Fungal and bacterial communities across meadow–forest ecotones in the western Cascades of Oregon

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Abstract: Meadows are natural dynamic features of forested mountain landscapes of the Pacific Northwest. Proportions of meadows and forests change with environmental conditions and disturbance history. We investigated the belowground microbial communities associated with these two vegetation types and how they change across the meadow–forest transition at two sites in Oregon. Soils were sampled along replicate transects extending from meadow into forest. We quantified total bacterial and fungal biomass using direct microscopy and described the composition of bacterial and fungal communities using a DNA-based fingerprinting technique. Bacterial biomass was similar in meadow and forest soils, but fungal biomass was significantly higher in forest soil. Meadow and forest soils had distinct communities of bacteria and fungi. Bacterial communities near the meadow–forest boundary reflected current vegetation, but fungal communities under meadow vegetation near the forest edge were intermediate in composition between those found in meadow and forest soils. The more gradual transition observed with fungal communities may reflect the influence of tree roots and their associated ectomycorrhizal fungi or possibly colonization by saprotrophic fungi associated with tree litter accumulating near the forest edge. Invasion of forest-associated fungi into the meadow soils may presage subsequent expansion of forest vegetation into meadows.

Résumé : Les prés sont des particularités naturelles et dynamiques des paysages montagneux du Pacific Northwest. Les proportions de pré et de forêt varient selon les conditions environnementales et l'historique des perturbations. Nous avons étudié les communautés microbiennes du sol associées à ces deux types de végétation et la façon dont elles changent dans la zone de transition entre le pré et la forêt à deux endroits en Oregon. Le sol a été échantillonné le long de transects répétés allant du pré vers la forêt. Nous avons quantifié la biomasse totale de bactéries et de champignons à l'aide de la microscopie directe et nous avons décrit la composition des communautés de bactéries et de champignons à l'aide d'une technique d'identification génétique. La biomasse bactérienne était semblable dans les sols du pré et de la forêt mais la biomasse fongique était significativement plus importante dans le sol de la forêt. Des communautés distinctes de bactéries et de champignons occupaient les sols du pré et de la forêt. Les communautés bactériennes situées près de la limite entre la forêt et le pré reflétaient la végétation actuelle mais les communautés fongiques situées sous la végétation du pré, près de la lisière de la forêt, avaient une composition intermédiaire entre la composition observée dans les sols du pré et de la forêt. La transition plus graduelle observée dans le cas des communautés fongiques reflète possiblement l'influence des racines des arbres et des champignons ectomycorhiziens qui leurs sont associés ou la colonisation par des champignons saprophytes associés à la litière des arbres qui s'accumule en bordure de la forêt. L'invasion de champignons associés à la forêt dans le sol des prés pourrait présager une expansion subséquente de la végétation forestière dans les prés.

[Traduit par la Rédaction]

Introduction

Meadows are prominent features in the mountains of the Pacific Northwest. Ninety-five percent of the central western Cascade Mountains of Oregon (hereinafter the Cascades) are forested (Hickman 1976). Much of the remaining 5% of non-forested land is composed of meadows. Although meadows compose a small percentage of the geographic area, they contain the majority of the plant diversity, up to 85%, in the Cascades (Hickman 1976). Some meadows are relatively stable; whereas, others are ephemeral. Pollen records indicate that meadows in the Olympic Range in Washington

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have persisted for thousands of years (Gavin and Brubaker 1999). Recent studies of conifer invasion of meadows indicate that other meadows are subject to change over time. Conifer invasion of meadows in the Cascades has been attributed to various factors, such as the cessation of sheep grazing (Hickman 1976; Miller and Halpern 1998; Griffiths et al. 2005).

The boundary areas, or ecotones, between mesic meadows and forests in the Pacific Northwest are dynamic (Hickman 1976; Miller and Halpern 1998). It is likely that many factors contribute to the ebb and flow of the forest boundary. Abiotic differences exist between the forests and meadow, grassland, or pasture, such as temperature, moisture, wind, and exposure to solar radiation (Murcia 1995). Until recently, the majority of the studies of forest edges have explored only aboveground factors, but belowground processes can be equally dynamic. Ectomycorrhizal fungi are of particular interest, because a source of ectomycorrhizal inoculum may be required for conifers to become established in meadows. For example, Cline et al. (2005) planted Douglas-fir seedlings (*Pseudotsuga menziesii* (Mirb.) Franco) <6 m and >16 m from mature trees and found that the diversity of ectomycorrhizal fungi on roots of seedlings declined with distance from the mature trees. The ectomycorrhizal communities on the roots of seedlings planted near mature trees were similar to those of the mature trees; whereas, seedlings planted far away from the mature trees had communities similar to what one would expect to find on conifer seedlings grown in a greenhouse. Similarly, Dickie and Reich (2005) found that the presence of ectomycorrhizal mycelium at blue oak (*Quercus macrocarpa* Michx.) forest edges was an important factor in facilitating tree seedling colonization and the invasion of trees into open habitat. These works suggested that there is a gradient of ectomycorrhizal fungal inoculum radiating out from the forest edge.

Studies have also shown that soil properties can change as conifers invade meadows. Griffiths et al. (2005) found that conifer invasion into meadows in the Cascades changed N availability and cycling in the transition zones to be more like those of the forest. These observations were supported by subsequent work on nitrification and denitrification in adjacent forests and meadows (Mintie et al. 2003; Rich et al. 2003). The goal for this study was to link belowground to aboveground communities by examining the corresponding changes in fungal and bacterial communities with changes in vegetation across meadow–forest ecotones in the Cascades.

Materials and methods

Site characteristics and sampling

Study sites were located 1500 m a.s.l. at the H.J. Andrews Experimental Forest Long Term Ecological Research site (44.2°N, 122.2°W) in the Cascades, Oregon, USA. Two high montane sites with adjacent meadow and forest communities were selected and will be referred to as Carpenter and Lookout. General vegetation and soil characteristics of these sites have been reported previously as part of studies on the effect of contrasting vegetation types on the activity and composition of ammonia-oxidizing and denitrifying bacteria (Mintie et al. 2003; Rich et al. 2003). The following provides more detailed descriptions of the ground vegetation and soil properties across the vegetation transitions at these sites.

The dominant grass at Lookout meadow was *Festuca idahoensis* Elmer, which was in very low abundance at Carpenter meadow. Other dominant meadow vegetation at both sites included arbuscular mycorrhizal taxa such as *Bromus carinatus* Hook. & Arn., *Elymus glaucus* Buckl., *Pteridium aquilinum* (L.) Kuhn, *Eriophyllum lanatum* (Pursh.) Forbes, and *Anaphalis margaritacea* (L.) Benth. & Hook. Vegetation at Lookout meadow was more diverse than at Carpenter meadow. Several species, including *Solidago canadensis* L., *Phacelia heterophylla* Pursh, *Gilia capitata* Sims, *Polygonum bistortoides* Pursh, and *Zigadenus venenosus* Wats., occurred only at Lookout meadow. In addition, soil was more disturbed at Carpenter meadow, presumably because of large animal activity (we observed several large burrows >30 cm in diameter).

Dominant forest trees at both sites were the ectomycorrhizal taxa *P. menziesii*, Amabilis fir (*Abies amabilis* (Dougl. ex Loud.) Dougl. ex J. Forbes), grand fir (*Abies grandis* (Dougl. ex D. Don) Lindl.), and mountain hemlock (*Tsuga mertensiana* (Bong.) Carrière). *Achlys triphylla* (Sm.) DC and *Xerophyllum tenax* (Pursh) Nutt. were the most common understory plants at both Carpenter and Lookout forests, although both were found in lower abundance at Lookout forest. The understory of Carpenter forest was more developed and had a greater diversity of plant species than Lookout forest. Understory plants found only in Carpenter forest include *Anemone lyallii* Britton, *Maianthemum dilatatum* (Alph. Wood) A. Nelson & J.F. Macbr., *Ribes lacustre* (Pers.) Poir., and *Vaccinium parvifolium* Sm.

The soils at both sites were relatively young, poorly developed, well-drained, and rich in organic matter. Meadow soils were Lithic Cryandepts with 15% surface rock at Lookout meadow; forest soils were Pachic Haplumbrepts (Rich 2003). In general, soil organic C and C/N ratios were higher in forest soils, whereas inorganic N concentrations were higher in meadow soils (Table 1).

Three parallel transects were established perpendicular to the meadow–forest boundary at each site. Transects were spaced 20 m apart and sampled at eight evenly spaced points along each transect with one exception: transect 1 at Carpenter was placed 100 m above transect 2, owing to the presence of a hiking trail. Three sampling points along each transect were in the meadow, two in the meadow–forest transition zone, and the final three were in the forest. Sampling points were 20 m apart at Carpenter and 10 m apart at Lookout, to account for the wider transition zone and greater forest canopy height at Carpenter. At the end of the growing season in September, we collected five soil cores (6 cm inner diameter, 15 cm depth) within a 0.5 m radius of each sampling point. Litter and humus were removed from all samples and the remaining mineral soil from the cores was pooled into a composite sample for each sampling point. There were 48 samples in total. Samples were stored on ice following collection in the field, refrigerated at 4 °C in the laboratory, and sieved (using a 4.75 mm mesh) within 24 h after sampling. Gravimetric water content was determined for each of the pooled transect samples by drying at 105 °C

Table 1. Soil characteristics (mean ± SE, n = 3) along transects at Carpenter and Lookout.

Transect position (m)	Organic C ($g \cdot kg^{-1}$)	C/N ratio	pH	NH_4^+ (mg $N \cdot kg^{-1}$)	NO_3^- (mg $N \cdot kg^{-1}$)	H_2O ($kg \cdot kg^{-1}$)
Carpenter						
–70	91.5±14.3	13.4±0.02	6.0±0.2	4.1±0.5	3.0±0.7	0.32±0.05
–50	86.7±16.4	12.7±0.13	5.5±0.0	4.1±0.7	2.7±0.5	0.26±0.02
–30	88.3±12.5	13.0±0.35	5.8±0.1	5.9±1.5	3.7±0.7	0.30±0.01
–10	94.6±6.6	14.0±0.58	6.1±0.1	4.2±0.6	2.2±0.7	0.32±0.02
10	128.4±7.1	18.7±1.43	5.7±0.1	1.5±0.1	1.5±0.2	0.34±0.02
30	116.6±7.8	23.0±1.26	5.8±0.1	1.3±0.1	1.2±0.2	0.31±0.00
50	147.8±25.6	24.7±1.36	6.0±0.1	1.5±0.1	1.7±0.2	0.31±0.02
70	125.6±12.0	23.7±1.41	5.8±0.1	1.4±0.1	1.4±0.2	0.30±0.02
Lookout						
–35	112.4±7.1	12.7±0.95	5.6±0.1	2.7±0.8	4.4±0.9	0.24±0.08
–25	103.8±6.4	13.1±0.44	5.7±0.2	2.1±0.5	3.4±0.9	0.24±0.04
–15	117.9±14.9	12.0±0.43	5.8±0.1	2.6±0.6	3.3±0.8	0.25±0.06
–5	125.2±16.8	12.7±0.98	5.8±0.0	3.0±0.8	2.7±0.5	0.28±0.08
5	119.0±8.2	16.0±2.32	5.7±0.1	1.6±0.2	2.0±0.5	0.30±0.05
15	133.8±16.4	15.0±1.18	5.8±0.0	1.6±0.2	2.1±0.2	0.26±0.05
25	173.6±11.7	17.8±2.11	5.6±0.1	2.1±0.3	1.9±0.3	0.30±0.08
35	155.1±10.0	15.4±0.74	5.7±0.1	1.5±0.1	1.7±0.0	0.32±0.06

Note: Positions reflect distances from the meadow–forest boundary, with negative numbers representing points under meadow vegetation and positive numbers representing points under forest vegetation.

for 24 h. Aliquots of soil for DNA extraction were frozen at –80 °C immediately after sieving and remained frozen until DNA extraction was carried out (in 2000 for the 16S dataset and in 2004 for the fungal ITS dataset).

Microscopic estimates of microbial biomass

Microscopy was used to determine biomass for bacteria and fungi. Fresh soil (1 g) was placed in 9 mL of 0.2 $mol \cdot L^{-1}$ PO_4 buffer at pH 7.2. For fungi, an agar film was prepared using 0.5 mL of the 1:10 soil suspension and 1 mL of liquefied 1.5% (w/v) agar (Lodge and Ingham 1991). Differential interference contrast microscopy (200×) was used to count hyphal lengths. To quantify bacteria, a 1:100 soil suspension was made. Cells were stained with fluorescein isothiocyanate and filtered onto a 0.4 μm polycarbonate filter. Epifluorescent microscopy at 1000× with oil immersion was used to examine filters (Babiuk and Paul 1970). Bacterial and fungal biomass were calculated from the volume of bacterial cells or fungal hyphae in 1 g of dry soil, using visual microscopic estimates. We assumed a mean bacterial cell density of 330 $mg \cdot cm^{-3}$ and a mean fungal tissue density of 410 $mg \cdot cm^{-3}$ (Ingham et al. 1991). Data were analyzed using a blocked, one-way analysis of variance with Tukey's all pairs comparison at $\alpha = 0.05$ in SAS version 8.2 (SAS Institute Inc., Cary, North Carolina).

Soil DNA extraction

DNA was extracted from 0.5 g of soil (fresh mass) using the FastDNA® kit (Bio 101, Inc., Irvine, California) according to the manufacturer's directions. Extracted DNA was checked by running 10 μL of extract on an agarose gel (1%) stained with ethidium bromide (0.5 $\mu g \cdot mL^{-1}$). DNA was quantified on a BioSpec-1601 DNA/Protein/Enzyme Analyzer (Shimadzu Corporation, Kyoto, Japan). Extracts were diluted to 25 ng $DNA \cdot \mu L^{-1}$ for PCR amplification.

Fungal length heterogeneity (LH) PCR

DNA was amplified using primers for the internal transcribed spacer (ITS) region of the nuclear ribosomal gene: ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). Reaction mixtures (50 μL) contained soil DNA (100 ng), AmpliTaq DNA polymerase (2.5 U), GeneAmp PCR buffer (1X), $MgCl_2$ (2 $mmol \cdot L^{-1}$), deoxynucleoside triphosphates (0.2 $mmol \cdot L^{-1}$ each), forward and reverse primers (0.2 $\mu mol \cdot L^{-1}$ each), and bovine serum albumin (0.064 $g \cdot mL^{-1}$). The forward primer, ITS1-F, was labeled with 6-FAM (6-carboxyfluorescein) for LH-PCR (Suzuki et al. 1998). PCR amplification was conducted as follows using a PTC-100 hot bonnet thermocycler (MJ Research, Inc., Waltham, Massachusetts): 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min. A final extension followed at 72 °C for 2 min. PCR products were visualized with electrophoresis on 1% agarose gels stained with ethidium bromide. Samples were diluted to 1 $ng \cdot \mu L^{-1}$ and 1 μL of PCR product was submitted for sequencing on an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Inc., Foster City, California) capillary sequencer in the Central Services Laboratory (Center for Genome Research and Biocomputing, Oregon State University, Corvallis, Oregon). PCR products were run on the Genetic Analyzer along with X-Rhodamine MapMarker™ 1000 internal lane size standard (BioVentures, Inc., Murfreesboro, Tennessee).

Bacterial 16S LH-PCR

Purified DNA (10 ng) was amplified by PCR as described by Ritchie et al. (2000). A 6-FAM (6-carboxyfluorescein) labeled forward primer 27F (Hicks et al. 1992) and an unlabeled reverse primer 338R (Amann et al. 1990), which are considered specific for eubacteria, were used to amplify the 16S rRNA gene. The reactions were performed using 50 μL (final volume) mixtures containing PCR buffer (1X), bovine

serum albumin (0.06%), $MgCl_2$ (1.5 mmol·L^{-1}), deoxynucleoside triphosphates (0.2 mmol·L^{-1} each), forward and reverse primers (0.2 mmol·L^{-1} each), and *Taq* DNA polymerase (2U). Initial denaturation at 94 °C for 3 min was followed by 25 cycles consisting of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s, and extension at 72 °C for 2 min. There was a final extension step that consisted of 72 °C for 7 min. For each sample, 1 ng of amplified DNA was separated by length by using Long Ranger (FMC, Rockland, Maine) polyacrylamide gel electrophoresis and an ABI automated DNA sequencer with GeneScan v2.1 software (Applied Biosystems, Inc., Foster City, California) at the Central Services Laboratory. Lengths (in base pairs) were calculated using a size standard (GeneScan 400Rox or GeneScan 2500Rox; Applied Biosystems, Inc.). The size standard was added to every lane of the gel for increased precision.

Fungal ITS and Bacterial 16S community data analysis

Size and relative abundance of LH-PCR fragments were quantified using GeneScan® version 3.5 software and Genotyper® version 2.5 software (Applied Biosystems, Inc.). Sequence length and peak area data were obtained using Genotyper® version 2.5. Data were downloaded into Microsoft® Excel and manually binned. Ordinations of LH-PCR fragment data were run in PC-ORD Version 4.36 (MJM Software, Gleneden, Oregon) using nonmetric multidimensional scaling (NMS) using the "slow and thorough" autopilot feature with the Sørensen distance measure. NMS was chosen because it avoids the assumption of linear relationships among variables and allows the use of any relativization method (McCune and Grace 2002). Analyses were run on the entire data set (both Lookout and Carpenter), by individual site (Lookout or Carpenter only), and then by site excluding samples collected in the transition zones. For the dataset containing all sites, columns (LH-PCR fragments) containing fewer than three nonzero values were deleted. The dataset was then relativized by row totals (samples) to adjust for differences in fluorescence levels of amplified fragments.

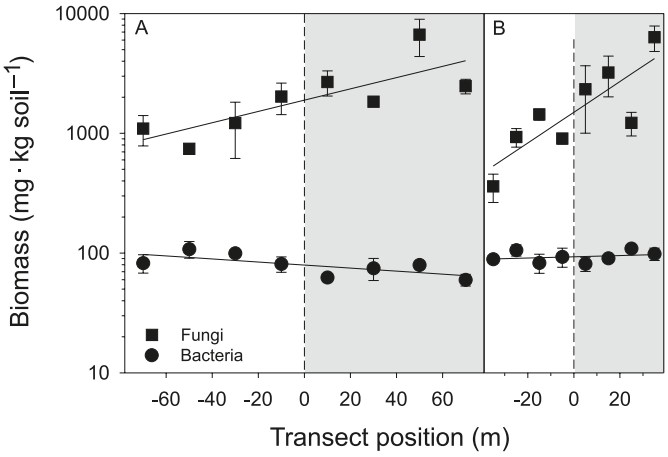
Multiresponse permutation procedures (MRPP) were used to test for differences in fungal and bacterial community composition between vegetation types and sites. We tested for the presence of edge effects by comparing meadow and forest samples to samples collected from each side of the meadow–forest boundary. MRPP is a nonparametric method that is used to test for group differences (McCune and Grace 2002). MRPP in the statistics package PC-ORD Version 4.36 (MJM Software) was used with the Sørensen (Bray–Curtis) distance measure.

Results

Fungal and bacterial biomass

Fungal biomass was 10–50× greater than bacterial biomass (Fig. 1). There were significant trends between transect position and fungal biomass at both sites, with fungal biomass significantly greater under forest compared with meadow vegetation at both sites. Bacterial biomass was not significantly correlated to transect position, although bacterial biomass at Carpenter was significantly lower under forest than meadow vegetation.

Fig. 1. Biomass of bacteria and fungi as a function of transect position at Carpenter (A) and Lookout (B). Numbers on the *x*-axis represent distance from the meadow–forest boundary. Data are given as mean ± SE (n = 3). The meadow–forest boundary is designated by the broken line, with the white area representing soils sampled under meadow vegetation and the shaded area representing soils sampled under forest vegetation. Lines represent linear regressions of biomass with transect position. At Carpenter, r = 0.272 and p = 0.515 for bacteria and r = 0.822 and p = 0.012 for fungi; at Lookout, r = –0.705 and p = 0.051 for bacteria and r = 0.791 and p = 0.019 for fungi.



LH-PCR: fungi

We examined a range of LH-PCR ITS fragments from 400 to 900 base pairs in length. NMS ordinations of fungal community data yielded a three-dimensional solution for the combined sites. Meadow and forest sites separated along Axis 1, which explained 38.2% of the variation in the ordination; Axis 2 explained an additional 16.0% of the variation but was not related to transect position (Fig. 2). Fungal communities at the two meadow sites grouped together in the NMS ordination, whereas fungal communities differed between Carpenter and Lookout forest sites despite greater heterogeneity of fragments. Samples collected in the meadow transition zone fell between the meadow and forest samples, whereas samples collected in the forest transition zone grouped with the forest samples. The Lookout transition samples were more variable than those from Carpenter.

Indicator species analysis of fungal LH-PCR ITS fragments showed differences in several fragments going from meadow to transition to forest (Fig. 3). There were several indicator fragments for the meadows (520, 578, 614, and 688 base pairs), no indicator fragments for the meadow or forest transition zones, and one indicator fragment for the forests (692 base pairs). In addition, there were some differences in indicator fragments between meadow sites (520 and 578 base pairs).

LH-PCR: bacteria

We examined a range of 16S rRNA gene fragments from 314 to 362 base pairs in length. NMS analysis yielded a two-dimensional ordination. Meadow and forest samples separated along Axis 1, which explained 83.0% of the variation in the ordination (Fig. 4). Bacterial communities of meadows differed between Carpenter and Lookout, as did

Fig. 2. NMS ordination plot of fungal communities based on LH-PCR of the ITS region of the ribosomal gene. The first two axes of a three-dimensional solution are shown; values in parentheses represent the variance explained by each axis. Data are given as mean ± SE (n = 9 for forest and meadow samples, n = 3 for transition zone samples).

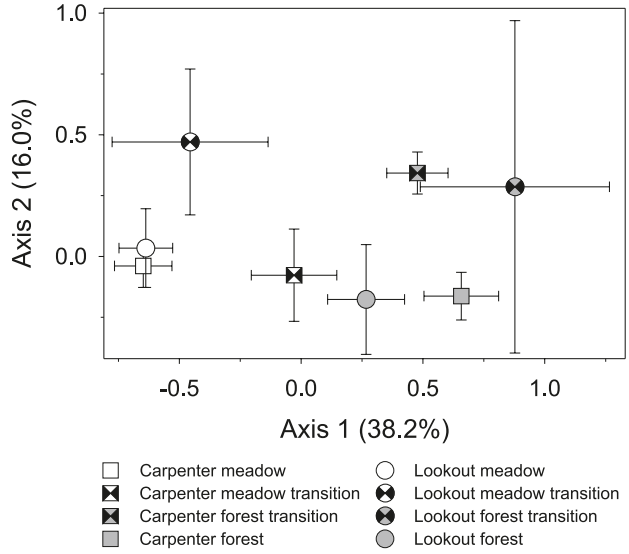
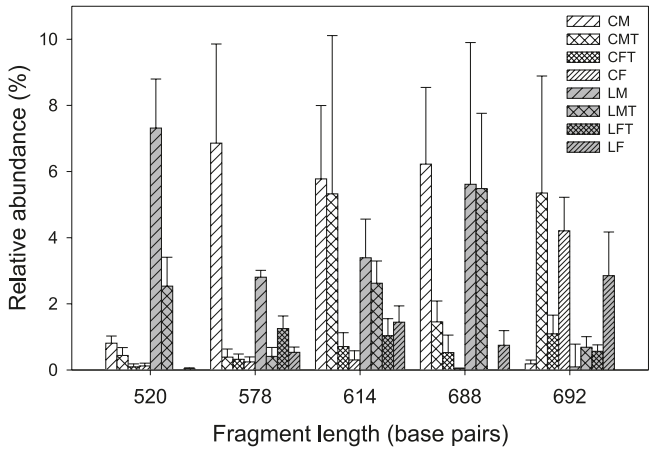


Fig. 3. Examples of indicator LH-PCR fragments of the fungal ITS region that differentiated between soils from meadow, transition, and forest sites. Data are given as mean ± SE (n = 9 for forest and meadow samples, n = 3 for transition zone samples). CM, Carpenter meadow; CMT, Carpenter meadow transition; CFT, Carpenter forest transition; CF, Carpenter forest; LM, Lookout meadow; LMT, Lookout meadow transition; LFT, Lookout forest transition; LF, Lookout forest.

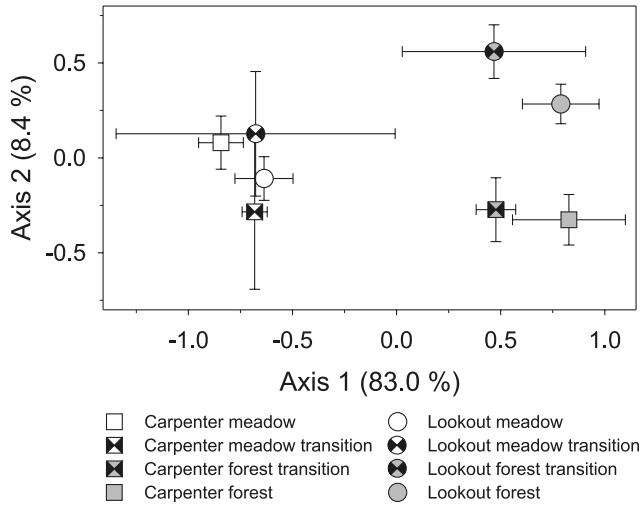


the forest bacterial communities. Meadow transition samples were similar to their respective meadow sites; forest transition samples were similar to their respective forest sites.

Analysis of edge effects at individual sites

MRPP confirmed the edge effects apparent in the ordinations of the fungal communities at both Carpenter and Lookout (Fig. 2). Meadow and forest fungal communities were different at both Carpenter and Lookout ($p < 0.0001$); however, the meadow and forest transition fungal communities

Fig. 4. NMS ordination plot of bacterial communities based on LH-PCR of the 16S rRNA gene. The two axes represent a two-dimensional solution; values in parentheses represent the variance explained by each axis. Data are given as mean ± SE (n = 9 for forest and meadow samples, n = 3 for transition zone samples).



were not different from each other at either Carpenter or Lookout ($p > 0.05$) or from their respective meadow and forest sites ($p > 0.05$), indicating a gradual transition in fungal communities from meadow to forest. For bacterial communities, significant differences were also found between meadows and forests ($p < 0.0001$). Meadow and forest transition bacterial communities were different from each other at Carpenter ($p < 0.05$) but not different from each other at Lookout ($p > 0.05$), which indicates an abrupt change at Carpenter but a more gradual change in communities at Lookout. Transition zones were not different from their respective meadow and forest sites ($p > 0.05$) for bacterial communities.

Discussion

Bacterial biomass was similar in meadows and forests, but fungal biomass tended to increase along the transects from meadows to forests. Fungal-to-bacterial biomass increased 3.5-fold at Lookout and 5-fold at Carpenter along the transects from meadow to forest. A study of microbial biomass along a subtropical coniferous forest to subalpine meadow gradient in Taiwan found highest values of microbial biomass C in the forest, intermediate values in the transition zone, and the lowest values in the meadow (Imberger and Chiu 2001). They also found that ergosterol was highest in the forest, 4-fold higher than in the transition zone and 7-fold higher than in the meadow, indicating increasing fungal biomass from meadow to forest. In Finland, fungal phospholipid fatty acids (PLFA) increased in Scots pine (*Pinus sylvestris*) L. and Norway spruce (*Picea abies* (L.) Karst.) stands with stand age, although bacterial PLFAs were variable (Pennanen et al. 1999).

Although bacterial biomass was relatively unaffected by vegetation type, we found that bacterial community composition differed significantly between meadows and forests. This finding is consistent with many previous studies that have shown that bacterial communities vary among soils oc-

cupied by different plant communities (e.g., Kowalchuk et al. 2002; Kuske et al. 2002; Leckie et al. 2004; Wardle et al. 2004). Bacterial communities of the transition zones in this study reflected those found in soils under the current vegetation, resulting in an abrupt shift in community structure at the meadow–forest boundary. Although it is possible that the application of LH-PCR using the 16S rRNA gene in this study may not be as discriminating as other fingerprinting methods, LH-PCR has been used successfully in several studies (Ritchie et al. 2000; Mills et al. 2007). Furthermore, previous work along these same transects using terminal restriction fragment length polymorphisms (T-RFLP) analysis of functional genes found similar abrupt changes in communities of ammonia-oxidizing and denitrifying bacteria (Mintie et al. 2003; Rich et al. 2003).

Like bacterial communities, the spatial distribution of fungal communities at the study sites was closely associated with vegetation; that is, meadow fungal communities were different from forest fungal communities. In forests, DNA-based assays have showed that ectomycorrhizal communities vary spatially in relation to fruit bodies (Guidot et al. 2002) and proximity to established trees (Dickie et al. 2002; Cline et al. 2005; Dickie and Reich 2005) or arbuscular mycorrhizal competitors (Haskins and Gehring 2004). In contrast with bacterial communities, fungal communities in the meadow transition had a composition intermediate between those found in meadows and forests, although those of the forest transition did not differ from those found in the forest. Transition zones were highly variable, particularly at Lookout, with no indicator fragments unique to the transition zones. However, these findings are supported by those of Brodie et al. (2003), who used T-RFLPs and denaturing gel gradient electrophoresis to examine differences in soil fungal communities along a seminatural upland grassland and agricultural enclosure transect and found that the highest degree of variation occurred in the transition zone.

Plants clearly exert an important influence on the composition of the soil microbial community, as many studies have shown (e.g., Saetre and Bååth 2000). Vegetation influences microbial communities primarily through C input, either through aboveground litter input or belowground root input. For example, the microbial community of litter layers has been shown to differ among stands with different types of conifer or deciduous trees (Priha et al. 2001; Grayston and Prescott 2005). More directly, studies of litter decomposition have shown that microbial communities vary by litter type and stage of decomposition (Aneja et al. 2006; Williams et al. 2006). Belowground C inputs have also been shown to affect microbial communities (Grayston et al. 1998; Kuske et al. 2002). Recently, Phillips and Fahey (2006) suggested that arbuscular mycorrhizal and ectomycorrhizal tree species influence the soil microbial community through what they refer to as "rhizosphere effects." They found that microbial biomass C, N mineralization, C mineralization, nitrification, and phosphatase activity were higher in the rhizospheres of the ectomycorrhizal trees.

The relative importance of above- and below-ground plant inputs in structuring microbial communities is unclear and likely depends upon the type of vegetation and how long it has influenced soil microbial communities. A reciprocal transplant experiment using forest floors from aspen and white spruce stands in Alberta, Canada, found no evidence that microbial communities changed 1 year after transplantation, even in the presence of roots (Hannam et al. 2007). Conversely, a study in an old-growth Douglas-fir forest in Oregon showed that exclusion of roots for 7 years altered soil microbial community composition, but litter exclusion or additional inputs of litter or woody debris had no effect (Brant et al. 2006). Similarly, in Sweden, girdling a stand of *P. sylvestris* resulted in a shift in soil PLFAs measured 4 years later, including a decrease in the fungal biomarker that was associated with a decrease in extramatrical ectomycorrhizal mycelium (Högberg and Högberg 2002; Högberg et al. 2007).

Edge effects were observed at both Carpenter and Lookout with MRPP analysis of fungal ITS data. Fungal ITS data suggest the presence of a forest shadow effect on fungal communities on the meadow side of the forest edge, particularly at Carpenter. Roots and fungi associated with roots from the forest growing into the meadow and the presence of conifer seedlings in the meadow side of the transition zone appear to be contributing to differences in the fungal community along the forest edge. It is likely that ectomycorrhizal and saprotrophic fungi associated with conifers are contributing to the community differences. Ectomycorrhizal fungal DNA sequences have been found to make up the majority of fungal DNA sequences amplified using the fungal-specific ITS primers for DNA extracted from bulk soil (Chen and Cairney 2002; Landeweert et al. 2005) and from sand-filled hyphal ingrowth mesh bags incubated in ectomycorrhizal forest communities (Bastias et al. 2006; Kjøller 2006) and using basidiomycete-specific laccase primers for DNA extracted from bulk soil (Luis et al. 2005).

Boundaries or edges between forests and meadows can be dynamic areas. Ectomycorrhizal inoculum on the meadow side of the boundary may allow for movement of the forest edge farther into the meadow. The presence or absence of ectomycorrhizal inoculum along meadow–forest boundaries may play a role in expansion of the forest into high montane to subalpine meadows in the Cascades. It is difficult to predict whether the meadows used in this study will eventually become forested. Although we are seeing changes in the fungal community in the meadow along the forest edge, those changes have yet to appear in the bacterial community.

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