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Soil microbial responses to fire and interacting global change factors in a California annual grassland

Kathryn M. Docherty · Teri C. Balser · Brendan J. M. Bohannan · Jessica L. M. Gutknecht

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Abstract Wildfire in California annual grasslands is an important ecological disturbance and ecosystem control. Regional and global climate changes that affect aboveground biomass will alter fire-related nutrient loading and promote increased frequency and severity of fire in these systems. This can have long-term impacts on soil microbial dynamics and nutrient cycling, particularly in N-limited systems such as annual grasslands. We examined the effects of a low-severity fire on microbial biomass and specific microbial lipid biomarkers over 3 years following a fire at the Jasper Ridge Global Change Experiment. We also examined the impact of fire on the abundance of ammonia-oxidizing bacteria (AOB), specifically *Nitrosospira* Cluster 3a ammonia-oxidizers, and nitrification rates 9 months post-fire. Finally, we examined the interactive effects of fire and three other global change factors (N-deposition, precipitation and CO_2) on plant biomass and soil microbial communities for three growing seasons after fire. Our results indicate that a low-severity fire is associated with earlier season primary productivity and higher soil-NH_4^+ concentrations in the first growing season following fire. Belowground productivity and total microbial biomass were not influenced by fire. Diagnostic microbial lipid biomarkers, including those for Gram-positive bacteria and Gram-negative bacteria, were reduced by fire 9- and 21-months post-fire, respectively. All effects of fire were indiscernible by 33-months post-fire, suggesting that above and belowground responses to fire do not persist in the long-term and that these grassland

University of Oregon and University of Wisconsin-Madison—institutions where this research took place.

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K. M. Docherty
Department of Biological Sciences, Western Michigan University, 1903 West Michigan Ave., Kalamazoo, MI 49008-5410, USA

K. M. Docherty · B. J. M. Bohannan
Center for Ecology and Evolutionary Biology (CEEB), University of Oregon, P.O. Box 5289, Eugene, OR 97403, USA

T. C. Balser · J. L. M. Gutknecht
Department of Soil Science, University of Wisconsin-Madison, 1525 Observatory Drive, Madison, WI 53706, USA

T. C. Balser
Department of Soil and Water Science, University of Florida, Gainesville, FL 32611, USA

J. L. M. Gutknecht (✉)
Department of Soil Ecology, Helmoltz-Centre for Environmental Research—UFZ, Theodor-Lieser-Strasse 4, 06120 Halle, Germany
e-mail: jessica.gutknecht@ufz.de

communities are resilient to fire disturbance. While N-deposition increased soil NH_4^+, and thus available NH_3, AOB abundance, nitrification rates and Cluster 3a AOB, similar increases in NH_3 in the fire plots did not affect AOB or nitrification. We hypothesize that this difference in response to N-addition involves a mediation of P-limitation as a result of fire, possibly enhanced by increased plant competition and arbuscular mycorrhizal fungi–plant associations after fire.

Keywords Fire · Grassland · Soil microbiology · PLFA · Global change

Introduction

Many grassland ecosystems, especially those in the semi-arid western United States, rely on fire disturbance regimes to maintain plant community structure and ecosystem function (Vogl 1979; Sugihara et al. 2007). Fires are an important factor in maintaining and restoring plant community composition and avoiding ecosystem degradation by invasive species (Menke 1992; DiTomaso et al. 2006). Fires may also indirectly improve ecosystem functions such as increasing soil organic matter and maintaining soil fertility (Menke 1992).

Fire regimes have been altered greatly by human activity, and are likely to be altered further in the coming century by climate change in the western US (Fried et al. 2004; Westerling et al. 2006; Westerling and Bryant 2008). Specifically, fires in California are likely to burn more rapidly and with higher intensity in the coming century under warmer climate scenarios (Fried et al. 2004). Multiple interacting effects of regional and global climate change may also increase fire incidence. For instance, increases in nitrogen deposition and fertilization, altered precipitation and drought patterns, and higher surface temperatures may substantially increase aboveground plant biomass, thus increasing fuel for fires (Henry et al. 2006).

Changes in aboveground communities as a result of fire can indirectly affect belowground systems by altering nutrient inputs, increasing solar warming and surface soil temperatures, and changing evapotranspiration rates and soil moisture availability (Neary et al. 1999). Furthermore, fire can lead to altered production of greenhouse gases from soils, such as N_2O (Niboyet et al. 2011) or CO_2 (Schulze et al. 2000; Dooley and Treseder 2011), suggesting that increased incidence of fire could alter current global change predictions (Schulze et al. 2000; Dooley and Treseder 2011). To date, few studies have linked the effects of grassland fire and other interacting global change factors to belowground microbial dynamics. Microbial communities, which comprise the majority of Earth's biomass and diversity (Roesch et al. 2007; Schloss and Handelsmann 2006), are responsible for many important environmental processes (e.g. Kowalchuk and Stephen 2001; Hayatsu et al. 2008; Evans and Wallenstein 2011; Yavitt et al. 2011) and are central to the long-term response of ecosystems to global change (Hu et al. 1999; Singh et al. 2010; Brown et al. 2011, Todd-Brown et al. 2011, Treseder et al. 2011; Wallenstein and Hall 2011). Yet little is known about how wildfire affects microbial community structure and the subsequent ecosystem functions that microorganisms mediate.

Low-severity grassland fires alter both physical and chemical soil properties that affect microbial communities, particularly within the top 15-cm of soil where most aerobic microbial activity occurs (Raison 1979; Neary et al. 1999). In the short-term, direct heating can lead to microbial mortality in the soil (Raison 1979), though this effect is reduced in low-severity grassland fires where aboveground biomass (AGB) and litter are ashed quickly with little downward transfer of heat (Hart et al. 2005; Raison 1979). However, removal of AGB leads to increased soil warming and drying through direct solar irradiation (Raison 1979; Henry et al. 2006). This can have longer-term effects on plant productivity, microbial respiration, and decomposition rates (Fioretto et al. 2005; Hamman et al. 2007).

Chemically, low-severity fires deposit plant ash residues that contain macronutrients and organic carbon (Boerner et al. 2006). Specifically these include: carbonates of alkaline earth metals, silica, heavy metals, sesquioxides, phosphates, and organic and inorganic N (Raison 1979; Grogan et al. 2000; Wan et al. 2001; Picone et al. 2003; Li and Herbert 2004; Boerner et al. 2006). Additionally, heating from fire can volatilize nitrogen in the top few centimeters of the soil horizon (Wan et al. 2001). Particularly in low-severity summer grassland fires, which occur during the dry period after plants have died, this increase in nutrients through fire ash can have effects that emerge in the following growing season(s) (Henry et al. 2006).

Physical and chemical changes induced by fire can alter soil microbial dynamics in several ways. Depending on the severity of the fire, total soil microbial biomass can decrease dramatically immediately following the fire (Boerner et al. 2005). Generally, fires reduce fungal biomass, while bacterial biomass is unaltered (Dooley and Treseder 2011). The activity of microbial enzymes for organic carbon degradation often decreases after burning (Eivazi and Bayan 1996; Ajwa et al. 1999; Gutknecht et al. 2010). Similarly, activities of microbial enzymes involved in phosphorus metabolism can also decrease after burning (Ajwa et al. 1999; Gutknecht et al. 2010). However, the responses of microbial enzymes involved in organic nitrogen decomposition can vary after fire, with both increases (Ajwa et al. 1999) and decreases (Gutknecht et al. 2010) being reported. Few studies have examined the effects of fire on specific microbial functional groups linked to a particular biogeochemical rate. Phylogenetically distinct microorganisms show varied physiological responses to stress and disturbance, with different implications for microbial biogeochemical cycling (Schimel et al. 2007). Since fire can induce large changes, particularly in available N, responses of particular groups and sub-groups of microorganisms to a flush of available nutrients may result in long-term changes at an ecosystem level. For example, Yeager et al. (2005) show that high-severity forest fires can have multi-seasonal effects on the community structure of soil nitrifying bacteria and nitrification rates.

However, the mechanisms by which ecosystem changes conveyed by fire will affect soil microbial communities in combination with other global changes are almost completely unknown. Shifts in plant–microbe competition for novel resources deposited by fire can affect the timing of microbial responses to fire (Fiorietto et al. 2005; Hart et al. 2005; Gutknecht et al. 2010). However, shifts in competition can be further confounded by factors such as increased plant productivity as a result of nitrogen deposition, or suppressed plant productivity as a result of elevated atmospheric CO_2 (Shaw et al. 2002; Zavaleta et al. 2003; Henry et al. 2006).

In this study, we examined multi-year responses to a low-severity grassland fire interacting with three other global change manipulations (nitrogen deposition, elevated CO_2 and elevated precipitation) at the Jasper Ridge Global Change Experiment (JRGCE), near Palo Alto, CA. We examined microbial community responses for 3 years post-fire at several levels of resolution, including total microbial growth (lipid biomass), general microbial community structure (lipid composition), and the abundance and community structure of a bacterial gene involved in nitrification. Lipid analysis allows for the quantitative analysis of total microbial biomass and several ecologically distinct groups of microorganisms, including Gram-positive bacteria, Gram-negative bacteria and arbuscular mycorrhizal fungi (AMF) (Smithwick et al. 2005; Balser et al. 2005; Carney and Matson 2006; Gutknecht et al. 2011). Because fire has been shown to alter soil N inputs which can impact soil microbial N-transformations at JRGCE (Niboyet et al. 2011) and the JRGCE is traditionally N-limited (Henry et al. 2006), we also chose to examine ammonia-oxidizing bacteria (AOB) as a functional group that is likely impacted most quickly by both direct and indirect effects of fire. We compared microbial community parameters to multi-year responses in plant productivity and abiotic factors to fire in the context of other multi-factor global change manipulations. Additionally we examined the effects of fire on nitrification potential and AOB communities at one time point following the fire. Because fire can increase soil nutrient inputs, we predicted that the soil microbial community would respond to fire and nitrogen deposition treatments in a similar manner. We also predicted that the affects of fire would dampen over time as the soil community recovered. This study provides a whole-ecosystem context to the response of a California annual grassland to fire coupled with future global change scenarios.

Methods

Field site and experimental design

Soils were sampled for 4 years from the JRGCE from 2003 to 2006 (beginning 6 years after treatments began in 1998). The JRGCE is located in the eastern foothills of the Santa Cruz Mountains (37°40N, 122°22W) at the Jasper Ridge Biological Preserve (Stanford University). The region experiences a Mediterranean climate with a cool wet growing season and hot dry summer. The plant community at the site is typical of a Californian annual grassland, dominated by non-native annual grasses (*Avena barbata*, *Avena*

fatua, *Bromus hordeaceus*, and *Lolium multiflorum*; Zavaleta et al. 2003; Henry et al. 2006). Nonnative forbs (*Geranium dissectum*, *Erodium botrys*, and *Crpis vesicaria*) and native annual forbs (*Hemizonia congesta* ssp. *luzulifolia* and *Epilobium brachycarpum*) are also present (Henry et al. 2006). Native grasses (*Danthonia californica* and *Nasella pulchra*) are dominant perennials, but comprise less than 10% of total biomass at the JRGCE (Henry et al. 2006). The JRGCE is located on a fine, mixed, thermic Typic Haploxeralf soil type, consisting of weathered alluvium from the Franciscan complex (Kashiwagi 1985).

The JRGCE includes four global change treatments, each at two levels (ambient and elevated), in a full factorial design. The treatments consisted of: CO_2 (at ambient or elevated to 700 ppm); N-deposition (ambient or +7 g of N (as $Ca(NO_3)_2$) m^{-2}); precipitation (ambient or 50% above ambient); and warming (ambient or increased to approximately +1°C in the grass canopy); Shaw et al. 2002; Zavaleta et al. 2003). The JRGCE has been under continuous manipulation of all four treatments since the beginning of the 1998–1999 growing season (November 1998). The treatment levels are consistent with regional and global predictions over the twenty first century (Hayhoe et al. 2004). In July 2003, an accidental fire burned a portion of the experiment. A controlled burn was then conducted such that two complete experimental blocks were burned, adding a fifth treatment to the experimental system. The burns resulting in the 2-block fire treatment were rapid, low severity fire, typical of wildfires in California annual grasslands that ashed above-ground litter in the plots. Warming treatments in the burn blocks were removed to create a smaller 'burn experiment' which had four replicates each of burning, elevated CO_2, N-deposition, and precipitation, all fully crossed, for a total of 16 treatments. In order to test wildfire effects and interactive effects between wildfire and global change factors, we analyzed a subset of data including all plots in the two burn blocks (N = 32) and all un-warmed, unburned blocks under the other global change treatments (total N = 80 for our analysis).

Plant biomass and soil sampling

At the JRGCE there are two annual peaks (mid versus late spring) in plant AGB production that are dependent both on species and treatments (Zavleta et al. 2003; Dukes et al. 2005; Henry et al. 2006). Plant AGB data were analyzed from the first biomass peak on the following dates: 24 April, 2003 (4 months before the fire); 22–26 April, 2004 (9 months after fire); 25–26 April 2005 (21 months after fire), 9–11 May 2006 (33 months after fire). AGB data from the first biomass peak was thought to correlate more closely with our microbial analyses from soil samples taken on the same date. AGB was determined from the collection of aboveground plant matter from a 141 cm^2 area of each experimental plot. Litter mass was determined by collecting all senesced plant material from within the same 141 cm^2 area. Belowground biomass (BGB) was determined only at the first harvest date each year (the same dates as above) by separating live roots out of soil cores (15 cm depth) taken in the area of the first AGB harvest. All biomass was oven-dried (70°C) before weighing (Dukes et al. 2005). Directly after AGB was harvested, four replicate soil cores (2.5 cm wide and 15 cm deep) were taken from the same 141 cm^2 area of each experimental plot and thoroughly homogenized. Sub-samples were then stored appropriately for each analysis described below.

Abiotic soil measurements

We measured the percent soil moisture gravimetrically every year from each freshly collected soil sample. We determined the percent water content from 10 g of soil, dried for 1 week at 105°C (Klute 1986). We measured soil N-NH_4^+ concentrations only in April 2004 with an Alpkem analyzer (Alpkem, Wilsonville, OR, USA). Ammonium extracts were prepared from 15 g fresh soil shaken in 1 M KCl and frozen at −20°C before analysis. We measured soil pH every year on each frozen soil sample. pH was determined from 5 g of thawed soil suspended in 10 ml deionized water, using an Accumet pH electrode and meter (Avrahami and Bohannan 2009). Soil temperature, soil moisture, pH and N-NH_4^+ concentrations were used to calculate molar concentrations of NH_3 per gram of soil, which are reported throughout the manuscript as the available form to soil ammonia oxidizers (Avrahami and Bohannan 2009).

Microbial lipid analysis

We extracted lipids as described in detail in Gutknecht et al. (2011). In brief, we extracted fatty acids from 3 g of freeze-dried soil using a chloroform and phosphate

buffer extraction, followed by reduction of the chloroform phase. We then performed saponification followed by strong acid methanolysis to extract the methyl-esterfied fatty acids. We identified lipid peaks using bacterial fatty acid standards and MIDI peak identification software ("Sherlock microbial identification system", MIDI Inc, Newark, DE). Peak areas were converted to nmol g soil^{-1} using internal standards (9:0 nonanoic methyl ester and 19:0 nonadecanoic methyl ester). The total nmol lipid g dry soil^{-1} (sum of all lipids present, 20 or less carbon atoms in length) was used as an index of microbial biomass (Vestal and White 1989; Zelles et al. 1992; Frostegård and Bååth 1996). We used individual lipids as biomarkers of broad groups of the microbial community: 16:1 ω5c for AMF (Balser et al. 2005), 18:2 ω6,9c for saprotrophic fungi (SF; Balser et al. 2005), 16:1 ω7c for Gram-negative bacteria (Wilkinson et al. 2002), and 15:0 iso for Gram-positive bacteria (Wilkinson et al. 2002). The fungal to bacterial ratio was calculated as the average biomass of fungal lipid biomarkers (16:1 ω5c, 18:1 ω9c, and 18:2 ω6,9c) divided by the average biomass of bacterial lipid biomarkers (15:0 iso, 15:0 anteiso, 16:0 2OH, 16:0 iso, 16:1 ω7c; 16:0 10 methyl; 17:0 iso, 17:0 anteiso, 17:0 cyclo, 18:1 ω5c, 18:0 10 methyl, and 19:0 cyclo) as described by Frostegård and Bååth (1996).

Ammonia oxidizer analysis

DNA extraction

Samples from the first growing season after fire (April 2004) were also examined for ammonia-oxidizer functional genes and nitrification rates. Soils for DNA analysis were stored at −80°C after field collection until laboratory analyses could be performed. We extracted DNA from 0.5 g frozen soil using the Fast DNA® SPIN® Kit for Soil (BIO 101, Carlsbad, CA, USA) and the FastPrep Instrument (BIO 101, Carlsbad, CA, USA), according to the manufacturer's instructions (Avrahami and Bohannan 2007). The DNA was re-suspended in a final volume of 50 µl and stored at −80°C.

PCR amplification of Bacterial amoA for T-RFLP analysis

We amplified the Bacterial *amo*A gene from DNA extracts according to Avrahami and Bohannan (2007). We used the modified forward primer *amo*A-1F* from Stephen et al. (1999) originally described in Rotthauwe et al. (1997). The 5′ end of this primer was labeled with 5-carboxyfluorescein for T-RFLP analysis. We used a degenerate reverse primer *amo*A-2R (Rotthauwe et al. 1997). Amplification was performed by using 1 µM of each primer (QIAGEN, Valencia, CA, USA), 0.48 µg µl^{-1} bovine serum albumin (Rosch, Penzberg, Germany), 0.625 units of AmpliTaq DNA polymerase (AmpliTaq, Applied Biosystems, Foster City, CA, USA), and 12.5 µl of MasterAmp 2′ PCR premix F containing 100 mM Tris–HCl (pH 8.3), 100 mM KCl, 7 mM $MgCl_2$, 400 µM of each deoxynucleoside triphosphate, and PCR enhancer betaine (Epicentre Biotechnologies, Madison, WI, USA). We added DNA template and sterile water to a final volume of 25 µl. All reactions were performed in triplicate. We started amplifications by placing PCR tubes into a preheated (94°C) DNA Engine thermal cycler (MJ Research, San Francisco, CA, USA). The amplification program included 5 min at 94°C, followed by 35 cycles of 45 s at 94°C, 1 min at 55°C, and 1 min at 72°C, and 10 min at 72°C for the last cycle. Following a gel electrophoresis check, the three replicates were pooled and cleaned together using a Wizard DNA Clean-Up Kit (Promega, Madison, WI, USA).

AOB community structure

We examined Bacterial amoA PCR products using terminal restriction fragment length polymorphism (T-RFLP) analysis. PCR products were restricted with Bsh1236I (Fermentas, Hanover, MD, USA), according to manufacturer's instructions (Horz et al. 2004; Avrahami and Bohannan 2007). This enzyme cuts at the sequence CG/CG, and produces a 434 bp product which is specifically diagnostic for phylogenetic Cluster 3a AOB most closely related to Nitrosospira multiformis (Horz et al. 2004; Avrahami and Bohannan 2007). Total peaks produced using this T-RFLP procedure ranged from 35 to 490 bp in size. We called peaks within an error of +5 and −5 bp in size as diagnostic for the same phylogenetic cluster. Thus, peaks ranging from 429 to 439 bp in size were considered to be in the same group as peaks that were 434 bp in size, and diagnostic for Cluster 3a ammonia-oxidizers. The relative proportion of the 434 bp-sized peak represents a metric for examining a shift in AOB

community structure, corresponding to an increase or decrease in the relative abundance of this specific phylogenetic Cluster 3a within the whole AOB community.

We performed T-RFLP fingerprinting analysis at the Genomics Technology Support Facility at Michigan State University (East Lansing, MI, USA), as described in Horz et al. (2004). The relative abundances of individual restriction fragments (or T-RFs) in a given *amo*A PCR product were calculated based on the peak height of the individual T-RFs in relation to the total peak height of all T-RFs detected in the respective T-RFLP community fingerprint pattern. The peak heights were automatically quantified by the GENESCAN software (Perkin-Elmer, Foster City, CA, USA).

Real-time PCR amplification of Bacterial amoA

For the DNA extracts, we also used a nested real-time quantitative PCR approach (Q-PCR) as described in Avrahami and Bohannan (2007) to determine the total abundance of Bacterial *amo*A in the test soils. In brief, the nested approach was performed with both rounds of amplification using the same primer set as described above for end-point PCR without the addition of a fluorescent label to the 5′ end of the forward primer (Stephen et al. 1999, modified from Rottahuwe et al. 1997). Thus, this method provides a measure of total abundance of all AOB, while the T-RFLP method provides a measure of relative abundance Cluster 3a AOBs only.

Nitrification potential measurements

Nitrification potential reflects the enzymatic potential of the soil nitrifying bacteria to oxidize NH_3 into NO_2^- or NO_3^- under optimal conditions (Barnard et al. 2005). For samples collected in April 2004, nitrifying enzyme activity was measured as described in Avrahami and Bohannan (2007). Sterile Erlenmeyer flasks containing 45 ml phosphate buffer (1 mM; pH 7.4), 0.04 ml $(NH_4)_2SO_4$ (0.25 M), and 5 g of soil were incubated at 25°C on a shaker for 46 h. Samples were taken at five time points, centrifuged for 5 min, and filtered through Acrodisc-CR 25-mm syringe filters (0.2 μm; Life Sciences). Samples were stored at −20°C until analysis of nitrite and nitrate in a DIONEX ion chromatograph system (Sunnyvale, CA) equipped with an AS11-HC analytical column (Avrahami and Bohannan 2007). The sum of NO_2^- and NO_3^- concentrations increased linearly with time. Rates of potential nitrification activity were determined from the slope of a linear regression of nitrite plus nitrate concentration on time (Avrahami and Bohannan 2007).

Statistics

We used Nonmetric Multidimensional Scaling (NMS), a multivariate analysis technique, to fingerprint the microbial community using the R software vegan package for multivariate analysis (R core team 2010). NMS is a useful method because it does not require data normality or equal variances, and also does not produce the artifacts of other ordination methods such as principal components analysis (McCune and Grace, 2002). Lipids present from all years of the study in greater than 0.005 mol fraction (moles individual lipid^{-1} × moles total lipid biomass^{-1}) were used to provide a breadth of information while also preventing "noise". Sørenson (Bray) distance was used to construct distance matrices for analysis. Thirty independent runs were performed with raw data to ensure that the best solution is global and not reflecting only local minima (McCune and Grace 2002; R core team 2010).

We performed Permanova analysis using R statistical software to test for treatment effects on the multivariate PLFA-based microbial community (R core team 2010; Anderson 2001). The analysis we used was based on the JRGCE split plot, fully crossed design. The dependent variables elevated CO_2, elevated N-deposition, warming, elevated precipitation, and all possible interactions were used on 2003 data to test main and interactive treatment effects. The dependent variables elevated CO_2, elevated N-deposition, fire, elevated precipitation, and all possible interactions were used on 2004–2006 data to test main and interactive treatment effects. As with NMS analysis, we used Sørenson (Bray) distance to construct the distance matrix for analysis. We performed 1000 permutations for the best possible sensitivity of calculated F-statistics and subsequent p values.

We used Analysis of Variance (ANOVA) on individual microbial indicators to complement the multivariate analysis. The dependent variables analyzed include total lipid biomass, the fungal to

bacterial ratio, Gram-negative, Gram-positive, AMF and general fungal lipids biomarkers, the proportion of AOB Cluster3a (T-RFLP), AOB abundance (Q-PCR), Nitrification Potential, AGB, BGB, litter, pH, and NH_3 data for all years these data were available. ANOVA analysis was performed using PROC MIXED in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) based on the fully blocked, split-plot design (factors nitrogen and water assigned randomly at the subplot level, nested within CO_2 and warming treatments assigned at the plot level) of the JRGCE including independent variables elevated CO_2, nitrogen addition, fire, water addition, and all possible interactions (in 2003 warming was included instead of fire). For 2003 data, $N = 128$, and in 2004, 2005 and 2006, $N = 80$ to reflect a balanced design to test for the effect of fire on these parameters. The only exception was that for April 2006, we only measured soils that were not influenced by the fire for [NH_3]. Thus, in April 2006, $N = 96$, and we were unable to assess the effect of fire on soil [NH_3] for this particular year. Means were calculated as least square means and the degrees of freedom were estimated using the Satterthwaite approximation. Transformations for all data for split-plot ANOVAs were as listed in the previous NMS section.

Results

Fire effects on plant biomass and abiotic measurements

We focused on biomass production at the first spring biomass peak measured at the same time as our microbial analysis [see Henry et al. (2006) for a detailed discussion of maximum biomass after fire]. The wildfire imposed at the JRGCE in July 2003 significantly altered AGB and litter quantity in the April 2004 sampling (Fig. 1; Table 1). In April 2004, 9 months after the fire, we found that the unburned (or ambient) plots had an average AGB of 281 g m^{-2} ($\pm$1.1) during the first harvest, while the burned plots were significantly higher, up to 508 g m^{-2} ($\pm$1.1). This trend was reversed in April 2005, 21 months after the fire, when the average AGB at the first harvest was significantly lower in the burned plots [382 g m^{-2} ($\pm$1.1)] than in the unburned plots [511 g m^{-2} ($\pm$1.1)] (Figs. 1, 2b; Table 1). By May 2006, 33 months after the fire, AGB in previously burned plots and ambient plots were indistinguishable (Table 1, Supplementary Table 1). Conversely, we found that average litter quantity was significantly lower in the burned plots during the April 2004 sampling, with 195 g m^{-2} ($\pm$1.4) litter in the unburned plots and only 8.3 g m^{-2} ($\pm$1.6) in the burned plots (Fig. 1; Table 1). This trend was still present in April 2005, though the difference between the burned and unburned plots was not statistically significant (Fig. 1; Table 1). By May 2006, there was no difference in litter quantity between the previously burned plots and the ambient plots (Table 1). BGB was not altered by the fire treatment alone in any of the years following the burn (Table 1). In April 2004, the only year we measured N-NH_4^+, we found that calculated NH_3 concentrations were significantly higher in the burned plots than in unburned plots and that pH was not affected by fire (Table 1; Fig. 2a). Fire also has been shown to lead to long-term increases in soil moisture in these plots, as described in Niboyet et al. (2011).

Other global change and interactive effects on plant biomass and abiotic measurements

While we saw many responses to global change that were only present sporadically (Table 1), we did not focus on how global change responses varied over time. A detailed examination of inter-annual variation at the JRGCE has been reported elsewhere on a variety of measurements (Shaw et al. 2002; Zavaleta et al. 2003; Barnard et al. 2005; Dukes et al. 2005; Gutknecht et al. 2010, 2011).

Increased N-deposition consistently increased plant AGB in all years of this study except for in May 2006, where it had no significant effect on AGB (Fig. 2b; Table 1, Supplementary Table 1). We also found that the elevated N-deposition treatment was related an increase in soil NH_4^+ concentrations compared to ambient plots in 2004 (Fig. 2a; Table 1). We did not find that N-deposition affected BGB or litter quantity significantly (Table 1). In April 2004, we found that an interaction between the fire and CO_2 treatments consistently affected several variables. AGB responded to an interactive fire by CO_2 effect, such that the combination of the fire and CO_2 treatments increased AGB beyond the increase exhibited by the fire treatment alone (Table 1, Supplementary Table 1). The BGB also responded to the fire by

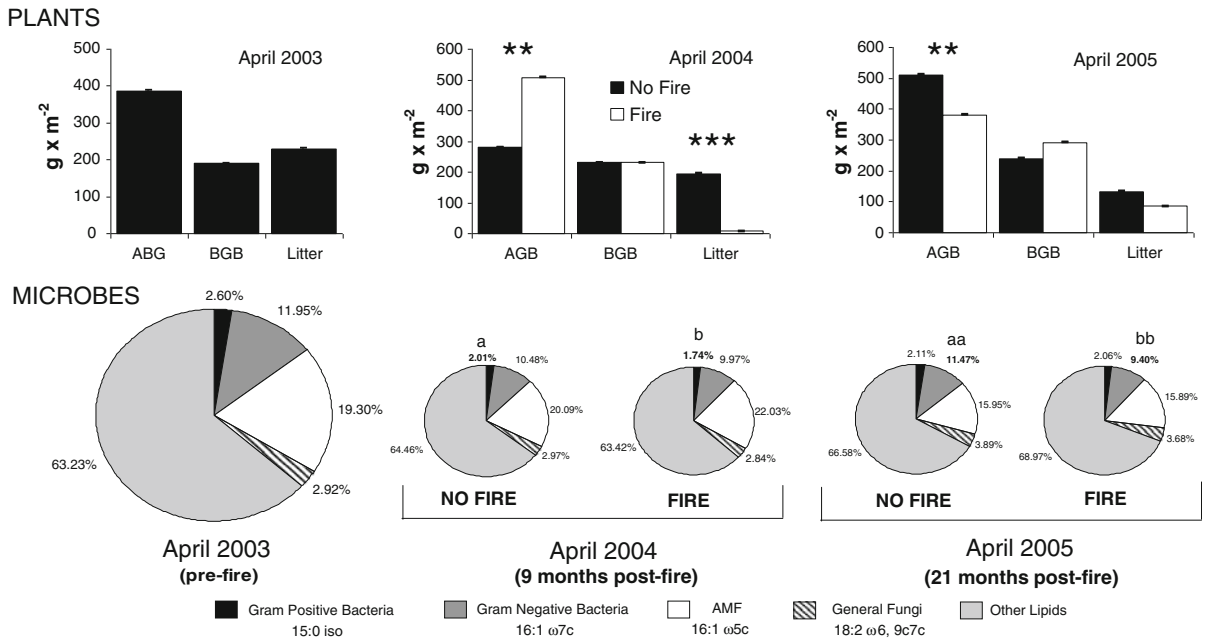


Fig. 1 Effects of fire on plant productivity (*top*) and microbial lipid composition (*bottom*) at JRGCE. Data collected in April 2003 were pre-fire. The fire occurred in July 2003. Data collected in April 2004 were 9 months post-fire; data collected in April 2005 were 21 months post fire. Stars reflect the level of significance between the least squared means derived from the split-plot ANOVA model. Error bars represent one standard error associated with the statistical least squared means model. Aboveground biomass (AGB) and litter were significantly different in burned (*white*) versus unburned (*black*) plots in April 2004 (AGB: $F_{1,11} = 12.25$, $p = 0.005$; litter: $F_{1,11} = 28.40$, $p = 0.0002$). AGB was significantly different in burned (*white*) versus unburned (*black*) plots in April 2005 ($F_{1,11} = 9.59, p = 0.003$). There were no significant differences in belowground biomass (BGB). Gram-positive bacterial lipid relative abundance (*black*, mol%) was significantly different in burned versus unburned plots in April 2004 ($F_{1,10.4} = 5.55$, $p = 0.039$) and Gram-negative bacterial lipid relative abundance (*dark gray*, mol%) was significantly different in burned versus unburned plots in April 2005 ($F_{1,10} = 11.49, p = 0.007$)

CO_2 interactive effect in April 2004, such that the fire and elevated CO_2 combined reduced the suppressive effect of elevated CO_2 alone on BGB (Table 1, Supplementary Table 1). Finally, the fire by CO_2 interactive effect in April 2004 influenced soil NH_4^+ concentrations, such that the combined two treatments negated a decreased calculated soil NH_3 concentration under CO_2 alone (Table 1, Supplementary Table 1).

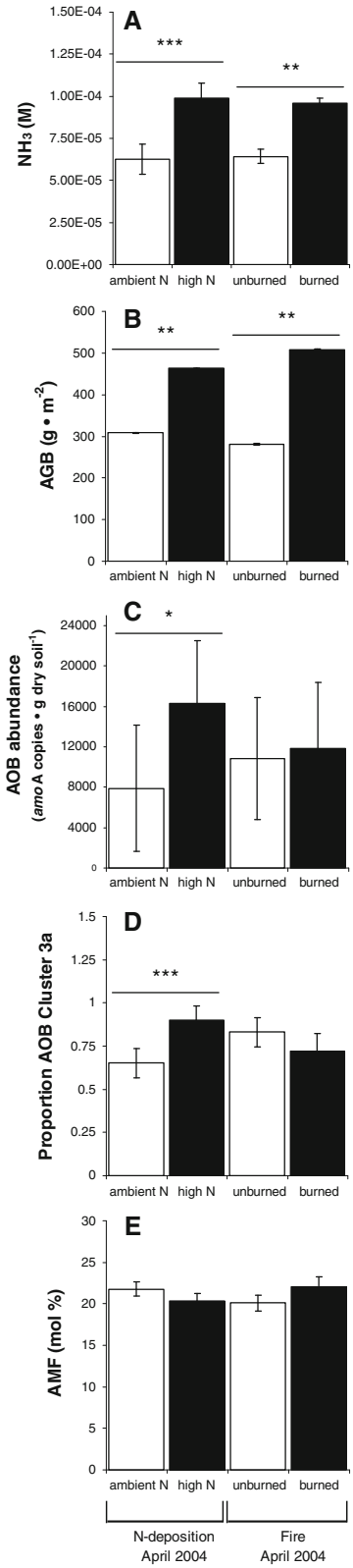
Fire effects on microbial biomass, the fungal to bacterial ratio, and lipid composition

We used several analyses to examine the effects of fire on soil microbial lipids, including multivariate community structure (Permanova analysis) and univariate measures of total microbial biomass (using the sum of all lipids present, 20 or less carbon atoms in length), the fungal to bacterial ratio, and analysis of several lipids diagnostic of broad microbial groups. In April 2004 (9 months after fire), our results indicate that fire affected the multivariate lipid composition (Table 2), as well as the Gram-positive bacterial lipid biomarker (15:0 iso). Specifically there was a lower relative abundance of Gram-positive lipid biomarkers in burned (1.74 mol% ± 0.06) than in unburned plots (2.01 mol% ± 0.08) (Fig. 1; Table 2). The AMF biomarker (16:1 ω5c) was also 1.94 mol% higher in burned than unburned, ambient plots in April 2004, but this effect was not significant (Table 2, Supplementary Table 2; Figs. 1, 2e). In April 2005 (21 months after the fire) the only lipid based parameter affected by fire was the Gram-negative bacterial lipid biomarker (16:1 ω7c), which was significantly lower in the previously burned plots (9.40 mol% ± 0.50) than in unburned plots (11.47 mol% ± 0.39) (Fig. 1; Table 2). By May 2006 (33 months after fire), no parameter based on microbial lipid analysis exhibited a response to fire (Table 2, Supplementary Table 2).

Table 1 Summary of p values from split-plot ANOVAs to test the treatment effects of fire and global changes on the following factors: above-ground biomass = log g m^{-2}; below-ground biomass = log g m^{-2}; litter = log g m^{-2}; pH = pH in water; soil moisture = gravimetric soil moisture (square root arcsin transformed %); NH_3 (2004 only) = calculated ammonia from extractable N-NH_4^+ (log M)

	Above-ground biomass	Below-ground biomass	Litter	pH	NH_3
April 2003 (pre-fire)					
CO_2	0.246	0.343	0.718	0.996	n.a.
Warming	0.406	0.962	0.748	0.507	n.a.
Precipitation	**0.049**	0.298	0.633	**<0.0001**	n.a.
N-deposition	**<0.0003**	0.873	0.074	**<0.0001**	n.a.
April 2004 (9 months post-fire)					
CO_2	0.947	**0.012**	0.934	0.698	0.247
Precipitation	0.358	0.728	0.383	**<0.0004**	0.973
N-deposition	**0.001**	0.263	0.495	0.587	**<0.0001**
Fire	**0.005**	0.993	**<0.0003**	0.253	**0.008**
Fire × CO_2	**0.015**	**0.042**	0.151	0.585	**0.006**
Fire × Precipitation	0.296	0.799	0.961	0.450	0.416
Fire × N-deposition	0.100	0.154	0.382	0.608	**0.001**
Fire × CO_2 × N-deposition	0.889	0.980	0.352	0.590	0.860
Fire × CO_2 × Precipitation	0.937	0.657	0.751	0.998	0.418
Fire × N-deposition × Precipitation	0.197	0.800	0.431	0.108	0.315
April 2005 (21 months post-fire)					
CO_2	**0.001**	0.070	**0.003**	n.a.	n.a.
Precipitation	0.904	0.115	0.915	n.a.	n.a.
N-deposition	**<0.0002**	0.731	**0.041**	n.a.	n.a.
Fire	**0.003**	0.336	0.093	n.a.	n.a.
Fire × CO_2	0.620	0.264	0.620	n.a.	n.a.
Fire × Precipitation	0.339	**0.005**	0.406	n.a.	n.a.
Fire × N-deposition	0.508	0.243	0.606	n.a.	n.a.
Fire × CO_2 × N-deposition	0.493	0.913	0.706	n.a.	n.a.
Fire × CO_2 × Precipitation	0.440	**0.016**	0.752	n.a.	n.a.
Fire × N-deposition × Precipitation	0.478	0.997	0.496	n.a.	n.a.
May 2006 (33 months post-fire)					
CO_2	0.675	0.654	**0.021**	n.a.	n.a.
Precipitation	0.265	0.451	0.145	n.a.	n.a.
N-deposition	0.148	0.492	**0.005**	n.a.	n.a.
Fire	0.285	0.898	0.095	n.a.	n.a.
Fire × CO_2	0.495	0.434	0.246	n.a.	n.a.
Fire × Precipitation	0.815	0.069	0.781	n.a.	n.a.
Fire × N-deposition	0.643	0.476	**0.004**	n.a.	n.a.
Fire × CO_2 × N-deposition	0.628	0.875	0.891	n.a.	n.a.
Fire × CO_2 × Precipitation	0.548	0.758	0.934	n.a.	n.a.
Fire × N-deposition × Precipitation	0.801	0.582	0.072	n.a.	n.a.

2003 values were based on all 128 plots of the JRGCE, while 2004 and 2005 data were based on the burn subset of data (see "Methods"). Designation of n.a. indicates that consistent data were not available. While all possible treatment interactions were tested, only treatments involving fire are shown (see Supplementary Table 1 for full information). Bold numbers indicate significance at $p < 0.05$



◀ **Fig. 2** Compared effects of fire and N-deposition 8-months post-fire. Least squared means from split-plot ANOVA tests are presented for **a** soil ammonia (NH_3) concentration, **b** aboveground biomass (AGB), **c** total ammonia-oxidizing bacterial (AOB) abundance as measured by quantitative PCR, **d** relative abundance of AOB Nitrosospira-like Cluster 3a, as measured by T-RFLP and **e** arbuscular mycorrhizal fungal (AMF) lipid relative abundance. *White bars* indicate either ambient N-deposition (*left*) or unburned (*right*); *black bars* indicate either elevated N-deposition (*left*) or burned (*right*). Error bars represent one standard error for the error associated with the statistical least squared means model. *Stars* indicate level of significance. NH_3 concentration increased significantly in both elevated N-deposition ($F_{1,48} = 21.77$, $p < 0.0001$) and burned ($F_{1,11} = 10.4$, $p = 0.008$) plots. AGB also increased significantly in both elevated N-deposition ($F_{1,48} = 11.40$, $p = 0.002$) and burned ($F_{1,11} = 12.25$, $p = 0.005$) plots. AOB abundance ($F_{1,48.8} = 6.11$, $p = 0.017$) and Cluster 3a abundance ($F_{1,43.3} = 33.95$, $p < 0.0001$) increased in elevated N-deposition plots only

Other global change and interactive effects on microbial biomass, the fungal to bacterial ratio, and lipid composition

No consistent global change factor affected multivariate lipid composition, total microbial biomass, or any individual lipid biomarker in every year examined (Table 2, Supplementary Table 2). One very consistent result was that the fungal to bacterial lipid ratio was significantly lower in N-deposition plots in all 4 years examined in this study (Fig. 3b). In April 2003 we found a significant effect of elevated precipitation and N-deposition on multivariate lipid composition (Table 2, separate multivariate clustering between treated and untreated plots). Gram-negative bacterial biomarker relative abundance was significantly higher in elevated N-deposition plots in 2004 and 2005. In April 2004 the Gram-negative bacterial lipid biomarker increased from 9.57 mol% (±0.36) in ambient to 10.87 mol% (±0.37) under N addition; in April 2005 it increased from 9.79 mol% (±0.39) in ambient to 11.08 mol% (±0.41) under N addition, suggesting a consistent increase in the Gram-negative bacterial community as a result of N-deposition. In 2004 the general fungal lipid indicator increased from 2.75 mol% (±0.11) in ambient plots to 3.06 mol% (±0.12) in N-deposition plots. In 2005 there were significant effects of CO_2 and N-deposition on multivariate lipid composition (Table 2). Additionally, AMF biomarker relative abundance was generally lower under N-deposition but this trend was only significant in April 2005, where relative abundance

Table 2 Summary of *p* values from split-plot ANOVAs to test the treatment effects of fire and global changes on microbial indicators

	Multivariate	Total microbial biomass	Fungal:bacterial lipids	General fungi	Gram-positive bacteria	Gram-negative bacteria	Arbuscular mycorrhizal fungi	Nitrification potential
April 2003 (pre-fire)								
CO_2	0.667	0.345	0.138	0.354	0.492	0.075	0.615	n.a.
Warming	0.1045	0.381	0.651	0.336	0.774	0.764	0.447	n.a.
Precipitation	**0.01**	0.914	**0.017**	0.517	0.582	0.830	0.053	n.a.
N-deposition	**0.01**	0.390	**0.004**	0.589	0.872	0.258	0.057	n.a.
April 2004 (9 months post-fire)								
CO_2	0.463	0.628	0.287	0.132	0.372	0.604	0.346	0.946
Precipitation	0.576	0.294	0.692	0.631	0.936	0.680	0.430	0.318
N-deposition	**0.025**	0.092	**0.029**	**0.042**	0.077	**0.002**	0.112	**0.005**
Fire	**0.001**	0.443	0.276	0.452	**0.039**	0.438	0.243	0.792
Fire × CO_2	0.323	0.762	0.157	0.388	0.233	0.403	0.195	0.334
Fire × Precipitation	0.922	0.132	0.545	0.418	0.811	0.326	0.619	0.678
Fire × N-deposition	0.418	0.057	0.726	**0.009**	**0.010**	0.225	0.721	0.072
Fire × CO_2 × N-deposition	0.737	0.642	0.969	0.814	0.884	0.595	0.811	0.135
Fire × CO_2 × Precipitation	0.441	0.976	**0.031**	0.264	0.367	0.236	**0.028**	0.235
Fire × N-deposition × Precipitation	0.925	0.621	0.502	0.648	0.877	0.867	0.977	0.347
April 2005 (21 months post-fire)								
CO_2	**0.003**	0.240	0.285	0.278	**0.020**	**0.006**	0.805	n.a.
Precipitation	0.829	0.157	0.539	**0.006**	0.698	0.742	0.642	n.a.
N-deposition	**0.002**	0.913	**<0.0001**	0.235	0.178	**0.010**	**<0.0001**	n.a.
Fire	0.346	0.697	0.525	0.510	0.674	**0.007**	0.965	n.a.
Fire × CO_2	0.495	0.388	0.804	0.605	0.700	0.999	0.829	n.a.
Fire × Precipitation	0.597	0.684	0.057	0.272	0.648	0.577	**0.033**	n.a.
Fire × N-deposition	0.736	0.767	0.359	0.325	0.805	**0.046**	0.919	n.a.
Fire × CO_2 × N-deposition	0.969	0.262	0.414	**0.035**	0.566	0.406	0.743	n.a.
Fire × CO_2 × Precipitation	0.770	**0.017**	0.109	0.667	0.252	0.417	0.139	n.a.
Fire × N-deposition × Precipitation	0.829	0.653	0.470	0.388	0.846	0.444	0.194	n.a.

Table 2 continued

	Multivariate	Total microbial biomass	Fungal:bacterial lipids	General fungi	Gram-positive bacteria	Gram-negative bacteria	Arbuscular mycorrhizal fungi	Nitrification potential
May 2006 (33 months post-fire)								
CO_2	0.130	**0.042**	0.482	0.100	0.749	**0.003**	0.101	n.a.
Precipitation	0.701	0.396	0.857	0.060	0.542	0.308	0.939	n.a.
N-deposition	0.732	0.502	**0.003**	0.377	0.770	0.980	0.131	n.a.
Fire	0.114	0.488	0.893	0.350	0.857	0.755	0.644	n.a.
Fire × CO_2	**0.042**	0.304	0.197	0.697	0.123	0.114	0.758	n.a.
Fire × Precipitation	0.374	0.496	0.928	0.353	0.580	0.145	0.923	n.a.
Fire × N-deposition	0.860	0.848	0.726	0.690	0.573	0.821	0.575	n.a.
Fire × CO_2 × N-deposition	**0.040**	0.009	0.301	0.062	0.216	**0.026**	0.082	n.a.
Fire × CO_2 × Precipitation	0.546	0.452	0.409	0.998	0.842	0.343	0.995	n.a.
Fire × N-deposition × Precipitation	0.715	0.713	0.973	0.740	0.888	0.549	0.456	n.a.

2003 values were based on all 128 plots of the JRGCE, while 2004–2006 data were based on the burn subset of data (see "Methods"). While all possible treatment interactions were tested, only treatments involving fire are shown (see Supplementary Table 2 for full information). Microbial indicators: Total microbial biomass (nmol lipid g $soil^{-1}$); fungal:bacterial lipids = ratio of fungal to bacterial lipids; general fungi = 18:2 ω6,9c mol%, Gram-positive bacteria = 15:0 iso mol%; Gram-negative bacteria = 16:1 ω7c mol%; arbuscular mycorrhizal fungi = 16:1 ω5c mol%; nitrification potential in 2004 only (ng N h^{-1} g dry $soil^{-1}$). The 'multivariate' column contains p values from Permanova nonparametric testing to determine treatment effects multivariately. Permanova analysis was performed on lipid relative abundance (mol fraction) data. The statistical model was based on the split-plot full factorial design of the JRGCE, run with 1000 permutations. Bold numbers indicate significance at $p < 0.05$

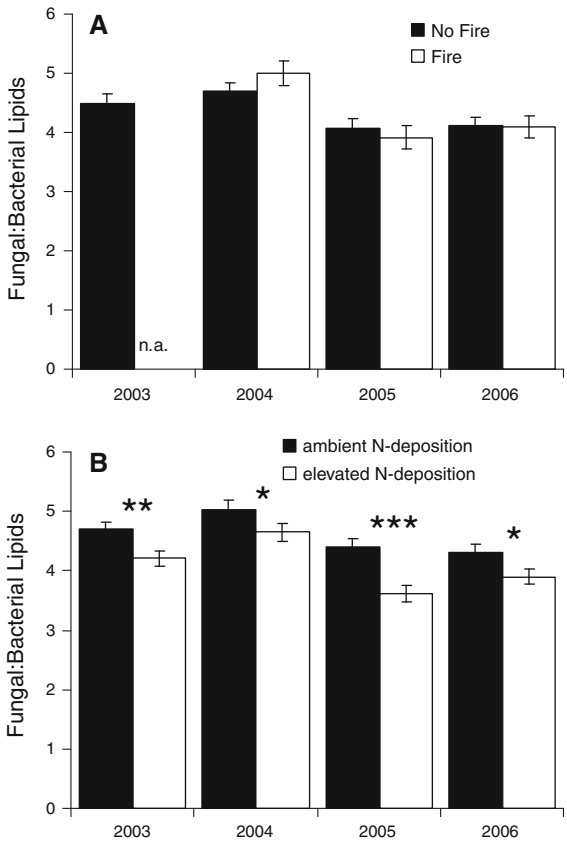


Fig. 3 Effects of fire and N-deposition on the ratio of fungal to bacterial lipids. Least squared means from split-plot ANOVA tests are presented for **a** burned (*white*) and unburned (*black*) plots and **b** ambient (*white*) and elevated (*black*) N-deposition treatments from April 2003, April 2004, April 2005, and May 2006. Error bars represent one standard error for the error associated with the statistical least squared means model. *Stars* indicate level of significance. The fungal to bacterial lipid ratio was significantly lower in all elevated N-deposition plots (2003: $F_{1,50.5} = 9.17$, $p = 0.004$; 2004: $F_{1,44.1} = 5.12$, $p = 0.029$; 2005: $F_{1,40.3} = 30.62$, $p < 0.0001$; 2006: $F_{1,42.2} = 10.8$, $p = 0.003$)

decreased from 17.89 mol% (±0.74) in ambient plots to 13.95 mol% (±0.75) in N-deposition plots (Table 2; Fig. 2e).

In addition to these single factors, we found a consistent interactive effect between the fire and N-deposition in the bacterial portion of the soil microbial community. In April 2004 (9 months after burning) elevated N-deposition treatments interacted such that when combined, the incidence of fire decreased the stimulatory effect of N-deposition on the Gram-positive bacterial lipid biomarker relative abundance (Supplementary Fig. 1a). Thus, under ambient levels of N-deposition, fire had no effect on Gram-positive bacterial lipids in April 2004, but under elevated N-deposition, fire decreased the Gram-positive bacterial lipid biomarker from 2.17 mol% (±0.08) to 1.70 mol% (±0.10) (Supplementary Fig. 1a). In April 2005 (21 months after burning) we found a similar fire by N-deposition interactive effect, except in the Gram-negative bacterial lipid biomarker instead of the Gram-positive bacterial biomarker (Supplementary Fig. 1b). Thus under ambient levels of N-deposition, fire had no effect on the Gram-negative bacterial lipid biomarker, but under elevated N-deposition, fire decreased the Gram-negative lipid biomarker from 12.61 mol% (±0.53) to 9.55 mol% (±0.60) (Supplementary Fig. 1b). By May 2006 (33 months after burning) this interactive effect of fire by N-deposition was no longer significant in any of the microbial lipids we measured (Table 2, Supplementary Table 2).

N-deposition and fire effects on a specific microbial functional group (ammonia-oxidizing bacteria) and rate (nitrification potential)

Our abiotic results show that both N-deposition and fire can significantly increase calculated NH_3 concentrations in soils (Fig. 2; Table 1). Since NH_3 is the direct substrate for the process of ammonia-oxidation and a rate-limiting step in nitrification, we chose to examine the similarities between these two global change factors further on the specific group of AOB and the process of nitrification in the first growing season following the fire (April 2004). We saw a response in both AOB and potential nitrification to nitrogen addition. The total AOB abundance (Fig. 2c) and the relative abundance of AOB Cluster 3a (Fig. 2d) were significantly higher in N-deposition plots than in the ambient (Fig. 2a). Nitrification potential measurements were also higher under N-deposition in April 2004. Nitrification potentials increased from 17.43 (±0.84) ng N h^{-1} g dry $soil^{-1}$ in ambient plots to 24.4 (±0.79) ng N h^{-1} g dry $soil^{-1}$ in N-deposition plots. No other global change factors or interactions significantly affected AOB abundance or Cluster 3a relative abundance. No other global change factors or interactions significantly affected AOB abundance or Cluster 3a relative abundance.

On the contrary, even though both the fire and nitrogen addition plots had similarly increased levels

of calculated soil NH_3, we found that after one growing season fire had no effect on AOB abundance (or quantity of ammonia-monooxygenase functional gene marker amoA, Fig. 2c) or community structure (relative abundance of Cluster 3a ammonia-oxidizers, Fig. 2d). We also found no effect of fire on nitrification potential measurements in April 2004 (Table 2). Nitrification potentials were approximately 475 ng N h^{-1} g^{-1} dry soil in both the ambient and burned plots.

Discussion

It should be noted that this is among the few studies to examine the response of microbial communities to grassland fires. Far more studies have focused on forest ecosystems (Sugihara et al. 2007; Dooley and Treseder 2011). Major differences between forest and grassland fires include the severity and heat of the fire, as well as the amount of resources deposited to the system as ash (e.g. Dooley and Treseder 2011; Hamman et al. 2007; Sugihara et al. 2007; Neary et al. 1999; Raison 1979). Additionally, forest and grassland ecosystems differ hugely in terms of soil formation, soil chemistry, and nutrient availability, which can directly affect microbial biodiversity (Brady and Weil 2008; Antonopoulos et al. in preparation). Forest microbial communities may also rely more on fermentation and organic acid pathways while grassland communities rely more on sugar alcohol and PO_4 metabolism pathways (Antonopoulos et al. in preparation). Thus, we focus our discussion mainly on responses to fire in grassland ecosystems.

Aboveground biomass was altered for 2 years after a summer wildfire at the JRGCE

Our results indicate that aboveground plant productivity (AGB) was stimulated 9 months following fire in April 2004 (Figs. 1, 2b). Such increases in primary production after fire are not typically observed in Mediterranean-climate annual grassland systems, such as this one, where litter accumulation is low and fires are typically of low intensity and severity (Launchbaugh 1964; Hurlbert 1969) but are more typical of tallgrass prairie systems (Abrams et al. 1986; Briggs and Knapp 1995). Even at the JRGCE, when plant biomass was analyzed over the entire growing season (as opposed to the first peak in April 2004), no differences were observed between burned and unburned plots (Henry et al. 2006).

We suggest that the stimulatory effect of fire we observed on first peak AGB in the April 2004 harvest is the result of several indirect effects of fire on the ecosystem. First, decreased litter in the burned plots allowed for earlier germination and plant growth. There is evidence for this from the JRGCE normalized difference vegetation index data measured through the 2003–2004 growing season after the burn, which showed earlier canopy greenness in the burned plots than the unburned plots (Henry et al. 2006), despite a decrease in soil moisture in burned plots also observed at this time (Niboyet et al. 2011). This response of earlier season, but not maximum, plant growth could be why there was no significant difference in litter mass the following year (April 2005). Second, calculated NH_3 concentrations significantly increased in the burned plots in April 2004, providing labile N to stimulate plant growth. The increases we and others have observed in AGB as a result of $Ca(NO_3)_2$ addition indicate that the Jasper Ridge system is N limited (Table 1; Shaw et al. 2002; Dukes et al. 2005; Menge and Field 2007). Productivity in N-limited systems with moderate fire frequencies can increase because of this functional N-fertilization effect of inorganic N-containing ash (Rissler and Parton 1982; Hurlbert 1988; Neary et al. 1999; Romanyá et al. 2001; Henry et al. 2006), though this is not always the case (Ajwa et al. 1999). In our study, increases in calculated soil NH_3 in April 2004 can be compared to a similar study where levels of inorganic N (NH_3 and NO_3^-) nearly doubled in burned grassland soils (Picone et al. 2003).

Henry et al. (2006) suggested that in addition to alleviating N-limitation, fire can alleviate limitation by other mineral nutrients such as phosphorus (P). This hypothesis was formed from the observation that an increase in available PO_4^- ions after fire may remove the suppressive effect that elevated CO_2 has on primary productivity at JRGCE (Henry et al. 2006; Shaw et al. 2002). In a similar study, available P increased significantly after burning as a result of the release of phosphates by heating, as well as calcium polyphosphates in ash (Picone et al. 2003). This explanation is corroborated by the observation that annual grasses in the burned plots in April 2004 had lower N:P ratios than in unburned plots, coupled with a shift in plant P-allocation to AGB (Henry et al. 2006).

While it was beyond the scope of the present study to directly measure soil P-availability, it is a reasonable hypothesis that more phosphorus becomes available following a grassland burn, and that P-limitation may become alleviated in the Jasper Ridge system as a result of this added phosphorus.

In April 2005, 21 months after the fire, primary productivity was significantly less in burned than in unburned plots. However, microbial extracellular enzyme activities per unit microbial biomass were lower in previously burned plots than in unburned plots, suggesting that the microbial community was less nutrient limited at that time (Gutknecht et al. 2010). It is possible that certain nutrients, especially micronutrients, were more limiting for plants than microbes 21 months after fire, though further investigation is necessary to validate this hypothesis. Alternatively, although we only saw a marginally significant increase in litter in burned plots 21 months after fire, it is possible that this litter or standing biomass from the previous season inhibited germination or delayed early season growth in the 2004–2005 growing season. By May 2006 there were no effects of fire on AGB, and microbial extracellular enzyme activities were similar to those in ambient plots (Gutknecht et al. 2010). This suggests that by 33 months post-fire, whole-ecosystem productivity had recovered completely from wildfire disturbance.

Microbial community structure, but not total microbial biomass, was affected for 2 years following a grassland fire

Total microbial biomass in our study was not affected by fire in any year (Table 2). In their meta-analysis of the effects of fire on microbial biomass presented in this special issue, Dooley and Treseder (2011) show that on average, microbial biomass tends to decline significantly following a wildfire, but that this decrease is often related to fire severity. In less severe fires, such as prescribed burns, that are similar to the JRGCE fire in July 2003, microbial biomass is not decreased by fire in any type of biome (Dooley and Treseder 2011). Several studies have shown that after a low-intensity grassland fire, soil chemical properties change (Sherman et al. 2005) but that total soil microbial biomass does not change long-term (Ponder et al. 2009; Hamman et al. 2007; Song et al. 2004; Picone et al. 2003).

While total microbial biomass was not altered in our study, multivariate lipid composition was affected by fire in April 2004, 9 months after fire, but only at this time point (Table 2). At a finer scale, our results revealed responses to fire in particular microbial groups both 9 months (April 2004) and 21 months (April 2005) following the fire (Fig. 1). All effects of the fire were then absent in the microbial community by 33 months following the fire (May 2006, Supplementary Table 2).

In April 2004, 9 months after fire, Gram-positive bacterial relative abundance was lower in burned plots, with similar non-significant trends in the Gram-negative bacteria and general fungi (not including AMF), while the AMF indicator relative abundance was not different in burned plots. We did see a marginal increase in the fungal to bacterial lipid ratio (Fig. 3; Table 2) in April 2004, which was due to a non-significant increase in the AMF indicator relative abundance. Several studies have shown the opposite, that within the year following a low-severity fire, soil fungal biomass decreases (Hamman et al. 2007; D'Ascoli et al. 2005; Ponder et al. 2009; Dooley and Treseder 2011), while bacteria recover rapidly and are more resilient to (D'Ascoli et al. 2005) or even stimulated by (Ponder et al. 2009) fire. Our results may differ from these other studies because most comparable studies do not distinguish between general fungal indicators (18:2 ω6,9c, 18:3 ω6c, 18:ω9c; Hamman et al. 2007; Ponder et al. 2009) from arbuscular mychorrhizal fungal indicators (16:1 ω5c; Balser et al. 2005), as we did. In studies that examined mycorrhizal biomass specifically, AMF abundance increased in low-frequency burns in African savannah systems (Hartnett et al. 2004), but no other studies have directly assessed the responses of AMF to grassland fire. In the next section we discuss in more detail the possible implications of these shifts in microbial and fungal communities in the first growing season post-fire.

In April 2005, 21 months post-fire, only the Gram-negative bacterial indicator was still affected by fire, being significantly lower in burned than in unburned plots, while other lipid indicators were unaffected by fire (Fig. 1). Gram-negative bacteria are the predominant rhizosphere bacteria, and are dependent on labile carbon and nutrients excreted from roots. Although we saw no change in BGB in 2005 in burned plots, the lower aboveground plant biomass we observed may

have corresponded to a decreased amount of labile carbon flow to the rhizosphere, thus limiting nutrients and growth to Gram-negative bacteria.

Specific nitrifying microorganisms are affected by nitrogen addition but not fire in the Jasper Ridge system

While lipid analysis is a good method for detecting proportional changes in bacterial and fungal communities, a drawback is that it cannot provide information about specific phylogenetic groups. Since N-deposition and fire both resulted in similar increases in AGB and calculated soil NH_3 (Fig. 2a) in the growing season immediately following fire (April 2004), we complemented the lipid analysis for that time point with a finer-scale examination of the ammonia-oxidizing bacterial (AOB) community. Through the ammonia-monooxygenase enzyme, AOB directly use NH_3 in the soil to produce NO_2^-. By comparing the abundances and community shifts in the gene encoding for ammonia monooxygenase (*amo*A), we examined fundamental differences in these two seemingly similar global change factors on AOB, and therefore, nitrification.

We expected, based on results from the N-deposition treatment and the analogous nutrient pulse after burning, that AOB would respond to burning. In the N-deposition treatment we found an overwhelming increase in AOB abundance and a shift in community structure toward Cluster 3a, increased nitrification potential (Fig. 2c, d; Supplementary Table 2), and increased Gram-negative biomarkers (AOB are Gram-negative microorganisms). Calculated soil NH_3 concentrations also increased in N-deposition plots (Fig. 2a), presumably the result of higher rates of N-mineralization resulting from increased plant productivity in N-deposition plots (Fig. 4b). These results are consistent with other studies that have shown increases in AOB abundance (e.g. Di et al. 2009; Shen et al. 2008; Schauss et al. 2009; Jia and Conrad 2009) and increases in the relative abundance of AOB Cluster 3a (Wang et al. 2009; Shen et al. 2008; Yeager et al. 2005; Bruns et al. 1999; Mendum and Hirsch 2002; Webster et al. 2002; Mintie et al. 2003; Horz et al. 2004; Kowalchuk et al. 2000a, b; Avrahami and Conrad 2003) in high N-NH_3 soils. In one notable case, NH_3 concentrations increased as a result of wildfire in a mixed conifer forest, which resulted in a long-term shift in the AOB community toward Cluster 3a ammonia-oxidizers (Yeager et al. 2005).

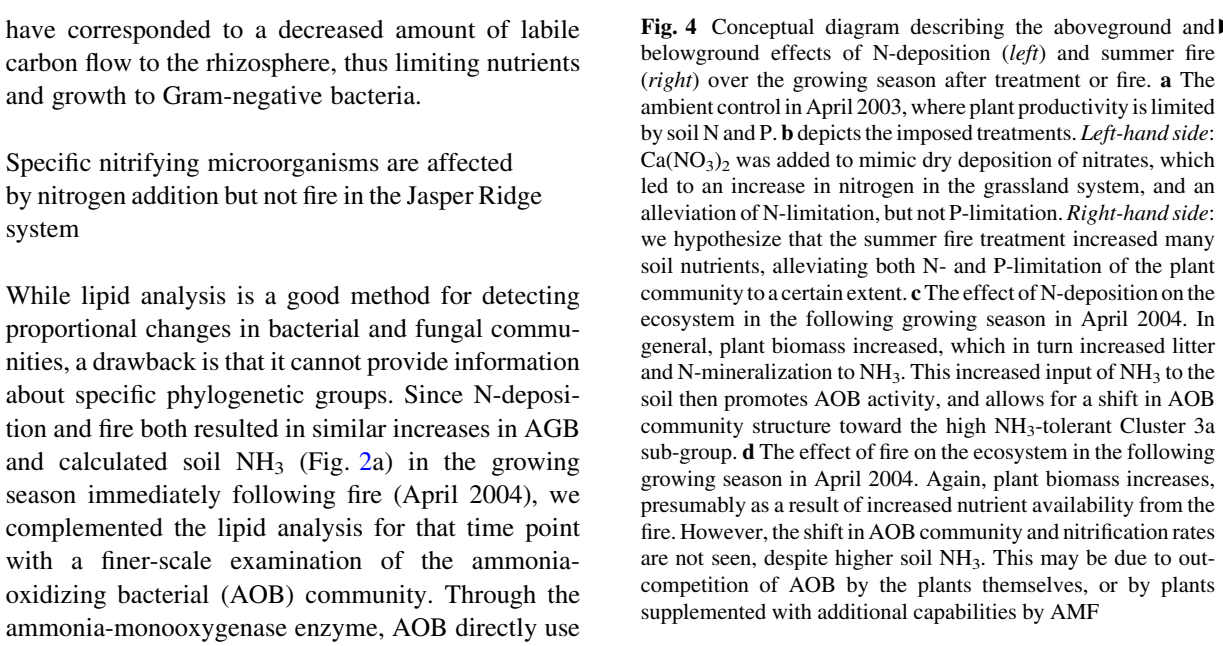
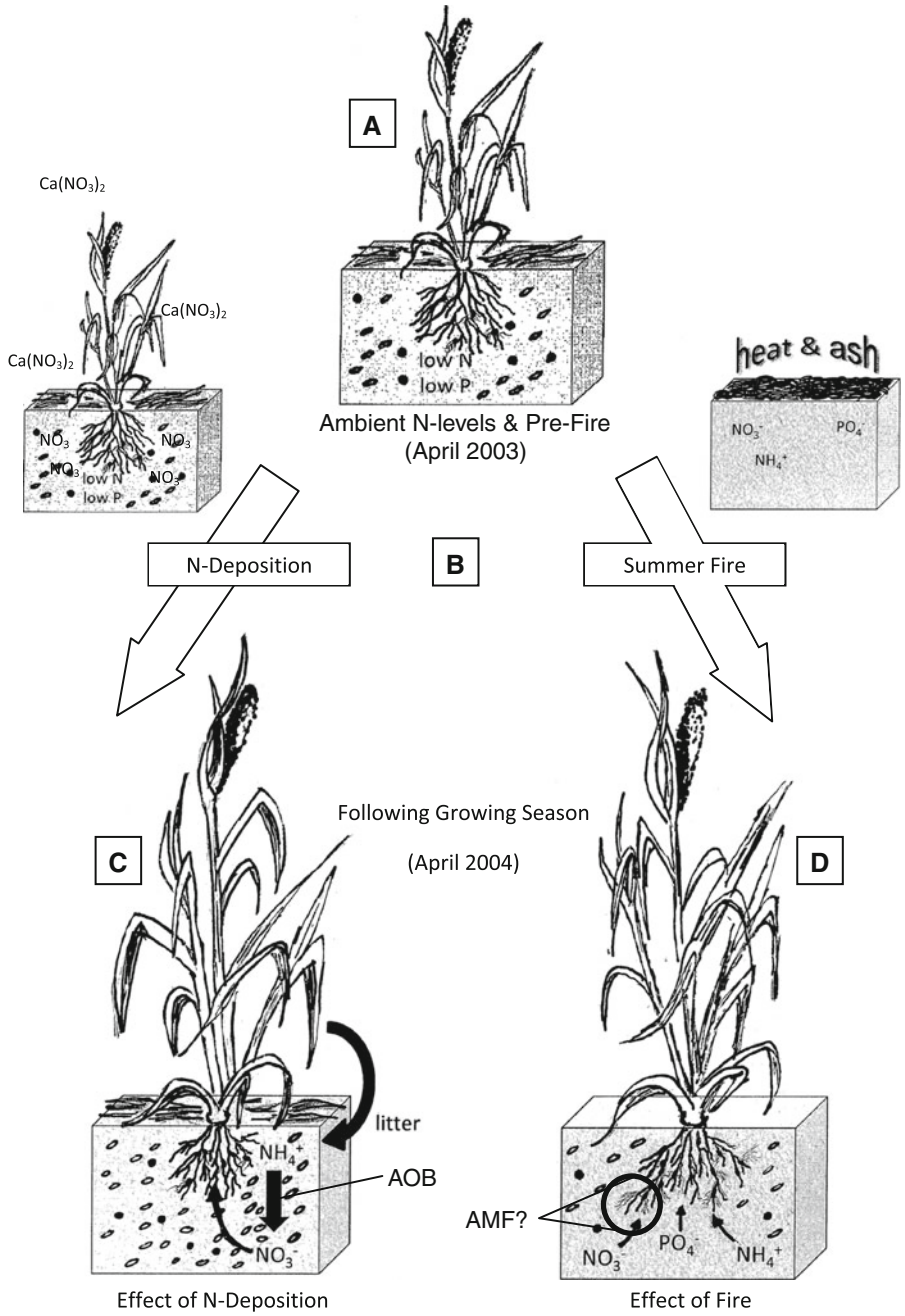


Fig. 4 Conceptual diagram describing the aboveground and belowground effects of N-deposition (*left*) and summer fire (*right*) over the growing season after treatment or fire. **a** The ambient control in April 2003, where plant productivity is limited by soil N and P. **b** depicts the imposed treatments. *Left-hand side*: $Ca(NO_3)_2$ was added to mimic dry deposition of nitrates, which led to an increase in nitrogen in the grassland system, and an alleviation of N-limitation, but not P-limitation. *Right-hand side*: we hypothesize that the summer fire treatment increased many soil nutrients, alleviating both N- and P-limitation of the plant community to a certain extent. **c** The effect of N-deposition on the ecosystem in the following growing season in April 2004. In general, plant biomass increased, which in turn increased litter and N-mineralization to NH_3. This increased input of NH_3 to the soil then promotes AOB activity, and allows for a shift in AOB community structure toward the high NH_3-tolerant Cluster 3a sub-group. **d** The effect of fire on the ecosystem in the following growing season in April 2004. Again, plant biomass increases, presumably as a result of increased nutrient availability from the fire. However, the shift in AOB community and nitrification rates are not seen, despite higher soil NH_3. This may be due to out-competition of AOB by the plants themselves, or by plants supplemented with additional capabilities by AMF

Given these results, we hypothesized that the similar increase in calculated soil NH_3 concentrations exhibited in April 2004 in the burn plots (Fig. 2a) would affect AOB abundance and Cluster 3a relative abundance in similar stimulatory ways. Surprisingly, our results show that AOB abundance and community structure do not change at all in the burned plots (Fig. 2c, d). Furthermore, there was no difference in nitrification potential or in Gram-negative biomarker relative abundance between the unburned and burned plots in April 2004. This provides further indication that the AOB community was not stimulated by the increased calculated concentrations of soil NH_3 resulting from the fire, or that stimulation occurred at a shorter or longer time scale than the first growing season post-fire. However, if AOB responses did exist at a shorter time scale post-fire, then our results suggest that AOB abundance and community structure recovered quickly within the 9-month period post-fire.

Plant competition for nutrients causes different microbial responses to wildfire induced nutrient pulses versus direct N-addition

Given the lack of response in AOB abundance and community to excess soil NH_3, it is reasonable to suggest that the excess NH_3 (calculated) present in the



fire plots was not used for autotrophic nitrification as it was in the N-deposition plots (Fig. 2a). We propose instead that NH_4^+ in the fire plots is converted into plant AGB both directly via plant uptake, and indirectly through a possible mechanism involving AMF (Fig. 4). Although we report a trend of decreased AMF under nitrogen addition, which has also been reported previously for this system (Gutknecht et al. 2011), we observed no change in AMF after burning, despite a similar increase in calculated soil NH_3. Since AMF have been shown to be stimulated by grassland fire (Hartnett et al. 2004), it is possible that AMF responses to fire are more immediate than our measurement 9 months post-fire could capture. Through growth of N-rich hyphae that increase plant root surface area, AMF can take up and transfer

significant amounts of nitrogen and phosphorus to their host plants in both inorganic and organic forms (Abbott et al. 1984; Sanders and Tinker 1971; He et al. 2003; Govindarajulu et al. 2005; Hodge and Fitter 2010). There is evidence that plant phosphorus uptake increased after fire (Henry et al. 2006) and AMF associations could have provided their plant symbionts with a competitive advantage for that uptake after fire. Gram-positive bacteria, which were lower in the fire plots in April 2004 (Fig. 1), could be poorer competitors against AMF mainly for two reasons. First, because Gram-positive bacteria have higher N requirements for glycine peptide interbridges and higher P requirements for teichoic acid in their peptidoglycan layers, they have corresponding slow growth and thus cannot respond as quickly to nutrient pulses (Prescott et al. 1996). Also, bacteria in general, likely including AOB and other Gram-positive bacteria, would have been more sensitive than fungi to the decreased soil moisture after fire at our sites, as reported by Niboyet et al. (2011). While pH increased in the second (21 months) and third (33 months) years after fire, there was no response in the first growing season, so pH did not likely play a role in microbial responses for the first growing season after fire (Table 1; Niboyet et al. 2011).

It is also possible that shifts in AMF biodiversity, instead of total abundance, could be involved in increased plant primary productivity through enhanced nutrient uptake (Maherali and Klironomos 2007). Further analysis of the AMF community that is more specific than PLFA would be necessary to determine if this mechanism is active. This conceptual model potentially also explains the fire by N-deposition interactions seen in April 2004 and April 2005 in the soil bacterial community, where we saw higher relative abundance of Gram-positive bacteria (2004) or Gram-negative bacteria (2005) in N-deposition plots but not in N-deposition plots that were also burned (Fig. 4, Supplementary Fig. 1). This interactive effect has been reported at our sites for other measures of microbial nitrogen cycling (Niboyet et al. 2011), strengthening the idea that after burning, plants and plant-associated AMF were capable of outcompeting soil bacteria for an influx of nutrients after burning. Unfortunately this conceptual model could not be verified with direct measures of phosphorus and nitrogen uptake by each component of our plant-microbial system, but this conceptual model offers potential mechanisms that could be tested in the future.

Conclusions

We examined the long-term effects of a low-severity fire in a California annual grassland ecosystem. In the first growing season following the fire, aboveground productivity increased as a result of fire, and litter decreased. However, we did not observe changes in belowground productivity or broad-scale microbial biomass. Instead, our results indicate that responses in specific portions of the soil microbial community occur as a result of fire, but that these responses do not persist past 3 years following fire occurrence. By examining even more specific microbial community members and functional genes using DNA/RNA sequencing, or metagenomics analysis, future studies may resolve microbial responses to fire still further. Also, though it was beyond the scope of this study, the decrease in Gram-negative lipids in April 2005 in the fire plots may be linked to a decrease in AOB abundance and nitrification rate, which could indicate longer-term effects of fire on soil microbial function and biogeochemistry.

Finally, we compared the fertilizing effect of the grassland fire to an N-deposition treatment in the same system, and found that autotrophic AOB are stimulated under N-deposition, but are not stimulated in fire treatments, despite similar impacts of the two treatments. We hypothesize that increased competition from plants and plant-associated AMF for N and P in the fire plots explains this difference in our observed effects on AOB.

In summary, although fire may act as a short-term disturbance to Mediterranean grassland ecosystems, the nutrient pulses specific to fire may affect microbial communities and above–belowground interactions in distinct ways. Our results indicate that microbial communities are more resilient to fire than aboveground plant biomass in this type of Mediterranean grassland. Overall though, we saw no distinguishable responses either above or belowground 33 months after fire, suggesting that these communities are resilient to this type of low-intensity grassland fire. As fire severity increases, due to increased deposition of atmospheric nitrogen or increased precipitation, it is unclear whether that resilience will continue in these systems.

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References

Abbott LK, Robson AD, DeBoer G (1984) The effect of phosphorus on the formation of hyphae in soil by the vesicular-arbuscular mycorrhizal fungus, *Glomus fasciculatum*. New Phytol 97(3):437–446

Abrams MD, Knapp AK, Hurlbert LC (1986) A ten-year record of aboveground biomass in a Kansas tallgrass prairie: effects of fire and topographic position. Am J Bot 73: 1509–1515

Ajwa H, Dell CJ, Rice CW (1999) Changes in enzyme activities and microbial biomass of tallgrass prairie soil as related to burning and nitrogen fertilization. Soil Biol Biochem 31:769–777

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46

Antonopoulos DA, O'Brien SL, Keegan KP, Amarr A, Bates BS, Brulc JM, Caporaso G, Domanus M, D'Souza M, Edwards R, Eshoo T, Gallery RE, Garoutte A, Glass EM, Jastrow JD, Kao RH, Kemner KM, Knight R, Skinner KA, Stevens R, Wilke A, Wilkening JR, Miller RM, Meyer F North American soil metagenomes cluster by ecosystem type and edaphic factors. Science (in preparation)

Avrahami S, Bohannan BJM (2007) Response of *Nitrosospira* sp. strain AF-like ammonia oxidizers to changes in temperature, soil moisture content and fertilizer concentration. Appl Environ Microbiol 73(4):1166–1173

Avrahami S, Bohannan BJM (2009) N_2O emission rates in a California meadow soil are influenced by fertilizer level, soil moisture and the community structure of ammonia-oxidizing bacteria. Global Chang Biol 15:643–655

Avrahami S, Conrad R (2003) Patterns of community change among ammonia oxidizers in meadow soils upon long-term incubation at different temperatures. Appl Environ Microbiol 69(10):6152–6164

Balser T, Treseder KK, Eklener M (2005) Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence. Soil Biol Biochem 37:601–604

Barnard R, Leadley PW, Hungate BA (2005) Global change, nitrification, and denitrification: a review. Global Biogeochem Cycles 19:GB1007

Boerner R, Brinkman JA, Smith A (2005) Seasonal variations in enzyme activity and organic carbon in soil of a burn and unburned hardwood forest. Soil Biol Biochem 37:1419–1426

Boerner R, Waldrop TA, Shelburne VB (2006) Wildfire mitigation strategies affect soil enzyme activity and soil organic carbon in loblolly pine (*Pinus taeda*) forests. Can J For Res 36:3148–3154

Brady NC, Weil RR (2008) The nature and properties of soils. Pearson Education Inc., Upper Saddle River

Briggs JM, Knapp AK (1995) Interannual variability in primary production in tallgrass prairie: climate, soil moisture, topographic position and fire as determinants of aboveground biomass. Am J Bot 82:1024–1030

Brown JR, Blankinship JC, Niboyet A, van Groenigen CJ, Dijkstra P, Leadley PW, Hungate BA (2011) Effects of multiple global change treatments on soil N_2O fluxes. Biogeochemistry. doi:10.1007/s10533-011-9655-2

Bruns MA, Stephen JR, Kowalchuk GA, Prosser JI, Paul EA (1999) Comparative diversity of ammonia oxidizer 16S rRNA gene sequences in native, tilled and successional soils. Appl Environ Microbiol 65:2994–3000

Carney KM, Matson PA (2006) The influence of tropical plant diversity and composition on soil microbial communities. Microb Ecol 52:226–238

D'Ascoli R, Rutigliano FA, De Pascale RA, Gentile A, De Santo AV (2005) Functional diversity of the microbial community in Mediterranean maquis soils as affected by fires. Int J Wildland Fire 14:355–363

Di JJ, Cameron KC, Shen JP, Winefield CS, O'Callaghan M, Bowatte S, He JZ (2009) Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. Nat Geosci 2:621–624

DiTomaso JM, Kyser GB, Miller JR, Garcia S, Smith RF, Nader G, Connor JM, Orloff SB (2006) Integrating prescribed burning and clopyralid for the management of yellow star thistle (*Centaurea solstitialis*). Weed Sci 54:757–767

Dooley SR, Treseder KK (2011, this issue) The effect of fire on microbial biomass: a meta-analysis of field studies. Biogeochemistry. doi:10.1007/s10533-011-9633-8

Dukes J, Chiariello NR, Cleland EE, Moore LA, Shaw MR, Thayer S, Tobeck T, Mooney HA, Field CB (2005) Responses of grassland production to single and multiple global environmental changes. PLOS Biol 3:1–9

Eivazi F, Bayan MR (1996) Effects of long-term prescribed burning on the activity of select soil enzymes in an oak-hickory forest. Can J For Res 26:1799–1804

Evans SE, Wallenstein MD (2011) Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter? Biogeochemistry. doi: 10.1007/s10533-011-9638-3

Fioretto A, Papa S, Pellegrino A (2005) Effects of fire on soil respiration, ATP content and enzyme activities in Mediterranean maquis. Appl Veg Sci 8:13–20

Fried JS, Torn MS, Mills E (2004) The impact of climate change on wildfire severity: a regional forecast for northern California. Clim Change 64:169–191

Frostegård A, Bååth E (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol Fertil Soils 22:59–65

Govindarajulu M, Pfeffer PE, Jin H, Abubaker J, Douds DD, Allen JW, Bucking H, Lammers PJ, Schachar-Hill Y

(2005) Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435:819–823
Grogan P, Bruns TD, Chapin FS (2000) Fire effects on ecosystem nitrogen cycling in a Californian bishop pine forest. Oecologia 122(4):537–544
Gutknecht JLM, Henry HAL, Balser TC (2010) Annual variations in soil extra-cellular enzyme activity in response to simulated climate change, nitrogen deposition, elevated CO_2, and burn disturbance. Pedobiologia 53:283–293
Gutknecht JLM, Field CB, Balser TC (2011, submitted) Long-term microbial responses to simulated multiple global changes. Global Chang Biol
Hamman S, Burke IC, Stromberger ME (2007) Relationships between microbial community structure and soil environmental conditions in a recently burned system. Soil Biol Biochem 39:1111–1120
Hart S, DeLuca TH, Newman GS, MacKenzie MD, Boyle SI (2005) Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. For Ecol Manag 220:166–184
Hartnett DC, Potgieter AF, Wilson GWT (2004) Fire effects on mycorrhizal symbiosis and root system architecture in southern African savanna grasses. Afr J Ecol 42:328–337
Hayatsu M, Tago K, Saito M (2008) Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification. Soil Sci Plant Nutr 54:33–45
Hayhoe K, Cayan D, Field CB, Frumhoff PC, Maurer DP, Miller NL, Moser SC, Schneider SH, Cahill KN, Cleland EE, Dale L, Drapek R, Hanemann RM, Kalkstein LS, Lenihan J, Lunch CK, Neilson RP, Sheridan SC, Verville JH (2004) Emissions pathways, climate change, and impacts on California. Proc Natl Acad Sci USA 101:12422–12427
He X-H, Critchley C, Bledsoe C (2003) Nitrogen transfer within and between plants through common mycorrhizal networks (CMNs). Crit Rev Plant Sci 22:531–567
Henry H, Chiariello NR, Vitousek PM, Mooney HA, Field CB (2006) Interactive effects of fire, elevated carbon dioxide, nitrogen deposition, and precipitation on a California annual grassland. Ecosystems 9:1066–1075
Hodge A, Fitter AH (2010) Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. Proc Natl Acad Sci USA 107(31):13754–13759
Horz H, Barbrook A, Field CB, Bohannan BJM (2004) Ammonia-oxidizing bacteria respond to multifactorial global change. Proc Natl Acad Sci USA 101:15136–15141
Hu FS, Slawinski D, Wright HE, Ito E, Johnson RG, Kelts KR, McEwan RF, Boedigheimer A (1999) Abrupt changes in North American climate during early Holocene times. Nature 400:437–440
Hurlbert LC (1969) Fire and litter effects in undisturbed bluestem prairie in Kansas. Ecology 50:874–877
Hurlbert LC (1988) Cause of fire effects in tallgrass prairie. Ecology 69:46–58
Jia Z, Conrad R (2009) Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil. Environ Microbiol 11(7):1658–1671
Kashiwagi J (1985) Soils map of the Jasper Ridge Biological Preserve. Soil Conservation Service Map, Jasper Ridge Biological Preserve Publication, Stanford
Klute A (ed) (1986) Methods of soil analysis part 1: physical and mineralogical methods, 2nd edn. American Society of Agronomy, Inc and Soil Science Society of America Inc, Madison
Kowalchuk GA, Stephen JR (2001) Ammonia-oxidizing bacteria: a model for molecular microbial ecology. Ann Rev Microbiol 55:485–529
Kowalchuk GA, Stienstra AW, Heilig GHJ, Stephen JR, Woldendorp JW (2000a) Changes in the community structure of ammonia-oxidizing bacteria during secondary succession of calcareous grasslands. Environ Microbiol 2(1):99–110
Kowalchuk GA, Stienstra AW, Heilig GHJ, Stephen JR, Woldendorp JW (2000b) Molecular analysis of ammonia-oxidising bacteria in soil of successional grasslands of the Drentsche A (The Netherlands). FEMS Microbiol Ecol 31:207–215
Launchbaugh JL (1964) Effects of early spring burning yields on native vegetation. J Range Manage 17:5–6
Li YZ, Herbert SJ (2004) Influence of prescribed burning on nitrogen mineralization and nitrification in grassland. Commun Soil Sci Plant Anal 35:571–581
Maherali H, Klironomos JN (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. Science 316:1746–1748
McCune B, Grace JB (2002) Analysis of ecological communities. MJM Software Design Press, Gleneden Beach, p 256
Mendum TA, Hirsch PR (2002) Changes in the population structure of B-group autotrophic ammonia oxidizing bacteria in arable soils in response to agricultural practice. Soil Biol Biochem 34:1479–1485
Menge DNL, Field CB (2007) Simulated global changes alter phosphorus demand in annual grassland. Global Chang Biol 13:2582–2591
Menke J (1992) Grazing and fire management for native perennial grass restoration in California grasslands. Fremontia 20:22–25
Mintie AT, Heichen RS, Cromack K, Myrold DD, Bottomley PJ (2003) Ammonia-oxidizing bacteria along meadow-to-forest transects in the Oregon cascade mountains. Appl Environ Microbiol 69(6):3129–3136
Neary DG, Klopatek CC, DeBano LF, Fgolliott PF (1999) Fire effects on belowground sustainability: a review and synthesis. For Ecol Manage 122:51–71
Niboyet A, Brown JR, Dijkstra P, Blankinship JC, Leadley PW, Le Roux X, Barthes L, Barnard RL, Field CB, Hungate BA (2011) Global change could amplify fire effects on soil greenhouse gas emissions. PLOS One. doi:10.1371/journal.pone.0020105
Picone LI, Quaglia G, Garcia GO, Laterra P (2003) Biological and chemical response of a grassland soil to burning. J Range Manage 56:291–297
Ponder F, Tadros M, Loewenstein EF (2009) Microbial properties and litter on soil nutrients after two prescribed fires in developing savannas in an upland Missouri Ozark forest. For Ecol Manage 257:755–763
Prescott LM, Harley JP, Klein DA (1996) Microbiology, 4th edn. WCB McGraw-Hill, Boston
R Development Core Team (2010) R: A language and environment for statistical computing, reference index version 2.9-2. R Foundation for Statistical Computing, Vienna. ISBN 3-900051-07-0. http://www.R-project.org

Raison RJ (1979) Modification of the soil environment by vegetation fires, with particular reference to nitrogen transformations—review. Plant Soil 51:73–108

Rissler P, Parton WJ (1982) Ecological analysis of a tallgrass prairie: nitrogen cycle. Ecology 63:1342–1351

Roesch L, Rulthorpe R, Riva A, Casella G, Hadwin A, Kent A, Daroub S, Camargo F, Farmerie W, Triplett E (2007) Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J 1:283–290

Romanyá J, Casals P, Vallejo VR (2001) Short-term effects of fire on soil nitrogen availability in Mediterranean grasslands and shrublands growing in old fields. For Ecol Manage 147:39–53

Rotthauwe JH, Witzel KP, Liesack W (1997) The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl Environ Microbiol 63(12): 4704–4712

Sanders FE, Tinker PB (1971) Mechanism of absorption of phosphate from soil by Endogone mycorrhizas. Nature 233:278–279

Schauss K, Focks A, Leininger S, Kotzerke A, Heuer H, Thiele-Bruhn S, Matthles M, Smalla K, Munch JC, Amelung W, Kaupenjohann M, Schloter M, Schleper C (2009) Dynamics and functional relevance of ammonia-oxidizing archaea in two agricultural soils. Environ Microbiol 11(2): 446–456

Schimel J, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. Ecology 88:1386–1394

Schloss P, Handelsmann J (2006) Toward a census of bacteria in soil. PLoS Comput Biol 2:0786–0793

Schulze E-D, Wirth C, Heimann M (2000) Managing forests after Kyoto. Science 289:2058–2059

Shaw M, Zavaleta ES, Chiariello NR, Cleland EE, Mooney HA, Field CB (2002) Grassland response to global environmental changes suppressed by elevated CO_2. Science 298: 1987–1990

Shen J-P, Zhang L-M, Zhu Y-G, Zhang J-B, He J-Z (2008) Abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea communities of an alkaline sandy loam. Environ Microbiol 10(6):1601–1611

Sherman LA, Brye KR, Gill DE, Koenig KA (2005) Soil Chemistry as affected by first-time prescribed burning of a grassland restoration on a coastal plain ultisol. Soil Sci 170(11):913–927

Singh BK, Bardgett RD, Smith P, Reay DS (2010) Microorganisms and climate change: terrestrial feedbacks and mitigation options. Nat Rev Microbiol 8:779–790

Smithwick EAH, Turner MG, Metzger KL, Balser TC (2005) Variation in NH_4^+ mineralization and microbial communities with stand age in lodgepole pine (Pinus contorta) forests, Yellowstone National Park (USA). Soil Biol Biochem 37:1546–1559

Song HG, Kim OS, Yoo JJ, Jeon SO, Hong SH, Lee DH, Ahn TS (2004) Monitoring of soil bacterial community and some inoculated bacteria after prescribed fire in mesocosm. J Microbiol 42(4):285–291

Stephen JR, Chang YJ, MacNaughton SJ, Kowalchuk GA, Leung KT, Flemming CA, White DC (1999) Effect of toxic metals on indigenous soil B-subgroup proteobacterium ammonia oxidizer community structure and protection against toxicity by inoculated metal-resistant bacteria. Appl Environ Microbiol 65(1):95–101

Sugihara NG, Van Wagtendonk JW, Shaffer KE, Fites-Kaufman J, Thode AE (eds) (2007) Fire in California's ecosystems. University of California Press, Berkeley

Todd-Brown K, Hopkins F, Kivlin S, Talbot J, Allison SD (2011) A framework for representing microbial decomposition in coupled climate models. Biogeochemistry. doi: 10.1007/s10533-011-9635-5

Treseder KK, Balser TC, Bradford MA, Brodie EL, Dubinsky EA, Eviner VT, Hofmockel KS, Lennon JT, Levine UY, MacGregor BJ, Pett-Ridge J, Waldrop MP (2011) Integrating microbial ecology into ecosystem models: challenges and priorities. Biogeochemistry. doi:10.1007/s10533-011-9636-5

Vestal JR, White DC (1989) Lipid analysis in microbial ecology—quantitative approaches to the study of microbial communities. Bioscience 39:535–541

Vogl RJ (1979) Some basic principals of grassland fire management. Environ Manage 3:51–57

Wan SQ, Hui DF, Luo YQ (2001) Fire effects on nitrogen pools and dynamics in terrestrial ecosystems: a meta-analysis. Ecol Appl 11:1349–1365

Wallenstein MD, Hall EK (2011) A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. Biogeochemistry. doi:10.1007/s10533-011-9641-8

Wang Y, Ke X, Wu L, Lu Y (2009) Community composition of ammonia-oxidizing bacteria and archaea in rice field soil as affected by nitrogen fertilization. Syst Appl Microbiol 32(1):27–36

Webster T, Embley TM, Prosser JI (2002) Grassland management regimens reduce small-scale heterogeneity and species diversity of B-proteobacterial ammonia oxidizer populations. Appl Environ Microbiol 68:20–30

Westerling AL, Bryant BP (2008) Climate change and wildfire in California. Clim Chang 87:S231–S249

Westerling A, Hidalgo HG, Cayan DR, Swetnam TW (2006) Warming and earlier spring increase western US forest wildfire activity. Science 313:940–943

Wilkinson SC, Anderson JM, Scardelis SP, Tisiafouli M, Taylor A, Wolters V (2002) PLFA profiles of microbial communities in decomposing conifer litters subject to moisture stress. Soil Biol Biochem 34:189–200

Yavitt JB, Yashiro E, Cadillo-Quiroz H, Zinder SH (2011) Methanogen diversity and community composition in peatlands of the central to northern Appalachian Mountain Region, North America. Biogeochemistry. doi:10.1007/s10533-011-9644-5

Yeager CM, Northup DE, Grow CC, Barns SM, Kuske CR (2005) Changes in nitrogen-fixing and ammonia-oxidizing bacterial communities in soil of a mixed conifer forest after wildfire. Appl Environ Microbiol 71:2713–2722

Zavaleta E, Shaw MR, Chiariello NR, Thomas BD, Cleland EE, Field CB, Mooney HA (2003) Grassland responses to three years of elevated temperature, CO_2, precipitation, and N deposition. Ecol Monogr 73:585–604

Zelles L, Bai QY, Beck T, Beese F (1992) Signature fatty-acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. Soil Biol Biochem 24:317–323