.09 (0.01) <sup>a</sup>	0.95 (0.12)
CO	0.49 (0.03)	35.6 (6.9)	15.1 (0.5)	21.3 (1.3)	6.7 (0.4)	1.1 (0.5)	5.4 (0.5) <sup>a</sup>	0.11 (0.01) <sup>a</sup>	0.74 (0.09)
NL	0.45 (0.02)	19.8 (4.9)	12.0 (1.4)	20.5 (0.8)	6.7 (0.1)	2.3 (1.0)	5.2 (1.3) <sup>a</sup>	0.11 (0.03) <sup>a</sup>	0.87 (0.06)
NR	0.49 (0.02)	28.3 (2.3)	15.5 (0.5)	21.3 (1.3)	6.0 (0.5)	1.3 (0.4)	2.5 (0.2) <sup>b</sup>	0.05 (0.01) <sup>b</sup>	0.86 (0.07)
NI	0.53 (0.07)	30.2 (3.5)	15.9 (1.2)	20.4 (0.7)	6.9 (0.4)	1.7 (0.5)	2.4 (0.3) <sup>b</sup>	0.05 (0.01) <sup>b</sup>	0.87 (0.10)
<b>BOU</b>									
DL	0.53 (0.04)	61.9 (17.9)	16.7 (0.5)	17.9 (1.0)	9.2 (0.3)	1.6 (0.3)	3.3 (0.7)	0.07 (0.02)	0.90 (0.03) <sup>a</sup>
CO	0.57 (0.04)	119.9 (66.7)	17.8 (0.1)	18.3 (0.4)	9.3 (0.3)	1.1 (0.3)	2.9 (0.2)	0.06 (0.01)	0.99 (0.12) <sup>ab</sup>
NL	0.59 (0.02)	114.2 (19.6)	16.0 (0.7)	17.9 (1.3)	8.7 (0.6)	1.2 (0.1)	4.1 (0.4)	0.08 (0.01)	1.01 (0.16) <sup>ab</sup>
NR	0.67 (0.03)	93.8 (27.9)	18.7 (0.6)	14.6 (2.2)	9.7 (0.9)	1.3 (0.1)	2.5 (0.5)	0.05 (0.01)	1.26 (0.14) <sup>ab</sup>
NI	0.65 (0.02)	65.4 (16.1)	18.4 (1.3)	11.9 (0.9)	10.3 (0.1)	1.3 (0.1)	4.0 (0.3)	0.09 (0.01)	1.42 (0.07) <sup>b</sup>
<b>SIK</b>									
DW	0.30 (0.01) <sup>a</sup>	79.0 (13.9) <sup>b</sup>	22.2 (1.0)	17.7 (1.0)	10.0 (0.3)	0.56 (0.2) <sup>ab</sup>	3.6 (0.3) <sup>a</sup>	0.07 (0.01) <sup>a</sup>	0.77 (0.04)
DL	0.26 (0.01) <sup>a</sup>	59.3 (15.3) <sup>ab</sup>	23.7 (1.0)	17.2 (1.0)	10.2 (0.3)	0.66 (0.2) <sup>ab</sup>	3.8 (0.8) <sup>a</sup>	0.08 (0.01) <sup>a</sup>	0.74 (0.03)
CO	0.27 (0.02) <sup>a</sup>	50.8 (6.0) <sup>ab</sup>	25.1 (0.1)	15.5 (0.6)	10.8 (0.1)	1.56 (0.4) <sup>a</sup>	2.5 (0.5) <sup>ab</sup>	0.05 (0.01) <sup>ab</sup>	0.94 (0.08)
NL	0.31 (0.01) <sup>ab</sup>	57.6 (5.4) <sup>ab</sup>	24.7 (1.3)	16.2 (0.8)	11.0 (0.2)	0.66 (0.3) <sup>ab</sup>	2.6 (0.2) <sup>ab</sup>	0.05 (0.01) <sup>ab</sup>	0.89 (0.03)
NR	0.36 (0.01) <sup>b</sup>	34.2 (5.5) <sup>a</sup>	25.3 (1.8)	16.4 (1.1)	10.9 (0.3)	1.07 (0.1) <sup>ab</sup>	1.2 (0.2) <sup>b</sup>	0.02 (0.01) <sup>b</sup>	0.91 (0.09)
NI	0.35 (0.01) <sup>b</sup>	38.5 (2.5) <sup>ab</sup>	24.3 (0.7)	16.5 (1.0)	11.5 (0.4)	0.23 (0.1) <sup>b</sup>	1.2 (0.2) <sup>b</sup>	0.02 (0.01) <sup>b</sup>	0.89 (0.04)

Data are means and 1 standard error. Letters denote statistical differences (ANOVA;  $P < 0.05$ ) among treatments

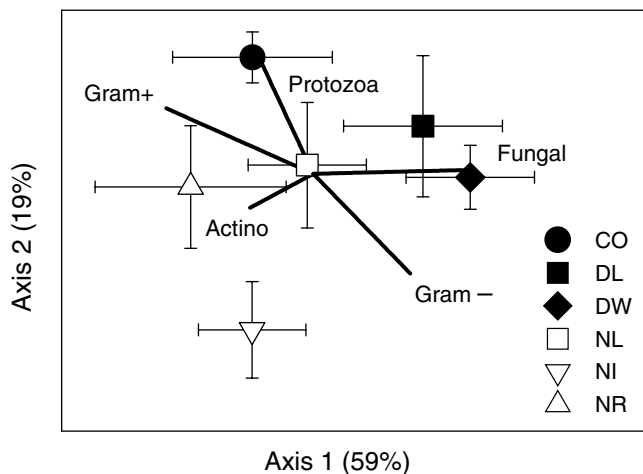




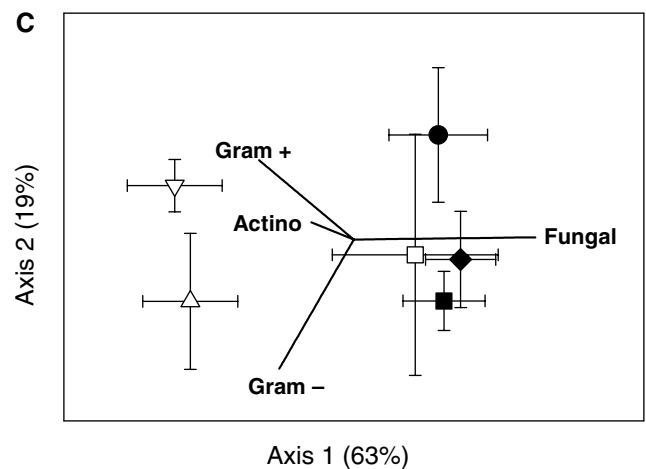
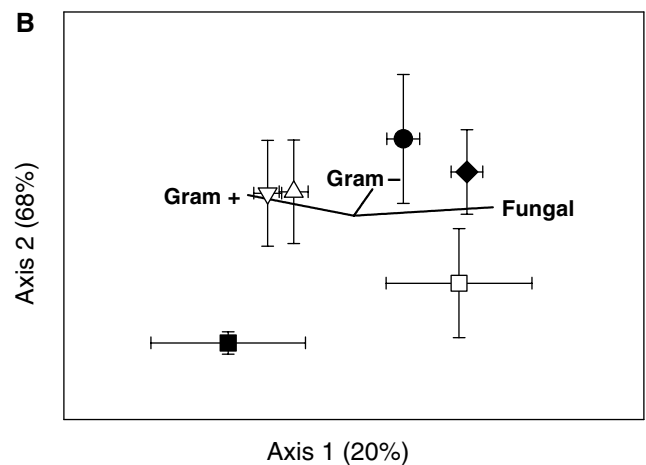
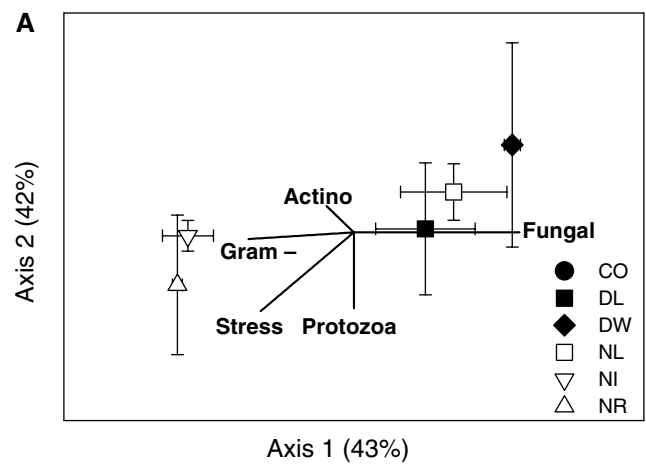
**Fig. 1** NMS plot of PLFA relative abundance at BOU from a two-dimensional solution. The percentage of variance explained by each axis is given in parentheses. Symbols are mean loading scores for each treatment ( $n=3$ ) with error bars showing  $\pm 1SE$ . Solid circle is CO, solid square is DL, open square is NL, inverted triangle is NI, triangle is NR. Joint plot vectors are based on summed abundances of specific PLFAs, with the length of the vector proportional to the correlation between that variable and the NMS axes

at 10 cm in the 14 days preceding sampling was highest in July and lowest in November (20.0 vs. 7.2 °C, respectively).

In general, the seasonal differences in microbial community structure at AND were larger than the treatment differences, as all the taxonomic groups showed significant changes by season (ANOVA;  $P < 0.05$ ), whereas only the fungi and fungal:bacterial



**Fig. 2** NMS plot of PLFA relative abundance at SIK. This figure shows the first two axes of a three-dimensional solution. The percentage of variance explained by each axis is given in parentheses. Symbols are mean loading scores for each treatment ( $n=3$ ) with error bars showing  $\pm 1SE$ . Solid circle is CO, solid square is DL, solid diamond is DW, open square is NL, inverted triangle is NI, triangle is NR. Joint plot vectors are based on summed abundances of specific PLFAs, with the length of the vector proportional to the correlation between that variable and the NMS axes



**Fig. 3** NMS plot of PLFA relative abundance at AND samples in: **a** July 2003, **b** November 2003, and **c** April 2004. A two-dimensional solution was found in July 2003 and November 2003, and a three-dimensional solution was found in April 2004. The percentage of variance explained by each axis is given in parentheses. Symbols are mean loading scores for each treatment ( $n=3$ ) with error bars showing  $\pm 1SE$ . Solid circle is CO, solid square is DL, solid diamond is DW, open square is NL, inverted triangle is NI, triangle is NR. Joint plot vectors are based on summed abundances of specific PLFAs, with the length of the vector proportional to the correlation between that variable and the NMS axes

ratio showed any evidence ( $P < 0.003$ ) of differences by treatment (Table 1). Total microbial biomass was also statistically different at all sampling dates (ANOVA;  $P < 0.05$ ), with the lowest biomass in November and the highest biomass in April (Table 1). The bacterial biomass showed a similar trend (data not shown), with a significant difference between April and the other two dates (ANOVA;  $P < 0.05$ ) and suggestive evidence of a difference in bacterial biomass in November and July (ANOVA;  $P < 0.1$ ).

Although there was no evidence of an interaction between season and treatment (ANOVA;  $P > 0.05$ ) for any of the taxonomic groups, there were consistent significant seasonal changes in the relative abundance of many microbial groups in all treatments (data not shown), therefore we use the control plots as an example. In the control plots there was significantly ( $P < 0.05$ ) lower relative abundance of the fungal biomarker and the fungal:bacterial ratio in November and a significantly higher relative abundance of the fungal biomarker (and fungal:bacterial ratio) in April when compared with July (Fig. 4). Gram-positive markers were significantly ( $P < 0.05$ ) lower in July than either November or April, whereas the relative abundance of the gram-negative biomarkers was significantly higher ( $P < 0.05$ ) in April than July, with no difference between the relative abundance in November and either of the two other sampling dates. Conversely, the actinomycete markers were significantly lower in the April sampling than either the July or November sampling. The protozoan markers

were significantly higher ( $P < 0.05$ ) in the July sampling than in the April sampling, with no differences between November and either of the other two sampling dates. Finally, the ratio of cyclopropyl PLFAs to their precursors was significantly lower in April than either July or November (data not shown).

## Discussion

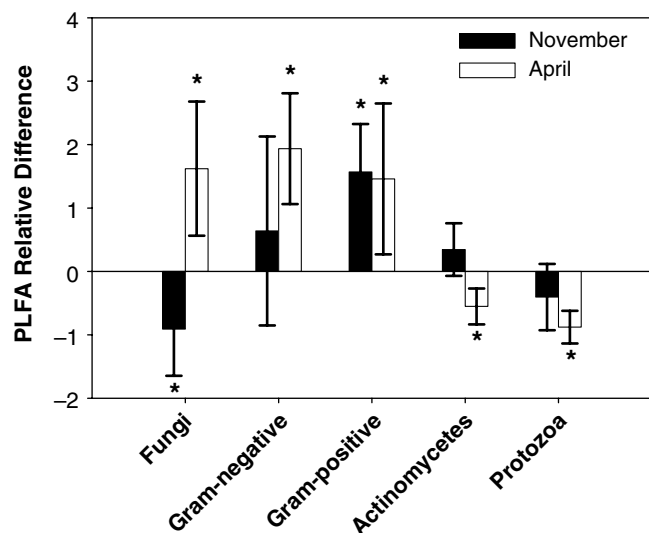
### Effects of C input manipulation on the microbial community

Neither total microbial biomass (measured as total PLFA) nor bacterial biomass were affected by manipulation of C inputs at any of our sites (Table 1). Our results are consistent with other studies, where similar C input manipulations resulted in no changes in mineral soil microbial or bacterial biomass (Fisk and Fahey 2001; Siira-Pietikainen et al. 2001, 2003; Spears et al. 2003; Nadelhoffer et al. 2004).

Phospholipid fatty acid analyses at the DIRT plots shows that belowground C inputs exert more influence on the soil microbial community than aboveground inputs in three very different forest ecosystems (Figs. 1, 2, 3). The small influence of chronic aboveground C inputs is somewhat surprising. Litter and wood additions at the AND DIRT plots, as well as those at Harvard Forest, have been shown to lead to an increase in dissolved organic matter fluxes from the organic horizon to the mineral soil after at least 5 years of manipulation (Nadelhoffer et al. 2004; Lajtha et al. 2005). Although the quantity of dissolved organic C entering the mineral soil was different among treatments, the chemistry of the soil solution leaving the organic horizon in the CO, DW, and DL treatments was the same at AND (Yano et al. 2005), suggesting microbial degradation of labile C before soil solution enters the mineral soil (Lajtha et al. 2005). Indeed, treatment differences in dissolved organic matter concentration entering the soil at AND disappeared between 0 and 30 cm, except in DW plots where the treatment differences disappear between 30 and 100 cm (Lajtha et al. 2005).

Root exclusion by trenching has significantly altered the microbial community structure in the mineral soil at all three sites (Figs. 1, 2, 3). These changes in community structure went beyond a loss of fungi, which might be expected as mycorrhizal fungi disappear, and include changes in the bacterial community at the two sites where the treatments have been in place for at least 5 years. The only other studies to look at the effects of trenching on microbial community structure concentrated on the organic horizon. Siira-Pietikainen et al. (2001, 2003) found a decrease in the relative abundance of fungi in the O layer, as well as a change in the bacterial community composition after only 3 years of treatment.

Under ectomycorrhizal tree species, root trenching has been shown to significantly decrease population size,



**Fig. 4** Relative differences in the PLFA mol% of taxonomic groups at the three AND sampling dates. The bars show the mean difference in the relative abundance between either the November (solid bar) or April (open bar) sampling dates and the July sampling date. Error bars show  $\pm$  1 SE of the differences between those two numbers. A star designates a bar is significantly different from zero (the July reference value); differences between November and April sampling dates occur when the error bars of these respective samples do not overlap

species richness, and root colonization of ectomycorrhizal fungi 16 months after trenching (Simard et al. 1997). The loss of ectomycorrhizal fungi appears to contribute to most of the observed differences in PLFA profiles at AND and SIK. On the other hand, at BOU, no difference was found in the fungal biomarker of the rootless plots compared to the other treatments (Table 1). This may be because sugar maple form only arbuscular mycorrhizal (AM) associations and AM fungi do not generally have a large portion of the standard 18:2 $\omega$ 6,9 fungal biomarker. The 16:1 $\omega$ 5 PLFA has been used as a biomarker for AM fungi (Olsson and Johansen 2000). We did observe a smaller, but not significant, relative abundance of the 16:1 $\omega$ 5 biomarker in the rootless plots at BOU (data not shown). Studies where root inputs and growth are increased by exposure to levels of atmospheric CO<sub>2</sub> above ambient showed an increase in the relative abundance of fungi after 2.5 years in a deciduous forest ecosystem (Zak et al. 2000) and after 4 years in a model beech-spruce ecosystem (Wiemken et al. 2001), although neither study found any changes in bacterial community structure. Any changes in the abundance and composition of mycorrhizal fungi could have a large influence on soil C processing rates and C input quality (Langley and Hungate 2003).

At SIK, BOU, and to a lesser extent AND, the actinomycete biomarkers tended to have larger relative abundances in the rootless treatments (Figs. 1, 2, 3), although none of these changes was significant. Actinomycetes are filamentous bacteria that have traditionally been associated with the degradation of more recalcitrant C compounds such as chitin and cellulose (McCarthy and Williams 1992). Actinomycetes have been shown to increase in relative abundance with lower resource availability either due to C addition in the form of synthetic root exudates (Griffiths et al. 1999) or an increase in soil depth (Fierer et al. 2003). These members of the soil microbial community might be filling the niche decomposing more recalcitrant C compounds usually filled by fungi in the plots without roots; however, more research into functional differences in the rootless plots is necessary to determine if this is the case.

The ratio of cyclopropyl PLFAs to their precursor PLFAs has been used as an indicator of microbial community stress (Bossio and Scow 1998). This ratio was higher in the rootless plots at BOU, implying that the lack of labile root inputs increased the physiological stress on the microbial community (Table 1). This ratio was also larger in the rootless plots at AND during July, although the difference was not statistically significant (Fig. 3a). Various studies have found that C limitation can lead to an increase in microbial community stress as determined by this ratio (Kieft et al. 1997; Bossio and Scow 1998; Lundquist et al. 1999; Fierer et al. 2003; Macdonald et al. 2004).

It is important to keep in mind that exclusion of roots may have additional effects beyond altering below-ground C inputs. For example, the loss of plant water

uptake in rootless plots should increase soil water content, which has been shown to have a major effect on microbial community structure (Kieft et al. 1997; Schimel et al. 1999; Wilkinson et al. 2002). Although we found that soil water content in the rootless plots was consistently higher than in the plots with roots, this difference was significant only at SIK (Table 1), the site that showed little effect of treatments on overall microbial community composition (Fig. 2) or in the ratio of cyclopropyl PLFAs to their precursors (Table 1), which might be expected to respond to water stress. As a result, it appears that C limitation plays a major role in the observed treatment differences, regardless of water content.

### Seasonal dynamics at AND

Seasonal changes in the soil microbial community were more pronounced than the effects of the DIRT treatments at AND (Table 1). Similar results have been found in other temporal studies of soil microbial communities (Bossio et al. 1998; Myers et al. 2001; Blume et al. 2002). Microbial biomass changed among the three sampling dates, with the highest biomass in April, the period of peak photosynthesis (Waring and Franklin 1979), and the lowest values in November, when the soils were saturated and fairly cold. Seasonal changes in microbial biomass have been reported in other studies in forest ecosystems, although the season of high biomass varied with respect to climate and tree physiology (Myers et al. 2001; Bohlen et al. 2002; Litton et al. 2003).

The relative abundance of fungi was significantly lower in November than either April or July (Fig. 4). Wallander et al. (2001) found that mycorrhizal biomass in a coniferous forest generally followed fine-root production, with high biomass during periods of high fine-root production and low biomass during periods of low fine-root production. In November, mycorrhizal biomass would be expected to decrease, as fine-root production in Douglas-fir forests is low during the winter (Tingey et al. 2005). Conversely, when the abundance of fungi was low, the relative abundance of actinomycetes increased (Fig. 4). This pattern could be further evidence that fungi and actinomycetes are competing for similar resources in this ecosystem.

The bacterial community also showed pronounced seasonal changes (Fig. 4). The mono-unsaturated PLFAs were most variable throughout the year (data not shown). These lipids are generally associated with gram-negative bacteria, which might be expected to be sensitive to seasonal changes in labile C availability and climate.

The rootless plots were significantly different from all other treatments at all three sampling dates (Fig. 3), although the specific PLFAs and microbial groups that were different in the rootless plots changed throughout the year. The fungal:bacterial ratio was lower in November, which implied a shift from fungal to bacterial dominance (Table 1). These changes were probably



due to changes in the abundance of mycorrhizal fungi, as no changes were seen in either of the treatments without roots (data not shown). At the point of lowest fungal biomass (November), there was no significant treatment effect on bacterial community structure. Soil water content can be ruled out as the cause of these treatment effects because there was no evidence of a difference in moisture content of the sampled soils between the April and November sampling dates (ANOVA;  $P = 0.77$ ), as well as no treatment effect on moisture content on either of these dates (Table 1).

## Conclusions

Our results suggest that root inputs are an important control of microbial community structure in the mineral soil of three distinct forest ecosystems. Changes were found in the bacterial community, especially actinomycetes, after accounting for the loss of mycorrhizal fungi following root exclusion. Additionally, seasonal differences in the PLFA profiles at AND were greater than any of the treatment differences, with the taxonomic biomarkers that drove treatment differences varying by season. This underscores the importance of seasonal sampling in any study looking at changes in microbial community composition in response to field experiments.

These data provide evidence that root C inputs exert a strong control on soil microbial community composition in forest soils. The lack of an effect of aboveground input manipulation was surprising, especially after 13 years of manipulation at BOU. Continued monitoring of these permanent plots could further our understanding of the litter controls on soil microbial community composition. Future research is needed into the mechanisms of these controls, as well as determining if compositional changes in soil microbial community structure lead to changes in ecosystem function and C utilization rates.

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