The ISME Journal (2012) 6, 2199–2218

www.nature.com/ismej

ORIGINAL ARTICLE

Significant and persistent impact of timber harvesting on soil microbial communities in Northern coniferous forests

This article has been corrected since Advance Online Publication and a corrigendum is also printed in this issue.

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Forest ecosystems have integral roles in climate stability, biodiversity and economic development. Soil stewardship is essential for sustainable forest management. Organic matter (OM) removal and soil compaction are key disturbances associated with forest harvesting, but their impacts on forest ecosystems are not well understood. Because microbiological processes regulate soil ecology and biogeochemistry, microbial community structure might serve as indicator of forest ecosystem status, revealing changes in nutrient and energy flow patterns before they have irreversible effects on long-term soil productivity. We applied massively parallel pyrosequencing of over 4.6 million ribosomal marker sequences to assess the impact of OM removal and soil compaction on bacterial and fungal communities in a field experiment replicated at six forest sites in British Columbia, Canada. More than a decade after harvesting, diversity and structure of soil bacterial and fungal communities remained significantly altered by harvesting disturbances, with individual taxonomic groups responding differentially to varied levels of the disturbances. Plant symbionts, like ectomycorrhizal fungi, and saprobic taxa, such as ascomycetes and actinomycetes, were among the most sensitive to harvesting disturbances. Given their significant ecological roles in forest development, the fate of these taxa might be critical for sustainability of forest ecosystems. Although abundant bacterial populations were ubiquitous, abundant fungal populations often revealed a patchy distribution, consistent with their higher sensitivity to the examined soil disturbances. These results establish a comprehensive inventory of bacterial and fungal community composition in northern coniferous forests and demonstrate the long-term response of their structure to key disturbances associated with forest harvesting.

The ISME Journal (2012) **6,** 2199–2218; doi:10.1038/ismej.2012.84; published online 2 August 2012
Subject Category: microbial population and community ecology
Keywords: timber harvesting; organic matter removal; soil compaction; microbial community structure; bacteria and fungi; massively parallel pyrosequencing

Introduction

Forest ecosystems are vital components of the biosphere, mediating fundamental nutrient and energy flow patterns with direct feedback on the climate system (Canadell and Raupach, 2008). Intact forests mitigate atmospheric increases in greenhouse gases and are among the largest global carbon sinks (Bonan, 2008; Fahey *et al.*, 2010), whereas poorly managed forests and deforested areas can become significant sources of greenhouse gases with the potential to accelerate global warming (Miles and Kapos, 2008). Furthermore, forest ecosystems are global hotspots for biodiversity, which is of

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Received 2 February 2012; revised 6 June 2012; accepted 11 June 2012; published online 2 August 2012

critical ecological and economic importance (Díaz *et al.*, 2006). Forest biodiversity is changing dramatically because of current management practices (Normile, 2010). Finally, forest ecosystems are of vast economic importance, and their significance is growing as a result of the need to shift to production of renewable materials and fuels (Kirschbaum, 2003). Conservation strategies and sustainable management practices are therefore essential to maintain forest functionality, preserve global biodiversity and meet socio-economic imperatives.

The cornerstone of sustainable forest management is a proper stewardship of soil. Soil provides fundamental ecosystem services, including decomposition and transformation of organic materials and toxic compounds, nutrient cycling, water regulation, physical substrate for plants and control of pests and disease (Dominati *et al.*, 2010). Soils are extremely complex and dynamic biological matrices containing diverse microorganisms that span all three domains of life. Interdependent constituents of this vast microbiota have essential roles in the above ecosystem services (Barrios, 2007; Singh *et al.*, 2010). At the system level, microbial processes regulate soil productivity and modulate resilience of the forest ecosystem to stresses. It is likely that microbial community structure, gene expression patterns and metabolic activities can serve as indicators of forest ecosystem status, which might improve our ability to monitor forest ecosystems, to evaluate effects of management practices, and perhaps, to detect changes in nutrient and energy flow patterns before they have irreversible effects. However, our understanding of how microbial soil communities respond to forest-management disturbances is limited. Studies of these highly diverse communities using previously available techniques yielded inconsistent or equivocal results (for example, Ponder and Tadros, 2002; Jones *et al.*, 2003; Busse *et al.*, 2006; Hannam *et al.*, 2006; Chatterjee *et al.*, 2008). Furthermore, information about long-term effects is largely missing (Marshall, 2000).

Site organic matter (OM) and soil porosity are two vital properties directly affected by management practices (Grigal, 2000; Marshall, 2000). These two properties regulate many important soil processes, including temperature, water regime, gas exchange, nutrient availability, carbon sequestration and energy supply (Powers *et al.*, 2005), and ultimately control a forest's productivity and functionality (Worrell and Hampson, 1997). Currently, there is a demand to maximize OM removal from forests, in order to divert previously unused OM to biofuel production, but the effect on long-term soil productivity (LTSP), biodiversity and ecosystem services is poorly understood (Berch *et al.*, 2011). Increased biomass removal as performed during whole-tree harvesting and site preparation for planting may affect the microbial communities and reduce forest productivity in the long term (Wei *et al.*, 2000; Walmsley *et al.*, 2009). Economically efficient harvesting requires the use of heavy machines, causing severe compaction of the soil, especially during wet conditions and in soils with low initial bulk density (Grigal, 2000; Marshall, 2000; Powers *et al.*, 2005). The soil system can suffer substantial and persistent damage, which ultimately reduces plant growth, forest productivity and ecosystem functioning (Greacen and Sands, 1980; Kozlowski, 1999). Soil compaction reduces water infiltration rates, hydraulic conductivity, aeration and rooting space, often resulting in increased surface runoff, soil erosion, nutrient leaching and greenhouse gas emission. Naturally, there will also be impact on the soil microbiota, but this impact is poorly understood. Microbial communities will be directly influenced by limited oxygen availability, altered water regime and reduced pore sizes in compacted soils (Wright *et al.*, 1995; Schnurr-Pütz *et al.*, 2006; Frey *et al.*, 2011).

Generally, short-term harvesting effects include changes in vegetation, nutrient availability, soil microclimate and structure, and litter quantity and quality (Keenan and Kimmins, 1993; Jurgensen *et al.*, 1997). However, adequately managed forest ecosystems are suggested to be highly resilient in the long-term perspective (Powers, 1999; Sanchez *et al.*, 2006a, b). In 1989, the USDA Forest Service launched the Long-Term Soil Productivity (LTSP) study in order to evaluate how OM removal and soil compaction affect soil processes and site productivity across major soil and forest types and to develop and validate the monitoring standards for sustainable forest-management practices throughout North America (Powers, 2006). Over the years, effects on ecosystem properties were found to be highly site-dependent, and forest productivity was generally not impaired by different levels of harvesting disturbances (Powers *et al.*, 2005; Ponder *et al.*, 2012). OM removal and soil compaction tended to change soil temperature, moisture, porosity, aeration, nutrient availability, carbon and nitrogen content, diversity of meso- and macrofauna and bulk density (Conlin and van den Driessche, 2000; Sanborn *et al.*, 2000; Battigelli *et al.*, 2004; Powers *et al.*, 2005; Page-Dumroese *et al.*, 2006). However, these effects were often variable among sites and soil horizons, precluding identification of general treatment effects. Relatively few microbial parameters, such as biomass, enzyme activity, nitrogen mineralization potential and litter decomposition rates, were measured (Kranabetter and Chapman, 1999; Li *et al.*, 2003; Busse *et al.*, 2006; Mariani *et al.*, 2006; Tan *et al.*, 2008) and, like other soil properties, they did not reveal consistent treatment effects. The few studies that examined microbial community composition were limited in scope and did not take advantage of the full-factorial experimental design and replicated experimental sites, or were limited in coverage and resolution of the microbial communities by the available methods (Axelrood *et al.*, 2002a, b; Chow *et al.*, 2002; Busse *et al.*, 2006;

Hartmann *et al*., 2009). Current high-throughput sequencing approaches have the capacity to survey forest soil microbial communities with a far greater sample throughput, sequence coverage and phylogenetic resolution than previously possible (Nacke *et al*., 2011; Baldrian *et al*., 2012) and, potentially, to identify indicators of forest ecosystem status.

Here we present a large-scale investigation of microbial community composition at six LTSP sites in British Columbia (BC), Canada, using massively parallel 454 pyrosequencing (Margulies *et al*., 2005) of over 4.6 million bacterial and fungal ribosomal sequence tags derived from 306 soil samples, representing different levels of OM removal and soil compaction. We employed an array of bioinformatic and statistical methods to analyze these data and determine changes in microbial community structure resulting from soil disturbances.

Materials and methods

Here we provide a summary of the methods. Detailed descriptions of the study sites, sequence processing pipeline and statistical analyses are provided as Supplementary Methods.

Study sites

Our study focused on six LTSP field sites established between 1992 and 2000 in BC (Holcomb, 1996; Hope, 2006). Three of the six BC LTSP sites are located in the sub-boreal spruce (SBS) biogeoclimatic zone and were harvested 15 years prior to sampling, whereas the other three sites are located in the interior Douglas-fir (IDF) biogeoclimatic zone and were harvested 10 years prior to sampling. The three SBS sites were established in different biogeoclimatic subzones with an average distance of 285 km between sites, whereas the three IDF sites were established in the same subzone with an average distance of 9 km between sites. Each LTSP site features nine treatment plots ($40 \times 70\,m^2$), including three levels of OM removal and three levels of soil compaction arranged in a randomized, full-factorial design, plus a tenth reference plot, which was left unharvested and allowed to develop naturally. The three levels of OM removal were defined as stem-only harvesting (OM1), whole-tree harvesting (OM2) and whole-tree harvesting plus forest floor removal (OM3), and resulted in average percent net carbon removal of 51 ± 4 (95% confidence interval), 65 ± 3 and 84 ± 2, respectively (Marty Kranabetter, BC Ministry of Forests, personal communication). The three levels of soil compaction were defined as none (C0), moderate (C1) and severe (C2), and resulted in average percent increases in bulk density of 4 ± 4, 20 ± 4 and 19 ± 4, respectively, when compared with the reference plots. Ten years after harvesting, the percent increase in bulk density in these soil systems remained at 4 ± 5, 14 ± 6 and 15 ± 6, respectively. During the spring following these treatments, all the harvested plots were planted with seedlings of lodgepole pine (*Pinus contorta*).

Soil sampling, DNA extraction and phospholipid fatty acid (PLFA) measurements

In each experimental plot, soil was sampled randomly at 9 (SBS) or 15 (IDF) locations. Sets of three SBS or five IDF samples from each plot were pooled, yielding three composite replicate samples per plot. The organic soil horizon (approximate thickness ranging between 0 and 20 cm) and the top 20 cm of the mineral soil horizon were sampled separately. In all the OM3 plots, the initially removed forest floor had not yet redeveloped after 10–15 years and thus could not be sampled. Therefore, we obtained a total of 306 soil samples for analysis (6 LTSP sites $\times$ 10 treatments/controls $\times$ 2 horizons $\times$ 3 replicates $=$ 360 $-$ 54 (6 LTSP sites $\times$ 3 OM3 treatments $\times$ 3 replicates) $=$ 306). Total nucleic acids were extracted from 0.5 g of sieved soil using the FastDNA SPIN Kit for soil (MP Biomedicals, Solon, OH, USA). DNA extracts were quantified using the Quant-iT PicoGreen kit (Invitrogen, Carlsbad, CA, USA). PLFAs were measured in the organic and mineral soil samples collected at the six reference plots. PLFAs were extracted, identified and quantified as described by Brockett *et al*. (2012). PLFA concentrations were converted to biomass according to Bååth and Anderson (2003). The fungal to bacterial biomass ratio was determined using the fungal signature 18:2ω6,9, and the sum of bacteria-specific signatures (Bååth and Anderson, 2003).

PCR amplification and sequencing of bacterial and fungal pyrotags

Partial bacterial small-subunit ribosomal RNA genes (16S) and eukaryotic ribosomal internal transcribed spacers (ITS) were amplified and sequenced using the 454-pyrosequencing GS-FLX Titanium technology (Roche 454 Life Sciences, Branford, CT, USA). The hypervariable region V1–V3 of the bacterial 16S gene was amplified using primers 27F/519R (Lane, 1991; Amann *et al*., 1995), whereas the hypervariable region ITS2 of the fungal ribosomal operon was amplified using primers ITS3/ITS4 (White *et al*., 1990). The phylogenetic content, sequence variability and representation in public data repositories make these genetic regions excellent targets for such surveys (Sundquist *et al*., 2007; Nilsson *et al*., 2009b; Jeraldo *et al*., 2011). PCR was performed using 50 ng soil DNA and the HotStar *Taq* amplification kit (Qiagen, Mississauga, ON, Canada). Each sample was amplified in triplicate and subsequently pooled prior to purification and quantification. Retrofit of barcodes and GS-FLX Titanium adapters followed by pyrosequencing were performed at the Génome Québec Innovation Center, Montréal, Canada. PCR products were unidirectionally sequenced from primers 27F and ITS4.

Sequence processing, clustering and taxonomic assignment

Reads with ambiguous base calls and average quality scores <25 were eliminated using MOTHUR (Schloss *et al.*, 2009). The bacterial $16S_{V1–V2}$ (that is, the region comprising segments V1 and V2) and the fungal ITS2 regions were verified and extracted using V-XTRACTOR (Hartmann *et al.*, 2010) and its ITS counterpart (Nilsson *et al.*, 2010), respectively. These tools extract defined gene segments that are comparable for community analysis and at the same time provide another quality control measure by confirming authenticity of the target. Sequences were clustered into operational taxonomic units (OTUs) using CRUNCHCLUST (http://code.google.com/p/crunchclust/), a novel, user-friendly, fast and efficient open-source software introduced in this study. CRUNCHCLUST clusters sequences into OTUs using a global exact Needlemann–Wunsch (Needleman and Wunsch, 1970) pairwise alignment and the Levenshtein distance (Levenshtein, 1966) to calculate pairwise sequence similarities while ignoring dissimilarities due to homopolymer lengths, a frequent sequencing error inherent to the 454 technology. Thus, CRUNCHCLUST represents a straightforward approach to avoid artificial inflation of OTU richness due to homopolymer sequencing errors, in case available resources do not allow for thorough data denoising with more sophisticated but resource-intensive and time-consuming algorithms such as Pyronoise (Quince *et al.*, 2009). Clustering was performed at a Levenshtein distance of 7 (approximately 97% sequence identity). All reads in a given OTU were assigned to a curated taxonomic database using a naïve Bayesian classifier (Wang *et al.*, 2007) implemented in MOTHUR and a minimum bootstrap support of 60%. Bacterial and fungal reads were queried against GREENGENES (DeSantis *et al.*, 2006) and EMERENCIA (Nilsson *et al.*, 2009a), respectively. The consensus taxonomy of each OTU was determined using MOTHUR as the taxonomic path represented by at least 51% of the sequences within the OTU. Based on the consensus taxonomies, abundance data for OTUs at specific taxonomic ranks (species, genus, family, order, class and phylum) were merged and used to generate taxonomic rank-specific matrices for community structure analysis. Based on the taxonomic information from species to phylum, a taxonomic dendrogram was generated using the SPECDIST routine in the PRIMER6+ software (Clarke and Gorley, 2006).

Multivariate analysis of community structures and diversity

Statistical analysis was performed according to the recommendations by Anderson and Willis (2003) who proposed four components in the analysis of multivariate ecological data: (1) a robust unconstrained ordination; (2) an appropriate constrained analysis with reference to a specific hypothesis; (3) a rigorous statistical test of the hypothesis; and (4) characterization of the species responsible for the multivariate patterns. In accordance with this strategy, we used the following techniques for the corresponding purposes: (1) principal coordinate analysis (Gower, 2005); (2) canonical analysis of principal coordinates (CAP; Anderson and Willis, 2003); (3) analysis of similarities (ANOSIM; Clarke, 1993) and permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001); and (4) indicator species analysis (De Cáceres and Legendre, 2009). Details on the four components are given in the following paragraphs.

A Bray–Curtis similarity matrix (Bray and Curtis, 1957) was calculated based on the standardized and square-root transformed read abundance data. Overall similarities in microbial community structures among samples were displayed using the unconstrained ordination technique, principal coordinate analysis. Differences in community structure related to the specific treatments were displayed using the constrained ordination technique, CAP. Tests of the multivariate null hypotheses of no differences among *a priori* defined groups were examined using ANOSIM, PERMANOVA and the CAP classification success rate. CAP ordination, CAP classification success rates and CAP $trace_{Q\_m'HQ\_m}$ statistics were examined in combination to draw conclusions about separation of *a priori* groups. The CAP classification rate, calculated as the ratio between source (known affiliation) and successfully classified (predicted affiliation) data in the CAP model, provides a quantitative estimate of the degree of discrimination among the groups achieved by the canonical axes (Anderson and Willis, 2003). Permutational analysis of multivariate dispersions (PERMDISP) (Anderson, 2006) was used to test for heterogeneity of community structure in *a priori* groups. ANOSIM, PERMANOVA, CAP and PERMDISP were performed with 9999 permutations and run as routines in PRIMER6+. Pearson's correlations between species abundance and CAP coordinates were calculated in PRIMER6+. All the ordinations were plotted with STATISTICA 8.0 (StatSoft, Tulsa, OK, USA). All the multivariate statistical tests performed in this study were considered significant using a threshold of $P<0.05$ unless indicated otherwise.

Good's coverage (Good, 1953) and Simpson's index for evenness ($E_{1/D}$; Simpson, 1949) were calculated in MOTHUR. Simpson's indices were normalized relative to the reference plots within each site in order to account for variability among different experimental sites. The effect of harvesting was examined by only including samples at compaction level C0 and using one-way analysis of variance followed by Fisher's least significant difference *post-hoc* test. Effects of the different treatment levels within the factorial design were examined by excluding the reference samples and using factorial analysis of variance followed by a desirability approach (Derringer and Suich, 1980) to

find the combination of OM removal and soil compaction where evenness is maximized.

Indicator species analysis
Taxon-treatment association analysis was used to determine the degree of preference (that is, correlation R) of each taxonomic unit for the target group compared with the other groups by using all possible group combinations (De Cáceres and Legendre, 2009; De Cáceres *et al.*, 2010). Correlations were calculated using the diagnostic species analysis routine implemented in GINKGO (Bouxin, 2005) with 99 999 permutations. Multiple hypothesis testing corrections of the *P*-values were performed using the false discovery rate (Benjamini and Hochberg, 1995). *q*-values were determined using the software QVALITY (Käll *et al.*, 2009) and associations were considered significant with an false discovery rate of 10% ($q < 0.1$). Differences in association strength (delta R) of each taxonomic unit among the whole comparison, for example, OM removal, were mapped onto the taxonomic tree generated in PRIMER6+ and displayed using ITOL (Letunic and Bork, 2011). The variability in abundance of each taxonomic unit among all samples was determined using the coefficient of variation (Lovie, 2005) and plotted with STATISTICA. The coefficient of variation is a measure of dispersion of data relative to the mean and reflects the chance of encountering a taxon in a particular sample.

Results

Pyrotag sequencing
We analyzed 306 samples with eight 454 runs, yielding a total of 3 597 269 bacterial 16S and 3 609 879 fungal ITS raw pyrotag reads. After quality filtering and target extraction, a total of 2 453 680 bacterial $16S_{V1–V2}$ and 2 224 307 fungal ITS2 sequences remained for community analysis. This corresponds to an average of 8019 ± 2034 bacterial $16S_{V1–V2}$ and 7269 ± 2155 fungal ITS2 pyrotags per sample, with an average read length of 247 ± 3 and 243 ± 9 bp, respectively. The quality-filtered bacterial and fungal pyrotag sequences are provided as Supplementary Data 1 and 2, respectively.

Sequence clustering yielded a total of 136 169 (average of 2017 ± 379 per sample) bacterial and 19 353 (383 ± 107) fungal OTUs. This represented an average Good's coverage of 0.83 ± 0.04 and 0.96 ± 0.01 for the bacterial and fungal data set, respectively. OTU abundance data were clustered at each major taxonomic level, that is, phylum, class, order, family, genus and species. This identified 41, 115, 204, 346, 697 and 1009 bacterial taxa and 7, 32, 101, 243, 669 and 1453 fungal taxa at the respective taxonomic levels. Full lists of the detected bacterial and fungal taxa, from phylum to OTU level, including abundance information, are provided in Supplementary Data 3 and 4.

Taxonomic composition and structural characteristics
Overall, Proteobacteria (55.5%), Actinobacteria (18.3%), Acidobacteria (13.3%), and to a lesser extent Chloroflexi (3.5%) and Gemmatimonadetes (2.1%) were the predominant bacterial phyla. Only 1.6% of the bacterial reads were unclassified at the phylum level. Among the Proteobacteria, class Alpha accounted for 48.2%, Beta for 3.4%, Gamma for 1.9% and Delta/Epsilon for 1.5% of the total abundance. A total of 17% of the bacterial OTUs, accounting for 40% of the total bacterial pyrotags, were identified at the genus level. The five most abundant genera were *Bradyrhizobium* (15.2%), *Rhodoplanes* (10.0%), *Candidatus solibacter* (2.5%), *Mycobacterium* (1.3%) and *Gemmatimonas* (1.0%). Only 2.3% of the bacterial OTUs were assigned at species level, accounting for 1.5% of the total abundance. The low species-assignment success was caused by the high number of sequences in the reference database not classified at this level. Six bacterial OTUs had a relative abundance $\geqslant 1\%$.

The overall fungal community was dominated by the phyla Ascomycota (64.0%) and Basidiomycota (31.1%), with the Zygomycota, Chytridiomycota and allies accounting for 2.2%. A total of 2.6% of the fungal pyrotags were unclassified at the phylum level. A total of 51% of the fungal OTUs, accounting for 65% of the total fungal pyrotags, were identified at the genus level. The five most abundant genera were *Oidiodendron* (10.5%), *Wilcoxina* (6.5%), *Piloderma* (4.2%), *Suillus* (4.0%) and *Hygrocybe* (3.7%). A total of 36% of the fungal OTUs, accounting for 58% of the total fungal pyrotags, were identified at the species level. The five most abundant fungal species were *Wilcoxina rehmii* (6.4%), *Oidiodendron chlamydosporicum* (6.2%), *Piloderma fallax* (3.3%), *Hygrocybe conica* (3.0%) and *Suillus brevipes* (2.1%). Of the fungal OTUs, 15 had a relative abundance of $\geqslant 1\%$.

Long-tailed rank-abundance curves characteristic of microbial communities were observed, but the two domains revealed different distribution patterns of OTUs across all samples when abundance was plotted against dispersion (Figure 1). In the bacterial community, the majority of abundant OTUs showed low dispersion and clustered around the inverse exponential curve. In the fungal community, many OTUs, including highly abundant ones had high dispersion and deviated from the inverse exponential curve. The different dispersion patterns were also maintained at lower than OTU-level resolution, such as species, genus and family levels (Supplementary Figure S1). These results indicate that the distribution of abundant populations was much more patchy for fungi than for bacteria.

Influence of geography and soil horizon
Major differences in community structure were driven by soil horizon and biogeoclimatic conditions

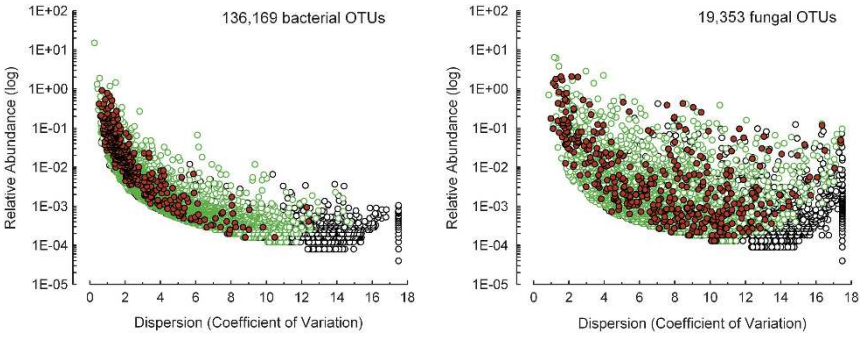

Figure 1 Abundance versus dispersion plots of bacterial (left) and fungal (right) OTUs across all samples. Colors denote OTUs that were significantly associated on the basis of indicator species analysis ($q < 0.1$) with either forest site or soil horizon (location indicators, open green circles) or with OM removal or soil compaction (treatment indicators, closed red circles); non significant OTUs are shown as open black circles. Some treatment indicators were also location indicators.

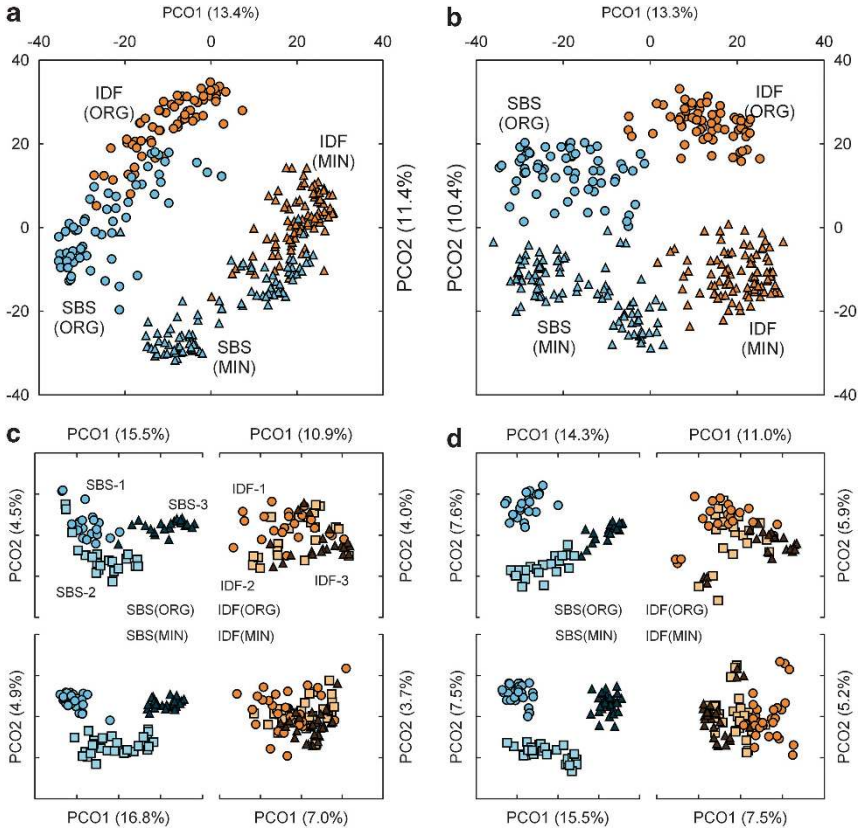

Figure 2 Principal coordinate analysis (PCO) ordinations of bacterial (**a**, **c**) and fungal (**b**, **d**) communities based on relative abundances of OTUs. Upper panels (**a**, **b**): ordinations of total samples from both organic (ORG) and mineral (MIN) soil horizons at six sites in both the SBS and IDF biogeoclimatic zones. Lower panels (**c**, **d**): separate ordinations for each soil horizon in each biogeoclimatic zone, with symbols for each site identified in panel **c**. Variance explained by each PCO axis is given in parentheses.

(Figure 2, Tables 1 and 2). Microbial communities strongly clustered according to soil horizon, biogeoclimatic zone and site. These three 'location' factors explained 16–17%, 14–18% and 19–20% of the total variance, respectively. Because forests in the two zones were harvested either 15 (SBS) or 10 (IDF) years prior to sampling, the zonal effect is driven by space and time. Differences in community structures among sites increased with increasing geographic distance (Figures 2c and d, Supplementary Table S1). Thus, intra-zone variability was much higher in the SBS than in the IDF zone, whereas the average intra-site variability was similar in both zones (Supplementary Figure S2). Intra-horizon variability was slightly higher in the organic horizon compared with the mineral horizon, but the difference was significant only for the bacterial community (data not shown). Principal coordinate analysis analyses were repeated over a range of taxonomic levels, from species to phylum (Supplementary

Table 1 Differences among *a priori* defined groups as assessed by analysis of similarity (ANOSIM)

	Bacteria (R[a]*)*	*Fungi (R)*
Horizon	0.696***	0.501***
	0.426***	0.591***
Zone		
ORG only	*0.657****	*0.748****
MIN only	*0.648****	*0.714****
Site[b]	0.427***	0.583***
SBS (ORG)	*0.621****	*0.675****
SBS (MIN)	*0.816****	*0.774****
IDF (ORG)	*0.175****	*0.325****
IDF (MIN)	*0.237****	*0.326****
OM[c]	0.057***	0.136***
SBS (ORG)	*0.050*[ns]	*0.200****
SBS (MIN)	*0.037*[ns]	*0.191****
IDF (ORG)	*0.182****	*0.346****
IDF (MIN)	*0.170****	*0.288****
C[c]	0.022*	0.084***
SBS (ORG)	*0.061**	*0.177****
SBS (MIN)	*0.030*[ns]	*0.130****
IDF (ORG)	*0.079**	*0.236****
IDF (MIN)	*0.042*[ns]	*0.183****

Abbreviations: C, compaction; IDF, interior Douglas-fir; MIN, mineral; ns, not significant; OM, organic matter removal; ORG, organic; SBS, sub-boreal spruce.
*$P<0.05$ and ***$P<0.001$.
[a]R, ANOSIM test statistic.
[b]This factor has multiple levels; results for pairwise tests of these levels can be found in Supplementary Table S1.
[c]This factor has multiple levels; results for pairwise tests of these levels can be found in Supplementary Table S2.

Table 2 Effect sizes and explained variances of the main factors assessed by permutational multivariate analysis of variance (PERMANOVA)[a]

Factor	*Bacteria*		*Fungi*	
	F/t	R^2	*F*/t	R^2
Horizon[b]	46.46***	17.4	38.20***	16.0
Zone[b]	32.06***	14.4	46.50***	17.7
Site[b]	12.75***	18.5	14.12***	19.8
OM	1.85***	5.0	4.31***	10.0
REF vs OM1	1.42***	NA	2.47***	NA
REF vs OM2	1.45***	NA	2.46***	NA
REF vs OM3	1.37***	NA	2.20***	NA
OM1 vs OM2	1.09[ns]	NA	1.32***	NA
OM1 vs OM3	1.39***	NA	1.88***	NA
OM2 vs OM3	1.28***	NA	1.73***	NA
C	1.59***	4.0	2.82***	6.8
REF vs C0	1.45***	NA	2.46***	NA
REF vs C1	1.37***	NA	2.37***	NA
REF vs C2	1.48***	NA	2.48***	NA
C0 vs C1	1.15*	NA	1.30***	NA
C0 vs C2	1.11*	NA	1.34***	NA
C1 vs C2	1.10*	NA	1.15*	NA
OM × C	1.01[ns]	0.8	1.28***	4.5

Abbreviations: C, compaction; C0, no compaction; C1, moderate compaction; C2, severe compaction; NA, not available; ns, not significant; OM, organic matter removal; OM1, stem-only harvesting; OM2, whole-tree harvesting; OM3, whole-tree harvesting plus forest floor removal; REF, reference plots.
*$P<0.05$ and ***$P<0.001$.
[a]Test statistics include F-ratio (F) for main PERMANOVA test, univariate t-statistic (*t*) for pairwise tests and estimation of variance components (R^2).
[b]Horizon, zone and site were defined as covariates in order to factor out the variance explained by these factors.

Figures S3 and S4), and the structuring of the data according to the location factors was maintained up to high taxonomic levels.

Influence of timber harvesting treatments

OM removal and soil compaction had significant effects on bacterial and fungal community structure, with much stronger effects on fungi. CAP ordination revealed distinct clustering of samples from each treatment group (Figure 3), whereas ANOSIM and PERMANOVA demonstrated that treatment effects were significant, though smaller than differences caused by the above location factors (Tables 1 and 2). In order to demonstrate that treatment effects were independent of the spatial effects, the 'location' factors were defined as covariates in PERMANOVA in order to factor out the variance explained by spatial factors. Timber harvesting treatments explained between 4% and 10% of the variance among bacterial and fungal community structures. The only communities not significantly different between treatments on the basis of pairwise PERMANOVA were the bacterial communities in OM1 versus OM2. Effects of OM removal were stronger than effects of soil compaction and both were slightly greater in the organic versus the mineral horizon. There was no interactive effect of the two disturbances on the bacterial community, but such interactions explained 4.5% of the variance in the fungal community. ANOSIM results for treatment effects were somewhat obscured by the large variability caused by location factors, resulting in significant treatment effects on bacterial communities within the geographically close IDF sites, but not within the distant SBS sites (Table 1).

The clustering of treatment groups in the CAP ordinations was supported by significant $trace_{Q\_m'HQ\_m}$ statistics and the classification success rates that ranged between 48–89% and 57–97% for bacteria and fungi, respectively (Figure 3). Most of the misclassifications of OM removal treatments (73% for bacteria and 84% for fungi) occurred between OM1 and OM2, supporting the comparatively lower separation strength between these two treatments as indicated by PERMANOVA (Table 2). As for the compaction treatment, misclassifications were similarly distributed among the three compaction levels, whereas few misclassifications were observed for the reference group (11% for bacteria and 1% for fungi).

Because interaction between OM removal and compaction was significant for the fungal

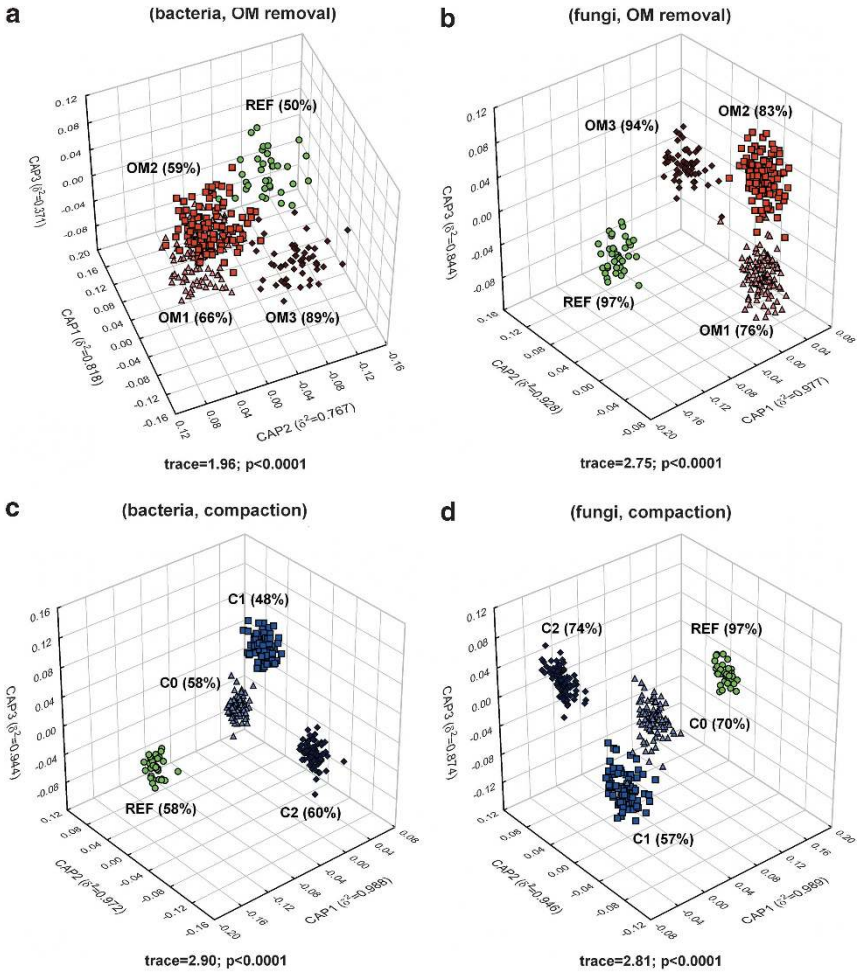

Figure 3 Canonical analysis of principal coordinates (CAP) ordinations of bacterial (**a**, **c**) and fungal (**b**, **d**) communities based on relative abundances of OTUs. These constrained ordinations show maximized differences among the different levels of OM removal (**a**, **b**) and soil compaction (**c**, **d**). Classification success rates for each treatment group are given in parentheses. Levels of OM removal: reference plots (REF), green circles; OM1, light-red triangles; OM2, red squares; and OM3, dark-red diamonds. Levels of soil compaction: reference plots (REF), green circles; no compaction (C0), light-blue triangles; moderate compaction (C1), blue squares; and severe compaction (C2), dark-blue diamonds. The canonical correlation (δ^2) of each CAP axis is given in parentheses. $Trace_{Q\_m'HQ\_m}$ test statistics are given at the bottom of each ordination.

community, CAP was used to test separation of OM removal at each compaction level and vice versa. Separating the OM removal treatments, the overall classification success was highest at level C0 (94%), followed by C1 (92%) and C2 (88%). Separating the compaction treatments, the overall classification success was the highest at level OM1 (86%), followed by OM3 (74%) and OM2 (63%). Thus, the influence of OM removal appears to be the strongest at low compaction levels, whereas the influence of compaction appears to be the strongest with low OM removal. Importantly, all OM-removal treatments yielded distinct communities at each level of compaction and vice versa.

The large differences in community structure between the two soil horizons influenced differences between OM3 and other levels of OM removal, because the organic horizon had not redeveloped in these plots and the OM3 treatment was therefore exclusively represented by samples from the mineral horizon. Therefore, CAP analyses were additionally performed separately on data sets from the organic (Supplementary Figure S5) and mineral (Supplementary Figure S6) horizons. The results confirmed the differences between OM3 and the other OM-removal treatments independent of the soil horizon.

CAP was repeated to evaluate treatment effects at each taxonomic level, from species to phylum (Supplementary Figures S7 and S8). The magnitude of treatment effects gradually decreased with increasing taxonomic level, showing overall classification success rates that dropped from 63% and 75% at species level to 43% and 26% at phylum level for bacteria and fungi, respectively. For soil-compaction treatments, overall classification rates dropped from 53% and 62% to 33% and 30% for bacteria and fungi, respectively. The most robust

separation was observed for reference versus harvested samples, whereas differences among the distinct levels of OM removal and compaction were less pronounced at higher taxonomic levels.

CAP was also used to determine the effect of OM removal on major fungal and bacterial phyla, that is, Basidiomycota, Ascomycota, Proteobacteria (split by class), Actinobacteria, Acidobacteria and Chloroflexi (Figure 4). For this purpose, the OTU data matrix was split according to the respective taxonomic affiliation. The fungal phyla, Basidiomycota and Ascomycota, were strongly affected by

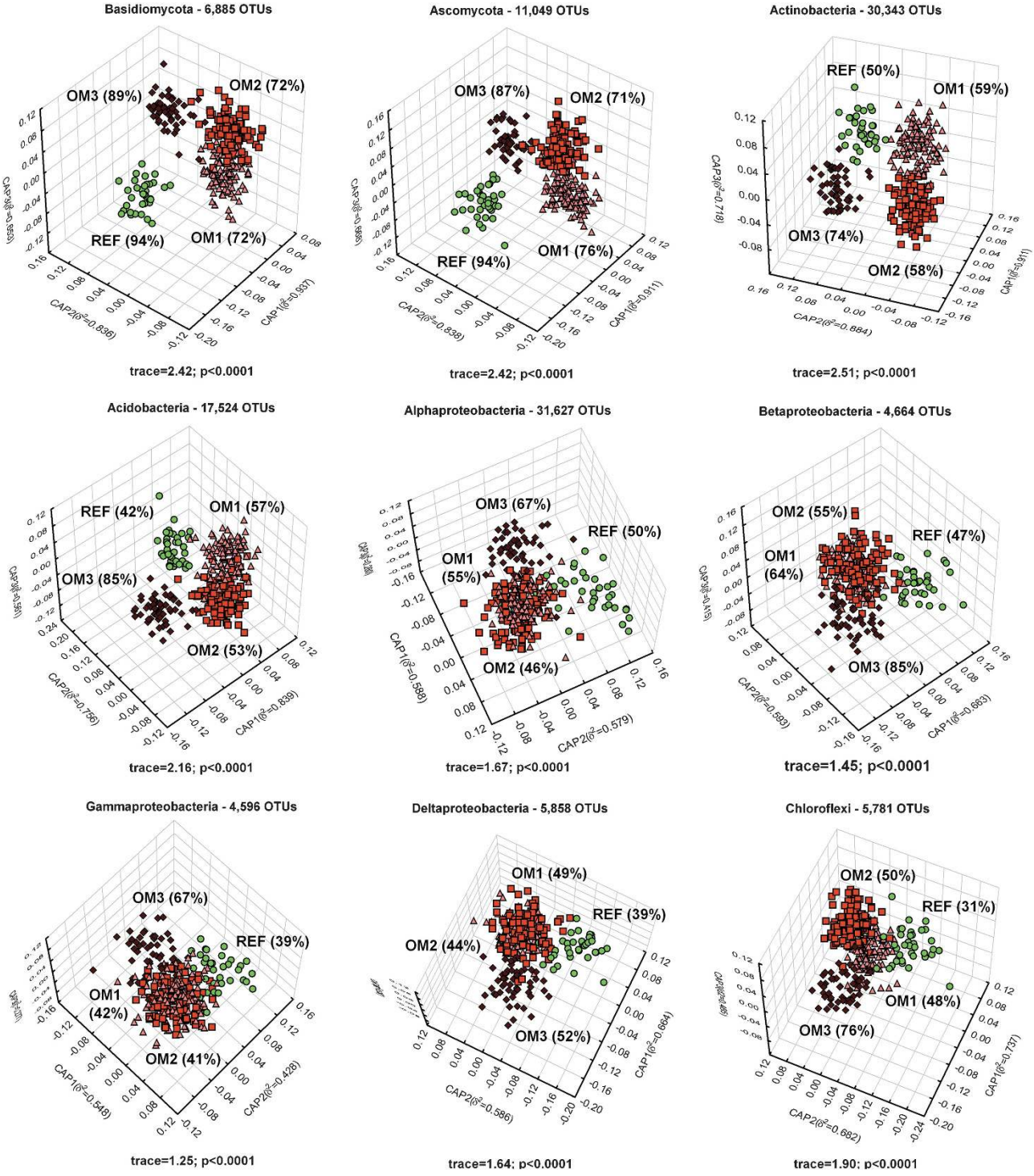

Figure 4 CAP ordinations of major fungal and bacterial phyla based on relative abundances of OTUs. These constrained ordinations show maximized differences among the different levels of OM removal. Levels of OM removal are represented by symbols and colors as in Figure 3. Classification success rates for each treatment group are given in parentheses. $Trace_{Q\_m'HQ\_m}$ test statistics are given at the bottom of each ordination.

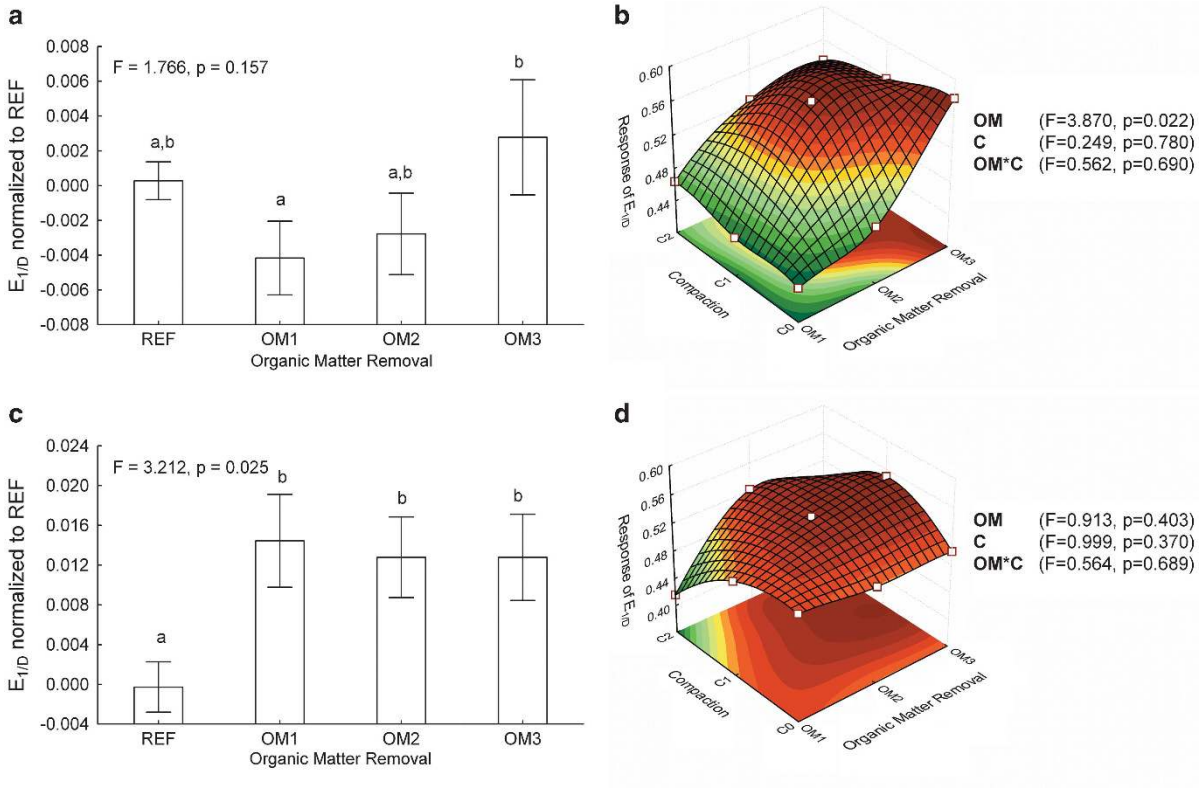

Figure 5 Simpson's evenness ($E_{1/D}$) of the bacterial (**a**, **b**) and fungal (**c**, **d**) communities based on OTUs. Indices were normalized within each site relative to the reference plots. Panels **a** and **c** compare evenness among different levels of OM removal with no compaction. Values are displayed as treatment-specific averages including s.e. Different letters indicate significant differences based on one-way ANOVA (F-test and *P*-value) followed by Fisher's least significant difference test ($P < 0.05$). Panels **b** and **d** compare evenness within the 3×3 factorial design of OM removal and soil compaction, excluding the reference plots. Significant differences among *a priori* defined that groups were evaluated using factorial analysis of variance (F-test and *P*-value) followed by a desirability approach.

OM-removal treatments, revealing high classification success rates between 71% and 94%. All bacterial phyla had lower classification success rates (31–85%) and weaker treatment separation relative to the fungal phyla. Bacterial communities in the reference samples were highly variable, resulting in consistently low classification success rates.

Bacterial evenness did not differ between unharvested and harvested treatments (Figure 5a), whereas fungal evenness significantly increased in harvested plots (Figure 5c). Within the factorial design of harvested treatments, increasing levels of OM removal corresponded to a significant increase in bacterial evenness (Figure 5b). This phenomenon was driven by the fact that the mineral soil, which was the only horizon in OM3 treatments, revealed significantly higher evenness ($E_{1/D} = 0.025$) than the organic soil ($E_{1/D} = 0.019$). Increasing compaction had no significant effect on bacterial evenness. There was no significant effect among the OM-removal and soil-compaction treatments on fungal evenness (Figure 5d).

Microbial indicators of timber harvesting

At each taxonomic level from species to phylum, indicator species analysis was used to examine the association between taxon abundance and location factors (soil horizon and geographic location) or treatment factors (OM removal and soil compaction). For every taxon, information about average abundance in each treatment group, maximum association strength (correlation R), significance of association (*P*-value) and false discovery rate correction (*q*-value) can be found in Supplementary Data 3 and 4. Associations were mapped onto taxonomic trees ranging from phylum to species. The complete interactive bacterial and fungal indicator trees can be accessed online at http://itol.embl.de/external.cgi?tree=1281891142522736131473214 40 and http://itol.embl.de/external.cgi?tree=1281891142522503131473175 30, respectively.

Complete discussion of indicator taxa is beyond the scope of this study, and we show only salient cases, which also serve as examples of how indicator species analysis can be used to describe trends for individual taxonomic groups in such large-scale data sets. The key findings among all fungal species and bacterial genera that were significantly influenced by OM removal are presented in Figures 6 and 7, respectively. Bacterial indicators are identified at the genus level, because only few bacterial OTUs could be identified at the

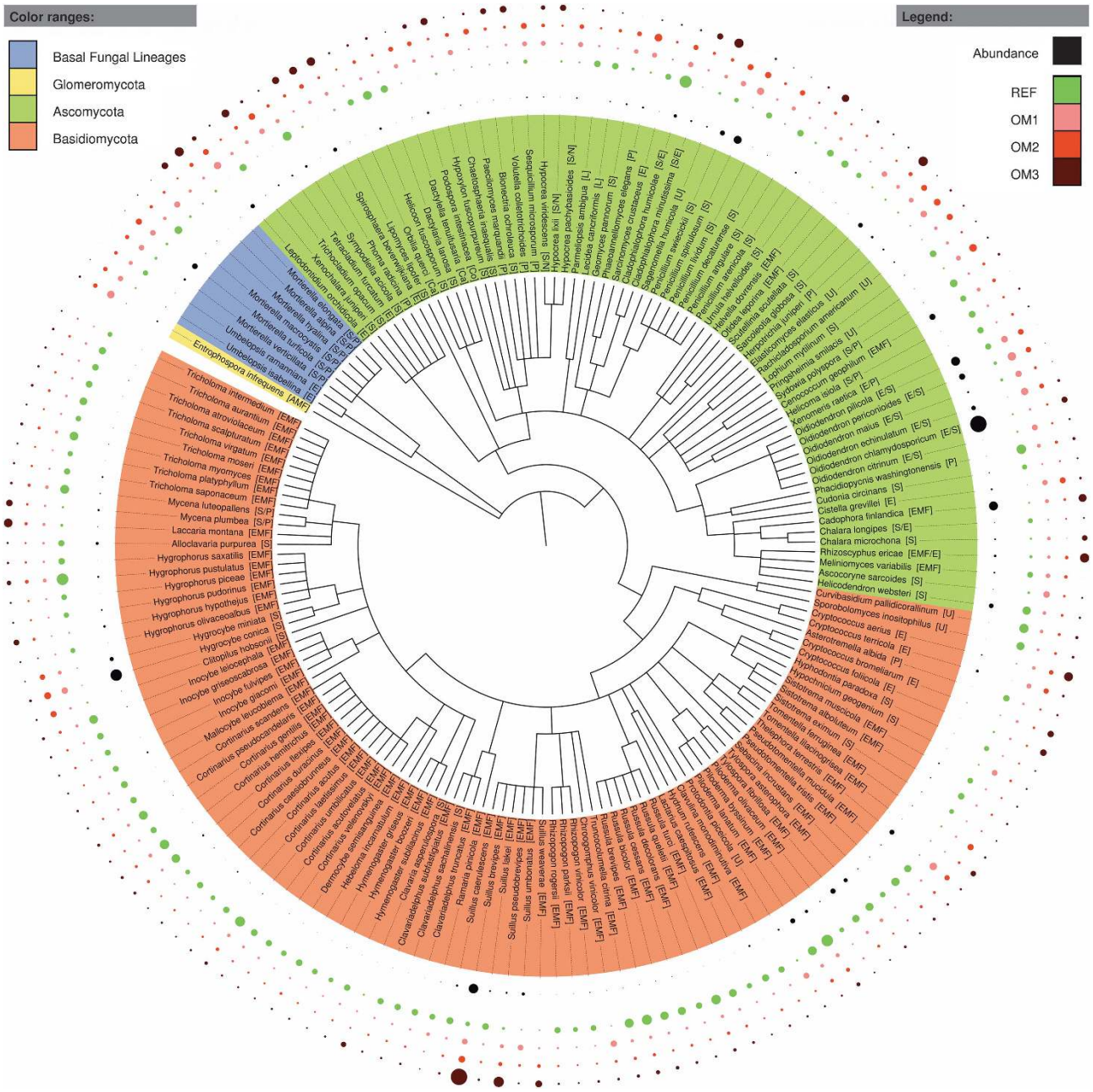

Figure 6 Indicator tree showing the taxonomy and taxon-treatment association strength of all 168 fungal species significantly associated with OM removal. Color ranges identify phyla within the tree. Legend colors identify circles. Diameters of black circles in the most inner ring represent the square-root transformed abundance of the corresponding species in the overall community. Diameters of the remaining circles represent the relative, significant (q <0.01) association strength between the corresponding species and the OM removal treatments identified by color. The putative lifestyle of each species is indicated in square brackets and was predicted based on available literature as listed in Supplementary Table S3. AMF, arbuscular mycorrhizal fungi; Ca, Carnivore; Co, Coprophile; E, endophyte; EMF, ectomycorrhizal fungi; P, pathogen/parasite; S, saprobe; U, unknown status. The full interactive tree is accessible at http://itol.embl.de/external.cgi?tree=1281891142522503131473175 30.

species level. For both fungi and bacteria, OTU-level analysis would have given many indicators redundant at the species level. In order to support the results of the indicator species analysis, Pearson's correlation coefficients were calculated between abundance of the significant fungal species or bacterial genera and the CAP ordination scores in Figure 3. These correlations are provided as Supplementary Tables S3 and S4, respectively.

Among the 1129 identified fungal species, 168 were significantly associated with OM-removal treatments (Figure 6). These 168 indicator species represented 28% of the total fungal pyrotags. Basidiomycetes revealed consistent responses to OM removal, whereas ascomycetes revealed variable responses. Overall, Ascomycota increased and Basidiomycota decreased in harvested plots. Most (80%) of the basidiomycetes indicator species were

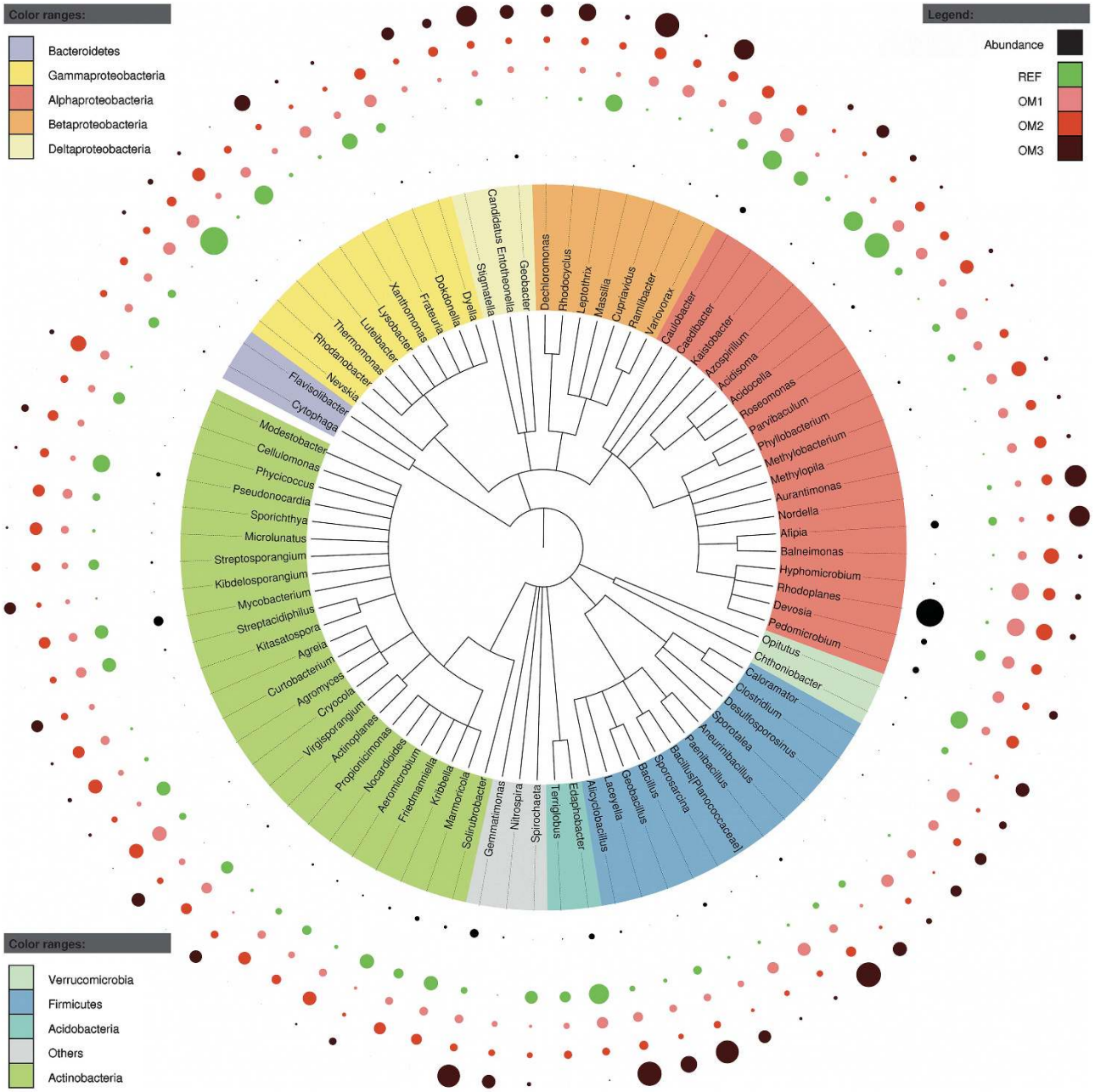

Figure 7 Indicator tree showing the taxonomy and taxon-treatment association strength of all 83 bacterial genera significantly associated with OM removal. Color ranges identify phyla (and proteobacterial classes) within the tree. Legend colors identify circles. Diameters of black circles in the innermost ring represent the square-root transformed abundance of the corresponding genera in the overall community. Diameters of the remaining circles represent the relative, significant ($q < 0.01$) association strength between the corresponding genus and the OM removal treatments identified by color. The full interactive tree is accessible at http://itol.embl.de/external.cgi?tree=1281891142522736131473214 40.

identified as putative ectomycorrhizal species with the remainder being putative saprobes (11%), putative endophytes (4%) or of unknown status (5%) (Supplementary Table S3). The majority (86%) of the ectomycorrhizal species (for example, members of the genera *Tricholoma*, *Inocybe*, *Cortinarius*, *Hymenogaster*, *Rhizopogon*, *Russula*, *Piloderma* and *Tylospora*) was associated with the unharvested reference stands and thus consistently reduced in harvested plots (Figure 6, Supplementary Table S3). Only few ectomycorrhizal species, such as the highly abundant *S. brevipes*, had the opposite response. Among the significantly associated ascomycetes, there were only 9% putative ectomycorrhizal species, which again were mainly reduced in harvested plots (Figure 6).

Non-mycorrhizal ascomycete species showed variable responses to OM removal (Figure 6,

Supplementary Table S3). For example, some putative saprobes (for example, *Xenochalara juniper*, *Sympodiella acicola*, *Helicoon fuscosporum* and *Helicodendron websteri*) had a positive association with the reference plots, whereas other putative saprobes (for example, *Trichocladium opacum*, *Lipomyces lipofer*, *Penicillium arenicola* and *Sarcoleotia globosa*) had a positive association with OM3. Furthermore, some species, such as *Oidiodendron maius*, *Pringsheimia smilacis*, *Scutellinia scutellata*, and *Paecilomyces marquandii*, differed between OM1 and OM2.

Among the 394 identified bacterial genera, 83 were significantly associated with OM-removal treatments (Figure 7). These 83 indicator genera represented 18% of the total bacterial pyrotags. None of the represented phyla had a consistent response. The second most abundant genus of the bacterial community, *Rhodoplanes*, was an indicator strongly associated with OM1 and OM2 plots. Genera with the strongest overall correlations to the CAP ordination scores were among the Proteobacteria, including *Rhodanobacter*, *Acidisoma*, *Luteibacter*, *Massilia* and *Hyphomicrobium*.

Several fungal and bacterial taxa significantly responded to soil-compaction treatments (see online trees, Supplementary Data 3 and 4). However, the main effects were largely related to differences between unharvested versus harvested plots. Effects of OM removal and soil compaction cannot be distinguished when comparing reference versus harvested plots, because even without soil compaction, removal of trees and the associated partial loss of soil structure induced a large increase in bulk density. On average, the uncompacted C0 treatments experienced an increase in bulk density equivalent to 20% of the maximum bulk density observed in C1. Excluding the reference samples and running the indicator species analysis on only the three compaction levels among the harvested plots revealed that the bacterial genera *Geobacter*, *Ramlibacter* and *Rhodanobacter* were the most strongly affected by the different compaction intensities (data not shown). *Suillus pseudobrevipes*, *Verticillium leptobactrum* and *Rhizopogon subbadius* were the most strongly affected fungal species. For details about taxa significantly associated with particular locations or horizons, we refer to the online trees and Supplementary Data 3 and 4.

Biomass

The average bacterial and fungal biomass in each soil horizon at each forest site was estimated by measuring PLFA contents in soil samples from the unharvested plots. The estimated bacterial biomass (average ± s.d.) was 3.3 ± 1.3 and 0.4 ± 0.2 mg C per gram soil dry weight in the organic and mineral horizons, respectively. The estimated fungal biomass was 0.6 ± 0.4 and 0.08 ± 0.12 mg C per gram soil dry weight, respectively. Differences in bacterial and fungal biomass were statistically significant between horizons, but not among forest sites or between biogeoclimatic zones. Fungal/bacterial biomass ratios were 0.17 ± 0.04 and 0.16 ± 0.09 in the organic and mineral horizons, respectively. The higher biomass in the organic horizon was confirmed by nucleic acid concentrations determined across all 306 soil samples, yielding 70 ± 30 and 22 ± 14 μg DNA per gram soil dry weight in the organic and mineral horizons, respectively. Based on DNA concentrations, soils from the SBS zone had significantly higher (36%) biomass than soils from the IDF zone. The northern sites SBS1 and SBS2 featured 36% and 88% higher concentrations than the average of the southern sites SBS3, IDF1, IDF2 and IDF3. There was no difference in DNA concentration between unharvested and harvested plots or among the different OM removal and soil-compaction treatments.

Discussion

We found that environmental changes associated with timber harvesting alter soil microbial communities over the long term. Effects of clear-cut harvesting *per se* far exceed any differences observed among harvested treatments with varied disturbance levels. However, different degrees of OM removal and soil compaction did lead to distinct communities. Long-term effects beyond 10 years have been poorly studied (Marshall, 2000), but soil microbial communities were suggested to possess a high potential for resilience or tolerance following intensive harvesting (Li *et al.*, 2004; Busse *et al.*, 2006; Hannam *et al.*, 2006; Mariani *et al.*, 2006). For the first time, we present data from new high-resolution analyses that challenge the assumption of resilience and demonstrate that soil microbial communities remain strongly affected 15 years after harvesting. This finding is surprising because no robust treatment effects on soil chemistry, nitrogen mineralization or growth of lodgepole pine have been observed in these BC LTSP sites 10–15 years post harvest (Marty Kranabetter, personal communication, and Kranabetter *et al.*, 2006; Sanchez *et al.*, 2006b; Ponder *et al.*, 2012). Correlating these changes in the microbial community structure, including information on functional groups, with forest productivity and soil processes over the long term will ultimately be required to predict the implications of these changes for soil management and biofuel-harvesting strategies.

We observed major variations in the microbial communities among the different forest sites and between the organic and mineral soil horizons. These findings demonstrate that local biogeoclimatic conditions, possibly including differences in tree productivity, result in characteristic microbial communities and provide useful baseline

information for assessing environmental change. Monitoring these communities over time can potentially help to assess ecosystem effects of climate change and to understand and facilitate migration of plant species. The variability among communities highlights the need for large-scale ecosystem studies to assess management effects across diverse climatic conditions, soils and vegetation. It is noteworthy that we found patterns of community disturbance spanning the different sites and soil horizons, despite the large natural variability among these communities. Robust indicators of forest disturbances can only be determined when the natural variability occurring among forest sites and soil horizons is sampled. This is only possible when communities are analyzed at high resolution and ecologically meaningful scales.

In this study, microbial communities proved to be sensitive measures of environmental change associated with timber harvesting, supporting the general notion that microbial communities are useful indicators of disturbances (Allison and Martiny, 2008). This is an important finding, because measures of many physical, chemical and other biological characteristics generally failed to consistently detect disturbance effects in the LTSP experimental series (Powers *et al.*, 2005). Sensitive measures are needed to detect changes in forest ecosystems before they have detrimental and irreversible effects, but because of potential functional redundancies in microbial communities, it remains to be determined if these measures indeed predict changes in ecosystem functioning. Important ecosystem properties such as soil displacement, erosion and loss of OM are poor indicators because they possess limited predictive power and reflect the end result of environmental degradation (Staddon *et al.*, 1999). Ideally, indicators should predict soil degradation before it has serious consequences. Long-term monitoring of tree growth, soil processes and microbial community structures in the LTSP sites and other long-term studies, ideally over decades and multiple rotations, will ultimately be necessary to relate particular patterns of change in soil community structures to long-term outcomes with respect to soil productivity, ecosystem functioning and sustainable timber harvesting.

Harvesting effects on fungi

Functional diversity of bacteria and fungi is high, often distributed among phylogenetically distinct groups, and we know little about the ecological roles of most taxa (Tedersoo *et al.*, 2010a). Therefore, summarizing the potential effects of harvesting on the microbial community is challenging. Here, we focus on some of the most robust effects observed in this study and their environmental implications. Although no genetic marker features the same phylogenetic resolution and allows absolute comparability between bacteria and fungi, clustering at 97% identity is commonly applied to both bacterial 16S and fungal ITS to delineate species (for example, Tedersoo *et al.*, 2010b; Turnbaugh *et al.*, 2010), suggesting that direct comparisons are warranted with caution. Our results support this conclusion, showing that differences in ecological patterns between bacteria and fungi, particularly the greater patchiness of fungi, are maintained at different taxonomic levels (Supplementary Figure S1).

Fungi showed a much stronger response to harvesting than bacteria. Clear-cut harvesting increased fungal evenness and altered fungal community structure. The patchy distribution of fungal populations may make them more susceptible to harvesting effects, because it has been hypothesized that populations with high spatial variability may be more prone to disturbances of their habitat (Vucetich *et al.*, 2000). It has been reported that clear-cut harvesting generally reduces fungal biomass (Bååth *et al.*, 1995; Busse *et al.*, 2006; Chatterjee *et al.*, 2008; Mummey *et al.*, 2010). Because fungal communities are vital to forest ecosystems (Dighton *et al.*, 2005), these pronounced harvesting effects are likely of environmental importance. The increased fungal evenness in harvested stands may reflect the loss of functional organization of this community, and the reduction of most ectomycorrhizal fungi in harvested stands suggests a change in functional potential. In contrast to mycorrhizal fungi, saprobic fungi did not show a consistent response. Thus, while mycorrhizal communities are likely affected by any harvesting procedure, saprobic communities might respond differently to various harvesting procedures. Because extirpation of fungal populations might be linked to biomass removal (Berch *et al.*, 2011), fungi appear to be excellent indicators of forest-harvesting impacts.

There are several potential explanations why fungi are more affected than bacteria by harvesting disturbances (Jones *et al.*, 2003). Harvesting eliminates plant hosts and therefore the energy source of symbiotic organisms. Removal of woody debris and forest floor eliminates the major habitat for many saprobic and ectomycorrhizal fungi, and associated loss of organic substrate will limit growth of saprobes. Furthermore, soil compaction can disrupt mycorrhizal networks, and subsequent increases in bulk density may similarily impede hyphal penetration through the soil. Although our findings strongly support the hypothesis that mycorrhizal fungi are negatively affected by timber harvesting (Marshall, 2000), they should not be generalized. Whereas harvesting consistently reduced the majority of putative ectomycorrhizal species, some others, such as *Suillius* species, exhibited a highly variable response, probably due to their high host-specificity (Bruns *et al.*, 2002). Our ability to detect these species-level effects on a large scale highlights the power of high-throughput sequencing approaches. Although effects are strong after more than a decade post harvest, mycorrhizal communities might recover

simultaneously with plant communities when canopy crown closure occurs (Simard, 2009). We now have molecular tools at hand to adequately monitor the response of soil microbial communities over longer time frames, including multiple tree rotations.

Harvesting effects on bacteria

Bacterial communities showed a smaller response to harvesting compared with fungal ones. It remains to be determined whether bacteria are more resistant, more resilient or both. Bacteria might be more tolerant of greater variability in moisture, temperature and aeration occurring in harvested plots (Ballard, 2000; Powers *et al.*, 2005). Many bacteria do not rely on symbiotic hosts, are ubiquitous throughout the soil environment and tolerate anoxic conditions resulting from soil compaction. Furthermore, bacteria might benefit from reduced soil pore space, affording protection from protozoan grazing (Wright *et al.*, 1995; Schnurr-Pütz *et al.*, 2006). All these factors could reduce the initial sensitivity and increase recovery of bacteria in harvested stands.

Actinobacteria, Acidobacteria as well as Alpha- and Betaproteobacteria were moderately affected by harvesting, whereas the other groups were mostly resilient or of low abundance. The affected bacterial groups are metabolically versatile, and we know little about the ecology of the genera most strongly associated with harvesting disturbances. Actinobacteria in general are specialized in degrading recalcitrant compounds of high molecular weight, such as lignin or cellulose (McCarthy and Williams, 1992; Kirby, 2005), and were suggested to prefer OM-derived carbon sources (Kramer and Gleixner, 2008). This makes them likely sensitive to changes in OM in harvested forests, as previously suggested (Hartmann *et al.*, 2009). As for Proteobacteria, the high relative abundance (35%) of Rhizobiales, and its genus *Bradyrhizobium* in particular (15%), suggests a potentially important contribution of these taxa to ecosystem services, such as nitrogen fixation. Given the low abundance of leguminous plants, such as alder or lupine in these plots (Marty Kranabetter, personal communication), it is likely that these *Bradyrhizobium*-like species are either non-symbiotic free-living representatives or are associated with tuberculate ectomycorrhizae formed with lodgepole pine. These tumor-like mycorrhizal structures (for example, between *Suillus* and pine) have been suggested to have a significant role in nitrogen fixation (Paul *et al.*, 2007), and sequences related to the genus *Bradyrhizobium* were recently recovered from such tubercules (Kretzer *et al.*, 2009). Given the high abundance of these taxa and the potential importance for ecosystem functioning, this observation warrants further investigation. Other key functional groups that could be affected by harvesting, such as nitrifiers (Belser, 1979) or methanotrophs (Bowman, 2006), were of very low abundance or not detected at all in our data set, highlighting the need to specifically monitor functional groups in these systems. Others, such as diazotrophs (Zehr *et al.*, 2003) and denitrifiers (Zumft, 1997), are phylogenetically diverse, making them difficult to assess via our analysis based on ribosomal genes, which again warrants investigations based on functional genes.

Increased biomass harvesting

We found that forest floor removal had a significant effect on the community structure of Basidiomycota, Ascomycota, Actinobacteria, Acidobacteria and Betaproteobacteria, as well as on total bacterial evenness. Practices like scalping the forest floor are used to manage early succession tree species, but cause soil OM losses and compaction (Jurgensen *et al.*, 1997). It has been shown that scalping can change the composition of ectomycorrhizae and alter their colonization of seedling roots, affecting seedling survival and growth (Simard *et al.*, 2003; Simard, 2009). Regeneration of the organic horizon is slow (Michel and Williams, 2011), and no forest floor had redeveloped in the present OM3 plots after more than a decade. Because microbial communities are highly distinct between organic and mineral horizons, removal of the former has a great impact on overall community composition. Furthermore, microbial biomass was reduced in these plots, because we found that the organic layer had around eight times higher biomass when compared with the mineral soil. Interestingly, the variability of bacterial communities was significantly higher in the organic horizon, which is likely to reflect greater fluctuations in the physical, chemical and biological parameters in the upper and more organic soil horizons (Fierer *et al.*, 2003; Will *et al.*, 2010).

Beyond eliminating a habitat and the associated populations, removal of the forest floor also had an impact on the community structure in the underlying mineral horizon. A significant loss of nutrients (Simard *et al.*, 2003) and the lack of the insulating organic layer influencing soil moisture and temperature (Powers *et al.*, 2005) may have contributed to this effect. Removal of recalcitrant compounds located in the forest floor might particularly affect saprobic populations, such as ascomycetes and actinomycetes, located in the underlying mineral soil. Our observations imply that retention and protection of the forest floor during site preparation are crucial processes for maintaining important soil properties and microbial metabolic potential, but measurement of other edaphic parameters, including process information, will be required to predict changes in soil functioning.

The sustainability of whole-tree harvesting is an important, yet largely unresolved management question. Residue management and retention of woody debris were previously shown to shape biodiversity (Nordén *et al.*, 2004; Josefsson *et al.*, 2010; Verschuyl *et al.*, 2011), and different components of dead wood

have been shown to harbor different microbial populations (Barengo *et al.*, 2000; Nordén *et al.*, 2004). Alterations of OM quality and quantity have been proposed to threaten forest biodiversity (Berch *et al.*, 2011). Despite these reports, previous studies showed small effects of residue retention on overall microbial community structure (Hannam *et al.*, 2006) or fungal saprobes (Allmér *et al.*, 2009). Similarly, compared with microbial community differences among the other OM treatments, we found relatively small differences between the OM2 and OM1 treatments, which were statistically significant for only the fungal community. Both basidiomycetes and ascomycetes appeared to be similarly affected by OM2 versus OM1, supporting the suggestion that saprobic and mycorrhizal fungi are among the organisms most susceptible to increasing biomass removal (Berch *et al.*, 2011). Overall, results of this and other studies indicate a relatively small difference between effects of OM1 versus OM2 harvesting, but that difference is detectable, and it remains to be determined whether it has functional consequences for ecosystem processes.

Increased soil compaction

Our results contradict the suggestion that the microbial community is generally resilient to soil compaction (Shestak and Busse, 2005; Busse *et al.*, 2006). We observed long-lasting effects of different compaction levels on microbial community structures. As with OM removal, effects of compaction on bacteria were smaller than that on fungi. This difference is likely due in part to bacterial tolerance of low oxygen concentrations and increased protection of bacteria from protozoan grazing as a result of compaction. Given that bulk density in the BC LTSP sites has recovered little in the 10–15 years because of soil compaction associated with harvesting, the long-term environmental consequences of compaction are likely significant. In fact, once a soil has been compacted, a return to the initial state might be very slow (von Wilpert and Schäffer, 2006) and recovery of a soil from severe compaction might take centuries rather than decades (Attiwill and Weston, 2008). In our study, although compaction effects on the communities were clear, no strong indicators were identified. This may indicate that compaction has small, variable effects on many populations. Additionally, detecting compaction indicators was limited by our inability to completely isolate OM removal and compaction treatments, because of the unavoidable increase in bulk density in C0 treatments caused by loss of soil structure after tree removal.

Conclusion

Our results indicate that bacterial and fungal communities, although varying greatly across soil horizons and biogeoclimatic regions, manifest significant long-term responses to OM removal and soil compaction, with distinct responses to different levels of disturbance persisting more than a decade after tree harvesting. Symbiotic and saprobic species appear to be the most sensitive microbial groups and are potential indicators for monitoring the recovery of forest systems. Long-term monitoring over the course of a forest stand rotation will be required to determine if the microbial community structure is resilient over the very long term. Given the high potential for functional redundancy within soil ecosystems, it remains to be determined if observed changes in microbial community structure predict changes in ecosystem processes and loss of soil productivity over multiple harvesting rotations, which would make such indicators invaluable forest-management tools. Future studies exploring soil processes and metabolic properties of these forest soil communities are now needed to determine the functional significance of the observed compositional effects and indicator dynamics with respect to higher-order ecological and biogeochemical processes.

Acknowledgements

We gratefully acknowledge the LTSP team from the British Columbia Ministry of Forests, that is, Shannon Berch, Bill Chapman, Stephane Dubé, Graeme Hope and Marty Kranabetter, for providing access to the LTSP experimental sites, assistance in sampling and their helpful discussions. We thank Andrew Yamada and Claudia Hartmann-Fischler for soil processing and DNA extraction. We are grateful to Pedro Dimitriu for soil sampling and helpful discussions during data analysis. We thank Ivica Letunic for implementing required features into the iToL software tool. We also wish to acknowledge the contribution of scientists at the McGill University and Génome Québec Innovation Center, Montréal, Canada, for the 454 pyrosequencing. We are grateful for the valuable comments on this manuscript provided by the three anonymous reviewers. This work was supported by grants from Genome British Columbia, the Canadian Institute for Advanced Research and the Tula Foundation to the Center for Microbial Diversity and Evolution.

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