Recovery of the Nitrogen Cycling Bacterial Community in Soils Following the Cerro Grande Fire in NM

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Abstract

The Cerro Grande Fire burned 50,000 acres of forest surrounding Los Alamos, NM in early May 2000, and created regions of badly burned, moderately burned, and unburned soils. To investigate the community recovery of nitrogen cycling bacteria in soil following burns of different heat intensities, samples were taken to a depth of 10 cm, from four plots within each burn treatment type at one month, three months, five months, and 14 months after the fire. DNA was extracted from samples using a bead-mill homogenization technique, and was quantified. The total amount of DNA increased during the three-month time point in all samples, possibly due to seasonal precipitation. Unburned soils usually contained more extractable DNA, while moderately and badly burned soils had comparable amounts of extractable DNA. Using primers specific for the *amoA* (ammonia-oxidizing bacteria) and the *nifH* (nitrogen fixing bacteria) genes, nested PCR results showed a general trend of fewer samples with amplifiable *amoA* (54% of samples) from the one and three month time-points. Nitrogen fixers showed a strong presence (88% of samples based on amplifiable *nifH*) during this same time period. The number of samples from which *amoA* could be amplified increased to 91% and 92% at five and 14 months, respectively. The number of samples from which *nifH* could be amplified remained relatively constant at five and 14 months. The *amoA* PCR products were digested with TaqI for terminal restriction fragment analysis. Resultant patterns showed the presence of one peak suggesting that the dominant population consists of *Nitrosospira* spp. These preliminary results suggest that nitrogen cycling bacterial populations differentially respond to fire disturbance.

Introduction

Fire plays an important role in many ecosystem processes and is particularly crucial for the release of nutrients that are tied up in plants and litter (Wright and Bailey 19782). Depending on the degree of heating, fire can produce a range of short term effects on soil ecosystems including: an increase in soil temperature that can kill many microorganisms, lower relative humidity close to the ground due to loss of insulating vegetation and litter, destruction of much of the N and C in the litter, and an increase in soil ammonium levels. Many studies have examined the impact of fire on plants and animals, but few studies have examined the impact of fire on soil microorganisms, and few of these used culture-independent methods (an example is Burgos et al. 2000). From these limited studies it appears that the intensity of the burn is a key factor in determining which microbial species survive. In general, it has been observed that spore-formers and acidophilic bacteria survive better, and cyanobacterial, fungal, and algal populations are depressed by fire (Vazquez et al. 1993). The release of nutrients and concommitant elimination of vegetation (removing a major N sink) can allow microbial species, especially nitrifiers, to flourish. Indeed, previous studies found strong effects of fire on ammonifiers and nitrifiers (Abril and Gonzalez 1999), including decreases (Klopatek et al. 1990) and increases in nitrogen-fixing species. However, specific information on the fate of ammonia-oxidizers and nitrogen-fixers following fires of differing intensities, their recovery over time, and the nature of the species composition of surviving and colonizing ammonia-oxidizers and nitrogen-fixers is currently lacking. The goals of the current study are to investigate whether ammonia oxidizers and N_2-fixing bacteria persist and whether changes in the species composition of these groups are observed following fires of differing intensities.

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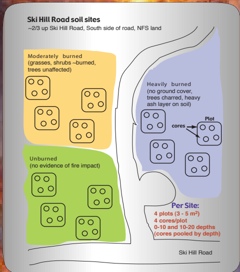
Research Questions

* Are nitrogen-fixing and ammonia-oxidizing bacteria present in burned soils after fires of different intensities?
* Are there differences in the presence/absence of nitrogen-fixing and ammonia-oxidizing bacteria in burned soils over time?
* Are there differences in the species of ammonia-oxidizing bacteria present in burned and control soils?



Sampling Date	Amplification Efficiency *amoA*	*nifH*
6/7/00+8/7/00	13/24 (54.2%)	21/24 (87.5%)
10/11/00	10/11 (90.9%)	9/11 (81.8%)
7/18/01	11/12 (91.7%)	9/12 (75.0%)

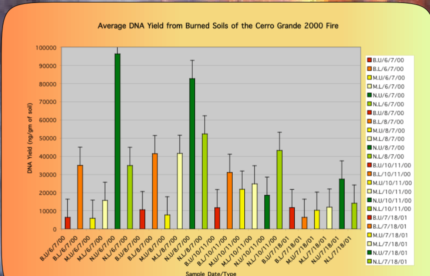
Nested PCR results showed a general trend of fewer samples with amplifiable *amoA* (54%) from the earlier time-points. The number of samples from which *amoA* could be amplified increased to 91% and 92% at five and 14 months respectively, while the number of samples from which *nifH* could be amplified remained relatively constant.

Methods

Sampling:
Four random 1" diameter soil cores were taken and pooled from within each of four plots in a badly burned, moderately burned, and non-burned areas on 6/7/00 (one month after the fire), 8/7/00, 10/11/00 and 7/18/01. Soil cores were stored at -80C.

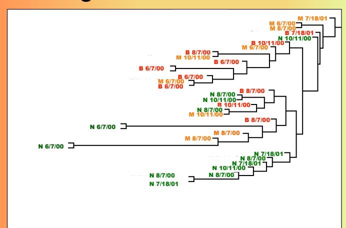
DNA Studies:
* DNA extraction followed the method of Kuske et al. 1998, utilizing bead mill homogenization and Sephadex purification. Amount of DNA recovered was quantified using standards and plotted using Excel.
* Nested PCRs: *amoA* nested PCRs were performed with 2F/5R, followed by 1F/2R primers. *nifH* nested PCRs were performed with 19F/nifH3, followed by nifH11/nifH22 primers; *amoA* amplification products were gel purified using the Qiagen Gel Extraction kit.
* Amplification products were cloned using the TOPO TA plasmid vectors (Invitrogen, Carlsbad, CA). 48 random clones were sequenced from each of four timepoints: B2U 8/7/00; B27 7/18/01; M1U 7/18/01; and N1U 7/18/01 by the JGI at LANL.
* Closest relatives were identified using the Blast database.

Terminal Restriction Fragment (TRF) Analysis:
PCRs were performed as above, but a fluorescently labelled forward primer was substituted. The *amoA* PCR products were digest with TaqI for TRF analysis. The *nifH* PCR products were digested with RSAI for TRF analysis. To create UPGMA dendograms, TRF profiles were converted to binary data for distance comparisons using the Jaccard distance measure.



Yellow= Moderately Burned; Red/Orange = Badly Burned; Green = Non-burned

Dendogram of *nifH* TRF Profiles

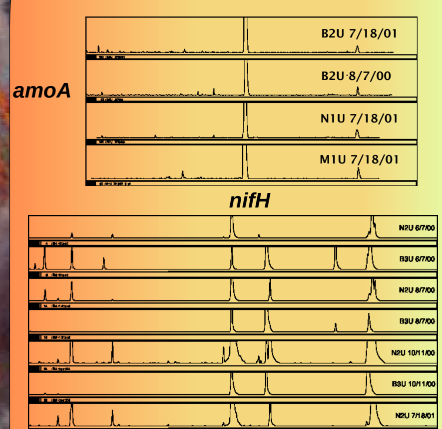


Dendogram of T-RFLP profiles reveals a tendancy for the *nifH* sequences retrieved from non-burned sites (green) to group separately from *nifH* sequences retrieved from moderately (orange) and badly (red) burned areas. These results suggest that the composition of the diazotrophic community is altered by fire.

Conclusions

* Intensely burned soils yielded less DNA from surface samples (0-10cm) than the corresponding lower (10-20 cm) samples. Three out of four time-points yielded greater DNA from non-burned than from burned soil samples.
* A lower amplification efficiency was observed for *amoA* (54%) than for *nifH* (88%) in samples collected one and three months after the fire.
* The number of samples from which *amoA* could be amplified increased to 91% and 92% in samples collected 5 and 14 months after the fire, respectively, while the number of samples from which *nifH* could be amplified remained relatively constant at 5 and 14 months.
* Clone libraries of *amoA* from badly, moderately, and non-burned soils showed no discernable pattern of species distribution.
* T-RFLP profiles of the nifH PCR pool obtained from non-burned samples tended to group separately from the *nifH* profiles of moderately and badly burned samples as determined by statistical analysis.
* Taken together, our results suggest that the fire severely decreased the number of ammonia-oxidizing bacteria in the surface soils, yet did not have a lasting effect on the composition of these bacteria. On the other hand, it appears that fire does alter the composition of the diazotrophic community.

T-RFLP Profiles of *amoA* and *nifH*



amoA T-RFLP profiles show no major differences among timepoints. The major peak at approximately 280 corresponds to Nitrosospira spp. *nifH* profiles reveal differences between burned and non-burned samples. Note the peak at approximately 325 in the burned samples, that diminishes over time and is not present in the non-burned samples.

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