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ORIGINAL PAPER

Responses of soil bacterial and fungal communities to reciprocal transfers of soil between adjacent coniferous forest and meadow vegetation in the Cascade Mountains of Oregon

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Abstract Little information exists on the responses of soil fungal and bacterial communities in high elevation coniferous forest/open meadow ecosystems of the northwest United States of America to treatments that impact vegetation and soil conditions. An experiment was conducted in which soil cores were reciprocally transplanted between immediately adjacent forests and meadows at two high elevation (~1,600 m) sites (Carpenter and Lookout) in the H.J. Andrews Experimental Forest located in the Cascade Mountains of Oregon. Half of the cores were placed in PVC pipe (closed) to prevent new root colonization, whereas the other cores were placed in mesh bags (open) to allow recolonization by fine roots. A duplicate set of open and closed soil cores was not transferred between sites and was incubated in place. After 2 year, soil cores were removed and changes in fungal and bacterial biomasses determined using light microscopy, and changes in microbial community composition determined by PLFA analysis, and by length heterogeneity PCR of the internal transcribed spacer region of fungal ribosomal DNA. At both sites soil microbial community structures had responded to treatments after 2 year of incubation. At Carpenter, both fungal and bacterial community structures of forest soil changed significantly in response to transfer from forest to meadow, with the shift in fungal community structure being accompanied by a significant decrease in the PLFA biomarker of fungal biomass,18:2ω6,9. At Lookout, both fungal and bacterial community structures of forest soil changed significantly in response to open versus closed core treatments, with the shift in the fungal community being accompanied by a significant decrease in the 18:2ω6,9 content of closed cores, and the shift in the bacterial community structure being accompanied by a significant increase in bacterial biomass of closed cores. At both sites, fungal community structures of meadow soils changed differently between open and closed cores in response to transfer to forest, and were accompanied by increases in the18:2ω6,9 content of open cores. Although there were no significant treatment effects on the bacterial community structure of meadow soil at either site, bacterial biomass was significantly higher in closed versus open cores regardless of transfer.

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Introduction

In recent years, considerable effort has been devoted to unraveling the composition of soil microbial communities, and several attempts have been made to place community composition into context with soil microbial functions (Balser and Firestone 2005; Waldrop and Firestone, 2004a, b). Although many publications have been devoted to determination of bacterial community composition (Axelrood et al. 2002; Fierer et al. 2005b), fewer papers have appeared that describe fungal community composition in soil ecosystems (Anderson and Cairney 2004; Brodie et al. 2003; Schadt et al. 2003). Soil microbiologists have recognized for many years that both fungal and bacterial biomass make variable contributions to the soil community (Anderson and Domsch 1973; Bååth and Anderson 2003; Schnürer et al., 1985), yet, little is known about what controls the relative abundance of these two major groups of microorganisms in soils. Recently, it was documented that fungal to bacterial ratios and community composition can be influenced by site fertility and N availability in forest and grassland soils (Grayston and Prescott 2005; Kennedy et al. 2004, 2005; Leckie et al. 2004). In addition, several reports have appeared showing that the relative activities of bacteria and fungi in forest soils can change when trees are exposed to elevated levels of O_3, CO_2 or inorganic N (DeForest et al. 2004; Phillips et al. 2002). Previously, we reported on the composition of nitrifying and denitrifying bacterial communities in adjacent meadow and forest soils of contrasting N availability (Mintie et al. 2003; Rich et al. 2003), and their responses when soil was reciprocally transferred from one environment to the other (Bottomley et al. 2004; Boyle et al. 2006). We hypothesized that by controlling root in-growth (open vs. closed cores), and transferring cores between the shaded, wetter forests and the drier meadows, this would permit us to evaluate the relative impact of vegetation, and forest and meadow microclimates on the processes of nitrification and denitrification and the associated community dynamics. Our data clearly showed that after 2 year of exposing high elevation (~1,600 m) forest soils to the meadow environment, N mineralization and nitrification had increased to values that were similar to those of the meadow soils, while less influence was detected in the reciprocal transfer from meadow to forest. Furthermore, although there were some significant effects of treatments on the composition and/or size of the nitrifier and denitrifier communities, by and large, the structures of these microbial communities were well buffered against environmental effects. In this manuscript we describe the response of the overall soil fungal and bacterial communities to the same reciprocal transfers of the meadow and forest soils.

Materials and methods

Site description

The study sites were located in the H.J. Andrews Experimental forest (44.2° N, 122.2° S) in the Cascade Mountains of Oregon, USA. At high elevations (~1,600 m) on steep south facing slopes, well-drained open areas of meadow vegetation are interspersed among coniferous forests. Two locations, hereafter referred to as Carpenter and Lookout, were chosen because of the close proximity of open grassy areas and forests. Aspects and slopes were similar (210° SSW/50% and 180° S/35%) at Carpenter and Lookout, respectively. The soils at both sites are poorly developed sandy loams with the forest soils being generally higher in organic C (140 mg C vs. 100 mg C g^{-1} soil), and lower in pH (5.2 vs. 5.7) and extractable cations than meadow soils. Soil N was higher at Lookout than Carpenter (9.3 mg N vs. 7.2 mg N g^{-1} soil). Meadow and forest soils are classified as lithic cryandepts, and pachic haplumbrepts, respectively. At Lookout, meadow vegetation was dominated by a mixture of grasses, perennial herbs, and legumes of the *Vicia*, and *Lupinus* genera. At Carpenter, meadow vegetation con-

sisted of grasses, herbs, and bracken fern (*Pteridium aquilinum* L.) and sparse occurrence of legumes. Douglas fir, silver and grand fir were the dominant tree species at both sites averaging about 30–50 and 95–100 years old at Lookout and Carpenter, respectively. The experimental sites have been described in detail elsewhere (Mintie et al. 2003; Rich et al. 2003).

Experimental set up

In September 2000, a 35 m × 35 m grid was established in each meadow and forest area at both Lookout and Carpenter. The grid consisted of 64 sample locations evenly spaced at 5-m intervals. Positions within the array were randomly assigned. Each grid accommodated 12 cores of each of four treatments: cores remaining in place (open and closed), hereafter abbreviated OR and CR, and cores transferred from one environment to the other (open and closed), hereafter abbreviated OT and CT. Prior to excavation, the organic surface layers of the forest and meadow soils were carefully scraped away to expose the mineral soil. Closed cores (5 cm diameter × 15 cm length) were collected in PVC pipe and either put back in place (CR) or transferred (CT). The upper end of the pipe was covered with 2-mm gauge window screen in an attempt to prevent inputs of large litter debris, and animal disturbance. Open cores were excavated in a similar fashion but were extruded from the pipe and placed into mesh bags constructed of window screen. The mesh bags were stapled shut and either put back in place (OR) or transferred (OT) into the assigned holes in the core field.

Field sampling

In September 2002, soil cores were removed from their specific locations in the core field. Six cores were excavated from each treatment. To ensure that sufficient soil was available for the different assays carried out in the study, the six cores were randomly grouped into three pairs of cores, and the soil from each pair was thoroughly mixed to produce three replicates of each field treatment. A set of background cores was taken for determination of background community composition and fungal and bacterial biomasses. Soil was brought back to the laboratory on the same day of sampling, stored overnight at 4°C, and sieved to < 5 mm while field moist. Root material was recovered from the soil cores, live roots retained, washed, and dried at 65°C to determine treatment effects on live root dry weight. Gravimetric water content was determined on sub samples of soil, and all data are expressed on a dry weight of soil basis. Bacterial and fungal biomasses were determined by light microscopy, and community composition by PLFA and length heterogeneity-PCR analyses (see below).

Estimation of bacterial and fungal biomass

The numbers of bacteria and lengths of fungal hyphae were estimated by direct counts and cell volumes and dry weights determined as described elsewhere (Lodge and Ingham, 1991). Briefly, 1-g portions of fresh soil were placed in 9 ml of 0.2 M phosphate buffer (pH 7.2) and shaken vigorously. Fungal hyphae were quantified in an agar film prepared from 0.5 ml of the 1:10 soil suspension and 1 ml molten 1.5% (w/v) agar. Hyphal lengths were counted using differential interference contrast (DIC) microscopy (200×). Bacteria were enumerated by epifluorescence microscopy. Bacterial and fungal biomass were calculated from the volume of bacterial cells and fungal hyphae in 1 g dry soil using the visual estimates, and assuming bacterial and fungal cell densities of 0.33 g cm^{-3} and 0.41 g cm^{-3}, respectively (Ingham et al., 1991).

PLFA extraction and analysis

Soil phospholipid fatty acids (PLFA) were extracted and analyzed according to the procedure of White and Ringelberg (1998) as modified by Butler et al. (2003). Briefly, portions of soil (15 g wet weight) were extracted overnight in a mixture of chloroform, methanol, and 50 mM phosphate buffer (pH = 7.1). Lipids were extracted the following day by centrifugation and filtration. The phospholipid fraction was recovered and saponified to obtain fatty acid methyl esters (FAME). Samples were analyzed by capillary GC-FID.

DNA extraction and length heterogeneity PCR (LH-PCR) analysis

DNA was extracted from 0.5-g portions of soil using the Fast DNA kit (Bio 101, Inc., Irvine, CA). DNA was amplified using fungal primers for the internal transcribed spacer region of rDNA: ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). The forward primer was fluorescently labeled with 6-FAM (6-carboxyfluorescein). Reaction mixtures (50 µl) contained soil DNA (100 ng), AmpliTaq DNA polymerase (2.5 U), GeneAmp PCR buffer (1×), $MgCl_2$ (2 mM), deoxynucleoside triphosphates (0.2 mM each), forward and reverse primers (0.2 µM each), and bovine serum albumin (0.064 g ml^{-1}). PCR amplification was conducted as follows using a PTC-100 hot bonnet thermocycler (MJ Research, Inc., Waltham, MA): 94° for 2 min followed by 35 cycles of 94° for 30 s, 55° for 30 s, 72° for 1 min. A final extension followed for 72° for 2 min. PCR products were visualized with electrophoresis on 1% agarose gels stained with ethidium bromide. Samples of PCR product (1 µg DNA ml^{-1}) were submitted for analysis on an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA) in the Central Analytical Laboratory, Oregon State University. PCR products were run on the Genetic Analyzer along with X-Rhodamine MapMarker™ 1,000 internal lane size standard (BioVentures, Inc., Murfreesboro, TN).

Statistical analysis of fungal and bacterial biomass data

Responses of fungal and bacterial biomass levels to treatments were analyzed by analysis of variance (ANOVA) methods. The treatment effects of site, soil origin, incubation location, and core type were analyzed using the data from the open and closed, remaining and transferred cores (i.e., background cores were excluded). The factorial arrangement of site, soil origin, transfer effect, and core type was first analyzed using ANOVA. Because of the complexity of this multiple factorial design, we examined only specific contrasts when the ANOVA showed significant ($P < 0.05$) interactions.

PLFA data analysis

About 40 PLFA were identified in the soil profiles and the amounts of each PLFA were expressed as mol %, except when 18:2ω6,9 was used as a surrogate of fungal biomass and expressed as nmoles g^{-1} soil. Community composition data were analyzed by principal component analysis (PCA) using PC-ORD version 4.36 (MJM Software, Gleneden Beach, OR), a multivariate statistical package. Multi-response permutation procedures (MRPP) were used to test for significant differences in the proportional abundance of PLFA among treatments. MRPP is a nonparametric method for testing group differences (McCune and Grace 2002), and is similar to multivariate analysis of variance (MANOVA). For community data, a P-value < 0.05 and an A-statistic > 0.1 were considered significant. Indicator species analysis was performed with PC-ORD to identify specific PLFA responsible for the separation of groups.

LH-PCR data analysis

Size and relative abundance of LH-PCR fragments was quantified using GeneScan® v. 3.5 software and Genotyper® v. 2.5 software (Applied Biosystems, Inc., Foster City, CA). Ordinations of LH-PCR fragment data were carried out in PC-ORD using nonmetric multidimensional scaling (NMS) using the autopilot feature, ''slow and thorough'' with the Sørensen distance measure. We chose to use NMS because it avoids the assumption of linear relationships among variables and allows the use of relativization (McCune and Grace 2002).

Results

Site and vegetation effects on microbial biomass levels in soil

Fungal biomass determined by light microscopy was significantly higher in forest than meadow soil at both Carpenter (1.8 ± 0.7 vs. 0.3 ± 0.05 mg g^{-1} soil), and Lookout (2.5 ± 0.5 vs. 0.5 ± 0.2 mg g^{-1} soil); site differences were not

significant. Bacterial biomass was significantly higher in forest than meadow at Carpenter (0.14 ± 0.01 vs. 0.10 ± 0.01 mg g^{-1} soil) and at Lookout (0.12 ± 0.01 vs. 0.09 ± 0.01 mg g^{-1} soil).

Site and vegetation effects on microbial community composition

A principal component analysis of the PLFA data obtained from background cores taken at the same time as the reciprocally transferred cores were sampled showed a clear separation of the meadow and forest soil microbial communities at both sites (Fig. 1). MRPP analysis of the PLFA data revealed that bacterial community structure differed significantly between forest and meadow at Lookout ($A = 0.31$, $P = 0.02$), and Carpenter ($A = 0.24$, $P = 0.02$), and between the two meadow sites ($A = 0.14$, $P = 0.04$). Although indicator species analysis revealed that no PLFA were specific biomarkers of forest or meadow at either site, there was a clear trend for some gram-positive PLFA biomarkers (i14:0, a15:0, i16:1, 10Me17:0) and the marker for arbuscular mycorrhizal fungi (16:1ω5) to be higher in Carpenter meadow than forest, while15:0, cy17:0, cy19:0 were higher in forest than meadow. In addition, there was a clear trend for gram-positive biomarkers (i14:0, a15:0, i16:0, i16:1, 10Me17:0) to be higher in Lookout than Carpenter meadow.

Transfer effects at Carpenter

At Carpenter, fungal biomass was significantly higher in soils of forest (1.8 ± 0.3 mg g^{-1} soil) than meadow origin (0.4 ± 0.07 mg g^{-1} soil) and tended to be higher in soils incubated in forest (1.3 ± 0.4 mg g^{-1} soil) than in meadow environments (0.9 ± 0.2 mg g^{-1} soil), The fungal PLFA biomarker, 18:2ω6,9, did not differ between forest and meadow soils, but was significantly higher in soils incubated in the forest compared to meadow (Fig. 2). Although no significant difference was detected in the microscopy-derived fungal biomass of open and closed cores, the amount of 18:2ω6,9 significantly decreased in closed compared to open cores (Fig. 2). LH-PCR clearly indicated that the fungal community composition of both open and closed cores changed similarly upon transfer from forest to meadow, whereas in the case of the reciprocal transfer from meadow to forest, fungal community composition changed differently in open and closed cores (Fig. 3). Bacterial biomass levels were significantly higher

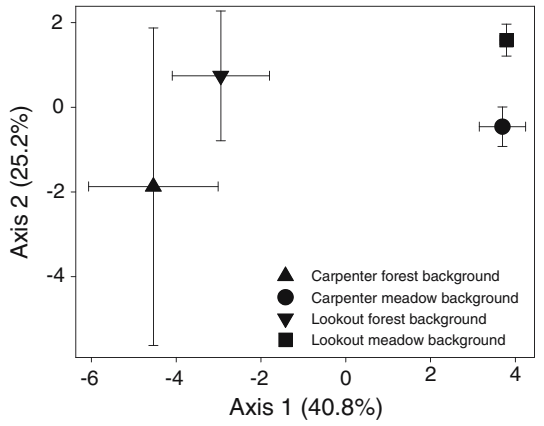

Fig. 1 An ordination plot derived from a principal components analysis of the mol % distribution of 40 PLFA extracted from background soil samples at Carpenter and Lookout forest and meadow sites, respectively. The percentages of variance explained by axes 1 and 2 are shown in parentheses. Each point represents the average of three replicate samples per site. A legend of symbols affiliated with each site is presented in the figure. Error bars represent the standard error of the mean

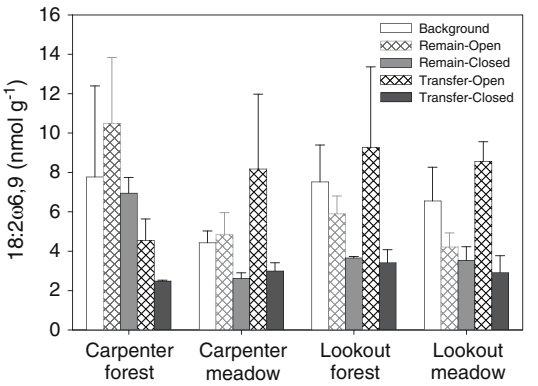

Fig. 2 Amount of the fungal biomarker PLFA 18:2ω6,9 extracted from soil of each treatment and at each of the sites. Label of *x*-axis designates soil origin. Shaded columns designate soil cores that were incubated in enclosed PVC pipe. Hatched columns represent soil cores that were incubated in open mesh screen bags. Error bars represent the standard error of the mean of three replicates

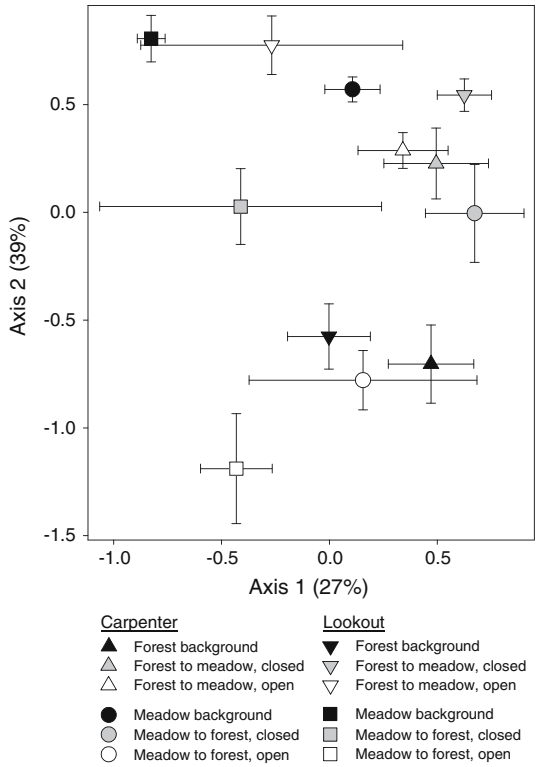

Fig. 3 An ordination plot derived from a nonmetrical multidimensional scaling (NMS) analysis of PCR amplified ITS fragments derived from fungal DNA extracted from soil of each treatment at each site. Error bars represent the standard error of the mean of three replicate samples per treatment per site. A legend of symbols affiliated with specific treatments at each site is presented in the figure. The definitions of the terms ''open'' and ''closed'' are the same as used in legend to Fig. 2

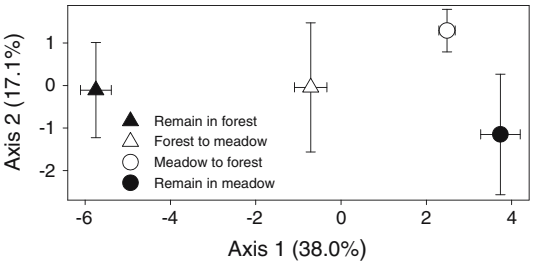

Fig. 4 An ordination plot derived from a principal components analysis of the mol% PLFA extracted from soil recovered from the various treatments at Carpenter. The percentages of variance explained by axes 1 and 2 are shown in parentheses. Each point represents the average of three replicate samples per treatment per site. Error bars represent the standard error of the mean. A legend of symbols describing the specific transfer treatments is presented in the figure

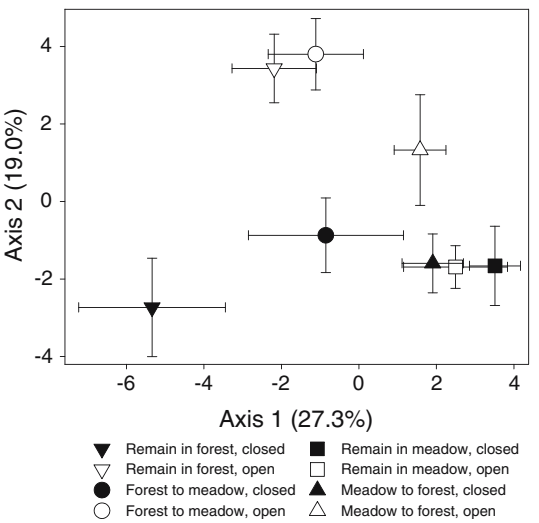

Fig. 5 An ordination plot derived from a principal components analysis of the mol% PLFA extracted from soil recovered from the various treatments at Lookout. The percentages of variance explained by axes 1 and 2 are shown in parentheses. Each point represents the average of three replicate samples per treatment per site. Error bars represent the standard error of the mean. A legend of symbols describing the specific transfer treatments is presented in the figure

($P < 0.05$) in closed vs. open forest soil cores regardless of transfer (0.14 ± 0.00 vs. 0.12 ± 0.00 mg g^{-1} soil). A PCA analysis of PLFA data clearly showed that transfer from forest to meadow had a major effect on the bacterial community, with a smaller effect of transfer from meadow to forest (Fig. 4). An MRPP analysis of PLFA data confirmed that the bacterial community of Carpenter forest soil cores changed in response to transfer to the meadow ($P = 0.003$, $A = 0.12$). Indicator species analysis showed that a significant decline ($P < 0.01$) occurred in the contributions of certain PLFA (16:1ω9, 16:1ω7, 17:0, cy17:0, 2-OH19:0) that was accompanied by a significant increase ($P < 0.01$) in the contributions of other PLFA (i16:0, 2-OH16:1, i17:0, 10Me18:0, 19:0) (Fig. 5). An MRPP analysis indicated no significant change in bacterial community structure of meadow soil cores transferred to the forest ($P = 0.51$, $A = –0.003$).

Transfer effects at Lookout

At Lookout, fungal biomass was higher in soils of forest (1.7 ± 0.2 mg g^{-1} soil) than meadow

origin (0.8 ± 0.1 mg g^{-1} soil) and tended to be higher in soils incubated in forest (1.5 ± 0.2 mg g^{-1} soil) than meadow environment (0.9 ± 0.2 mg g^{-1} soil). The fungal PLFA biomarker (18:2ω6,9) did not differ between forest and meadow soils or by location of incubation (Fig. 2), however. Although no significant difference was detected in the microscopy-derived fungal biomass of open and closed cores, the amount of 18:2ω6,9 significantly decreased in closed compared to open cores (Fig. 2). LH-PCR of the fungal community of cores transferred from forest to meadow showed that fungal community composition of open and closed cores changed differently, with open cores resembling meadow background whereas closed did not (Fig. 3). In the case of the reciprocal transfer from meadow to forest, the fungal community composition changed in both open and closed cores, with neither achieving the composition of Lookout forest. Bacterial biomass was significantly higher in closed vs. open cores regardless of soil origin or transfer (0.14 ± 0.00 vs. 0.18 ± 0.00) mg g^{-1} soil). A PCA of PLFA data suggested that the forest bacterial community structure changed in response to open and closed treatments; in addition, the community of closed cores showed some response to transfer (Fig. 5). This was confirmed by MRPP analysis, which revealed a significant open vs. closed core effect ($P = 0.06$, $A = 0.12$), with no particular indicator PLFA accounting for the effect; the effect of transfer on the community structure of closed forest soil cores was not significant ($P = 0.13$, $A = 0.04$). MRPP analysis indicated no significant change in bacterial community structure of meadow soil transferred to forest ($P = 0.23$, $A = -0.012$).

Root dry weight

There were clear trends for root dry weights to be significantly greater at Lookout than at Carpenter (Fig. 6). At Carpenter, root weights were significantly higher in cores of forest than meadow soil and when incubated in the meadow compared to the forest. Root weights were lower in cores transferred from meadow to forest, suggesting that root decomposition was much

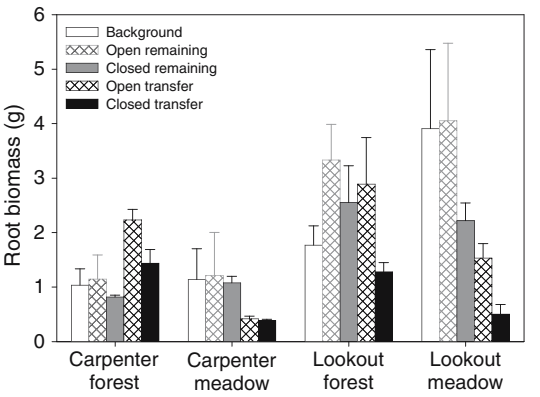

Fig. 6 Plant root dry weight biomass recovered from soil cores of the different treatments. Error bars represent the standard error of the mean of three replicates. Shaded columns designate soil cores that were incubated in enclosed PVC pipe. Hatched columns represent soil cores that were incubated in open mesh screen bags

greater than root in-growth; conversely, root weights of forest cores increased when transferred into the meadow, indicating root in-growth was greater than root decomposition. As at Carpenter, root dry weight was greater in Lookout meadow soil incubated in the meadow than in meadow soil incubated in the forest; however, forest soil at Lookout did not respond to location of incubation. In addition, at Lookout, root dry weight was significantly greater in open than closed cores, indicating that root decomposition was either more rapid in the closed cores or that root in-growth had occurred into open cores. A similar trend between open and closed cores was also observed at Carpenter.

Discussion

Meadow and forest bacterial community differences

Despite the levels of meadow and forest bacterial biomass being similar at Carpenter and Lookout, PLFA composition clearly separated the structures of the bacteria communities of the forests and the meadows. There was a strong trend for gram-positive PLFA to be higher in the meadow soils with high N mineralizing potential than in the forest soils with lower N

availability, and for some of the gram-positive PLFA biomarkers to be higher in the relatively N-rich soil of Lookout (higher total N, lower C:N ratio) than Carpenter meadow (Mintie et al. 2003; Rich et al. 2003). These findings can be compared to other reports that showed the relative amounts of total bacterial and fungal biomass, and the relative contribution of gram positive bacteria to the total bacterial community in forest soils correlated with differences in N availability (Grayston and Prescott 2005; Leckie et al. 2004). Kennedy et al. (2004) emphasized that N availability had a greater impact on bacterial community structure than did plant species composition in a grassland. It remains to be determined what might account for the success of the gram-positive bacterial populations in high elevation, moderately low pH meadow soils, and if they had a role in the ability of meadow bacterial populations to resist our experimental treatments more effectively than did the forest soil bacterial communities.

Bacterial community dynamics

At both sites bacterial biomass increased substantially in closed versus open forest remaining cores, and in closed versus open meadow cores inferring that enhanced C availability in closed cores allowed more growth to occur. Recently, several papers have shown that bacterial communities associated with tree roots are different from those in bulk soil (Frey-Klett et al. 2005; Sowerby et al. 1998), and these communities change in response to plant root senescence (Gomes et al. 2003), root trenching (Brant et al. 2006; Siira-Pietikäinen et al. 2001), and tree defoliation or felling (Cullings et al. 2005; Hernesmaa et al. 2005). While it is reasonable to believe that C availability is a major factor driving the increase in bacterial biomass, we also observed greater nitrification potential, inorganic N levels, and water content in closed vs. open forest remaining cores, and in meadow to forest transferred cores (Bottomley et al. 2004; Boyle et al. 2006), indicating that enhanced N availability and/or water status might also have played role(s) in the growth response of the bacterial communities in closed cores.

The different treatment responses of Carpenter and Lookout forest soil bacterial community structures indicates that vegetation and microclimate have different degrees of influence over community structure at the two sites. For example, at Carpenter, a similar bacterial community shift occurred in both open and closed transferred cores that was unaccompanied by bacterial biomass change, indicating that the meadow environment prevented the biomass increase observed in closed cores, and was the primary driver of community structural shift. On the other hand, at Lookout, the increase in bacterial biomass of closed and open remaining forest cores was accompanied by community structural shifts between both core types, yet, the shift occurring in closed transfer cores was unaccompanied by biomass increase and thereby resembled the situation at Carpenter. Because the transfer induced shift was not accompanied by significant change in any specific indicator PLFA, we conclude that Lookout meadow environment placed enough pressure on the forest soil bacterial community to prevent biomass increase but not enough to cause significant advantage or disadvantage to specific community members as occurred at Carpenter meadow. It is intriguing to wonder why the bacterial community structure of the younger Lookout forest was more responsive to vegetation disturbance than the bacterial population of the older Carpenter forest. Further studies are needed to determine if bacterial population structure in coniferous forest soils changes with successional stages, and if the responses of these soils to disturbance might differ because of their community structure.

Forest soil fungal community dynamics

Transfer of soil from forest to meadow also caused responses in the fungal communities that are noteworthy. At Carpenter, the similar transfer-induced shift of community structure in both core types resembled the response of the bacteria, further substantiating that the Carpenter meadow environment is much more influential than its vegetation on the composition of the microbial community. In contrast, at Lookout, fungal community structure changed differently in open and

closed cores (as did the bacteria), but in this case, while the fungal community of the open transferred cores resembled the meadow background after 2 year, the bacterial community of open cores did not. Interestingly, a major vegetation difference between the two meadow sites was the occurrence of bracken fern (*Pteridium aquilinum* L.) at Carpenter and legume species at Lookout. Further work is needed to determine what influence specific components of the vegetation might have had on the different community responses obtained at the two sites.

Meadow soil fungal community dynamics

Both meadow fungal communities were quite responsive to transfer to the forest. These changes in fungal community were mirrored by similar increases in both microscopy-derived fungal biomass, and 18:2ω6,9 content of open cores by 170–200%. In closed cores, however, there was little change (80–110%) in either measure of fungal biomass. Future studies should be directed at determining the relative contributions of ectomycorrhizal and saprotrophic fungi to the new communities (Wu et al. 2003, 2005). Second, we need to determine if the community shift is dependent or independent of: (a) tree root penetration, (b) hyphal invasion from adjacent forest soil, or (c) if the shift is due to germination of spores of forest fungal species that had lain dormant in the meadow soil. Interestingly, at Carpenter, we noted a correlation between fungal biomass decrease (measured by a decline in 18:2ω6,9 content), and change in fungal community structure, with a corresponding decrease in mineralizable C upon forest to meadow transfer (Boyle et al. 2006). In the reciprocal transfer, mineralizable C of meadow soil increased to forest soil levels and was accompanied by increases of fungal biomass in open cores and of bacterial biomass in closed cores (Boyle et al. 2006). Further work is needed to determine if the transfer-induced changes in C mineralization at Carpenter are linked to the changes in fungal and/or bacterial biomass and community composition.

Balser and Firestone (2005) obtained evidence of transfer effects on the composition of a forest soil microbial community when soil cores were transferred from a high-elevation coniferous forest (1,240 m) to a lower-elevation meadow site (~470 m), and also saw little effect of the reciprocal transfer from meadow to forest. The authors suggested that the site differences might be explained if the forest community was less able to deal with the extremes of temperature and water content associated with the meadow environment. Although we observed similar overall trends as Balser and Firestone (2005), our cores were transplanted without elevation change, and community responses were unlikely to be caused by large changes in temperature range. Several studies have reported that small changes in air/soil temperature can have large influences on soil processes and litter decomposition (Bottner et al. 2000; Fierer et al. 2005a; Hart and Perry 1999). Further experiments are required to examine the temperature and moisture sensitivity of mineralizing processes in these soils and their associated community dynamics.

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References

Anderson JPE, Domsch KH (1973) Quantification of bacterial and fungal contribution to soil respiration. Arch Microbiol 93:113–127

Anderson IC, Cairney JWG (2004) Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. Environ Microbiol 6:769–779

Axelrood PE, Chow ML, Radomski CC, McDermott JM, Davies J (2002) Molecular characterization of bacterial diversity from British Columbia forest soils subjected to disturbance. Can J Microbiol 48:655–674

Bååth E, Anderson T-H (2003) Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biol Biochem 35:955–963

Balser TC, Firestone MK (2005) Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. Biogeochemistry 73:395–415

Bottner P, Couteaux M-M, Anderson J M, Berg B, Billes G, Bolger T, Casabianca H, Romanya J, Rovira P (2000) Decomposition of ^{13}C-labelled plant material in a European 65–40° latitudinal transect of coniferous forest soils: simulation of climate change by translocation of soils. Soil Biol Biochem 32:527–543

Bottomley PJ, Taylor AE, Boyle SA, McMahon SK, Rich JJ, Cromack Jr. K, Myrold DD (2004) Responses of nitrification and ammonia oxidizing bacteria to reciprocal transfers of soil between adjacent coniferous forest and meadow vegetation in the Cascade Mountains of Oregon. Microb Ecol 48:500–508

Boyle SA, Rich JJ, Bottomley PJ, Cromack Jr. K, Myrold DD (2006) Reciprocal transfer effects on denitrifying community composition and activity at forest and meadow sites in the Cascade Mountains of Oregon. Soil Biol Biochem 38:870–878

Brant J B, Myrold DD, Sulzman EW (2006) Root controls on soil microbial community structure in forest soils. Oecologia DOI:10.1007/s00442-006-0402-7

Brodie E, Edwards S, Clipson N (2003) Soil fungal community structure in a temperate upland grassland soil. FEMS Microbiol Ecol 45:105–114

Butler JL, Williams MA, Bottomley PJ, Myrold DD (2003) Microbial community dynamics associated with rhizosphere C flow. Appl Environ Microbiol 69: 6793–6800

Cullings K, Raleigh C, New MH, Henson J (2005) Effects of artificial defoliation of pines on the structure and physiology of the soil fungal community of a mixed pine-spruce forest. Appl Environ Microbiol 71:1996–2000

DeForest JL, Zak DR, Pregitzer KS, Burton AJ (2004) Atmospheric nitrate deposition and the microbial degradation of cellobiose and vanillin in a northern hardwood forest. Soil Biol Biochem 36:965–971

Fierer N, Craine JM, Mclauchlan K Schimel JP (2005a) Litter quality and the temperature sensitivity of decomposition. Ecology 86:320–326

Fierer N, Jackson JA, Vilgalys R, Jackson RB (2005b) Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl Environ Microbiol 71:4117–4120

Frey-Klett P, Chavatte M, Clausse M-L, Courrier S, Le Roux C, Raaijmakers J, Giovanna Martinotti M, Pierrat J-C, Garbaye J (2005) Ectomycorrhizal symbiosis affects functional diversity of rhizosphere fluorescent pseudomonads. New Phytol 165: 317–328

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Gomes NCM, Fagbola O, Costa R, Rumjanek N G, Buchner A, Mendona-Hagler L, Smalla K (2003) Dynamics of fungal communities in bulk and maize rhizosphere soil in the tropics. Appl Environ Microbiol 69:3758–3766

Grayston SJ, Prescott CE (2005) Microbial communities in forest floors under four tree species in coastal British Columbia. Soil Biol Biochem 37:1157–1167

Hart SC, Perry DA (1999) Transferring soils from high to low-elevation forests increases nitrogen cycling rates: climate change implications. Global Change Biol 5:23–32

Hernesmaa A, Björklöf K, Kiikkilä O, Fritze H, Haahtela K, Romantschuk M (2005) Structure and function of microbial communities in the rhizosphere of Scots pine after tree-felling. Soil Biol Biochem 37:777–785

Ingham ER, Griffiths RP, Cromack Jr. K, Entry JA (1991) Comparison of direct vs. fumigation incubation microbial biomass estimates from ectomycorrhizal mat and non-mat soils. Soil Biol Biochem 23:465–471

Kennedy N, Brodie E, Connolly J, Clipson N (2004) Impact of lime, nitrogen and plant species on bacterial community structure in grassland microcosms. Environ Microbiol 6:1070–1080

Kennedy N, Connolly J, Clipson N (2005) Impact of lime, nitrogen and plant species on fungal community structure in grassland microcosms. Environ Microbiol 7:780–788

Leckie SE, Prescott CE, Grayston SJ, Neufeld JD, Mohn WW (2004) Characterization of humus microbial communities in adjacent forest types that differ in nitrogen availability. Microb Ecol 48:29–40

Lodge DJ, Ingham E (1991) A comparison of agar film techniques for estimating fungal biovolumes in litter and soil. Agric Ecosys Environ 34:131–144

McCune B, Grace JB (2002) Analysis of ecological communities. MjM Software, Gleneden Beach, Oregon

Mintie AT, Heichen RS, Cromack Jr. K, Myrold DD, Bottomley PJ (2003) Ammonia oxidizing bacteria along meadow-to-forest transects in the Oregon Cascade Mountains. Appl Environ Microbiol 69:3129–3136

Phillips RL, Zak DR, Holmes WE, White DC (2002) Microbial community composition and function beneath temperate trees exposed to elevated atmospheric carbon dioxide and ozone. Oecologia 131:236–244

Rich JJ, Heichen RS, Bottomley PJ, Cromack Jr. K, Myrold DD (2003) Community composition and functioning of denitrifying bacteria from adjacent meadow and forest soils. Appl Environ Microbiol 69:5974–5982

Schadt CW, Martin AP, Lipson DA, Schmidt SK (2003) Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science 301:1359–1361

Schnürer J, Clarholm M, Rosswall T (1985) Microbial biomass and activity in an agricultural soil with different organic matter contents. Soil Biol Biochem 17:611–618

Siira-Pietikäinen A, Haimi J, Kanninen A, Pietikäinen J, Fritze H (2001) Responses of decomposer community to root isolation and slash. Soil Biol Biochem 33:1993–2004

Sowerby A, Emmett B, Beier C, Tietema A, Penuelas J, Estiarte M, Van Meeteren JM, Timonen S, Jørgensen KS, Haahtela K, Sen R (1998) Bacterial community

structure at defined locations of *Pinus sylvestris-Suillus bovinus* and *Pinus sylvestris-Paxillus involutus* mycorrhizospheres in dry pine forest humus and nursery peat. Can J Microbiol 44:499–513

Waldrop MP, Firestone MK (2004a) Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities. Oecologia 138:275–284

Waldrop M P, Firestone MK (2004b) Altered utilization patterns of young and old soil C by microorganisms caused by temperature shifts and N additions. Biogeochemistry 67:235–248

White DC, Ringelberg DB (1998) Signature lipid biomarker analysis. In: Burlage RS, Atlas D, Stahl D, Geesey G, Saylor G (eds) Techniques in microbial ecology. Oxford University Press, New York, pp 255–272

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninksky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, New York, pp 315–322

Wu T, Sharda JN, Koide RT (2003) Exploring interactions between saprotrophic microbes and ectomycorrhizal fungi using protein-tannin complex as an N source by red pine (*Pinus resinosa*). New Phytol 159:131–139

Wu T, Kabir Z, Koide RT (2005) A possible role for saprotrophic microfungi in the N nutrition of ectomycorrhizal *Pinus resinosa*. Soil Biol Biochem 37:965–975