Plant Soil (2013) 372:459–471
DOI 10.1007/s11104-013-1760-7

REGULAR ARTICLE

Effects of disturbance scale on soil microbial communities in the Western Cascades of Oregon

Stacie A. Kageyama · Nancy R. Posavatz · Sarah S. Jones · Kirk E. Waterstripe · Peter J. Bottomley · Kermit Cromack Jr. · David D. Myrold

Received: 26 February 2013 / Accepted: 3 May 2013 / Published online: 12 May 2013

Abstract
Aims To gain a better understanding of how rapidly microbial communities respond to different magnitudes of perturbation that mimic minor or catastrophic disturbances.
Methods Two montane sites in the western Cascade Mountains of Oregon with adjacent areas of forest and meadow vegetation were studied. A reciprocal transplant experiment evaluated both minor (soil cores remaining in the same vegetation type) or more severe disturbance (soil cores transferred to a different vegetation type). The biomass and composition of the bacterial and fungal communities were measured for 2 years following the establishment of the experiment.
Results Minor disturbance (coring) had little impact on microbial biomass but transferring between vegetation type showed greater fungal biomass in soil incubated in the forest environment. The composition

Responsible Editor: Klaus Butterbach-Bahl.

Electronic supplementary material The online version of this article (doi:10.1007/s11104-013-1760-7) contains supplementary material, which is available to authorized users.

S. A. Kageyama
USDA Agricultural Research Service, Fort Keogh Livestock and Range Laboratory,
243 Fort Keogh Road,
Miles City, MT 59301, USA

N. R. Posavatz
The Mannik & Smith Group, Inc.,
234 Midtown Dr.,
Traverse City, MI 49684, USA

S. S. Jones
Oregon Department of Agriculture,
635 Capitol Street NE,
Salem, OR 97301, USA

K. E. Waterstripe
Biology Program, Health Science Building HS111A ML,
Northwestern Michigan College,
Traverse City, MI 49686, USA

P. J. Bottomley
Departments of Microbiology and Crop and Soil Science,
Oregon State University, Agriculture and Life Sciences Building 3017,
Corvallis, OR 97331, USA

K. Cromack Jr.
Department of Forest Ecosystems and Society, Oregon State University,
321 Richardson Hall,
Corvallis, OR 97331, USA

D. D. Myrold (✉)
Department of Crop and Soil Science, Oregon State University, Agriculture and Life Sciences Building 3017,
Corvallis, OR 97331, USA
e-mail: David.Myrold@oregonstate.edu

of bacterial communities was not influenced by coring but responded strongly to transfers between vegetation sites, changing to reflect their new environment after 2 years. Fungal community composition responded somewhat to coring, probably from disrupting mycorrhizal fungal hyphae, but more strongly to being transferred to a new environment.

Conclusions The response of the microbial community to major disturbance was rapid, showing shifts reflective of their new environment within 2 years, suggesting that microbial communities have the capacity to quickly adjust to catastrophic disturbances.

Keywords Bacteria · Fungi · Reciprocal transfer · Forest soil

Introduction

Forest ecosystems in the Pacific Northwest have evolved with a history of disturbance ranging in scale from infrequent catastrophic (Agee 1993) to frequent small-scale disturbances. Infrequent catastrophic disturbances include wildfire and volcanic eruptions. Natural fire intervals in the Pacific Northwest are several hundred years (Franklin and Halpern 2000). Volcanic eruptions are extremely rare occurrences but volcanic legacies, such as soils, are major influences in the ecology of the Pacific Northwest. Although catastrophic disturbances can result in dramatic changes to the landscape, smaller scale disturbances still play a large role in vegetation dynamics and ecosystem function.

Meadows occur throughout the upper montane and subalpine forests in the Cascade Range (Franklin and Halpern 2000). Many meadows in the Cascades have been persistent features on the landscape, some for more than 1,000 years (Hickman 1976). Numerous factors have contributed to the creation and maintenance of these features on the landscape (Franklin and Halpern 2000; Halpern et al. 2010; Haugo et al. 2011; Miller and Halpern 1998; Zald et al. 2012). Some meadows have remained in place due to the presence of shallow rocky soils on extremely steep slopes (Hickman 1976), while others are maintained by small-scale disturbance such as animal burrowing (Franklin and Halpern 2000). It is likely that microbial communities in these systems are well-adapted to small-scale disturbances.

Reciprocal transplant experiments, which have the advantage of examining effects of both environment and microbial community composition on ecosystem function (Reed and Martiny 2007), have been used to examine process-level changes in carbon (C) and nitrogen (N) cycling to disturbances. Studies using reciprocal transplants have examined the changes in C- and N-cycling that occur with succession (Clein and Schimel 1995), the effects of climate change on N dynamics (Balser and Firestone 2005; Hart and Perry 1999; Jonasson et al. 1993; Link et al. 2003), the changes in N mineralization and nitrification due to clearcutting (Carmonsini et al. 2003; Matson and Vitousek 1981), the effects of partial cutting on nitrate concentrations (Prescott et al. 2003), and the effects of plant invasion on N cycling (Funk 2005). Recently, several litter decomposition studies have used this approach, with the results leading to the concept of "home-field advantage", that microbial communities are adapted and optimized to their native substrate and environmental conditions (Ayres et al. 2009; Strickland et al. 2009).

The response of microbial communities to the reciprocal transplant approach has been found to vary from study to study. Transplanting forest floor material or plant litter between vegetation sites did not show significant shifts in overall microbial communities based on phospholipid fatty acids (PLFA) analysis after 1 year (Hannam et al. 2007; St. John et al. 2011) or of fungal communities based on terminal-restriction length polymorphism (T-RFLP) analysis (St. John et al. 2011). After 15 months, Lazzaro et al. (2011) found that reciprocal transfer of two geologically different glacial soils had no effect on bacterial communities as measured by T-RFLP. Longer running soil transplant studies have more often seen shifts in microbial abundance or composition, but the nature of the response has been variable. Balser and Firestone (2005) used PLFA and found an increase in the relative abundance of fungi in soils moved from an annual grassland to a mixed-conifer forest 2 years after transplanting, but little change in bacterial PLFA biomarkers. Waldrop and Firestone (2006) observed shifts in microbial PLFA profiles after 2 years when soils of an oak savanna were moved from under the trees to the adjacent grassland, but not vice versa. With PLFA analysis, Bottomley et al. (2006) found overall microbial communities shifted in soil cores moved from high montane meadow to forest and vice versa

2 years after transplant, but in the same experiment Boyle et al. (2006) found no effect of reciprocal transfer on communities of denitrifying bacteria using T-RFLP. Vanhala et al. (2011) measured a shift in PLFA biomarkers 2 years after the organic layer of boreal forests was transferred south to a warmer site; however, the effect was about the same as just excising the organic layer, suggesting root disturbance was the cause of the shift. Eleven years after transferring soil cores to a lower, warmer elevation in the Swiss Alps, Budge et al. (2011) found a modest shift in microbial biomass and PLFA biomarkers to values intermediate between that of soils in the original and current locations. Collectively, these results suggest that when using a reciprocal transplant design, it is important to control for the severity of disturbance caused by the transplantation, measure multiple microbial groups, and sample the experiment long enough for effects to be manifested; the methodology chosen for microbial community analysis seems less important.

Our goals for this study were to gain a better understanding of how microbial communities respond to disturbance and how quickly microbial succession occurs after disturbance. In the present study, we address disturbance on two different scales, small-scale root disturbance but no change in environment, and large-scale root disturbance and environmental change. The first part of the experiment addressed small-scale disturbance and the changes in microbial communities that occur when plants roots and mycelial connections are severed. We hypothesized that severing roots and mycelial connections would result in short-term changes that would disappear once soil cores were recolonized by roots and fungal hyphae. We hypothesized that we would see a sampling effect, i.e., a change in community composition due to disturbance occurring during sampling. We also hypothesized that we would see a decomposition effect as roots and hyphae decompose. Finally, we hypothesized that we would see an effect of root presence or absence. The second part of the study examined disturbance on a larger scale and the changes in microbial community that occur when plant communities drastically change. We hypothesized that we would see changes in microbial community once soil cores were transferred to new environments as microbial communities from their original communities would not persist. For this study, we examined the changes in microbial communities that occurred during the 2 years following disturbance. We expected to see differences in microbial community composition between the forest and meadow sites. We also expected to see rapid colonization of transferred cores by bacteria and fungi in their new environments.

Methods

Site characteristics

Study sites were located at the H. J. Andrews Experimental Forest, a Long-term Ecological Research site (44.2°N, 122.2°W) in the Cascade Mountain Range of Oregon, USA. Two high montane sites (~1,500 m), designated Lookout and Carpenter, were selected. Both sites had adjacent meadow and forest communities. Dominant meadow vegetation at both sites included *Bromus carinatus* Hook. & Arn., *Elymus glaucus* Buckl., *Pteridium aquilinum* (L.) Kuhn, and *Anaphalis margaritacea* (L.) Benth. & Hook. Dominant forest trees at both sites included *Pseudotsuga menziesii* (Mirb.) Franco, *Abies amabilis* Douglas ex Forbes, *A. grandis* (Dougl. ex D. Don.) Lindl., and *Tsuga mertensiana* (Bong.) Carr. Large stumps and charcoal at the Carpenter meadow site indicate that the site was harvested and burned in the past. More detailed descriptions of the Carpenter and Lookout sites have been published (Mintie et al. 2003; Rich et al. 2003; Kageyama et al. 2008).

Reciprocal transplant study design and sampling

The details of this reciprocal transplant experiment have been fully described previously (Bottomley et al. 2004; Bottomley et al. 2006; Boyle et al. 2006). Briefly, 35×35 m grids were installed in adjacent meadow and forest locations at Carpenter and Lookout in September 2000. Each grid had 64 sampling points spaced 5 m apart. At 48 randomly selected points, the superficial litter layer was removed from the soil surface and soil cores (5 cm diameter, 15 cm length) were collected in PVC pipe. Roots were left intact in the soil cores. This allowed for 12 cores for each of four treatments: open remaining, open transfer, closed remaining, and closed transfer. The open remaining and open transfer cores were placed in bags (1-mm mesh fiberglass screening) that allowed root growth into the cores. The closed remaining and closed

transfer cores remained enclosed in PVC pipe in order to exclude roots and were capped with 1-mm mesh screening to exclude litter inputs. Open and closed remaining cores were replaced into the hole from which they were taken. Transfer cores were transplanted from the meadow and moved to the adjacent forest and vice versa at both the Carpenter and Lookout sites. The remaining 16 points were designated for the collection of background cores, which were collected at each sampling date. Four of these background cores were collected at the time of installation in 2000 for characterization of the microbial community at the start of the experiment. In September of 2001 and 2002, six cores of each type (background, open remaining, open transfer, closed remaining, and closed transfer) were collected after incubating in situ for 1 and 2 years. To ensure adequate soil for subsequent analysis, two randomly selected cores of each treatment were pooled into a composite sample and homogenized through a 4-mm sieve. The three composite soil samples of each treatment served as replicates for statistical analysis.

Microscopic estimates of microbial biomass

Light microscopy was used to determine the biomass of fungi and bacteria. Fresh soil (1 g) was added to 9 ml of 0.2 M phosphate buffer at pH 7.2. 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 (Ingham and Klein 1984). Differential interference contrast microscopy (200X) was used to measure 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 1000X with oil immersion was used to examine filters (Babiuk and Paul 1970). Bacterial and fungal biomass was calculated by converting the volume of bacterial cells or fungal hyphae in 1 g of dry soil, assuming that bacterial cell density averages 330 mg cm^{-3} and fungal tissue density averages 410 mg cm^{-3} (Ingham et al. 1991).

Data for bacterial and fungal biomass were log transformed prior to analysis by ANOVA using PROC GLM and selected treatment comparisons made with PROC LSMEANS using Tukey's adjustment for multiple comparisons (SAS Institute Inc., Cary, NC, USA). Only results with $p \leq 0.05$ are reported as significant.

DNA extraction and length heterogeneity PCR

Soil aliquots were frozen and stored at −20 °C until DNA extraction was done within a few months. DNA was extracted from 0.5 g of soil (fresh weight) using the FastDNA® kit (Bio 101, Inc., Irvine, CA, USA) according to the manufacturer's directions. Extracted DNA quality was checked by agarose gel electrophoresis and was quantified with a BioSpec-1601 DNA/Protein/Enzyme Analyzer (Shimadzu Corporation, Kyoto, Japan). Extracts were diluted to 25 ng of DNA per μl for PCR amplification. DNA was amplified using 6-carboxyfluorescein (6-FAM) labeled forward primers for Length Heterogeneity PCR (LH-PCR). Primers for the internal transcribed spacer region of ribosomal DNA, 6-FAM labeled forward primer ITS1F (Gardes and Bruns 1993) and unlabeled ITS4 (White et al. 1990), were used for the amplification of fungi from soil, producing amplicons of about 500–750 bp. Eubacterial primers for the 16S rRNA gene, 6-FAM labeled forward primer 27F (Hicks et al. 1992) and an unlabeled reverse primer 338R (Amman et al. 1990), were used to amplify bacterial communities from soil, producing amplicons of about 300–360 bp. Reaction mixtures (50 μl) contained soil DNA (100 ng), AmpliTaq DNA polymerase (2.5 U), GeneAmp PCR buffer (1x), $MgCl_2$ (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}). For the ITS primers, PCR amplification was conducted as follows using a PTC-100 hot bonnet thermocycler (MJ Research, Inc., Waltham, MA, USA): 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. For the 16S primers, 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. Amplicons were visualized with electrophoresis on 1 % agarose gels stained with ethidium bromide. One μl of each amplicon (~1 ng of DNA) was submitted for capillary sequencing with an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA, USA) at the Core Labs of the Center for Genome Research and Biocomputing (Oregon State University, Corvallis, OR, USA). Amplicons were run on the Genetic Analyzer along with X-Rhodamine MapMarker™ 1000

internal lane size standard (BioVentures, Inc., Murfreesboro, TN, USA).

LH-PCR data analysis

Size and relative abundance of LH-PCR fragments were quantified using GeneScan® v. 3.5 software and Genotyper® v. 2.5 software (Applied Biosystems, Inc., Foster City, CA, USA). Sequence length and peak area data was obtained using Genotyper. Data were downloaded into Microsoft® Excel and manually binned (±2 bp). We excluded fragments that were below 1.5 % of the total peak area for each sample. We also excluded rare peaks that occurred in less than 5 % of our samples. The dataset was then relativized by the total fluorescence of each sample to adjust for differences in fluorescence levels between samples.

Community data were analyzed with PC-ORD v. 5.15 (MJM Software, Gleneden, OR, USA). Ordinations of LH-PCR fragment data were run 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 any relativization method (McCune and Grace 2002). Multi-response permutation procedures (MRPP) were used to test for treatment differences of the bacterial and fungal communities. MRPP is a nonparametric method that is used to test for group differences (McCune and Grace 2002). Only results with $p \leq 0.05$ are reported as significant.

Results

Natural temporal variation

Data collected from the background cores provides an insight into the natural temporal variation of the microbial communities. During the study, bacterial biomass averaged about 100 mg kg^{-1} soil across sites and vegetation types (Table 1), with a significant three-way interaction among year of sampling, site, and vegetation. Bacterial biomass was about 30 % lower in 2000 compared to the following 2 years. Within a year there were only a few significant effects of site and vegetation; however, bacterial biomass was higher in forest compared to meadow vegetation in all but one case where there was not a significant difference. Fungal biomass was more variable than bacterial biomass, and showed no differences with year of sampling or by site but was more than four-fold higher under forest than meadow vegetation (Table 1). The strong influence of vegetation type on fungi compared to bacteria resulted in fungal:bacterial biomass ratios averaging 22 for forest soil versus 5 for meadow soil (Table 1). Forest sites had significantly higher fungal:bacterial ratios in 2000 compared to 2001 and 2002 because of the lower bacterial biomass in 2000.

Nonmetric multidimensional scaling ordination indicated that LH-PCR fragments of both the bacterial 16S rRNA gene and fungal ITS region from background cores showed variation among years, sites, and vegetation (Fig. 1). In general, temporal variation was greater for bacterial than fungal communities, with bacterial communities in 2000 differing from other years for Carpenter meadow soil, between 2000 and 2002 for Carpenter forest soil, and between 2000 and 2001 for Lookout forest soil (MRPP: $p<0.05$); however, overall temporal variation was less than variation between sites or vegetation type. In each year, both bacterial and fungal communities differed between the two meadow sites, but forest sites did not differ (MRPP: $p<0.05$). The most pronounced difference for bacterial and fungal communities, however, was between vegetation types, with forest and meadow soil communities differing at each site for each year (MRPP: $p<0.05$).

Temporal response to disturbance

Disturbance treatments can be viewed at two scales, or degrees. The remaining cores reflect the response of soils to excluding litter and roots in the closed cores and of excluding litter while allowing root regrowth to occur in open cores. The transferred cores added the additional stress of being moved to a new environment, which included differences in microclimate and vegetation.

Microbial biomass Averaged across sites and transfer treatments, bacterial biomass was 8–19 % higher in closed cores compared to background or open cores; however, this was statistically significant only in 2002 (data not shown). Bacterial biomass was significantly higher in 2002 than 2001, ranging from 10 to 42 % higher depending upon transfer treatment (Fig. 2a). Source of soil and location of incubation interacted

Table 1 Microbial biomass as measured by direct microscopy in undisturbed locations at the Carpenter and Lookout sites sampled in 2000, 2001, and 2002. Means±standard error, n=3. Within the same column, letters designate significant differences at $p \leq 0.05$. Note these comparisons reflect a significant year by site by vegetation effect for bacteria, no significant interactions and only a significant vegetation main effect for fungi, and a significant year by vegetation effect for the fungal:bacterial ratio

Year	Site	Vegetation	Bacteria (mg kg^{-1} soil)	Fungi (mg kg^{-1} soil)	Fungal:bacterial ratio
2000	Carpenter	Forest	83±2 ^{c}	2465±293 ^{a}	29.87±3.75 ^{a}
		Meadow	77±5 ^{c}	509±33 ^{b}	6.63±0.15 ^{bc}
	Lookout	Forest	82±6 ^{c}	2862±574 ^{a}	34.18±4.46 ^{a}
		Meadow	71±6 ^{c}	438±59 ^{b}	6.17±0.71 ^{bc}
2001	Carpenter	Forest	102±5 ^{b}	1675±767 ^{a}	16.08±6.95 ^{bc}
		Meadow	110±1 ^{ab}	438±114 ^{b}	4.00±1.08 ^{c}
	Lookout	Forest	139±4 ^{a}	1994±670 ^{a}	14.48±5.04 ^{bc}
		Meadow	103±7 ^{b}	685±175 ^{b}	6.96±2.33 ^{c}
2002	Carpenter	Forest	139±6 ^{a}	1829±675 ^{a}	13.49±5.48 ^{b}
		Meadow	104±5 ^{b}	333±52 ^{b}	3.22±0.56 ^{c}
	Lookout	Forest	118±8 ^{ab}	2484±568 ^{a}	21.23±5.00 ^{b}
		Meadow	97±5 ^{b}	551±193 ^{b}	5.51±1.68 ^{c}

significantly, with bacterial biomass consistently lower when incubated in forest environments, although this was significant only for forest soil at Lookout in 2002 (Fig. 2a).

Neither year of sampling nor core type had a significant effect on fungal biomass or on fungal to bacterial ratios but fungal biomass was significantly higher (~50 %) at Lookout compared to Carpenter. The patterns of fungal biomass were consistent across years and sites, with higher fungal biomass in forest than meadow soils (~150 %) and in soils incubated in forest compared to meadow locations (~150 %; Fig. 2b). In effect, fungal biomass increased when meadow soils were moved into the forest, whereas it decreased when forest soils were moved into the meadow. Fungal to bacterial ratios largely reflected the dynamics of the fungal biomass (Fig. 2c), although only the effect of soil origin was statistically significant.

Microbial communities Bacterial communities based on LH-PCR of the 16S rRNA gene did not differ among background, closed, or open core types, therefore data for closed and open cores were combined for analysis. Nonmetric multidimensional scaling

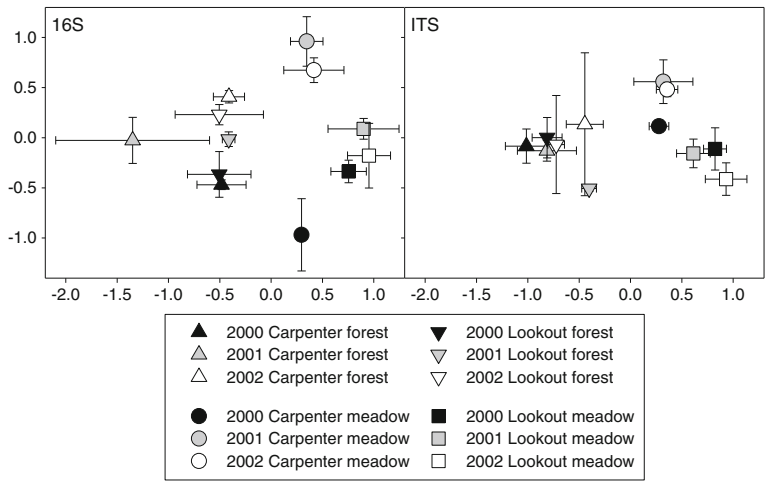

Fig. 1 Nonmetric multidimensional ordination of bacterial (16S) and fungal (ITS) communities from undisturbed forest and meadow soils sampled from the Carpenter and Lookout sites in 2000, 2001, and 2002. Data are means with standard error bars (n=3). The ordination explained >85 % of the variation in the bacterial community data (58.8 % by the x-axis, 28.8 % by the y-axis) and >65 % of the variation in the fungal community data (49.5 % by the x-axis, 18.9 % by the y-axis)

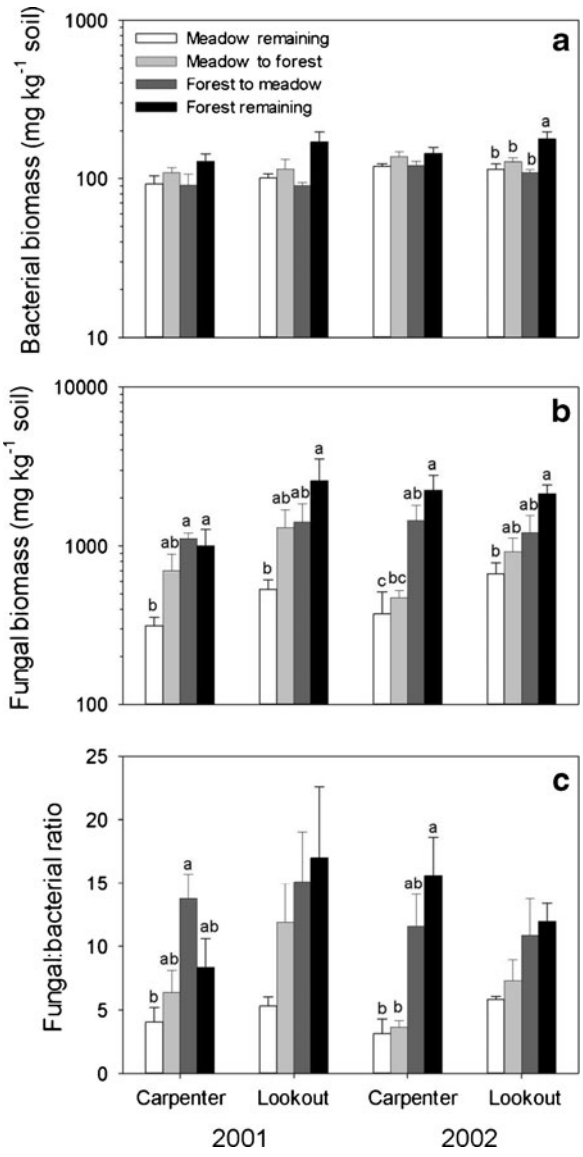

Fig. 2 Microbial biomass as measured by direct microscopy for Carpenter and Lookout sites one (2001) and two (2002) years after transferring cores: (**a**) bacterial biomass, (**b**) fungal biomass, and (**c**) fungal:bacterial biomass ratio. Data are means with standard error bars (n=6) for open and closed cores combined, because there was not a significant effect of core type, except for bacterial biomass in 2002. Significant differences ($p \leq 0.05$) among the four treatments at a site within a year are shown by *lower case letters*

ordinations clearly show that bacterial communities of forest soils remaining in place were distinct from meadow soil remaining in place in both years at both sites (Fig. 3; MRPP: $p<0.05$). This was the only statistical difference among the bacterial communities at the Carpenter site in 2001, but in 2002 cores collected from the meadow were different from those collected from the forest, suggesting that bacterial communities had become indistinguishable from their current environment regardless of initial origin. At the Lookout site in 2001, the bacterial community of the meadow soil differed from all other treatments, suggesting that the bacterial community of meadow soil transferred to the forest environment had already assumed a distinct composition that was similar to that of the forest soil. The bacterial community of the forest soil transferred to the meadow was still similar to that of its origin and had not yet shifted towards that of its new environment, but in 2002 it had transitioned to being intermediate, and not statistically separable, from that of either the meadow or forest soils.

As with bacterial communities, fungal communities based on LH-PCR of the ribosomal ITS region were statistically different between forest soils remaining in place and meadow soils remaining in place (Fig. 4; MRPP: $p<0.05$). Unlike bacterial communities, however, fungal communities showed some influence of core type. In both years at Lookout forest, the fungal community in the background (undisturbed) core was significantly different than the closed core and in 2001 from the open core, but fungal communities in the open and closed cores did not differ. Core type did not significantly affect fungal communities in the remaining cores in the meadow at either site or the forest site at Carpenter in either year. There was no effect of core type on fungal communities of forest soils transferred to the meadow environment at either site in either year; in contrast, fungal communities of open and closed cores did differ in meadow soil transplanted into forests in both years at the Carpenter site and in 2002 at the Lookout site.

The pattern of response of fungal communities to soil transfers differed somewhat from that of bacterial communities, generally showing more significant effects of movement to a new environment (Figs. 3 and 4). At both sites in 2001, fungal communities of soils moved from meadows to forests were significantly different from their original meadow soils, although based on MRPP analysis the fungal communities of only the open cores at the Carpenter site were similar (i.e., not significantly different) than those of their new, forest environment. By 2002, fungal communities of Carpenter meadow soil moved into the forest

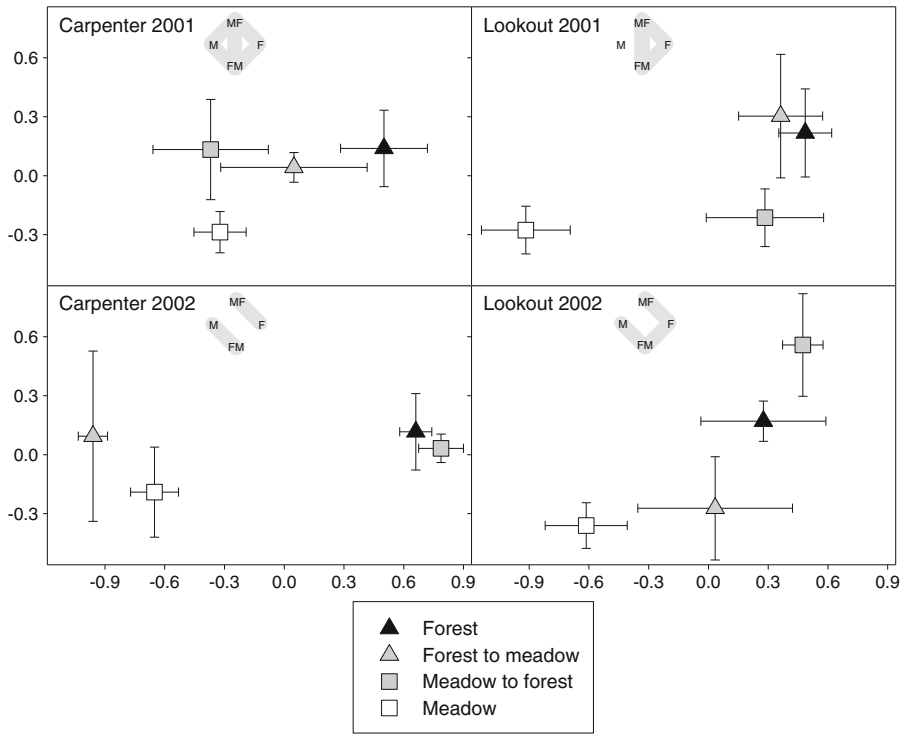

Fig. 3 Nonmetric multidimensional ordination of bacterial (16S) communities of the Carpenter and Lookout sites one (2001) and two (2002) years after transferring cores. Data are means with standard error bars (n=6) for open and closed cores combined, because there was not a significant effect of core type. The diamond-shaped diagram adjacent to each panel title represents statistical significance based on MRPP: treatment abbreviations (*F* forest remaining, *FM* forest to meadow, *MF* meadow to forest, *M* meadow remaining) connected by a gray line are *not* significantly different (p<0.05). The variation explained by the x- and y-axes were: 57.6 % and 18.5 % for Carpenter in 2001, 55.7 % and 39.3 % for Carpenter in 2002, 69.1 % and 23.4 % for Lookout in 2001, and 73.5 % and 22.7 % for Lookout in 2002

had shifted to be similar to those of the forest soil. This was also the case for closed cores at the Lookout site. After 2 years, the fungal communities of most forest soils transferred to the meadow environment were significantly different from their original environment, with the closed cores at Carpenter being the only exception. Most of these forest-to-meadow fungal communities were still significantly different than meadow fungal communities, with the exception of open cores transferred from the forest to meadow at the Carpenter site.

Discussion

Measuring microbial community composition

The LH-PCR fingerprinting method we used to measure bacterial and fungal community composition has been shown to work well for this purpose (Ritchie et al. 2000; Mills et al. 2007) and to distinguish between the background microbial communities at our study sites (Kageyama et al. 2008); however, it does have several shortcomings. Fingerprinting techniques do not provide the taxonomic resolution of current high-throughput sequencing approaches (e.g., Roesch et al. 2007; Caporaso et al. 2012) nor do they allow for the direct identification of taxa. Because comparisons of community composition are dependent mainly on the dominant taxa, fingerprinting methods are often adequate and we found them to be sensitive to treatment differences in this study, which was our emphasis. We were restricted, however, in identifying the taxa associated with the shifts in microbial communities that we observed.

Natural variations in microbial communities

Vegetation type had the greatest effect on bacterial and fungal abundance, and on the composition of their

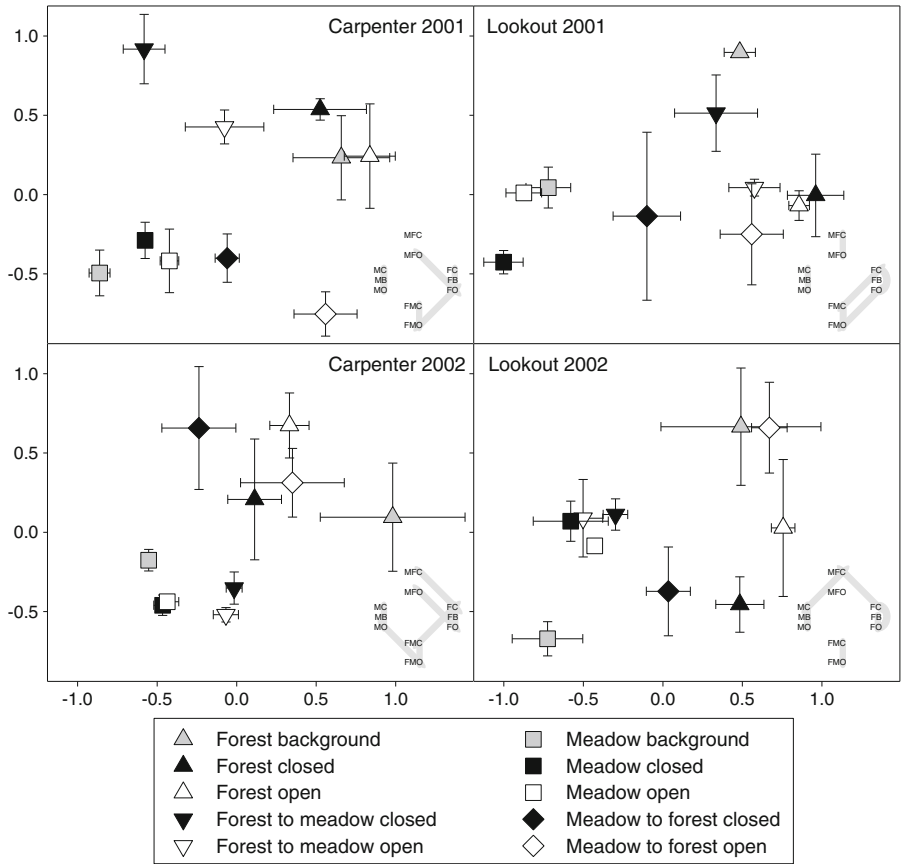

Fig. 4 Nonmetric multidimensional ordination of fungal (ITS) communities of the Carpenter and Lookout sites one (2001) and two (2002) years after transferring cores. Data are means with standard error bars (n=3). The *diamond-shaped diagram* in the lower right of each panel represents statistical significance based on MRPP: treatment abbreviations (*F* forest remaining, *FM* forest to meadow, *MF* meadow to forest, *M* meadow remaining for transfer type, *B* background, *C* closed, *O* open for core type) connected by a gray line are *not* significantly different ($p<0.05$). The variation explained by the x- and y-axes were: 35.5 % and 26.3 % for Carpenter in 2001, 31.2 % and 27.3 % for Carpenter in 2002, 57.5 % and 17.3 % for Lookout in 2001, and 33.0 % and 26.1 % for Lookout in 2002

communities. This agrees with a transect study done a year earlier at the same sites (Kageyama et al. 2008), as does the similarity of the bacterial and fungal communities of the two forest sites and difference between these communities of the two meadow sites. These trends were consistent during all 3 years of the current study.

There is likely seasonal variability in microbial communities, as observed by others in alpine ecosystems (Lazzaro et al. 2011; Lipson and Schmidt 2004); however, by sampling in September when soils reached their annual nadir in soil moisture, we were able to determine the degree of interannual variability in the microbial community of meadow and forest soils. The fungal community was stable, both in terms of biomass and community composition, perhaps because of a dominance of plant-associated mycorrhizal fungi that act to buffer it against environmental perturbations. The bacterial community did shift some from year to year, being lower in biomass the first year, and showing a consistent trend in compositional shift throughout the 3 years of the study. We do not know the cause of this trend, but it was not related to obvious climatic differences among years, based on temperature and rainfall data for the site (http://andrewsforest.oregonstate.edu/).

Small-scale root disturbance

By having open and closed cores remaining at their site of origin, it was possible to determine if severing roots had a lasting effect on microbial communities.

After 1 year, physical disruption caused by taking cores and, in the case of closed cores, the exclusion of roots had little effect on bacterial biomass; however, after 2 years bacterial biomass was significantly higher in closed cores. This may have been associated with the soil in these cores being wetter, particularly when incubated in the forest (Supplemental Table 1), thereby potentially providing a better habitat for bacterial growth and decomposition of severed roots. This increased biomass did not appear to be due to the increased growth of particular members of the bacterial community, because no effect of core type was observed on the composition of the bacterial community. The minimal impact of excluding roots on bacterial communities that we observed contrasts with other studies in forest ecosystems that have shown that interruption of root inputs of C for months to years have affected bacterial communities (Brant et al. 2006; Chen et al. 2012; Rasche et al. 2011; Yarwood et al. 2009).

Based on several prior studies, we had anticipated that soil fungi would be more adversely impacted than bacteria by the physical disruption caused by taking cores and the exclusion of roots in the closed cores, mainly because of the impact on mycorrhizal fungi (Wallander et al. 2001; Högberg et al. 2007; Yarwood et al. 2009); however, this is not what we observed. Neither fungal biomass nor fungal community composition was affected, with the exception of Lookout forest. At Lookout forest, coring caused a shift in the fungal community in 2001, which persisted in the closed cores in 2002. This suggests that severing roots did alter the fungal community at this site but that the fungal community ultimately recovered as roots recolonized the open cores in the second year (Bottomley et al. 2006) and ectomycorrhizal hyphal connections were reestablished.

Large-scale disturbance effects

The goal of the transfer treatment was to mimic a large-scale disturbance such as clearcutting or wildfire. Although there was a general trend for bacterial and fungal biomass to increase when soil cores were transferred from the meadow to the forest, or to decrease when soil cores were transferred from the forest to the meadow environment (Fig. 2), this was statistically significant only for bacterial biomass at the Lookout site in 2002. Collectively, these data on microbial biomass suggest that the forest environment was generally more favorable for supporting a higher microbial biomass, which is consistent with the data for undisturbed soil (Table 1) and using the PLFA fungal biomarker (Bottomley et al. 2006). Although we cannot definitively separate the effect of different vegetation types from that of microclimate, we think that differences in microclimate are more likely because there were few differences in biomass between open and closed cores and because forest soils were moister than meadow soils at the time of sampling (Supplemental Table 1).

The influence of reciprocally transplanting soil cores was more marked on the composition of bacterial and fungal communities than on their biomasses. Two years after transplantation, bacterial communities resembled those of their new habitat at both sites, a trend that was already apparent after 1 year (Fig. 3). Less consistent results were obtained in previous studies of ammonia oxidizers and denitrifiers at these sites, with communities of transplanted cores resembling those of their new habitat less than half of the time (Bottomley et al. 2004; Boyle et al. 2006). One explanation may be that significant shifts in general heterotrophic bacteria overshadowed a lack of response by these two groups. Clearly it would be interesting to identify those members of the microbial community that did respond to the core transfers, but sequencing was beyond the scope of the study when the reciprocal transplant treatment was implemented.

Fungal communities generally responded more slowly than bacterial communities to core transfers (Fig. 4). This was contrary to our expectations because severing roots should quickly impact mycorrhizal fungi (Wallander et al. 2001; Jones et al. 2003; Högberg et al. 2007; Yarwood et al. 2009). Although mycorrhizal fungi no longer receive carbon from plants once roots are killed, it is known that some ectomycorrhizal mycelium may persist for a short time after clear-cuts (Hagerman et al. 1999; Harvey et al. 1980; Perry et al. 1987). Such persistence may partially explain why soil cores transferred from the forest to meadow environment responded more slowly than cores transferred from meadow to forest. The more rapid response of meadow soils transferred to the forest likely reflected colonization by ectomycorrhizal fungi, although we cannot discount a shift in the saprotrophic fungal

community in response to different microclimatic conditions. As with the bacterial community, sequencing of the fungal community would have provided a more definitive explanation for the community shifts we observed.

Microbial community response to disturbance

Disturbance events open up new habitat for colonization and result in increased heterogeneity in the system (Denslow 1985), e.g., the catastrophic Yellowstone Fires of 1988 resulted in a mosaic pattern on the landscape (Platt and Connell 2003; Turner et al. 1998). Similarly, the composition of a microbial community following small- or large-scale disturbance depends on the ability of individuals to survive, adapt to new conditions, and recolonize disturbed sites (Zak 1992; Friese et al. 1997; Reed and Martiny 2007; Allison and Martiny 2008; Chaer et al. 2009). Our results show that bacterial and fungal communities are resilient and adapt within a few years to small- or large-scale disturbances.

Acknowledgements Support for this work was provided by grants from the National Science Foundation Microbial Observatory Program (MCB-9977933 and MCB-0348689) and by the Oregon Agricultural Experiment Station. We acknowledge the NSF Long-term Ecological Research program for infrastructure support at the H.J. Andrews Experimental Forest. Numerous present and past members of our laboratory assisted in site establishment, soil sampling, and generation of supporting data. We thank the staff of the Core Labs of the Center for Genome Research and Biocomputing for their help and guidance with Gene Scan analyses. The insights provided by the reviewers and editor helped us to improve the final product.

References

Agee JK (1993) Fire ecology of Pacific Northwest forests. Island Press, Washington, DC

Allison SD, Martiny JBH (2008) Resistance, resilience, and redundancy in microbial communities. Proc Natl Acad Sci U S A 105:11512–11519

Amman RI, Krumholz L, Stahl DA (1990) Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. J Bacteriol 172:762–770

Ayres E, Steltzer H, Simmons BL, Simpson RT, Steinweg JM, Wallenstein MD, Mellor N, Parton WJ, Moore JC, Wall DH (2009) Home-field advantage accelerates leaf litter decomposition in forests. Soil Biol Biochem 41:606–610

Babiuk LA, Paul EA (1970) The use of flourescein isothiocyanate in the determination of the bacterial biomass of grassland soil. Can J Microbiol 16:57–62

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

Bottomley PJ, Taylor AE, Boyle SA, McMahon SK, Rich JJ, Cromack K Jr, 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

Bottomley PJ, Yarwood RR, Kageyama SA, Waterstrip KE, Williams MA, Cromack K Jr, Myrold DD (2006) 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. Plant Soil 289:35–45

Boyle SA, Rich JJ, Bottomley PJ, Cromack K Jr, Myrold DD (2006) Disturbance 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 JB, Myrold DD, Sulzman EW (2006) Root controls on soil microbial community structure in forest soils. Oecologia 148:650–659

Budge K, Leifeld J, Egli M, Fuhrer J (2011) Soil microbial communities in (sub)alpine grasslands indicate a moderate shift towards new environmental conditions 11 years after soil translocation. Soil Biol Biochem 43:1148–1154

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6:1621–1624

Carmonsini N, Devito KJ, Prepas EE (2003) Net nitrogen mineralization and nitrification in trembling aspen forest soils on the Boreal Plain. Can J For Res 33:2262–2268

Chaer GM, Fernandes MF, Myrold DD, Bottomley PJ (2009) Shifts in microbial community composition and physiological profiles across a gradient of induced soil degradation. Soil Sci Soc Am J 73:1327–1334

Chen DM, Zhou LX, Wu JP, Hsu JN, Lin YB, Fu SL (2012) Tree girdling affects the soil microbial community by modifying resource availability in two subtropical plantations. Appl Soil Ecol 53:108–115

Clein JS, Schimel JP (1995) Nitrogen turnover and availability during succession from alder to poplar in Alaskan taiga forests. Soil Biol Biochem 27:743–752

Denslow JS (1985) Disturbance-mediated coexistence of species. In: Pickett STA, White PS (eds) The ecology of natural disturbances and patch dynamics. Academic Press, Inc., New York, pp 307–323

Franklin JF, Halpern CB (2000) Pacific Northwest Forests. In: Barbour MG, Billings WD (eds) North American terrestrial vegetation, 2nd edn. Cambridge University Press, Cambridge, pp 123–159

Friese CF, Morris SF, Allen MF (1997) Disturbance in natural ecosystems: scaling from fungal diversity to ecosystem functioning. In: Wicklow DT, Söderström BE (eds) The

Mycota IV: environmental and microbial relationships, vol IV. Springer, Berlin, pp 47–63

Funk JL (2005) *Hedychium gardnerianum* invasion into Hawaiian montane rainforest: interactions among litter quality, decomposition rate, and soil nitrogen availability. Biogeochemistry 76:441–451

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

Hagerman SM, Jones MD, Bradfield GE, Gillespie M, Durall DM (1999) Effects of clear-cut logging on the diversity and persistence of ectomycorrhizae at a subalpine forest. Can J For Res 29:124–134

Halpern CB, Antos JA, Rice JM, Haugo RD, Lang NL (2010) Tree invasion of a montane meadow complex: temporal trends, spatial patterns, and biotic interactions. J Veg Sci 21:717–732

Hannam KD, Quideau SA, Kishchuk BE (2007) The microbial communities of aspen and spruce forest floors are resistant to changes in litter inputs and microclimate. Appl Soil Ecol 35:635–647

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

Harvey AE, Jurgensen MF, Larsen MJ (1980) Clearcut harvesting and ectomycorrhizae: survival of activity on residual roots and influence on a bordering forest stand in Western Montana. Can J For Res 10:300–303

Haugo RD, Halpern CB, Bakker JD (2011) Landscape context and long-term tree influences shape the dynamics of forest-meadow ecotones in mountain landscapes. Ecosphere 2:art91

Hickman JC (1976) Non-forest vegetation of the western Cascade Mountains of Oregon. Northwest Sci 50:145–155

Hicks RE, Amann RI, Stahl DA (1992) Dual staining of natural bacterioplankton with 4′,6-diamidino-2-phenylindole and fluorescent oligonucleotide probes targeting kingdom-level 16S rRNA sequences. Appl Environ Microbiol 58:2158–2163

Högberg MN, Högberg P, Myrold DD (2007) Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150:590–601

Ingham ER, Klein DA (1984) Soil fungi: measurement of hyphal length. Soil Biol Biochem 16:279–280

Ingham ER, Griffiths RP, Cromack 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

Jonasson S, Havström M, Jensen M, Callaghan TV (1993) In situ mineralization of nitrogen and phosphorus of arctic soils after perturbations simulating climate change. Oecologia 95:179–186

Jones MD, Durall DM, Cairney JWG (2003) Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. New Phytol 157:399–422

Kageyama SA, Ritchie NR, Waterstripe KE, Jones SJ, Bottomley PJ, Cromack K Jr, Myrold DD (2008) Fungal and bacterial communities across meadow-forest ecotones in the western Cascades of Oregon. Can J For Res 38:1053–1060

Lazzaro A, Gauer A, Zeyer J (2011) Field-scale transplantation experiment to investigate structures of soil bacterial communities in pioneering sites. Appl Environ Microbiol 77:8241–8248

Link SO, Smith JL, Halvorson JJ, Bolton H (2003) A reciprocal transplant experiment within a climatic gradient in a semi-arid shrub-steppe ecosystem: effects on bunchgrass growth and reproduction, soil carbon, and soil nitrogen. Glob Chang Biol 9:1097–1105

Lipson DA, Schmidt SK (2004) Seasonal changes in an alpine soil bacterial community in the Colorado Rocky Mountains. Appl Environ Microbiol 70:2867–2879

Matson PA, Vitousek PM (1981) Nitrogen mineralization and nitification potentials following clearcutting in the Hoosier National Forest, Indiana. Forest Sci 27:781–791

McCune B, Grace JB (2002) Analysis of Ecological Communities. MJM Software Design, Gleneden Beach

Miller EA, Halpern CB (1998) Effects of environment and grazing disturbance on tree establishment in meadows of the central Cascade Range, Oregon, USA. J Veg Sci 9:265–282

Mills DK, Entry JA, Gillevet PM, Mathee K (2007) Assessing microbial community diversity using amplicon length heterogeneity polymerase chain reaction. Soil Sci Soc Am J 71:572–578

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

Perry DA, Molina R, Amaranthus MP (1987) Mycorrhizae, mycorrhizospheres, and reforestation: current knowledge and research needs. Can J For Res 17:929–940

Platt WJ, Connell JH (2003) Natural disturbances and directional replacement of species. Ecol Monogr 73:507–522

Prescott CE, Hope GD, Blevins LL (2003) Effect of gap size on litter decomposition and soil nitrate concentrations in a high-elevation spruce-fir forest. Can J For Res 33:2210–2220

Rasche F, Knapp D, Kaiser C, Koranda M, Kitzler B, Zechmeister-Boltenstern S, Richter A, Sessitsch A (2011) Seasonality and resource availability control bacterial and archaeal communities in soils of a temperate beech forest. ISME J 5:389–402

Reed HE, Martiny JBH (2007) Testing the functional significance of microbial composition in natural communities. FEMS Microbiol Ecol 62:161–170

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

Ritchie NJ, Schutter ME, Dick RP, Myrold DD (2000) Use of length heterogeneity PCR and fatty acid methyl ester profiles to characterize microbial communities in soil. Appl Environ Microbiol 66:1668–1675

Roesch LF, Fulthorpe RR, Riva A, Casella G, Hadwin AKM, Kent AD, Daroub SH, Camargo FAO, Farmerie WG, Triplett EW (2007) Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J 1:283–290

St. John MG, Orwin KH, Dickie IA (2011) No 'home' versus 'away' effects of decomposition found in a grassland-forest

reciprocal litter transplant study. Soil Biol Biochem 43:1482–1489

Strickland MS, Osburn E, Lauber C, Fierer N, Bradford MA (2009) Litter quality is in the eye of the beholder: initial decomposition rates as a function of inoculum characteristics. Funct Ecol 23:627–636

Turner MG, Baker WL, Peterson CJ, Peet RK (1998) Factors influencing succession: lessons from large, infrequent natural disturbances. Ecosystems 1:511–523

Vanhala P, Karhu K, Tuomi M, Bjorklof K, Fritze H, Hyvarinen H, Liski J (2011) Transplantation of organic surface horizons of boreal soils into warmer regions alters microbiology but not the temperature sensitivity of decomposition. Glob Chang Biol 17:538–550

Waldrop MP, Firestone MK (2006) Response of microbial community composition and function to soil climate change. Microb Ecol 52:716–724

Wallander H, Nilsson LO, Hagerberg D, Bååth E (2001) Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. New Phytol 151:753–760

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, New York, pp 315–322

Yarwood SA, Myrold DD, Högberg MN (2009) Termination of belowground C allocation by trees alters soil fungal and bacterial communities in a boreal forest. FEMS Microbiol Ecol 70:151–162

Zak JC (1992) Response of soil fungal communities to disturbance. In: Carroll GC, Wicklow DT (eds) The fungal community: its organization and role in the ecosystem, 2nd edn. Marcel Dekker, Inc., New York, pp 403–425

Zald HSJ, Spies TA, Huso M, Gatziolis D (2012) Climatic, landform, microtopographic, and overstory canopy controls of tree invasion in a subalpine meadow landscape, Oregon Cascades, USA. Landsc Ecol 27:1197–1212