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Changes in Nitrogen-Fixing and Ammonia-Oxidizing Bacterial Communities in Soil of a Mixed Conifer Forest after Wildfire

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This study was undertaken to examine the effects of forest fire on two important groups of N-cycling bacteria in soil, the nitrogen-fixing and ammonia-oxidizing bacteria. Sequence and terminal restriction fragment length polymorphism (T-RFLP) analysis of *nifH* and *amoA* PCR amplicons was performed on DNA samples from unburned, moderately burned, and severely burned soils of a mixed conifer forest. PCR results indicated that the soil biomass and proportion of nitrogen-fixing and ammonia-oxidizing species was less in soil from the fire-impacted sites than from the unburned sites. The number of dominant *nifH* sequence types was greater in fire-impacted soils, and *nifH* sequences that were most closely related to those from the spore-forming taxa *Clostridium* and *Paenibacillus* were more abundant in the burned soils. In T-RFLP patterns of the ammonia-oxidizing community, terminal restriction fragments (TRFs) representing *amoA* cluster 1, 2, or 4 *Nitrosospira* spp. were dominant (80 to 90%) in unburned soils, while TRFs representing *amoA* cluster 3A *Nitrosospira* spp. dominated (65 to 95%) in fire-impacted soils. The dominance of *amoA* cluster 3A *Nitrosospira* spp. sequence types was positively correlated with soil pH (5.6 to 7.5) and NH_3-N levels (0.002 to 0.976 ppm), both of which were higher in burned soils. The decreased microbial biomass and shift in nitrogen-fixing and ammonia-oxidizing communities were still evident in fire-impacted soils collected 14 months after the fire.

Recent droughts in the western United States, in combination with heavy fuel loads, have led to an increase in the occurrence of large, stand-replacing forest fires in the region. Intense fires can produce long-lasting effects in the below-ground portion of the ecosystem, including changes in soil hydrology and physical properties, disruption of biological processes, loss of organic matter, and nitrogen transformations and volatilization (15, 16, 23, 40). Although soil bioprocesses are likely drivers of ecosystem recovery from catastrophic fires (40), little attention has been devoted to fire impacts on soil microorganisms.

Fire can have numerous and various effects on different groups of bacteria, algae, microfauna, and fungi within the soil community (2, 4, 11, 18, 19, 39, 40, 46). The immediate effect of fire on the soil microbial biomass depends on the intensity and duration of the fire and can range from complete sterilization to little or no effect (4, 55). Reductions in the total soil microbial biomass due to fire can persist for decades (21, 22, 42). However, rapid recolonization of specific microbial groups has also been observed, and fire has even been reported to stimulate microbial numbers and activity shortly after the burn, potentially through the release of readily utilizable C and N substrates (2, 4, 20, 55).

To date, studies that have examined the effects of fire on soil microbes have primarily relied on culture- and activity-based methods. These studies have provided solid data regarding microbiological activity and microbial population sizes within fire-impacted soils but little information on the effect of fire on the composition of the total soil microbial community or specific functional groups. Molecular techniques can provide more comprehensive examination of the effects of fire on the composition of the microbial community (48). For example, phospholipid fatty acid analysis has been used to demonstrate shifts in the total microbial community in burned and heated soils (10, 41). However, studies examining the effects of fire using DNA- or RNA-based techniques that target specific functional groups have not been published.

Coniferous forests of the western United States are often N limited (5, 20). This investigation focused on the effects of wildfire on two groups of soil bacteria important in N cycling: the N-fixing bacteria that are responsible for exogenous input of NH_4^+ and the ammonia-oxidizing bacteria that produce NO_2^-/NO_3^-. The specific goal of the current study was to use *nifH* and *amoA* gene sequence analyses in combination with terminal restriction fragment length polymorphism (T-RFLP) profiles to compare the nitrogen-fixing and ammonia-oxidizing communities in unburned, moderately burned, and severely burned soils of a mixed conifer forest following the Cerro Grande Fire (an intense crown fire) near Los Alamos, New Mexico. The topologies of phylogenetic trees based on both *nifH* and *amoA* sequences are largely congruent with those from the corresponding sets of 16S rRNA gene data (1, 43, 60), and *nifH* and *amoA* genes have been sequenced from a number of cultured species. For these reasons, analysis of the two functional genes provides a robust, culture-independent method of examining nitrogen-fixing and ammonia-oxidizing bacterial diversity and community composition in the environment (12, 30, 59–61).

MATERIALS AND METHODS

Study area, sampling, and soil chemistry. Soil samples were collected from fire-impacted areas of Pajarito Mountain, Los Alamos County, New Mexico,

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following the Cerro Grande fire of May 2000. Prefire vegetation consisted of ponderosa pine and Douglas fir, with a sparse understory of grasses, at an elevation of 8,570 ± 15 feet. Sampling sites were within an area bounded by coordinates 13S 376.780, 376.890, 3971.710, 3971.620 (Universal Transverse Mercator, North American Datum 1983). Samples were collected approximately 1 month, 3 months, 5 months, and 14 months postfire (collection dates were 7 June 2000, 7 August 2000, 11 October 2000, and 18 July 2001, respectively). Three sampling sites were selected within a 9,710-m^2 area based on the degree of fire impact. In the severely burned (B) site, all vegetation was severely burned, with the removal of all low-lying grass and the killing of all trees. A heavy ash layer was present on top of the soil. In the adjacent moderately burned (M) site, grasses were burned, but larger trees were unaffected. Some ash was present, primarily from burned pine needle litter. In the unburned (U) site, there was no evidence of burning, but there was a light dusting of ash on top of the pine needle litter. Within each site, four replicate plots of approximately 3 to 5 m^2 were chosen. Within each plot, four 1-in.-diameter soil cores were taken to a depth of 10 cm. In areas with heavy ash or pine needle layers, these were removed prior to coring. Cores from each plot were pooled, mixed, and sieved to yield four samples per severely burned, moderately burned, or unburned site. Samples were coded B, M, or U for burn severity, followed by 1, 3, 5, or 14 for the time in months after the fire, followed by a letter from a to d to denote the field replicate plot. For example, B3c designates a sample that was collected from the severely burned site 3 months postfire (7 August 2000) from plot "c." Samples were transported on ice to the laboratory (approximately 30 min) and stored at −70°C before analysis.

Analysis of soil chemistry was performed by the Central Analytical Laboratory of the Department of Crop and Soil Science at Oregon State University, Corvallis, Oregon, using standard procedures (http://cropandsoil.oregonstate.edu/Services/Plntanal/CAL/index.html). Total carbon and nitrogen were measured with a Leco CNS-2000 macroanalyzer, and NH_4^+ and NO_3^- were analyzed with an ALPKEM rapid flow analyzer following extraction with KCl.

Soil DNA extraction. A bead-beating protocol with a sodium dodecyl sulfate-based DNA extraction was used to obtain DNA from soil samples (34). Using selective bead sizes that optimally disrupt small particles, our method attempted to preferentially recover microbial DNA (http://www.biospec.com; C. R. Kuske, unpublished results). Although this measurement is affected by extraneous factors such as intersample extraction efficiency and the amount of free DNA found in the soil, we feel that it is useful as a relative measure of soil microbial biomass in surface soils between trees, where root biomass is low.

One milliliter of TENS (50 mM Tris [pH 8.0], 20 mM disodium EDTA, 100 mM NaCl, 1% [wt/vol] sodium dodecyl sulfate) and 0.5 g of soil were added to a 2-ml bead-beating tube containing 0.1-mm and 0.5-mm beads (450 mg each) and incubated at 70°C for 30 min. Bacterial cells were disrupted by bead beating the soil mix at 5,000 rpm for 3 min at room temperature. The mix was then centrifuged for 10 min at 12,000 × *g*. The supernatant was collected, and DNA was precipitated with ice-cold ethanol and pelleted by centrifugation for 10 min at 13,000 × *g*. The pellet was air dried and suspended in 200 μl of TE buffer (10 mM Tris [pH 8.0], 1 mM EDTA). Subsamples of the soil DNA stock solutions were purified for PCR by passage through Sephadex G-200 in 96-well plates as previously described (34).

To quantify the DNA yield from soil samples, each DNA stock solution was diluted 10-fold, and 5-μl aliquots were analyzed by electrophoresis in ethidium bromide-stained 3% SeaKem agarose gels (FMC Bioproducts, Rockland, Maine). High-molecular-weight DNA was visualized under UV light and quantified by analyzing band intensities with Science Lab 99 Image Gauge version 3.3 software (Fuji Photo Film Co., Tokyo, Japan) using lambda DNA as a calibration standard.

***nifH* and *amoA* PCR.** In all PCRs, hot-start reactions were carried out in an MJ Research (Waltham, Mass.) PTC-200 Peltier thermal cycler. PCR amplification of *nifH* gene fragments from soil DNA was performed using a nested protocol and degenerate primers designed to amplify the majority of known *nifH* genes that encode the reductase subunit of dinitrogenases with an active site containing iron and molybdenum. This primer pair also amplifies *anfH* genes that encode the reductase subunit of alternative iron-containing dinitrogenases and *vnfH* genes that encode the reductase subunit of alternative iron- and vanadium-containing dinitrogenases (57). Briefly, forward primer 19F (5′-GCIWTYTAY GGIAARGGIGG) (54) and reverse primer nifH3 (5′-ATRTTRTTNGCNGC RTA) (58) were used in the first amplification reaction, which contained 40 pmol of each primer, 30 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 10 μg bovine serum albumin, 200 μM of each deoxynucleoside triphosphate, 2.5 U of AmpliTaq DNA polymerase (low DNA; Applied Biosystems, Foster City, California), and 0.2 to 2.0 ng of soil DNA in a final reaction mixture volume of 50 μl. Parameters for the amplification were as follows: 95°C for 5 min, followed by 20 cycles of 48°C for 1 min, 72°C for 1 min, and 94°C for 45 sec, with a 72°C final extension step for 10 min. For the second PCR, slight variations of primers nifH1 and nifH2 (58) were designed. Primers nifH11 (5′-GAYCCNAARGCNGA CTC) and nifH22 (5′-ADWGCCATCATYTCRCC) (40 pmol each) were used in a 32-cycle PCR with a 55°C annealing temperature. Reaction cocktail components were identical to those for the first reaction, except that this cocktail contained 2.0 mM $MgCl_2$ and 2 μl of a 1:10 dilution of the first PCR cocktail as a template to amplify a final 358-bp fragment. To generate *nifH* amplicons for T-RFLP analysis, 5-carboxyfluorescein-labeled nifH11 primer was used in the second PCR.

Amplification of *amoA* sequences from soil DNA was also achieved with hot-start nested PCR. In the first PCR, forward primer amoA-2F (5′-AARGC GGCSAAGATGCCGCC) and reverse primer amoA-5R (5′-TTATTTGATCC CCTC) (56) were used (7 pmol each) in a reaction cocktail containing 30 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 10 μg bovine serum albumin, 200 μM of each deoxynucleoside triphosphate, 1.5 U of AmpliTaq DNA polymerase (low DNA), and 0.2 to 2.0 ng of the soil DNA in a final reaction mixture volume of 30 μl. The touchdown method of Webster et al. (56) was used, with the following parameters: 95°C for 5 min, followed by 20 cycles of 55°C for 5 s, ramp to 45°C at 0.2°C/s, 72°C for 1 min, and 94°C for 40 s; next, 5 cycles of 45°C for 50 s, 72°C for 1 min, and 94°C for 40 s; and lastly, a 72°C final extension step for 10 min. For the second PCR, forward primer amoA-1F (5′-GGGGTTTCTAC TGGTGGT) and reverse primer amoA-2R (5′-CCCCTCKGSAAAGCCTTC TTC) (44) were used (20 pmol each) to amplify a 491-bp product with 2 μl of a 1:10 dilution of the first PCR mixture serving as the template; otherwise, the reaction cocktail was the same as described above. The amplification parameters were as follows: 95°C for 5 min, followed by 40 cycles of 60°C for 1 min, 72°C for 1 min, and 94°C for 45 s, followed by a 72°C final extension step for 10 min. To generate *amoA* amplicons for T-RFLP analysis, 5-carboxyfluorescein-labeled amoA-1F primer was used in the second PCR.

***nifH* and *amoA* T-RFLP analysis.** T-RFLP analysis of *nifH* PCR amplicons was performed as previously described (57). Fifty nanograms of gel-purified *nifH* amplicons was digested with 8.0 U RsaI (New England Biolabs, Beverly, Massachusetts). One microliter of the restriction digestion was heated to dryness, suspended in 2.25 μl of loading buffer (0.25 μl of Genescan 500 6-carboxytetramethylrhodamine size standard [ABI], a 5:1 mixture of deionized formamide-blue dextran, and 25 mM EDTA), denatured at 95°C for 2.5 min, and immediately placed on ice. Samples (1.5 μl) were then loaded onto a 5% denaturing polyacrylamide gel (Long Ranger Singel Packs; Cambrex Bio Science Rockland Inc., Rockland, ME), and fragments were separated by electrophoresis with an ABI Prism model 377 DNA sequencer. Genescan version 3.1 software (ABI) was used to analyze fragment sizes and peak fluorescence intensities. Duplicate T-RFLP profiles were generated for each sample. T-RFLP of *amoA* fragments was performed similarly except that AvaI (5.0 U) (New England Biolabs) was used to digest the *amoA* PCR amplicons. Analysis of T-RFLP peak data was performed as previously described (57). To determine the expected terminal restriction fragment (TRF) sizes, sequences of the *nifH* and *amoA* PCR products were imported into Webcutter 2.0 (http://www.firstmarket.com/cutter/cut2.html) and analyzed for the presence and position of RsaI and AvaI restriction sites, respectively.

Cloning, sequencing, and phylogenetic analysis. PCR products (*nifH* and *amoA*) of the correct size were purified by electrophoresis on SeaKem agarose gels, and the bands were excised and purified using a QIAquick gel extraction kit (QIAGEN, Inc., Chatsworth, Calif.). Small clone libraries for sequencing of the purified amplicons were then generated with the TOPO TA cloning kit and TOP10 chemically competent *E. coli* (Invitrogen). One *nifH* clone library was generated for each of six field samples (B1c, 10 sequences; M1a, 16 sequences; M1b, 13 sequences; U1b, 7 sequences; M5b, 29 sequences; U14b, 16 sequences). One *amoA* clone library was constructed for each of four field samples (B3b, 17 sequences; B14b, 21 sequences; M14a, 14 sequences; U14a, 16 sequences). Alignment of the DNA sequences was performed using ClustalX v1.81 (53) and visually inspected with the BioEdit sequence alignment editor (24). Translations and phylogenetic analysis of sequences were performed with MEGA software version 2.1 (33). The *amoA* (gene) and NifH (derived amino acid) dendrograms were constructed using the minimum evolution function to analyze initial trees obtained by the neighbor joining (NJ) method. Percent dissimilarity distances used for the analysis were determined with pairwise deletion of gaps and missing data.

Nucleotide sequence accession numbers. Sequences were deposited in GenBank with accession numbers AY819559-AY819604 (*nifH*) and AY819605-AY819626 (*amoA*).

TABLE 1. DNA yield and PCR results from unburned, moderately burned, and severely burned soils

Sample[a]	DNA yield (μg DNA g soil^{-1})[b]	Amplification results[c]	
		nifH PCR	*amoA* PCR
U1	18.4 ± 8.4 abc	4/4	4/4
U3	17.7 ± 4.8 bc	4/4	4/4
U5	26.1 ± 10.8 ab	4/4	4/4
U14	30.5 ± 3.8 a	4/4	4/4
M1	0.8 ± 0.9 d	3/4	1/4
M3	4.0 ± 1.0 d	4/4	3/4
M5	5.8 ± 3.1 cd	3/3	3/3
M14	6.1 ± 3.7 cd	3/4	3/4
B1	1.8 ± 0.5 d	3/4	0/4
B3	2.5 ± 1.2 d	3/4	1/4
B5	3.0 ± 1.4 d	2/4	3/4
B14	2.8 ± 0.8 d	2/4	4/4

[a] Samples were collected from U, M, or B sites 1, 3, 5, or 14 months following the fire (e.g., B1 represents samples collected from severely burned sites 1 month after the fire).

[b] DNA yields represent the averages ± standard deviations of four soil samples. Letters represent significant differences ($p < 0.05$) across all samples using Tukey-Kramer's mean separation analysis.

[c] Amplification results are presented as the number of soil samples yielding a product of the expected size divided by the number of soil samples analyzed.

RESULTS

DNA extraction/quantification and results of the *amoA* and *nifH* PCRs. Soil DNA was extracted from four replicate U, M, and B soil samples from each site. The mean DNA yields ($n = 4$) for each site at a given sampling date ranged from 0.8 to 30.5 μg DNA g soil^{-1} (Table 1). DNA yields were 4- to 23-fold higher from U soils than from B or M soil samples that had been collected at a given sampling date up to 14 months following the fire. By this measure, fire resulted in a substantial loss of soil microbial biomass within our study area, and recovery of the lost biomass was minimal during the 14-month period following the fire.

The fire also had a negative impact on the population size of the nitrogen-fixing and ammonia-oxidizing bacteria at our study area. With both *nifH* and *amoA* PCRs, amplification was less successful with M and B soil samples than with U soil samples when similar template concentrations were used (Table 1). This negative effect was most pronounced with the *amoA* PCR. Indeed, *amoA* PCR failed to yield a product from all B1 samples processed, and only one reaction yielded a product using B3 samples as the template. However, amplification of the *amoA* gene from M and B soil samples was increasingly successful beginning 3 months after the fire. For samples that yielded a negative result with either the *nifH* or *amoA* PCR, multiple reaction conditions were tried, including the following: several initial template concentrations, additional cleanup of DNA samples to remove PCR inhibitors, and lower primer annealing temperatures. Consistently negative results were obtained (data not shown). Also, 16S rDNA genes were successfully amplified in each of the samples analyzed using universal eubacterial primers (data not shown). These efforts gave us some confidence that the results presented in Table 1 were likely influenced by the levels of target genes within the soil samples rather than simply by differences in amplification efficiencies between samples due to PCR inhibitors.

***nifH* clone sequencing and T-RFLP analysis.** It is difficult to quantify the relative abundance of sequence types within a population using small clone libraries. However, with certain functional genes or limited-scope 16S rRNA gene surveys, T-RFLP analysis can provide a semiquantitative representation of sequence distribution (richness) and relative abundance (evenness) between samples. To associate T-RFLP peak(s) from a sample with a specific sequence type(s), sequence information from that sample must first be obtained. Therefore, we cloned and sequenced *nifH* PCR products from six different soil samples at our study area spanning the U, M, and B sample sets. These soil samples were chosen to provide reasonable coverage of the sequence diversity found at our sites, based on the information from the initial T-RFLP analysis.

Phylogenetic analysis of the amino acid sequences derived from the *nifH* clones (91 total; 49 unique sequences) placed them into six distinct groups, labeled as clusters NF1 to NF6 in Fig. 1. The majority of sequences (88%) belonged to one of three clusters. Clusters NF1, NF4, and NF5 comprised 22, 18, and 48% of the total NifH sequences, respectively. Most of the cluster NF1 sequences were closely related to NifH sequences from cultured members of the groups *Alpha-* or *Gammaproteobacteria*, cluster NF4 sequences were most closely related to NifH sequences from *Paenibacillus* spp., and cluster NF5 sequences were most closely related to those from a number of environmental clones. Other NifH sequences identified in the current study were most closely related to NifH from *Nostoc* spp. (NF3) and AnfH from *Clostridium pasteurianum* (NF6). Three sequences retrieved in the current study (cluster NF2) were not closely related to any NifH sequences accessible by BLAST analysis.

Analysis of *nifH* gene sequences obtained from B, M, and U soils led us to use RsaI to generate T-RFLP profiles for optimal differentiation of the various *nifH* sequence types observed. By determining an expected RsaI TRF size for each *nifH* sequence type identified in our *nifH* clone libraries (clusters in Fig. 1), TRFs generated with RsaI could be presumptively assigned to *nifH* clusters (Fig. 2 legend). For example, all of the cluster NF6 clones yielded a 325-bp RsaI TRF via in silico analysis. NF3 cluster sequence analysis also yielded a single expected TRF size (133 bp). Although a 133-bp TRF was not detected in any of our T-RFLP profiles, this result was not totally unexpected, as the cluster NF3 sequences comprised only 2% of the *nifH* clones recovered in our libraries. Clone sequences belonging to clusters NF1, NF2, NF4, and NF5 had multiple expected TRF sizes which sometimes overlapped. However, sequences from each of these clusters could be assigned to unique TRFs with some level of confidence (Fig. 2 legend). For example, in silico analysis predicted that the 261- and 55-bp TRFs were represented solely by cluster NF4 sequences and that the 123-bp TRF represented only sequences from cluster NF1. Also, because cluster NF5 sequences comprised 48% of our *nifH* gene library and cluster NF2 only 3%, it is likely that the bulk of sequences represented by the 228-bp TRF are of cluster NF5 origin.

The *nifH* T-RFLP profiles were primarily composed of two TRFs of 228 and 358 bp in length (Fig. 2). In unburned sites, each of these TRFs comprised, on average, close to 50% of the total T-RFLP profile, suggesting that NF1-, NF2-, NF4-, and NF5-type sequences dominated prior to the fire. Two obser-

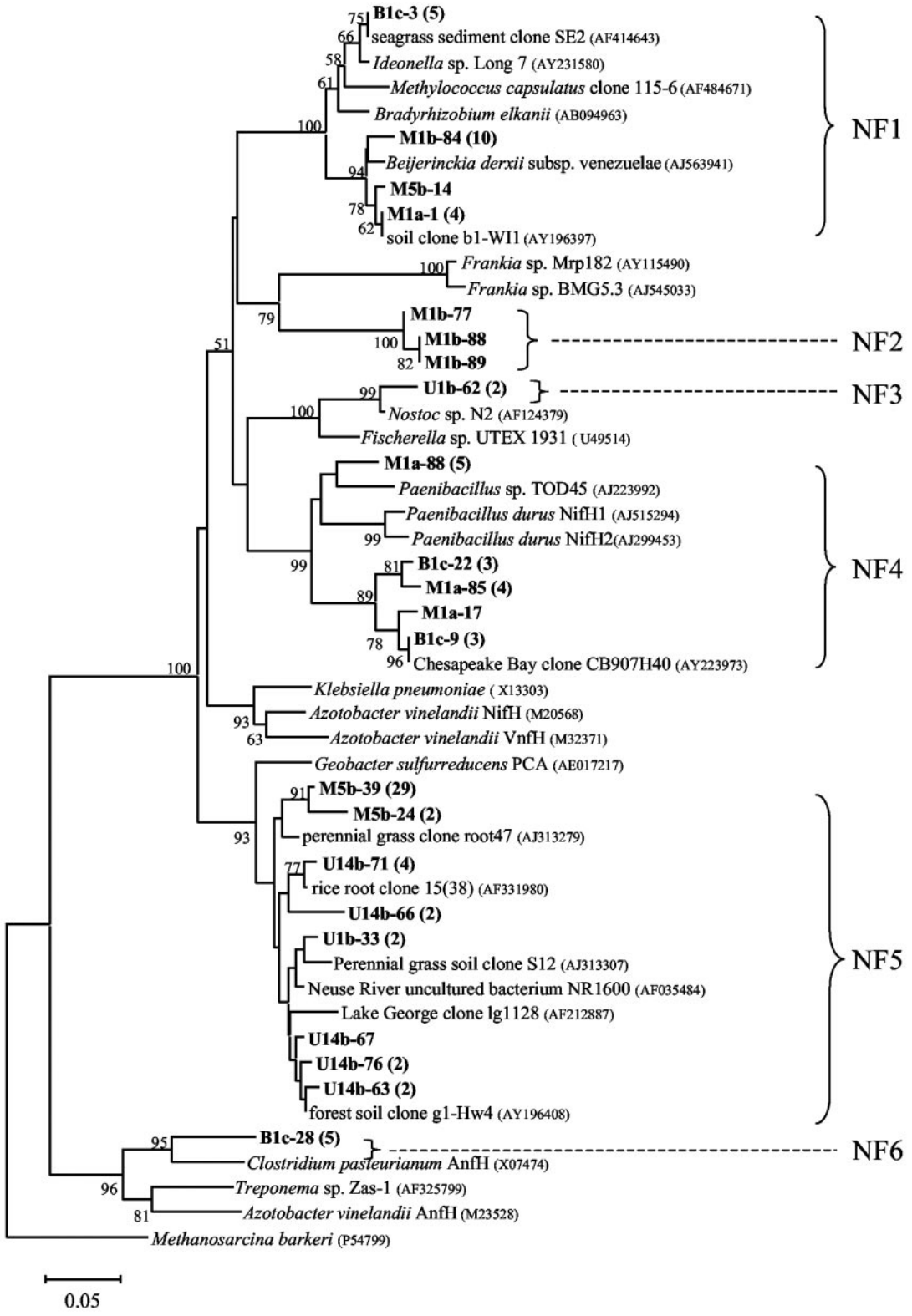

FIG. 1. Dendrogram of representative NifH sequences (109 derived amino acid positions) translated from *nifH* clones obtained from six field samples: B1c, M1a, M1b, U1b, M5b, and U14b (shown in boldface and followed by clone number; e.g., B1c-3). Boldface numbers in parentheses denote the numbers of additional *nifH* clones identified in this study that share ≥95% DNA similarity with the corresponding representative sequence. Sequences were grouped into six clusters (NF1 to 6) based on the individual inspection of alignments, distance data, and NJ trees. The dendrogram was constructed as described in Materials and Methods. Bootstrap percentage values from 100 resamplings are shown above the internal nodes if larger than 50. The cluster IV-type NifH sequence from *Methanosarcina barkeri* was used as the out-group. Accession numbers are listed next to previously described sequences.

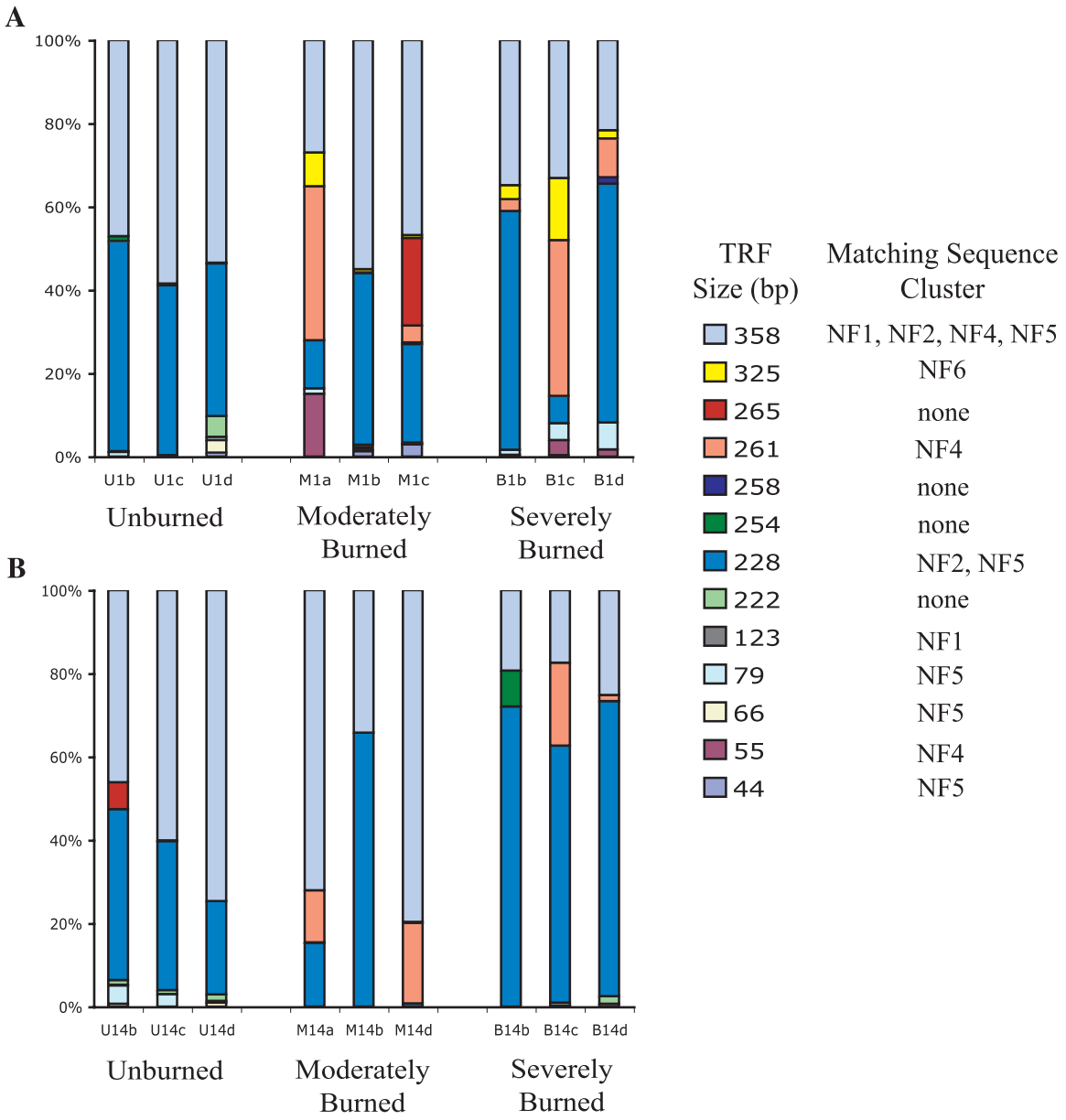

FIG. 2. Relative abundance of *nifH* TRFs generated from samples collected from unburned, moderately burned, and severely burned sites 1 (A) and 14 (B) months after the fire. The bars represent the abundance (percentage of total fluorescence) of each TRF (classified by size) identified in the T-RFLP profiles of the listed samples. The matching sequence cluster(s) from Fig. 1 (as determined by in silico RsaI digestion of all *nifH* clone sequences recovered in this study) is listed at right next to the corresponding TRF.

vations can be made from comparative analysis of the T-RFLP profiles from U versus M and B soil samples. First, the relative abundance of the 358-bp TRF decreased from roughly 50% of the total profile in U soils to 20 to 25% of the total profile in B soils. Second, the number of different TRF types was greater in soils impacted by the fire. In particular, the relative abundance of the 261- and 325-bp TRFs was greater in the T-RFLP profiles generated from M and B soils than in those from U soils, and a variety of additional rare types were present in the M and B profiles. These trends were also observed in T-RFLP profiles obtained from the 3-month and 5-month soil samples (data not shown). We observed four TRFs of 222, 254, 258, and 265 bp that did not match any of the expected TRFs obtained by in silico analysis of our *nifH* clones. These TRFs were detected only sporadically and, when present, generally comprised a small proportion of the total peak area of the T-RFLP profile (with the exception that the 265-bp TRF comprised ~20% of sample M1c).

***amoA* clone sequencing and T-RFLP analysis.** Clone sequencing and T-RFLP analysis was also used to investigate the diversity and distribution of *amoA* sequences at our study area. The *amoA* sequences (68 total, 22 unique) were analyzed from four clone libraries prepared from four individual soil samples. All *amoA* sequences were classified as belonging to the *Ni-*

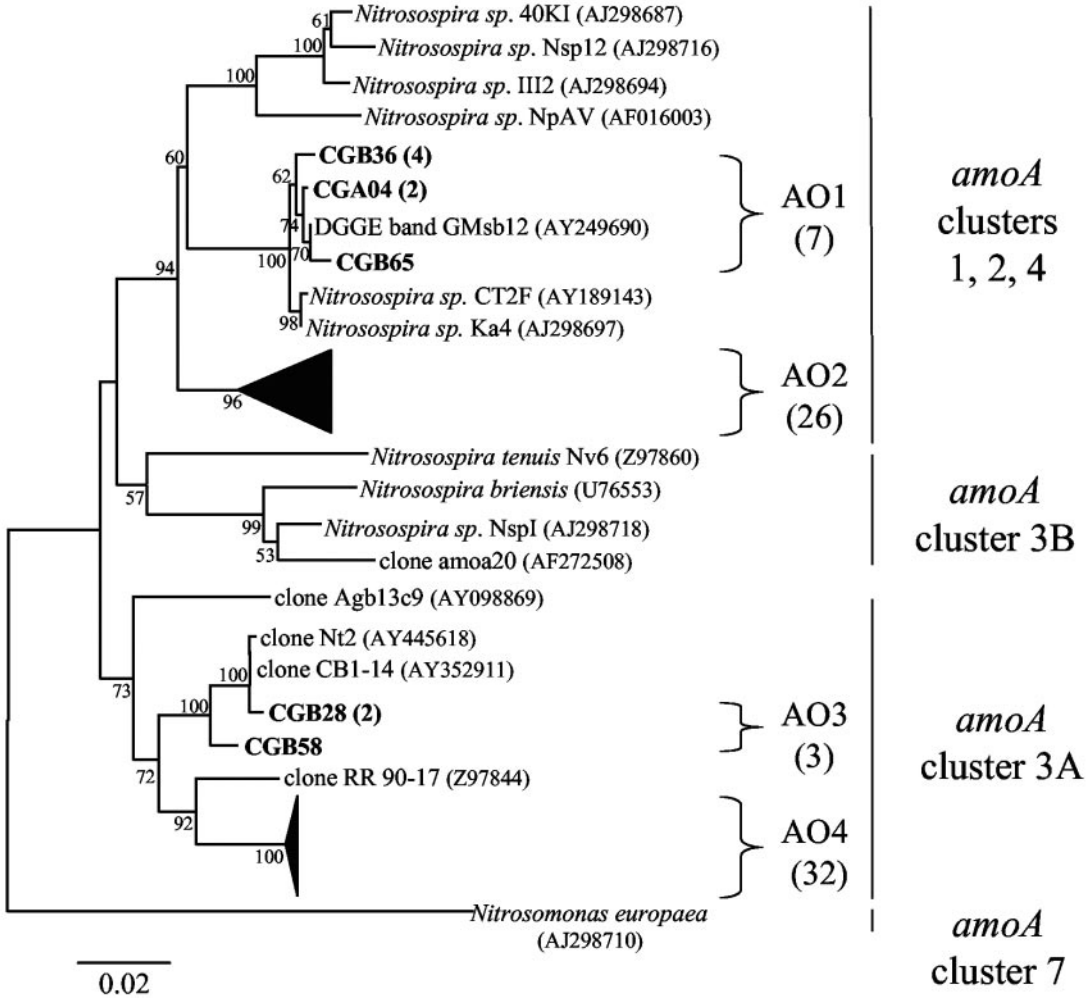

FIG. 3. Dendrogram of representative *amoA* gene sequences recovered from B, M, and U soils (boldface). The dendrogram was constructed as described in Materials and Methods. Bootstrap values from 100 resamplings are shown if larger than 50. An *amoA* sequence from *Nitrosomonas europaea* was used as the out-group. Accession numbers for *amoA* sequences included in the tree from public databases are shown in parentheses next to the corresponding sequences. Sequences grouped into four clusters (AO1 to AO4) based on individual inspection of alignments, distance data, and NJ trees. The number of *amoA* sequences identified in this study that fall within each cluster is shown in parenthesis below the corresponding cluster name. Numbers in parentheses after sequence names are numbers of identical sequences. Clusters AO2 and AO4 were collapsed into triangles, with distance data still represented along the horizontal axis. Placement of the *amoA* sequences in this tree within previously described AmoA protein clusters (8, 9) is shown on the far right.

trosospira type by phylogenetic analysis (Fig. 3). The sequences clustered into four distinct groups, the majority of which belonged to one of two distinct clusters, AO2 (26/68 = 38%) or AO4 (32/68 = 47%). The remaining *amoA* sequences were designated either as cluster AO1 (7/68 = 10%) or as cluster AO3 (3/68 = 4%). In comparison to *amoA* sequences from cultured isolates, cluster AO1 and AO2 sequences were most similar to those from *Nitrosospira* sp. strain CT2F (97 to 98 and 92% similarities, respectively) (37). Cluster AO4 and AO3 sequences were most similar to *amoA* sequences from *Nitrosospira* sp. strain Nsp2 (97 to 98% and 89 to 92% similarities, respectively; the sequence for *Nitrosospira* sp. strain Nsp2 falls within the collapsed cluster AO4 in Fig. 3 [1]). The diversity of *amoA* sequences was much greater within cluster AO2 than within any of the other three clusters identified in this study.

To examine the relative abundance of these *amoA* genotypes within B, M, and U soils at our study area, an AvaI T-RFLP method that could discriminate between the sequences in clusters AO1+AO2 versus clusters AO3+AO4 was developed. In silico digestion of all 68 *amoA* sequences from our libraries with AvaI resulted in 94% of the AO1+AO2 sequences producing either a 480-bp TRF or the uncut 491-bp PCR product (6% produced a 155-bp TRF) and 91% of the AO3+AO4 sequences producing a 155-bp TRF (3% produced a 480-bp TRF and 6% produced a 267-bp TRF). There was no recognizable relationship between the AO1 or AO2 sequence type and the AvaI restriction pattern (480-bp fragment versus 491-bp uncut PCR product). In the *amoA* T-RFLP profiles, the 155-, 480-, and 491-bp TRFs were the only peaks detected (Fig. 4a).

Using the AvaI T-RFLP assay, a remarkable difference was

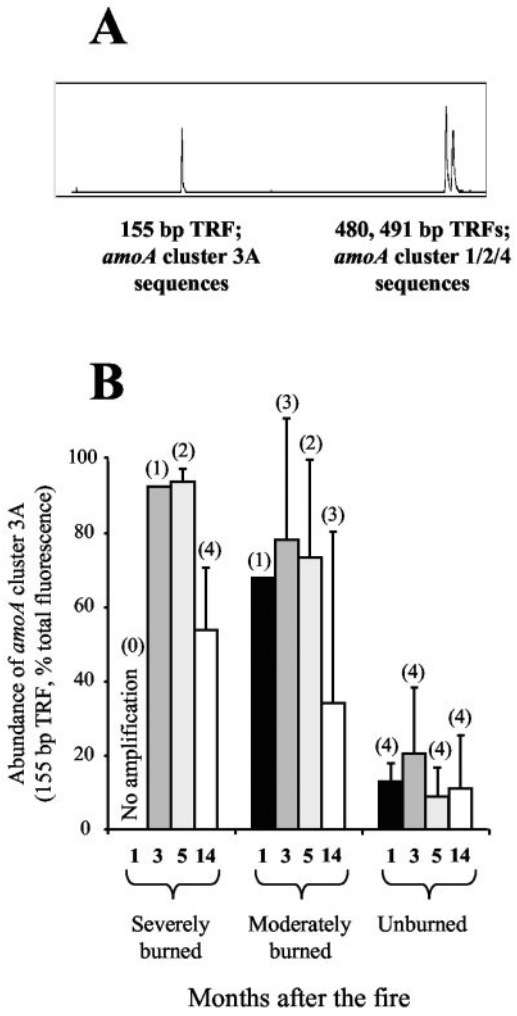

FIG. 4. (A) Typical *amoA* T-RFLP profile obtained from forest soil DNA. The 155-bp TRFs contain primarily *amoA* cluster 3A sequences, whereas the 480- and 491-bp TRFs contain primarily *amoA* cluster 1/2/4 sequences (see Results). (B) Mean abundance (percent total fluorescence) of *amoA* cluster 3A sequences (155-bp TRF) in the T-RFLP profiles generated from soil samples collected from U, M, and B sites 1 (black bars), 3 (dark gray bars), 5 (light gray bars), and 14 (white bars) months after the fire. The numbers in parenthesis above each symbol indicate the number of soil samples that yielded successful PCRs.

found in the distribution of the AO1+AO2 versus AO3+AO4 sequence types within U, M, and B soils at our study area. On one hand, the 155-bp TRF (AO3+AO4 sequences) averaged only 10 to 20% of the total peak area of each T-RFLP profile obtained from U soil samples, regardless of the sampling date (Fig. 4b). On the other hand, the 155-bp TRF comprised 65 to 95% of the total peak area of the T-RFLPs profiles obtained from M and B soils collected up to 5 months after the fire. Averaged over time, the relative abundance of *amoA* cluster 3A sequences was significantly greater in DNA extracted from both M and B than in that from U soils by Tukey-Kramer's mean separation analysis ($P < 0.001$).

Soil chemistry. Soil chemical analysis was performed on samples collected from U, M, and B soils within our study area

TABLE 2. Soil pH, NH_4-N, and NO_3-N from unburned, moderately burned, and severely burned sites

Sample[a]	Soil chemistry[b]		
	pH	NH_4-N (ppm)	NO_3-N (ppm)
U1	6.3 ± 0.5	17.1 ± 3.5	1.4 ± 1.2
U3	6.1 ± 0.2	5.9 ± 2.2	6.0 ± 6.8
U5	6.1 ± 0.4	6.5 ± 3.0	2.1 ± 3.0
U14	6.3 ± 0.4	5.1 ± 3.0	2.0 ± 2.4
M1	7.0 ± 0.1	49.9 ± 33.4	0.9 ± 0.4
M3	7.0 ± 0.2	47.7 ± 44.9	0.4 ± 0.1
M5	6.7 ± 0.3	33.1 ± 30.7	8.5 ± 9.2
M14	6.7 ± 0.1	8.6 ± 7.0	1.5 ± 1.3
B1	7.2 ± 0.6	57.2 ± 11.8	0.8 ± 0.2
B3	7.1 ± 0.7	44.7 ± 9.7	4.3 ± 3.3
B5	6.6 ± 0.9	34.3 ± 13.8	3.7 ± 3.0
B14	6.6 ± 0.7	23.3 ± 10.1	13.5 ± 16.4

[a] Samples were collected from U, M, or B sites 1, 3, 5, or 14 months following the fire (e.g., B1 represents samples collected from burned sites 1 month after the fire).

[b] Soil chemistry values represent the averages ± standard deviations of four soil samples (except for the NO_3-N measurements for samples U5, M5, and B14 [$n = 3$], and sample M3 [$n = 2$]; in these cases, soil samples were depleted prior to NO_3-N analysis).

(Table 2). The means ($n = 4$) for NO_3^--N, the percent total carbon, and the percent total N did not vary significantly between U, M, and B soils 1 month after the fire (data not shown for the percent total C and the percent total N). The mean ($n = 12$) values for NO_3^--N, the percent total carbon, and the percent total N in all soils at our study area were 1.06 ± 0.70 ppm, 3.91% ± 0.75%, and 0.20% ± 0.04%, respectively. Soil pH and NH_4^+-N levels were consistently higher in samples collected from burned sites (M and B) than in those from unburned sites (U). On average, soil from M and B sites had pH values between 0.5 and 1.0 log units higher than found in site U soil, and NH_4^+-N levels were approximately three- to eightfold higher in soils from the fire-impacted sites. Both pH and NH_4^+-N levels for the M and B samples were highest during the first 3 months following the fire. Over time, however, the pH and NH_4^+-N levels of the M and B soils decreased.

Correlation of T-RFLP peak abundance with soil factors. The relative abundance of the 155-bp *amoA* TRF varied dramatically among the B, M, and U soils. For this reason, linear regression analysis was used to determine the correlation of the relative abundance of this fragment with each of four soil variables that could affect the distribution of ammonia-oxidizing bacteria: pH and the levels of NH_4^+-N, calculated NH_3, and NO_3^--N. The relative abundance of the 155-bp TRF was positively correlated with an increase in pH from 5.6 to 7.5 (Fig. 5A). The relative abundance of the 155-bp TRF was also positively correlated with log-transformed values of calculated NH_3 levels from 0.0017 to 0.9760 ppm (Fig. 5B), soil NH_4^+-N levels from 2.9 to 110.8 ppm, and soil NO_3^--N levels from 0.05 to 18.8 ppm (data not shown).

DISCUSSION

The consequences of forest fire can affect important nutrient-cycling functions both above and below ground. Our study demonstrated that forest fire altered the abundance and com-

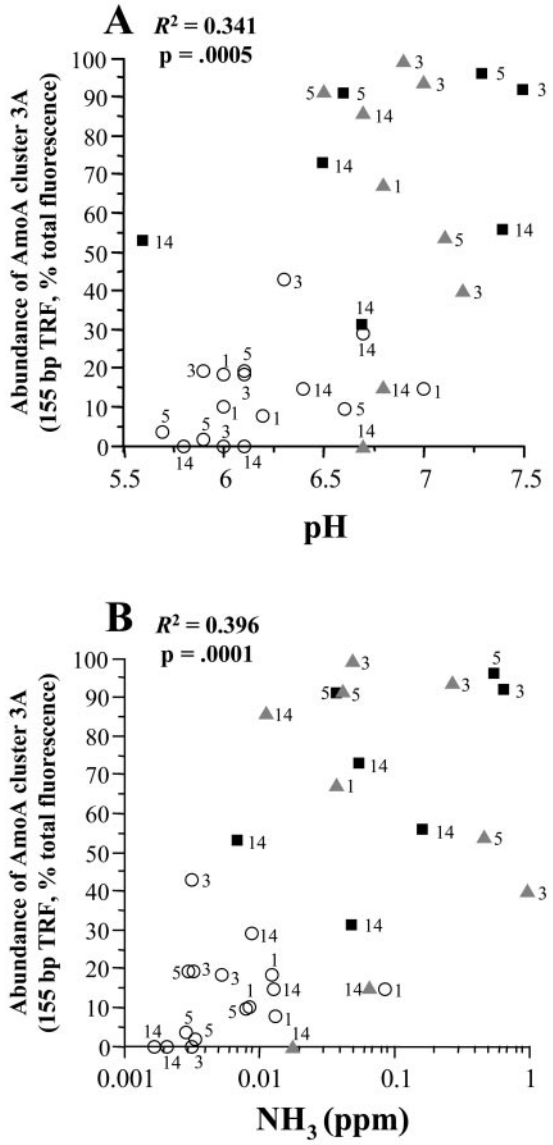

FIG. 5. Mean abundance (percent total fluorescence) of *amoA* cluster 3A sequences (155-bp TRF) in the T-RFLP profiles generated from soil samples collected from unburned (○), moderately burned (▲), and severely burned (■) sites plotted against soil pH (A) and log-transformed, calculated NH_3 concentration (B). The numbers next to each symbol denote the number of months postfire that the corresponding sample was collected. The R^2 and *P* values were determined using linear regressions of the raw (pH) or log-transformed (calculated NH_3) data.

position of two important groups of N-cycling bacteria in soil, i.e., the nitrogen-fixing and ammonia-oxidizing bacteria. In addition, the severity of fire affected the composition of N-cycling bacteria and their recovery after fire. This study is also the first culture-independent analysis of the indigenous populations of nitrogen-fixing and ammonia-oxidizing bacteria in a semiarid, mixed conifer forest. The results indicated a decline in the total soil biomass, including nitrogen-fixing and ammonia-oxidizing bacteria, in soils impacted by the fire. However, the effects of fire on the community composition of these two functional groups of bacteria were quite different. The dominant portion of the nitrogen-fixing community, as determined by *nifH* T-RFLP analysis, became more diverse within a month after the fire. In contrast, the fire resulted in a large shift in the composition of the ammonia-oxidizing community (as determined by analysis of *amoA* gene sequences) from dominance by one genotype to dominance by another.

The 10- to 20-fold difference in soil biomass (DNA yield) observed between moderately or severely burned soils and unburned soils 1 month after the Cerro Grande Fire is similar to estimates of microbial biomass loss due to other past fires, including those in ponderosa pine/Douglas fir, Mediterranean, and Atlantic pine forests (15, 26, 42, 55). This indicated an immediate and drastic effect on the indigenous soil microbial community in the burned soils. More important in terms of ecosystem recovery, the biomass remained low in the burned soils over the course of our 14-month postfire sampling period (10 to 20% of the biomass found in nonburned soils). Our data also indicate that the fire-impacted soils contained diminished percentages of nitrogen-fixing and ammonia-oxidizing bacteria within the soil community (Table 1 shows amplification results for *nifH* and *amoA* PCRs).

Reestablishment of microbial soil populations following fire can be limited by a myriad of factors. These include immediate changes to the physiochemical properties of the soil in addition to postfire effects such as increases in mean soil temperatures resulting from canopy loss and blackening of the soil surface, excessive erosion, and increased nutrient loss through leaching or volatilization (40). In arid and semiarid landscapes, it is generally thought that moisture is a critical factor in postfire recovery (15, 29). Our study area is within a semiarid climate zone and received only 515 mm of precipitation over the 14-month period following the fire. Thus, it is quite possible that moisture played a key role in the relatively slow recovery of microbial biomass (including nitrogen-cycling bacteria) in the M and B soils.

The N_2-fixing community in surface soils at our study area included sequences related to *nifH* sequences from *Proteobacteria* spp., *Cyanobacteria* spp., and *Firmicutes* spp. and to *anfH* sequences from *Firmicutes* spp. Additionally, we amplified several *nifH* sequences that were not closely related to any in the public databases (cluster NF2). Forty-nine percent of the *nifH* sequences identified in the current study (cluster NF5) could be grouped within "subcluster 1A" as described by Zehr et al. (60) (equivalent to "cluster A" as described by Hamelin et al. [25] and "*nifH* cluster 3" as described by Bürgmann et al. [14]). Subcluster 1A contains over one hundred unique *nifH* sequences that have been amplified from a range of environments, including lakes, the open ocean, estuaries, forests, and rice paddies, and yet it contains only one sequence from a cultured diazotroph, the δ-proteobacterium *Geobacter metallireducens* (3, 14, 54, 58). In each of the environments listed above, less than 10% of the *nifH* sequences retrieved could be classified as subcluster 1A types. In contrast, our results are more closely aligned with those of Hamelin et al. (25), who reported that half (56%) of the total number of *nifH* clones analyzed from the roots and surrounding soil of the perennial grass *Molinia coerulea* in a littoral meadow in Switzerland belonged to subcluster 1A.

The Cerro Grande Fire had an interesting effect on the composition of the amplifiable *nifH* population from surface

soils at our study area. T-RFLP analysis indicated that the diversity of major *nifH* sequence types (TRFs comprising at least 10% of the total profile) was greater in B and M soil samples than in U soil samples immediately after the fire. Over time, the compositions of the diazotrophic communities in M and B soils appeared to be reverting to that seen in U soil, which was comprised of two dominant TRF peaks (228- and 358-bp TRFs). Interestingly, the two *nifH* sequence types (55-, 261-, and 325-bp TRFs; clusters NF4 and NF6) that were clearly abundant in T-RFLP profiles from B and M soils, but not U soils, were most closely related to *nifH* (or *anfH*) from the spore-forming *Clostridium* spp. and *Paenibacillus* spp. Increases in nitrogen-fixing *Azotobacter* spp. and *Clostridium* spp. have been previously observed in other ecosystems following fire (4). From such observational evidence, researchers have suggested that spore-forming bacteria thrive after fires because they either survive the heat generated by fires or rapidly colonize recently burned soils (21, 55).

In contrast to the diversity of *nifH* sequence types recovered at our study area, amplifiable *amoA* sequences formed only four separate clusters (AO1, AO2, AO3, and AO4), all of which were most closely related to *Nitrosospira* spp. *amoA* sequences (Fig. 3). These sequences could be placed within a broader taxonomic framework for AmoA protein sequences that was developed by Avrahami et al. (8, 9) and is based upon corresponding 16S rRNA sequence clusters (43, 52). Within this framework, sequences of clusters AO1 and AO2 from the current study could be grouped within *amoA* clusters 1, 2, or 4 (16S rRNA clusters 2 and 4; referred to as *amoA* cluster 1/2/4 hereafter), and clusters AO3 and AO4 belong in *amoA* cluster 3A (16S rRNA cluster 3).

The fire greatly affected the ratio of *amoA* cluster 1/2/4 to *amoA* cluster 3A sequences found at our study area, as determined by T-RFLP analysis. Whereas cluster 3A sequences (155-bp TRF) comprised 10 to 20% of the amplifiable *amoA* population in U soils, they comprised 65 to 95% of the *amoA* population in M and B soils up to 5 months after the fire. Fourteen months after the fire, cluster 3A sequences still comprised 35 to 55% of the total *amoA* population retrieved from the M and B soils. The shift of the ammonia-oxidizing population in fire-impacted soils of a mixed conifer forest from a predominance of cluster 1/2/4 *Nitrosospira* spp. to one of cluster 3A *Nitrosospira* spp. could be attributed to a number of factors. However, our results in combination with an emerging picture from the literature on ammonia oxidization suggest that members of cluster 3A *Nitrosospira* spp. respond favorably to environmental perturbation and higher concentrations of soil NH_4^+-N, whereas cluster 1/2/4 *Nitrosospira* spp. are typically dominant in undisturbed later-successional-stage soils with lower concentrations of soil NH_4^+-N (13, 31, 32, 36, 37, 56). As such, it may be useful to monitor the ammonia-oxidizing community as a partial measure of soil health following a fire.

In fire-impacted soils within our study area, the dominance of cluster 3A *Nitrosospira* spp. was associated with increases in KCl-extractable NH_4^+-N and NO_3^--N, calculated levels of soil NH_3, and pH relative to unburned soils. The relative abundance (%) of cluster 3A *Nitrosospira* spp. *amoA* sequences amplified from soil samples was most positively correlated with soil pH (5.6 to 7.5) and NH_3 concentration (0.0017 to 0.9760 ppm). An abundance of cluster 3A *Nitrosospira* spp. in soils has been previously associated with higher concentrations of ammonium and with neutral pH (6, 13, 31, 32, 51, 52, 56). Indeed, in a recent study Horz et al. (27) found that elevated nitrogen deposition [$Ca(NO_3)_2$ application intended to mimic the increased nitrogen deposition expected with global climate change] resulted in a shift of the ammonia oxidizer population to one dominated by cluster 3A *Nitrosospira* spp. This trend has been corroborated using enrichment cultures where higher concentrations of ammonium selected for 16S rRNA cluster 3A *Nitrosospira* spp., whereas lower ammonium concentrations selected for 16S rRNA cluster 4 *Nitrosospira* spp. (*amoA* cluster 1 *Nitrosospira* spp.) (31). Soil temperature may also act as a selective factor for ammonia oxidizer populations, with warmer temperatures (>25°C) favoring cluster 3A *Nitrosospira* spp. (7). Physiologic studies of pure cultures are needed to further elucidate the association between pH, NH_3-NH_4^+ concentration, temperature, and growth or survival rates of different *Nitrosospira* spp. (28).

It has been suggested that increased levels of ammonium in fire-impacted soils lead to higher rates of nitrification and thus higher rates of NO and N_2O flux (35). Indeed, autotrophic ammonia oxidation can be a significant source of terrestrial NO and N_2O (17, 49, 50), and elevated concentrations of ammonium have been linked to increased rates of NO and N_2O flux through nitrification (8, 38, 45, 47). Our results suggest that fire-related increases in soil ammonium or pH may influence the structure of the indigenous ammonia-oxidizing community in a mixed conifer forest. In light of these observations, a systematic study investigating the association between soil temperature, ammonia levels, pH, the ammonia-oxidizing community structure, rates of nitrification, and flux of NO and N_2O in postfire ecosystems would be a worthwhile endeavor.

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