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Effects of Wildfire and Harvest Disturbances on Forest Soil Bacterial Communities▽†

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Wildfires and harvesting are important disturbances to forest ecosystems, but their effects on soil microbial communities are not well characterized and have not previously been compared directly. This study was conducted at sites with similar soil, climatic, and other properties in a spruce-dominated boreal forest near Chisholm, Alberta, Canada. Soil microbial communities were assessed following four treatments: control, harvest, burn, and burn plus timber salvage (burn-salvage). Burn treatments were at sites affected by a large wildfire in May 2001, and the communities were sampled 1 year after the fire. Microbial biomass carbon decreased 18%, 74%, and 53% in the harvest, burn, and burn-salvage treatments, respectively. Microbial biomass nitrogen decreased 25% in the harvest treatment, but increased in the burn treatments, probably because of microbial assimilation of the increased amounts of available NH_4^+ and NO_3^- due to burning. Bacterial community composition was analyzed by nonparametric ordination of molecular fingerprint data of 119 samples from both ribosomal intergenic spacer analysis (RISA) and rRNA gene denaturing gradient gel electrophoresis. On the basis of multiresponse permutation procedures, community composition was significantly different among all treatments, with the greatest differences between the two burned treatments versus the two unburned treatments. The sequencing of DNA bands from RISA fingerprints revealed distinct distributions of bacterial divisions among the treatments. *Gamma-* and *Alphaproteobacteria* were highly characteristic of the unburned treatments, while *Betaproteobacteria* and members of *Bacillus* were highly characteristic of the burned treatments. Wildfire had distinct and more pronounced effects on the soil microbial community than did harvesting.

Forests have always experienced natural disturbances, most notably fires. Such disturbances have important and, in many cases, beneficial long-term effects on forest ecosystems. Microbial communities, which mediate decomposition and nutrient cycling, play an important role in the resilience of forests to disturbances and in the regeneration process. In managed forests, harvesting has become the most important disturbance. One proposed approach to forest management is to emulate natural disturbance. To properly evaluate this approach and, more generally, to sustainably manage forests, it is critical that we understand the differences between fire and harvesting in terms of their effects on forest ecosystems, including soil microbial communities.

Fire has profound effects on forest ecosystems. Surface temperatures have been reported to reach 1,000°C (1), and a number of physicochemical properties of the soil are affected. Vegetation and litter removal reduce albedo and result in increased surface temperatures (31). Severe heating of soil breaks down the structures of the inorganic parent materials, destabilizing soil structure (52). Fire creates hydrophobic layers within the soil structure, decreasing water infiltration and increasing soil erosion by water runoff (2, 15). Nutrient transformations occur when excessive heat is applied to soils. The effects on ammonium and nitrate concentrations are variable (13, 29, 33), while concentrations of phosphorus, potassium, and magnesium are reported to increase (46). Finally, most studies report an increase in soil pH following fires, due to the release of basic cations during combustion and their deposition on the soil surface (2, 40). This increase in soil pH increases the availability of phosphorus, calcium, magnesium, and potassium.

Harvesting has physicochemical effects on forest ecosystems that resemble the effects of fire in some respects and are distinct in other respects. A major result of harvesting is the compaction of forest soils (10). Consequently, soil aggregates disintegrate, releasing clay and silt particles that plug soil pores and reduce infiltration capacity. Increased fluctuations in soil temperature and moisture follow harvesting because of the loss of the shading overstory (10, 19). Increased availability of magnesium (19) as well as short-term increases in NH_4^+ (6) has been reported for clear-cut treatments.

Predictably, fires and harvesting have effects on both the biomass and composition of forest soil microbial communities. Biomass most often decreases as a result of these disturbances, and this effect can last for many years (5, 16, 17, 35, 39, 42, 44). Culture-based analyses indicated effects of both fire and harvesting on the composition of microbial communities (18, 38, 54). More recently, phospholipid fatty acid (PLFA) analyses have confirmed and better characterized these effects (5, 21, 41, 45). However, not all studies using PLFA analysis showed a significant effect of harvesting on community composition (23), consistent with the hypothesis that harvesting has a less

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TABLE 1. Locations of sampling sites in the Chisholm-Slave Lake area of Alberta, Canada

Site name	Latitude/longitude	Area
C1	54°54′N, 114°3′W	Chisholm
C2	55°17′N, 115°6′W	Canyon Creek
C3	55°13′N, 114°42′W	Vanderwell Haul Road
H1	55°17′N, 115°5′W	Canyon Creek
H2	55°17′N, 115°6′W	Canyon Creek
H3	55°17′N, 115°6′W	Canyon Creek
B1	54°54′N, 114°6′W	Chisholm
B2	54°56′N, 114°6′W	Chisholm
B3	55°8′N, 114°12′W	Saulteaux
S1	54°55′N, 114°6′W	Chisholm
S2	55°1′N, 114°22′W	Five Corners Road
S3	55°1′N, 114°23′W	Five Corners Road

severe effect than does fire. DNA-based analyses have also demonstrated effects of both fire and harvesting on community composition (6, 34, 56), although relatively few such studies have been reported.

Despite substantial progress made in the above studies, knowledge of how forest soil microbial communities are impacted by wildfire and harvesting remains limited. One constraint of many studies on effects of fire is that they investigated managed fires (prescribed burns), which are neither as hot nor as disruptive as natural wildfires. Further, there is a lack of studies comparing the effects of fire and harvesting using comparable sites and time frames. In the present study, the bacterial community in a spruce-dominated boreal forest soil was examined 1 year postwildfire and 8 months postharvest. The effects on both microbial biomass carbon (C_{mic}) and microbial biomass nitrogen (N_{mic}) were determined. Bacterial community composition was analyzed by two DNA fingerprinting methods, denaturing gradient gel electrophoresis (DGGE) and ribosomal intergenic spacer analysis (RISA), to determine the effects on community composition and the relationships between community composition and abiotic environmental factors. DGGE is based on 16S rRNA gene fragments, which are highly conserved and distinguish populations at approximately the genus level. RISA is complementary to DGGE, as it is based on intergenic spacers, which are highly variable and distinguish populations at approximately the species-strain level. Populations critical for distinguishing treatment effects were identified by sequencing their bands from the DNA fingerprints. This means of identification permits greater taxonomic resolution than is possible with PLFA analysis. This is the first extensive molecular study to assess how wildfire and large-scale harvest treatments impact boreal forest soil microbial biomass and bacterial community composition.

MATERIALS AND METHODS

Sample sites. Soil samples were taken from the Chisholm-Slave Lake area, approximately 150 km north of Edmonton, Alberta, Canada (Table 1). A 116,000-ha wildfire occurred in this area between 23 May 2001 and 4 June 2001. Twelve sites, each approximately 2.5 to 5 ha, half burned and half unburned, were selected in a completely randomized design. Three unburned sites were harvested, and three burned sites were salvage harvested, both by clear-cutting over the 2001-2002 winter. Thus, the four treatments, in triplicate, were control (C), harvest (H), burn (B), and burn-salvage (S). Each site was surrounded by a 50-m buffer zone and had a 16-point grid with 20 m between plots, from which 10 sample plots were randomly chosen for sampling. Sample names were recorded as, for instance, C1 P10, where C indicates control treatment, 1 indicates the replicate number, and 10 indicates the plot number. All sites were comprised of productive mixed wood (at least a 60%:40% ratio of coniferous-to-deciduous species), predominantly (>80%) white spruce [*Picea glauca* (Moench) Voss] plus trembling aspen (*Populus tremuloides* Michx.) and balsam poplar (*Populus balsamifera* L.). The sites were classified as productive based on the size, density, and composition of trees located in the stand, as classified by the Alberta Vegetation Inventory. Optimum site characteristics consisted of a stand density of 51 to 70% crown closure with a tree height of 25 m. Most sites were level to nearly level (0 to 0.5% slope), while one (H1) had some plots (P11, P13, P14, and P16) on a west-facing aspect roughly classified as very gentle to gentle (2 to 9% slope). The soils in the area were generally brunisols and dark gray luvisols, with an average clay content of 19.6% and sand content of 37.7%.

Litter layers were completely incinerated in the two burned treatments. In the control and harvest treatments, litter was removed from the sample plots. Soil samples were taken from the top 0 to 10 cm of the mineral soil with a sterile scoop. Samples were homogenized in sterile plastic bags and placed in sterile sample bottles (I-CHEM certified) for transport. All samples were collected in July 2002 and kept cold prior to processing, which was done within 1 week of collection. The Canadian Forest Service analyzed samples for NO_3^-, NH_4^+, P, and pH by using standard methods.

DNA extraction. Total microbial community DNA (extracted microbial DNA [DNA_{ext}]) was extracted from 0.5-g (fresh weight) soil samples by using the FastDNA Spin kit for soil (Qbiogene, Irvine, CA) according to manufacturer's instructions. The optimization of soil DNA extractions entailed agitating samples in a FastPrep instrument for 40 s at 5.5 m/s and eluting them in 100 μl of DNA Elution Solution-Ultra Pure water. The integrity of the DNA_{ext} was checked on a 0.8% agarose gel stained with ethidium bromide and UV illuminated in an AlphaImager (Alpha Innotech, San Leandro, CA). DNA_{ext} was quantified using a known amount of 1-kb ladder, and solutions of 3 ng/μl of DNA_{ext} were prepared for PCR fingerprinting methods.

Chloroform fumigation extraction of soluble organics. Chloroform fumigation extraction was carried out using 15 g (fresh weight) of each soil sample, according to the standard technique (25). Briefly, samples were fumigated for 5 days with 50 ml of ethanol-free chloroform in moist paper towel-lined desiccators. Samples were extracted with 70 ml of 0.5 M K_2SO_4 on a reciprocal shaker on ice for 1 h. Samples were gravity filtered through presoaked Whatman ashless filter paper (grade 42; Whatman International Ltd., England), and then the extracts were vacuum filtered through 0.45-μm Millipore filters (Millipore Corporation, MA). Filtered extracts were stored at −20°C in acid-washed vessels until analyzed. Control samples without soil were also extracted to determine background carbon and nitrogen values for both the filter paper and the extractant.

Microbial biomass carbon. Soluble organic carbon was analyzed in extracts from fumigated and nonfumigated samples using the high-temperature combustion method with a Shimadzu TOC-500 carbon analyzer. C_{mic} was estimated as the difference between fumigated and nonfumigated samples (both minus blank values) divided by a K_{EC} value (extractable fraction of microbial biomass C) of 0.45 (28, 55). All microbial biomass data were expressed on a dry soil basis (oven dried at 105°C for 24 h).

Microbial biomass nitrogen. For the determination of total nitrogen, an alkaline persulfate oxidation, which oxidizes all the nitrogen species to NO_3^-, was carried out on the K_2SO_4 extracts (9). Preoxidation levels of NO_3^--N were determined for the nonfumigated samples on the Lachat QuikChem Ae autoanalyzer at the Soil Science Laboratory at UBC. Available NH_4^+ was determined colorimetrically for the nonfumigated samples by using a Technicon Autoanalyser II. Total NO_3^--N in the oxidized K_2SO_4 extracts was measured in the fumigated and nonfumigated samples. N_{mic} was estimated as the difference between fumigated and nonfumigated samples (both minus blank values) divided by a K_{EN} (extractable fraction of microbial biomass N) of 0.54 (8).

RISA. RISA fragments were amplified using the universal bacterial primers S926f and L189r, as previously described (57), yielding amplicons with the 3′ region of the 16S rRNA gene plus the length-variable RIS. The rRNA gene-RIS amplicons were separated electrophoretically and imaged. Fingerprint banding patterns (see Fig. S1 in the supplemental material) were analyzed using GelCompar II software (Applied Maths, Belgium) and normalized. Band classes (operational taxonomic units [OTUs]) were established from the band mobility and intensity data. Inevitably, some band classes were empty, while others contained the sum of two or, rarely, three bands. For further details of electrophoresis, imaging, and fingerprint analysis, see the supplemental material.

DGGE. DGGE fragments included the variable 3 region of the 16S rRNA gene, as previously described (37). Amplicons were checked for size, purity, and concentration with agarose gels and separated with polyacrylamide gels having a 40% to 60% gradient of denaturants. Fingerprint banding patterns (see Fig. S2

TABLE 2. Comparison of microbial biomass among treatments

Treatment	Concn in soil for[a]:						Ratio in soil for:	
	C_{mic} (mg/g of dry soil)		N_{mic} (mg/g of dry soil)		DNA_{ext} (μg/g of dry soil)		C_{mic}/N_{mic}	C_{mic}/C_{org}
	Mean	SD	Mean	SD	Mean	SD		
Control	0.55	0.28	0.077	0.025	10.2	5.27	8.0	3.9
Harvest	0.45	0.47	0.064	0.011	9.89	6.37	7.0	2.3
Burn	0.14	0.12	0.10	0.056	2.91	2.12	1.2	0.81
Salvage	0.26	0.20	0.089	0.033	11.2	7.32	3.5	2.0

[a] Values varied significantly among treatments (analysis of variance; $P < 0.05$) and differed significantly between each possible pair of treatments according to Bonferroni contrasts, where adjustments to the alpha level were made to maintain a constant probability of type I error.

in the supplemental material) were analyzed, and OTUs were established, as carried out for RISA fingerprints. For further details of amplification, electrophoresis, imaging, and fingerprint analysis, see the supplemental material.

Statistical analyses. Nonmetric multidimensional scaling (NMS) was used for ordination of the RISA and DGGE fingerprints plus soil chemistry data by using PC-ORD software (MjM Software, Gleneden Beach, OR). This nonparametric ordination method best handles ecological community data that are nonnormally distributed (11, 36). For ordination analysis, the Bray-Curtis nonmetric distance measure was used. Multiresponse permutation procedures (MRPP) were performed using PC-ORD software to test for significant differences between treatments of both RISA and DGGE fingerprints. Mantel tests were used to evaluate the agreement between RISA and DGGE ordination analyses. For details of statistical analyses, see the supplemental material.

Sequencing. Band sequencing was conducted mostly from the RISA DNA fingerprints. Correlation coefficients, indicating the relationship of each band class to the ordination scores, were the basis upon which bands were chosen for sequence analysis (see Table S1 in the supplemental material). Band classes with high positive or negative rankings with respect to the ordination scores and which also were represented by intense, well-resolved bands were selected. The selected bands were extracted, and gels were reimaged to ensure that the desired bands were removed. Extracted bands were amplified and electrophoresed to check for correct size and to quantify DNA prior to sequencing at the Nucleic Acids Protein Services Unit at the University of British Columbia. Bands that did not initially yield good sequence data were instead cloned using the TOPO TA cloning system (Invitrogen, Carlsbad, CA). At least three white clones were chosen from each plate, and the inserts were amplified by colony PCR. Amplicons were assayed for correct size, quantified, and sequenced, as described above.

Phylogenetic analysis. The resulting sequences of approximately 500 bp were evaluated using the Chimera Check program implemented in the Ribosomal Database Project (12), and no chimeric sequences were found. The sequences were then compared to those in the NCBI database by using BLAST, version 2.0, and the most similar rRNA gene sequence was used as the reference affiliation for subsequent phylogenetic analysis. ClustalX, version 1.83 (51), was used to align all rRNA gene-RIS sequences and reference affiliations. TREECON for Windows, version 1.3b (53), was used to construct the phylogenetic tree by using the neighbor-joining distance method (43), with Jukes and Cantor (30) correction. Bootstrap analysis consisted of 1,000 replications.

Nucleotide sequence accession numbers. The partial 16S rRNA gene sequences determined in this study were deposited in GenBank under accession numbers AY730464 to AY730531.

RESULTS

Soil contents of C_{mic}, N_{mic}, and DNA_{ext} were all significantly different among all four experimental treatments (Table 2). The C_{mic} and DNA_{ext} levels were higher in forest soil from the unburned experimental treatments (control and harvest) than the burned ones (burn and burn-salvage), while N_{mic} was lower in unburned than burned treatments. Accordingly, the C_{mic}/N_{mic} ratio was highest for the unburned treatments and substantially lower for the burned treatments.

Based on the soil DNA fingerprint data, burning had a major effect on bacterial community composition, while harvesting appeared to affect community composition to a lesser extent. NMS ordinations of the RISA and DGGE fingerprint patterns provided graphical representations of the patterns of the bacterial community composition, which clearly distinguished fingerprints of the unburned communities and the burned communities (Fig. 1 and 2). Similarities of RISA patterns were higher (maximum 93%) than those of DGGE patterns (maximum 73%). Consequently, the smaller variability in the RISA fingerprint patterns suggests that differences seen from this method can be interpreted with more confidence than those seen from the DGGE method. Community differences among the treatments were tested with an MRPP test, and overall, the RISA OTU space explained 76% of the total cumulative variance represented from the distances in the original unreduced OTU space, while the DGGE ordination explained 64% of the total cumulative variance.

The overall MRPP analysis of RISA bacterial fingerprint patterns indicated significant differences among the treatments

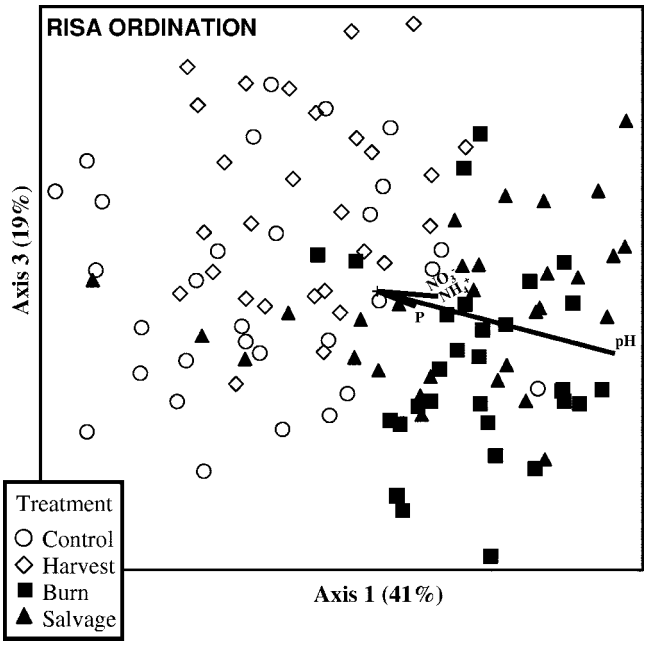

FIG. 1. RISA NMS ordination plot with superimposed joint plot of soil chemistry data. Vectors indicate the relative strength and direction of correlation of soil chemistry variables with the ordination. Three axes produced the best ordination. Values in parentheses indicate the percentages of variance explained by individual axes.

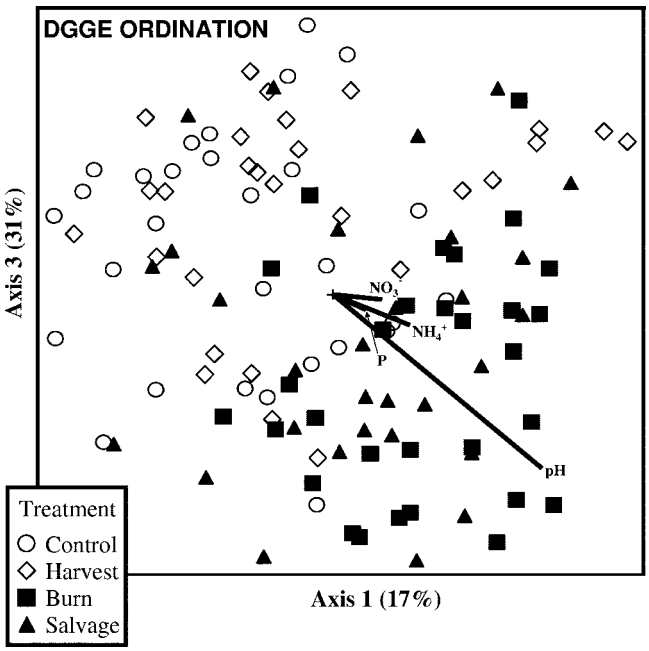

FIG. 2. DGGE NMS ordination plot with superimposed joint plot of soil chemistry data. Vectors indicate the relative strength and direction of correlation of soil chemistry variables with the ordination. Three axes produced the best ordination. Values in parentheses indicate the percentages of variance explained by individual axes.

(Table 3). Although differences between fingerprints of the two unburned treatments and between fingerprints of the two burned treatments were less obvious in the ordination plot (Fig. 1) they were highly significant on the basis of multiple pairwise comparisons. This clearly indicates that each treatment's fingerprint patterns reside in distinct areas of the ordination OTU space and that there are real differences between the compared groups. DGGE fingerprint similarity between pairs of treatments showed trends similar to those for RISA fingerprint similarity, with significant differences between the burned and unburned treatments and between the control and harvest fingerprint patterns. However, unlike the RISA fingerprints, the DGGE fingerprints were not significantly different between the burn and burn-salvage treatments. The RISA and DGGE analyses agree well with one another, as there is a significant relationship between the RISA and DGGE fingerprint distance matrices. A Mantel test of correlation between both RISA and DGGE fingerprint distance matrices indicated a positive correlation ($r = 0.1303$) that is statistically significant ($P < 0.001$).

Higher concentrations of NO_3^-, NH_4^+, and P as well as higher pH levels were found in soil from the burned treatments than in soil from the unburned treatments. A joint plot of these abiotic environmental factors was overlaid on the fingerprint community data (Fig. 1 and 2). The direction and length of the vectors radiating from the centroid of the fingerprint ordinations represent the degree of correlation between the abiotic factors and the fingerprint patterns. Thus, the burned sample fingerprints cluster and are positively correlated with increases in NO_3^-, NH_4^+, P, and pH. The unburned sample fingerprints also cluster but are negatively correlated with increases in NO_3^-, NH_4^+, P, and pH. The relative length of the vectors indicates that pH is four to five times more strongly correlated with the fingerprints than are the other abiotic factors. The strength of the relationship between the RISA or DGGE fingerprint distance matrix and the abiotic environmental factors distance matrix is positive in both cases but is 2.6 times greater for the RISA fingerprint data ($r = 0.1613$) than for the DGGE fingerprint data ($r = 0.0620$), with both comparisons being statistically significant (P values were <0.001 and <0.05, respectively).

Populations most responsible for distinguishing between RISA fingerprints from different treatments were identified by rRNA gene sequence analysis. Using correlation coefficients which indicate the relationship of each band class to the ordination scores, we selected band classes with highly positive or negative correlations to the ordination scores (see Table S1 in the supplemental material). In all cases where these band classes were represented by intense RISA bands, representative bands from samples of each treatment in which they were present were excised and sequenced (Fig. 3). A total of 37 bands, including bands of the same band class from different treatments, were chosen for sequencing. Out of the 37 highly correlated bands, 26 produced high-quality sequence data from amplicons and the remaining 11 were cloned for sequencing (see Table S1 in the supplemental material). A total of 23 amplicon sequences were affiliated with prokaryotes, while 3 were affiliated with eukaryotes from the *Basidiomycota*. From each clone library, at least three random clones were chosen for sequencing. For only one library, S3 P7, were all three sequenced clones most similar to the same reference affiliation. Thus, the inability to directly sequence some bands was probably due to representation of multiple populations in those bands. In most cases, two of three clones from a library represented a common division.

The most common reference affiliation that occurred was *Hydrocarboniphaga effusa*, a gammaproteobacterium (see Table S1 in the supplemental material). Sequences affiliated with this organism were from band class numbers >0.19 to 0.21, >0.37 to 0.39, >0.39 to 0.41, and >0.65 to 0.67, which were found in all treatments, but were most intense in fingerprints from the unburned treatments. Another organism to which several sequences were affiliated was *Bacillus aminovorans*. These sequences were found in band class numbers >0.53 to

TABLE 3. Comparison of the differences in RISA and DGGE community fingerprints among treatments using multiresponse permutation procedures[a]

Comparison	RISA		DGGE	
	T[b]	A[c]	T	A
Control vs harvest	−8.2**	0.27	−2.3**	0.03
Control vs burn	−23.6**	0.27	−15.6**	0.19
Control vs salvage	−16.2**	0.19	−9.2**	0.11
Harvest vs burn	−20.8**	0.24	−11.8**	0.15
Harvest vs salvage	−17.7**	0.21	−9.0**	0.11
Burn vs salvage	−3.9*	0.04	−1.8	0.02

[a] *, $P < 0.01$; **, $P < 0.001$.
[b] T statistic; the more negative the T value, the greater the separation among treatments.
[c] Chance corrected within group agreement; if A was 1, items within a group were identical, and if A was 0, heterogeneity within a group equaled that expected by chance.

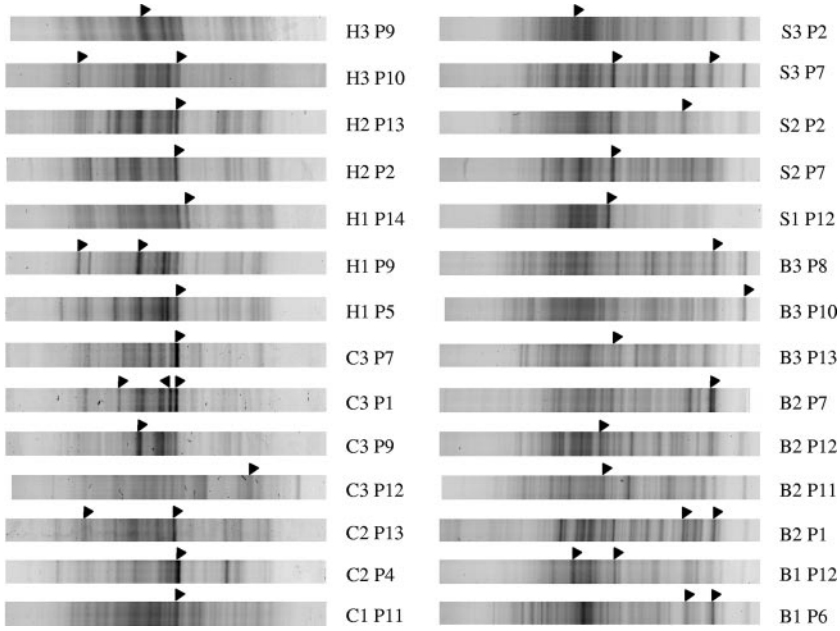

FIG. 3. RISA fingerprints indicating bands that were sequenced.

0.55 and >0.59 to 0.61, which were found only in fingerprints from the burned treatments.

RISA bands that were selected for sequence analysis ranged from 8.3% to 41.8% of the total intensity in their respective fingerprints (Fig. 3; see Table S1 in the supplemental material). Only in fingerprints from C2 P4, C3 P1, and C3 P7 did the sequenced bands contribute greater than 25% of the total intensity, suggesting that these are predominant phylotypes in the unburned control treatment. Of the remaining sequenced bands, about half comprised 12.5 to 25% of the total intensity of their fingerprints and about half comprised less than 12.5%.

Some DGGE bands that migrated identical distances were chosen visually to determine whether the bands corresponded to the same organism. Both C2 P4-1T and H2 P2-3T were most similar to sequences of *Gammaproteobacteria*, while both C2 P4-2B and H2 P2-4B were most similar to sequences of *Alphaproteobacteria* (see Table S1 in the supplemental material). However, two bands, B1 P1-5 and S2 P2-6, from the burn treatments that migrated to the same position were most similar to sequences of *Gamma-* and *Alphaproteobacteria*, respectively.

The sequences determined spanned eight different divisions (Fig. 4), with certain divisions found only in either the unburned or the burned treatment. For instance, the sequences affiliated with the *Betaproteobacteria*, *Bacillus*, *Parachlamydia*, and *Nitrospirae* divisions were exclusively derived from the burned treatments. Similarly, the sequences affiliated with the *Alphaproteobacteria* were exclusively from the unburned treatments. Sequences from both the unburned and the burned treatments were affiliated with the remaining divisions.

DISCUSSION

The Chisholm-Slave Lake area wildfire considerably reduced microbial biomass. In this study conducted 1 year after the fire, the burn treatment resulted in 74% less C_{mic} and the burn-salvage treatment resulted in 52% less C_{mic} than the control treatment (Table 2). Concentrations of DNA_{ext} generally confirmed the reduction in biomass due to burning, but DNA_{ext} did not correlate well with either C_{mic} or N_{mic}. The lack of correlation is not surprising, as DNA_{ext} is considered less reliable as a measure of microbial biomass estimate than is C_{mic}. In general, C_{mic} measurements following wildfires showed significant reductions and these reductions were sustained over periods of up to 13 years (16, 24, 35, 42). The heat of the Chisholm-Slave Lake area wildfire likely had a direct effect, killing a substantial fraction of microbial biomass, but we cannot rule out other indirect causes of reduction of microbial biomass, such as changes in nutrient supply due to loss of plant cover. The clear difference in C_{mic} between the burn-salvage and burn treatments may be due to the addition of organic matter to the forest floor in the form of tree residue left behind during the salvaging of marketable wood, which has been shown to provide nutrients for the recolonization of soil (26).

Harvesting is reported to have variable effects on forest soil microbial biomass. Summer clear-cutting was reported to reduce C_{mic} in a coniferous forest (39), while there was a slight increase in C_{mic} of a Norway spruce stand, in which logging residue from a winter clear-cut was evenly distributed over the harvested site (47). In the Chisholm-Slave Lake harvest sites, logging residue also remained on site; however, the mineral soils of the harvest treatment had a significantly lower C_{mic} (19%) than did those of the control treatment (Table 2).

In response to forest disturbances, microbial populations change faster than does soil organic matter (3). Sparling (48) observed that the ratio of C_{mic} to total organic carbon (C_{org}) in soil was consistently higher in long-term, undisturbed soils than in deforested or cropped soils and proposed the C_{mic}/C_{org} ratio as a monitoring index for the status of forest soils. The

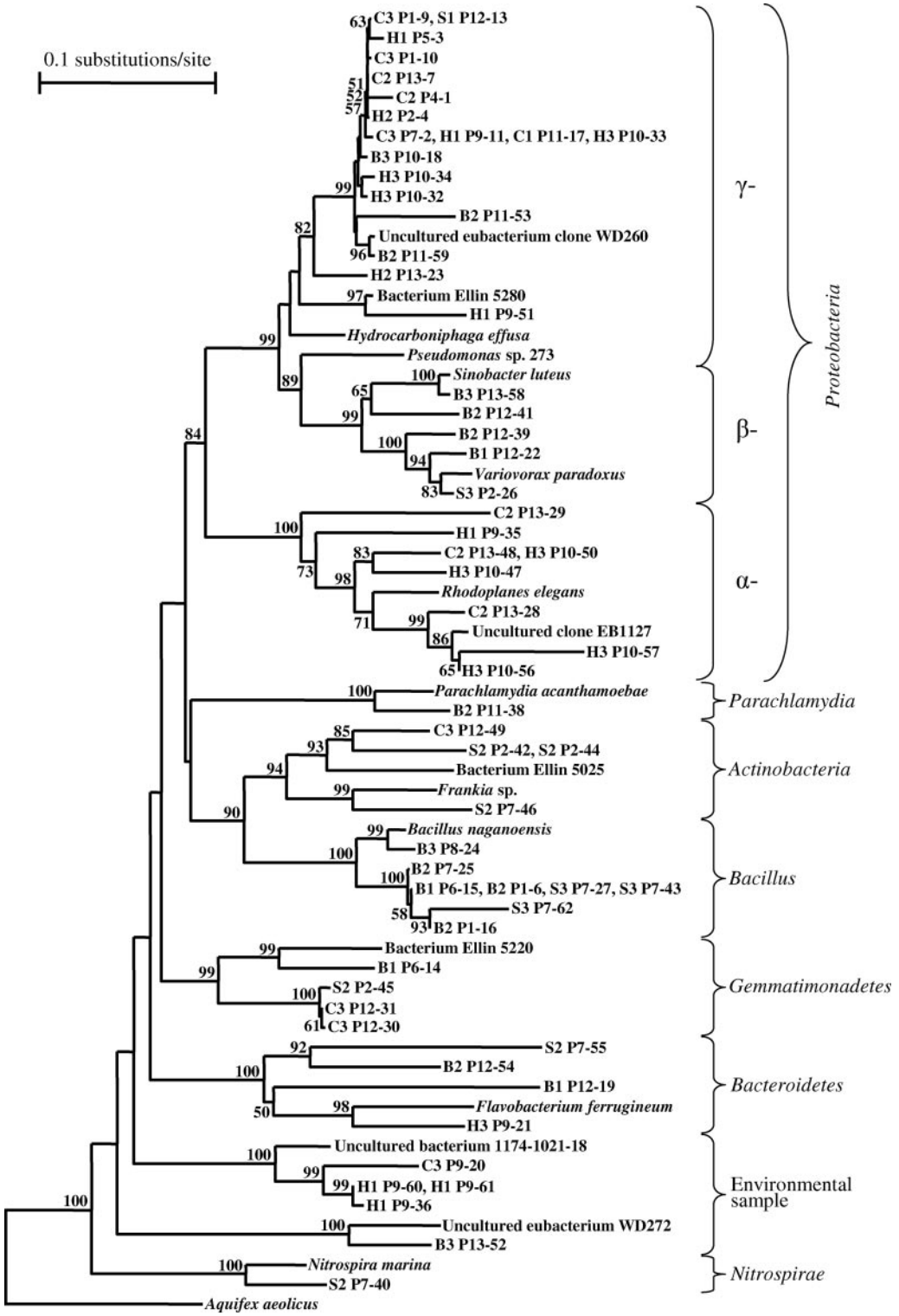

FIG. 4. Rooted neighbor-joining phylogenetic tree of all sequences of amplicons and clones from RISA gel bands with high values of correlation to the NMS ordination. Reference sequences used were uncultured eubacterium clone WD260 (AJ292673.1), uncultured eubacterium clone WD272 (AJ292684.1), uncultured bacterium clone 1174-1021-18 (AB128891.1), uncultured alphaproteobacterium clone EB1127-395446.1, bacterium Ellin 5280 (AY234631.1), *Hydrocarboniphaga effusa* (AY363244.1), *Pseudomonas* sp. strain 273 (AF039488.1), *Sinobacter luteus* (AY966001.1), *Variovorax paradoxus* (AF532868.1), *Rhodoplanes elegans* (AF487437.1), bacterium Ellin 5220 (AY234571.1), *Flavobacterium ferrugineum* (M62798.1), bacterium Ellin 5025 (AY234442.1), *Frankia* sp. (AF063641.1), *Bacillus naganoensis* (AB021193.1), *Parachlamydia acanthamoebae* (Y07556.1), *Nitrospira marina* (X82559.1), and rooted with *Aquifex aeolicus* (AJ309733.1). Numbers at the nodes are bootstrap values of >50 made with 1,000 bootstrap resamplings.

C_{mic}/C_{org} ratios provide not only an indication of carbon availability and quality (27, 48) but also an indication of microbial activity (24, 42). In this study, substantial decreases in C_{mic}/C_{org} for all treatments were mostly attributable to decreases in microbial biomass (Table 2). Fire had a much greater effect on this ratio than did harvesting, suggesting that fire had greater negative impacts than did harvesting on carbon availability and/or microbial activity.

One year after the burn, significant increases in N_{mic} occurred in both burned treatments in this study (Table 2). Burning may be responsible for rendering nitrogen available for microbial growth. In agreement with our results, 1 year postwildfire in a Mediterranean pine forest, N_{mic} levels were significantly higher (16). In contrast, lower N_{mic} levels were reported for a coniferous forest soil impacted by a wildfire in Spain (42) and for a eucalypt forest soil impacted by a moderate-intensity bushfire in South Australia (49).

While fire seems to consistently reduce C_{mic}, the effects of fire on N_{mic} and of harvesting on both C_{mic} and N_{mic} are variable. Significant decreases in N_{mic} were observed in the harvest treatment in this study (Table 2). In a balsam fir forest two growing seasons after precommercial thinning treatments, increases in N_{mic} levels were observed in organic horizons, while no significant changes were observed in mineral horizons (50). After 5 years, mean N_{mic} increased in soluble organic and inorganic nitrogen buried-bag incubations in high-elevation spruce-fir forest floor and mineral horizons in both a harvest treatment and a control treatment, but the net change in N_{mic} was greater for the clear-cut site than for the control (22). These comparisons are complicated by many variables differing between studies. This study is the first to directly compare the effects of wildfire and harvesting on C_{mic} and N_{mic} in a large-scale experiment, and it reveals clear differences in how the two disturbances impact both components of the microbial community.

The sterilizing effect of fire on soils is more lethal to fungi than to bacteria (5, 16, 35, 41, 54). Also, increases in pH caused by fires tend to favor bacteria over fungi (7). There is typically a positive correlation between the C_{mic}/N_{mic} ratio and the relative abundance of fungi in a microbial community (16). In this study, the C_{mic}/N_{mic} ratio was reduced by harvesting and more greatly reduced by burning (Table 2). These results suggest that both disturbances have a negative impact on fungal populations, with fire having a greater effect, as would be predicted.

Changes in bacterial community composition resulting from the various treatments in this study were clearly evident from the RISA and DGGE community fingerprint patterns (Fig. 1 and 2). Replicate treatments were up to 43 km apart, and spatial variability clearly contributed to variability within treatments. However, with the large number of samples analyzed in this study, the DNA fingerprints of each treatment were significantly different from those of every other treatment, with the sole exception of the DGGE fingerprints of the burn versus burn-salvage treatments (Table 3). Thus, the disturbances in this study had severe effects on bacterial community composition and distinguishing aspects of community composition were common over a large spatial scale in this environment. DNA fingerprint analysis was consistent with measurements of C_{mic} and N_{mic} in indicating a greater impact of fire than of harvesting on the soil community. Microbial communities at harvested sites may therefore recover faster than microbial communities at burned sites, and harvesting likely will not mimic natural wildfire disturbances at the level of microbial communities. Further studies are required to determine how long these trends persist following the disturbances and how consistent the effects in different forest types are.

Communities within treatments had significant similarity and were characterized by particular predominant ribotypes (Fig. 4; see Table S1 in the supplemental material). In this study, RISA was a more powerful tool for discerning treatment effects than was DGGE, as treatment comparisons based on RISA yielded lower *P* values than those based on DGGE and only RISA distinguished burn versus burn-salvage treatments. This result suggests that differences among treatments in community composition are greater at lower taxonomic levels (strain-species) than at a higher level (genus), as might be expected.

Bååth et al. (5) showed evidence that increased soil pH substantially accounts for the effect of fire on microbial community composition, measured by PLFA analysis. In that study, other factors, such as soil organic matter, also appeared to contribute to the effect of fire. The strong correlation between pH and community composition observed in the current study (Fig. 1 and 2) is consistent with the pH increase caused by fire having an important effect on microbial communities.

Sequence analysis identified the phylotypes that are most responsible for NMS axes and so are most important for distinguishing among bacterial communities in the various treatments (see Table S1 in the supplemental material). Individual RISA bands contributed a greater percentage of total intensity for the fingerprints of the unburned treatments than for those of the burned treatments, suggesting a greater predominance of particular populations in the unburned treatments. The rRNA gene sequences determined belong to eight different bacterial divisions (Fig. 4). Percent identities with reference affiliations were quite low for most submitted sequences, suggesting that they belong to unidentified species or even genera. One exception is the sequences from the burn treatments, which have high similarities to *Bacillus aminovorans* sequences (98% to 99%).

Some distinct RISA bands turned out to have very similar sequences. Sequences affiliated with the gammaproteobacterium *Hydrocarboniphaga effusa* were examples of this (Fig. 4). This group included four band classes that ranged in similarity from 86% to 97%, and it is impossible to say whether these ribotypes represented different strains of the same genus or different rRNA operons of the same strain, as interstrain sequence variability has been found at these levels (14, 20). These *Hydrocarboniphaga* ribotypes, which were present in all treatments, contributed from 9.0% to 41.8% of total band intensity for RISA fingerprints and were most predominant in the unburned treatments.

Overall, the dominant group found was the *Gammaproteobacteria*. The majority of these phylotypes were from the unburned treatments, although 4 of 18 were from the burned treatments. Most characteristic of the burned treatments were members of the *Firmicutes* (*Actinobacteria* and *Bacillus* groups), which were dominated by one *Bacillus* sp. sequence. *Bacillus* spp. and other gram-positive organisms are common

in burned soils (32, 35, 49, 54), likely due in part to survival of spores. It further appears that members of the *Alphaproteobacteria* were selected in the unburned treatments, while members of the *Betaproteobacteria* were selected in the burned treatments. Unique to the burned treatments were sequences affiliated with the divisions *Chlamydiae* and *Nitrospirae*. In a long-term soil productivity study in British Columbia, Canada, bacterial rRNA gene sequences from soil in harvested treatments with and without heavy soil compaction included those of *Alpha-*, *Beta-*, *Gamma-*, and *Deltaproteobacteria* and *Actinobacteria*, *Acidobacteria*, *Verrucomicrobia*, *Bacillus/Clostridium* group, *Cytophaga-Flexibacter-Bacteroides*, green nonsulfur bacteria, the *Planctomycetales*, candidate divisions TM6 and OP10, and unclassified divisions (4). In the long-term soil productivity study, *Betaproteobacteria* and bacilli were of low abundance in the mineral soil layer of both treatments, while *Betaproteobacteria* were more abundant and bacilli were not found in the organic horizon. The current study suggests that neither *Betaproteobacteria* nor bacilli are abundant in the harvest or control treatments from the Chisholm-Slave Lake region. However, because we selected RISA bands for sequencing, this result certainly does not indicate that these groups are not present at relatively low abundances in those treatments. The functional roles played by the specific phylotypes revealed in this study are unknown, particularly given the large number of ribotypes not affiliated with described species. However, it is clear that the experimental treatments, most notably the burned treatments, cause significant changes in the phylogenetic compositions of soil bacterial communities. Importantly, from a forest management perspective, the analysis of phylotypes confirms that harvesting does not resemble the natural disturbance of fire in its impact on bacterial community composition and indicates that predominant populations in the resulting communities are phylogenetically very different.

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