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Bacterial composition of soils in ponderosa pine and mixed conifer forests exposed to different wildfire burn severity

Carolyn F. Weber*, J. Scott Lockhart, Emily Charaska, Ken Aho, Kathleen A. Lohse

Idaho State University, Department of Biological Sciences, Pocatello, ID 83209, USA

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ABSTRACT

Soil microbial communities influence the rate and trajectory of ecosystem recovery after wildfire, but how their composition varies with burn severity in different vegetation types is largely unknown. This study utilized high throughput amplicon sequencing of a bacterial 16S rRNA gene fragment to determine the bacterial community composition in soils that were unburned, moderately burned ("low burn") and severely burned ("high burn") in ponderosa pine ('P') and mixed conifer ('M') forests, three months after the Las Conchas fire (New Mexico, USA; July 2011). Community composition was distinct in unburned M and P soils, but it was similar in high burn soils, despite differences in initial and post-burn M and P soil parameters (i.e. pH, moisture, organic matter, carbon and nitrogen content), which are known to correlate with shifts in bacterial community composition. Richness tended to be lower in the high burn M soils relative to unburned M soils, while it was similar across all P soils. Collectively, our findings indicate that high burn severity may result in bacterial communities shifting to similar compositions within a few months post-fire, even if the initial communities, as well as initial and post-burn soil physical and chemical properties are distinct.

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1. Introduction

The frequency and severity of wildfires in the Western U.S. have significantly increased over the last 25 years (Conard et al., 2001; Dombeck, 2001; Grissino-Mayer and Swetnam, 2000; Westerling et al., 2006). These changes have been attributed to both land management practices and climate change. Long-term fire suppression has led to biomass and organic matter accumulation that fuel wildfires of increased size, duration, and frequency that release large pulses of nutrients into the environment (Neary et al., 1999). The impacts of wildfire on overall ecosystem recovery and functioning, particularly nutrient cycling, are intimately tied to the composition and structure of soil microbial communities. As a result, there is an increased need for a thorough understanding of the resistance and resilience of soil microbial communities to climate change and associated disturbances, such as wildfire, and how this may influence the rate and characteristics of ecosystem recovery (Allison and Martiny, 2008; Bardgett et al., 2008; Fierer et al., 2010).

Previous studies have documented the impacts of wildfire on soil physical and chemical properties including increased soil pH, increased soil hydrophobicity and decreased soil structural stability (Certini, 2005; MacDonald and Huffman, 2004) as well as a reduction in the quantity and quality of soil organic matter (Certini, 2005; Neary et al., 1999). Additionally, organic nitrogen (N) may be volatilized or converted to inorganic forms through mineralization. Mineralization can increase biologically available N, but if it is not consumed, large fluxes from ecosystems can occur. Immediately after a fire, the predominant form of inorganic N is ammonium, a direct product of burning, which is subsequently converted to nitrate through the process of nitrification (Certini, 2005; Johnson et al., 2007; Neary et al., 1999; Prieto-Fernandez et al., 1998; Smithwick et al., 2005).

Soil microbial communities play a critical role in carbon (C) and N cycling. Thus, identifying associations between wildfire severity and community characteristics provides a foundation for gaining insights into the possible impacts of fire on ecosystem functioning. Surface soil temperatures in wildfires typically exceed 200 °C causing a substantial decrease in total microbial biomass and changes in soil physical and chemical characteristics that influence microbial community composition (Dumontet et al., 1996; Certini, 2005; Mabuhay et al., 2006; Hamman et al., 2007). To date, studies have documented that post-fire microbial communities are often less diverse and their composition may be influenced by

* Corresponding author. Idaho State University, Department of Biological Sciences, 921 S. 8th Avenue, Mail Stop 8007, Pocatello, ID 83209, USA. Tel.: +1 208 282 2149; fax: +1 208 282 4570.
E-mail address: webecaro@isu.edu (C.F. Weber).

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factors such as changes in soil pH, nutrient and water availability (Acea and Carballas, 1996; Dumontet et al., 1996; Prieto-Fernandez et al., 1998; Certini, 2005; Smithwick et al., 2005; Yeager et al., 2005; Jackson et al., 2006; Mabuhay et al., 2006; Hamman et al., 2007; Dangi et al., 2010; Barcenas-Moreno et al., 2011; Sun et al., 2011; Docherty et al., 2012; Ginzburg and Steinberger, 2012; Goberna et al., 2012). However, such studies have largely examined soil microbial communities in a single vegetation type exposed to wildfire, have often not considered burn severity and have utilized coarse-resolution DNA fingerprinting methods (e.g. T-RFLP) and phospholipid fatty acid (PLFA) profiles (Barcenas-Moreno et al., 2011; Mabuhay et al., 2006) in attempts to discern shifts in microbial community composition.

Following the trend of large, intense wildfires, the 2011 Las Conchas fire in Northern New Mexico burned over 150,000 acres (Forest Service, 2011) including large portions of the Valles Caldera National Preserve and within it, parts of the Jemez River Basin Critical Zone Observatory (CZO) (Chorover et al., 2011). In this system, we asked two questions:

1) How do soil properties vary with wildfire burn severity in ponderosa pine and mixed conifer forest soils?
2) How is the composition of soil microbial communities shifted in soils exposed to low and high burn severities relative to those outside the burn perimeter in the two vegetation types?

Three months after the Las Conchas fire, we expected that burn severity would be associated with shifts in N and C pool sizes as well as pH, water content and microbial communities, but that different responses would be observed in the two forest soil types. To test these hypotheses, we compared physical and chemical properties of unburned soils and those exposed to low and high burn severity in mixed conifer and ponderosa pine forests, as well as bacterial 16S rRNA gene sequence libraries generated from the same soils.

2. Materials and methods

2.1. Sample collection

All soil samples were collected from mixed conifer ("M") or ponderosa pine ("P") stands within the Jemez River Basin Critical Zone Observatory in the Valles Caldera National Preserve, New Mexico (106°33′23″W, 35°52′19″N). Mixed conifer forest stands consisted of Douglas fir (*Pseudotsuga menziesii*), white fir (*Abies concolor*), blue spruce (*Picea pungens*), corkbark fir (*Abies lasiocarpa* var. *arizonica*) and Englemann's spruce (*Picea englemannii*) (Parmenter et al., 2007). Objective dangers in the post-fire environment limited regions of safe sampling to relatively small areas (represented as symbols in Fig. 1) that met criteria of different burn severity as well as controlled for soil differences across burn and forest types. Areas classified as high and low burn severities were identified at larger scales using burn intensity maps and then locally identified following a classification scheme used in Turner et al. (2007) and with the aid of forest service staff in the field. In brief, high burn severity was associated with areas in which all vegetation was burnt and the ground was ashy and no pine needles were left. Areas classified as low burn severity had trees with red needles and some vegetation was still on the ground. Unburned sites were selected outside the burn perimeter established by the Forest Service (Fig. 1). Soil collections were restricted to the Calaveras–Anastacio (0–15% slope) Series (Fig. 1).

Soil sampling was performed in October 2011, three months after the Las Conchas fire. Within each vegetation type, three surface soil samples (0–5 cm) were randomly collected from each of the six individual areas that were representative of no burn ("M_N" or "P_N"), low burn severity ("M_L" or "P_L"), and high burn severity ("M_H" or "P_H") (18 soil samples total). The M_N and P_N areas were approximately 1 × 1 km^2, while remaining areas were approximately 0.5 × 0.5 km^2. We acknowledge that confident inferences can only extend to these randomly sampled regions. However, concurrent studies affirm the findings in this paper (see Discussion) indicating that the results are generalizable to larger areas affected by the fire (Lohse, unpublished data).

Each soil sample (identified as 1, 2 or 3; e.g. M_N1) was manually homogenized and kept on ice until it was processed in the laboratory. Subsamples for DNA analyses were collected in sterile two oz. (58 ml) Whirlpak bags (Nasco, Fort Atkinson, WI), immediately placed on ice, and then frozen at −20 °C within 24 h of field collection.

2.2. Soil properties

Soil physical and chemical properties (pH, moisture content and organic matter content and nutrient content) were determined using established methods. Immediately upon returning from the field, soils were sieved; the coarse fraction averaged less than 5% of the total weight of the surface soils and less than 1.5% by volume. The fine fraction (<2 mm) was used for all analyses. One 25 g soil subsample was dried at 105 °C to determine gravimetric soil moisture (Gardener, 1986) and then combusted in a muffle oven at 360 °C for 16 h to determine soil organic matter (OM) by loss-on-ignition (Nelson and Sommers, 1996). The pH was measured on a 10 g subsample in a soil slurry (2 parts water: 1 part soil) using a combination electrode (Thomas, 1996). Another 10 g subsample was extracted with 50 ml of 2 N potassium chloride (KCl) to determine exchangeable mineral N following methods described by Lohse and Matson (2005). Extracts were analyzed for ammonium and nitrate + nitrite following the salicylate ammonium method and nitrate reduction method on a SmartChem Discrete Analyzer (Westco Scientific, Brookfield, CT), respectively. A final soil subsample was dried at 60 °C for analysis of total C and N, ground using a ball mill, packed in a tin capsule, and analyzed on a Delta V Isotope Ratio Mass Spectrometer (ThermoElectron Corporation, Milan, Italy) at the Idaho State University Center for Archaeology, Materials and Applied Spectroscopy (CAMAS; Pocatello, ID).

2.3. Soil DNA extraction, PCR and sequencing

Total DNA was extracted from 0.25 g of frozen soil for each sample using the MoBio Powersoil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA). DNA extractions were performed according to manufacturer protocol, with the exception of using a final elution volume of 50 μl. Bacterial 16S rRNA gene fragments were amplified in triplicate from each of the 18 resulting DNA extracts using the primers 515F and 806R (Caporaso et al., 2011), which target the V4 hypervariable region (~254 bp). Each 25 μl reaction contained 1.5 units of AmpliTaq DNA polymerase (Applied Biosystems, Carlsbad, CA), 1× AmpliTaq Buffer II (Applied Biosystems, Carlsbad, CA), 800 μM dNTPs, 0.4 mM each primer, 1.5 mM $MgCl_2$ and 6 μg bovine serum albumin. Samples were initially denatured at 95 °C for 3 min followed by 25 cycles of 94 °C for 45 s, 59.5 °C for 20 s, and 72 °C for 30 s with a final extension at 72 °C for 10 min. Thermal cycling was carried out using an Eppendorf Mastercycler proS (Eppendorf, Westbury, NY). After confirming successful amplification by gel electrophoresis, PCR products from triplicate reactions were pooled and then purified with the Qiagen MinElute PCR Cleanup kit (Qiagen, Valencia, CA). Amplicons were prepared for multiplex sequencing on the Ion Torrent PGM (Life Technologies, Grand Island, NY) using manufacturer protocols (user manual 4471989 Rev. C). Unique Ion Xpress Barcode Adapters (Life Technologies, Grand Island, NY) were ligated to the fragments in each sample pool. Equal masses of