



Effects of substrate supply, pH, and char on net nitrogen mineralization and nitrification along a wildfire-structured age gradient in chaparral



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ARTICLE INFO

Article history:

Received 19 October 2015

Received in revised form

18 December 2015

Accepted 22 December 2015

Available online 8 January 2016

Keywords:

Wildfire

Char

Nitrification

pH

Substrate supply

Chaparral

ABSTRACT

Mediterranean-type ecosystems are structured by fire. In California chaparral, fires uncouple inorganic nitrogen (N) production and consumption by enhancing nitrification and reducing plant uptake. Nitrate (NO_3^-) that accumulates after fire is vulnerable to leaching. However, the controls over N metabolism can change as chaparral ecosystems recover from fire, and these mechanisms are not well understood. To evaluate how ammonium (NH_4^+) availability, pH, and char influence N cycling, we measured inorganic N concentration and microbial biomass in chaparral soils that burned 1.5, 4, 23, and 49 years prior to sampling. We then experimentally adjusted NH_4^+ concentration, pH, and char content for all soils in a factorial design, and incubated them for 8 weeks. Each week, we measured respiration, exchangeable NH_4^+ and NO_3^- content, nitrification potential, microbial biomass, and pH. Within each watershed, NO_3^- was higher in soils collected from recently burned sites, than in soils from neighboring mature sites. Also, NO_3^- concentrations increased over the course of incubation in soils from all sites, especially with the addition of NH_4^+ . When NH_4^+ was sufficiently high, pH determined the relative proportion of inorganic N that was nitrified. Char did not have a strong impact on N cycling, but in soils from 23- and 49-year-old sites, char treatments reduced the decline of microbial biomass C and N relative to control and fertilized treatments, perhaps by supplying C to soil microbes. These findings suggest that under some circumstances char may decelerate N cycling if it promotes immobilization and limits NH_4^+ supply to nitrifiers.

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1. Introduction

Recurring cycles of fire and recovery characterize most Mediterranean-type ecosystems, including California chaparral (Rundel et al., 1998). Regrowth after fire relies on a long-term balance between N-inputs and losses, both of which vary through succession (Gray and Schlesinger, 1981). Although fire immediately increases N availability in chaparral by depositing nutrient-rich ash, over time it tends to deplete total N through volatilization, convection of ash, erosion, and leaching (Debano and Conrad, 1978). N export to from burned slopes to streams can be severe immediately after fire (Coombs and Melack, 2013; Verkaik et al., 2013), while over time, the amount of N exported, and the rate that plants

recover, are driven by how rapidly soil microbes mineralize N into forms that can be taken up or leached (Hanan, unpublished).

One robust pattern is that fire stimulates net nitrification, while NO_3^- seldom accumulates in mature stands (Fenn et al., 1993; Hanan, unpublished). Rapid nitrification can intensify greenhouse gas emissions, threaten streams and watersheds, and interfere with plant recovery (Knicker, 2007). For instance, nitrification increases the leaching potential of bioavailable N: while both NH_4^+ and NO_3^- are plant-available, NO_3^- is more mobile in soil. Thus, nitrification after fire may stimulate N-loss before plant uptake can recover. Fires can enhance nitrification through both direct and indirect mechanisms, driven in large part by increases in substrate supply (Robertson and Vitousek, 1981; Carreira et al., 1994), elevated pH (Christensen, 1973; Ste-Marie and Paré, 1999), and interactions with soil organic compounds (Olson and Reiners, 1983). However the balance of these mechanisms is not clear in Mediterranean-type ecosystems, and their relative importance likely varies over the course of ecosystem recovery. Evaluating the factors driving

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postfire N metabolism is essential, not only to understand controls over N loss during recovery, but also to predict and model how ecosystems will respond to changing climate and disturbance regimes (Neff et al., 2002).

Chaparral fires increase substrate supply to nitrifying bacteria by depositing ash rich in NH_4^+ and readily decomposable organic N on soil surfaces, and by reducing competition with plants for NH_4^+ . They also tend to raise pH from around 5.5 to around 6.5 or 7 by combusting organic acids in soil (Kutiel and Inbar, 1993; Binkley and Fisher, 2012) and depositing basic cations with ash (Giovannini, 1990). These processes raise the solubility of soil organic matter, encouraging mineralization, and increasing NH_4^+ supply (Raison, 1979). pH increases also directly stimulate nitrification by increasing the ratio of ammonia (NH_3) to NH_4^+ ($\text{pK}_a = 9.25$; Bollmann and Laanbroek, 2001; De Boer and Kowalchuk, 2001). In burned chaparral, elevated pH means that more mineralized N occurs as NH_3 , which is the form available to NH_3 -oxidizing bacteria and archaea (Stein et al., 1997).

In chaparral, heterotrophic microbes may also interact with substrate supply and pH to influence nitrification rates. In an observational study in a California watershed, nitrification was elevated in burned plots two years after fire even when plant N-uptake was rapid and total inorganic N concentrations were similar to those in unburned plots (Hanan, unpublished). Sustained nitrification may have occurred in these plots because fire provided a short-term pulse of available N and increased soil pH, which activated nitrifier populations; nitrifiers may have remained active even as N availability and pH decreased. Alternatively, nitrification may have remained high if labile C was depleted and overall microbial biomass was suppressed, which would reduce competition with heterotrophs for NH_4^+ .

In addition to creating ash, fires also deposit charred materials on soil surfaces, which may affect NH_4^+ supply, pH, and microbial dynamics. Wildfire can produce char that varies from slightly scorched plant matter to completely graphitized debris, depending on the intensity of fire (Nocentini et al., 2010). As a consequence, the effects of char on N dynamics might vary, yet these effects have not been thoroughly explored. In some cases, wildfire-produced char can enhance nitrification by adsorbing soil organic compounds that would otherwise bind substrates and reduce soil pH (DeLuca et al., 2006). For example, char can adsorb phenolics such as tannins, which can bind soil proteins, making N-rich substrates unavailable to heterotrophs (Fierer et al., 2001). Char also contains alkali and inorganic salts that can increase pH, and improve conditions for nitrification (Yanai et al., 2007). In contrast, char might interfere with nitrification by supplying carbon (C) to heterotrophic microbes, counteracting the destruction of C substrates in vegetation, litter, and O-horizons that occurs with fire (Grasso et al., 1996; Choromanska and DeLuca, 2002). Increased populations of heterotrophs may then compete with nitrifiers for NH_4^+ . Moreover, char can persist in soil for centuries due to its aromatic nature (Nocentini et al., 2010), and though it slowly loses its sorptive capacity over time (Zackrisson et al., 1996), char may influence N cycling over longer timescales than ash or reductions in plant uptake.

Because the relative importance of substrate, pH, and char can vary over space and time, it is difficult to predict nitrification rates in fire-prone environments (Killham, 1990; Ste-Marie and Paré, 1999). Our objective was to gain a better understanding of the factors controlling N cycling in fire-dominated chaparral by addressing the following questions:

1. How do substrate, pH, and char influence net mineralization, nitrification, and microbial dynamics in chaparral soils?
2. How do the effects of these drivers change with time since fire?

We hypothesized that nitrification is regulated by the interaction of NH_4^+ and pH—these drivers can act independently, or amplify each other over the years following fire. Additionally, char can influence nitrification both positively and negatively, and the balance between positive and negative effects can vary over space and time. To answer the questions outlined above, we measured inorganic nitrogen and microbial dynamics in chaparral soils collected along a wildfire-structured age gradient. We then measured the response of those soils to experimental manipulations of NH_4^+ , pH, and char content in the laboratory.

2. Methods

2.1. Study site

We collected soil from two chaparral watersheds along the south facing slopes of the Santa Ynez Mountains in Santa Barbara County, California ($34^\circ 28' \text{N}$, $-119^\circ 40' \text{E}$). Regional climate is Mediterranean, with cool wet winters and warm dry summers. Chaparral watersheds typically receive less than 90 cm of rainfall annually. Warm season droughts span 5–7 months, during which vegetation dries enough to become highly flammable. When chaparral burns, the fire consumes most of the aboveground plant biomass, with fire return intervals typically ranging around 40–60 years for this region (Moritz, 2003). In the immediate years after fire, seeds germinate, and many species resprout from basal lignotubers (Keeley et al., 1981). Short-lived herbaceous and suffrutescent plants that germinate from seeds proliferate, and play an important role in nutrient retention and cycling during the early stages of recovery (Rundel and Parsons, 1984). Although these nitrogen-rich ephemerals dominate the immediate postfire landscape, sclerophyllous shrubs tend to recover closed canopies within ten years (Gray and Schlesinger, 1981); herbaceous and suffrutescent vegetation is absent in mature stands (Keeley et al., 1981).

Soils in the region are most commonly sandy loams, classified as Typic Dystrochrepts from the Maymen series (NRCS, 2015). They are shallow, highly drained, and are weathered from shale, schist, greenstone, sandstone and conglomerate. They are acidic and highly erosive (NRCS, 2015). Soil samples were collected from four regions that burned approximately 1.5, 4, 23, and 49 years prior to sampling. The 4 and 49-year old sites were located in the Rattlesnake Canyon watershed, and the 1.5- and 23-year old sites were located in the Painted Cave region of the Maria Ygnacio watershed. Three replicate 10 m by 10 m plots were delineated in each site except the 23-year-old site where only 2 plots could be laid out, resulting in 11 plots total. Plots in Rattlesnake Canyon had been established in 2009 for a field study that tracked N cycling and plant N-uptake after the Jesusita fire in 2009 (Hanan, unpublished). Elevations ranged from 702 to 1083 m in Rattlesnake Canyon, and from 686 to 807 m in Painted Cave.

Both watersheds were dominated by *Arctostaphylos* spp. (manzanita), *Adenostoma fasciculatum* (chamise), and *Ceanothus* spp., though other species such as *Malosma laurina* (laurel sumac), *Heteromeles arbutifolia* (Toyon), and *Prunus ilicifolia* (holly-leaved cherry) were also present. In 1.5-year-old plots, the 2012 fire had removed almost all vegetation and litter, leaving only small charred stumps, which were beginning to resprout. Post-fire herbs such as *Calystegia macrostegia* (coast morning glory) were also present in 1.5-year-old sites, but had mostly senesced during the time of sampling. In 4-year-old plots, the 2009 fire had similarly destroyed most aboveground vegetation, clearing the way for a thick layer of ephemeral herbs (Hanan, unpublished). At the time of sampling for the current study, shrub re-sprouting was vigorous, with most shrubs reaching more than 1.5 m in height, and few postfire herbs

remaining on site. In 23- and 49-year old sites, shrubs formed dense, nearly impenetrable, closed canopies that were approximately 2–4 m tall.

2.2. Field sampling

Soil samples were collected in June 2013 from each of the replicate 10 m by 10 m plots in each age class. This sampling date was well into the typical summer drought, which allowed us to capture the effects of rewetting at the start of our incubations. 25 samples were collected from the first 10 cm of the A-horizon in each plot, and combined to form a single representative composite sample for that plot. Thus plots were considered replicates for each age class. We selected the A-horizon in order to have comparable measurements between mature chaparral soils and those that recently burned, as the O-horizon had been consumed in recently burned sites. Samples were brought to the laboratory and processed immediately for preliminary analysis. Because soil samples were extremely dry at the time of collection, subsamples were stored in a cool dry place (to avoid freezing or condensation) for less than one week prior to starting the incubation.

2.3. Preliminary analysis of soil physical and chemical properties

Bulk densities were determined for all composite soil samples as oven-dry weight divided by soil core volume. Samples were then homogenized and run through a 2 mm sieve. Gravimetric water content was determined by drying 5 g subsamples at 105 °C until constant mass. Water-holding capacity was determined by saturating 5 g sub-samples for 2 h and then drying to constant mass. Soil pH was measured using 10 g subsamples combined with deionized water in 1:1 slurries (Jones, 2001). Total carbon (TC) and total nitrogen (TN) concentrations were determined using a Carlo Erba/Fisons, NA 1500 Series 2 elemental analyzer, and normalized to mg g⁻¹ dry soil weight.

2.3.1. Inorganic N pools

Exchangeable NH₄⁺ and NO₃⁻ were determined for all fresh soil samples by extracting 10 g field collected soil (wet weight) with 40 mL 0.5 M potassium sulfate (K₂SO₄) for 4 h, then vacuum filtering through a glass fiber filter (Pall Gelmann Type A/E 1.0 μm). Extracts were frozen until analysis. Inorganic N concentrations were analyzed using a Lachat autoanalyzer (Lachat, 1989); NH₄⁺ was analyzed using the diffusion method (Lachat method #31-107-06-5-A, Milwaukee, WI), and NO₃⁻ was reduced by Cd followed by Griess–Ilosvay reaction, and analyzed colorimetrically (Lachat method #12-107-04-1-B, Milwaukee, WI).

2.3.2. Nitrification potential

Nitrification potentials were determined as an index of autotrophic nitrifier biomass using the chlorate slurry inhibition assay (Belser and Mays, 1980). Five g of soil were incubated for 6 h in a solution containing 0.2 M dipotassium phosphate (K₂HPO₄), 0.2 M monopotassium phosphate (KH₂PO₄), and 0.2 M ammonium sulfate ((NH₄)₂SO₄) to maximize nitrification rates, and 1 M sodium chlorate (NaClO₃) to block the oxidation of nitrite to nitrate. Aliquots were drawn from the slurry at hour 0, 3 and 6, and NO₂⁻ concentrations were measured colorimetrically. Increases in NO₂⁻ over the course of the assay were used to estimate nitrification potential.

2.3.3. Microbial biomass C and N

Microbial biomass was determined using the chloroform slurry-extraction method (Fierer and Schimel, 2002). Ten g subsamples were extracted with 40 mL of 0.5 M K₂SO₄ and 0.5 mL of chloroform

(CHCl₃), and filtered as above. Non-purgeable organic carbon (NPOC) and total nitrogen (TN) content of CHCl₃-treated and untreated extracts were measured using a Shimadzu Corporation, Series V Model CSN analyzer. Extractable microbial biomass C and N were calculated by subtracting the NPOC and TN in untreated samples from the amount in treated samples. Because CHCl₃ releases only a fraction of total microbial biomass C and N, concentrations were corrected for extraction efficiency using conversion factors of 0.45 for C (Beck et al., 1997) and 0.54 for N (Brookes et al., 1985). Extractable organic nitrogen (EON) was estimated as extracted TN minus inorganic N.

2.4. Incubation and time series analyses

To evaluate the influence of substrate, pH, and char on mineralization, nitrification, and microbial dynamics we experimentally adjusted NH₄⁺ concentration at two levels (i.e., control, +NH₄⁺), pH at four levels (i.e., -1, control, +1, +2), and char content at two levels (i.e., control, +char) for all soil samples in a factorial design. Thus, 16 treatments were applied to each of the 11 composite soil samples. However, we did not acidify soils from the 23-year-old sites in Painted Cave because they were already acidic (average pH was 4.67). Similarly, we did not apply the +2 pH treatment to soils from 1.5-year-old sites, as they had recently burned and already had elevated pH. Thus, our sampling design resulted in 156 treatment combinations and constrained pH within bounds of 4.6–7.7.

For each incubation treatment, we placed eight replicate 50 mL uncapped centrifuge tubes, each containing 40 g of treated soil in a 1.89 L mason jar. Soils were incubated at 50% water holding capacity inside sealed mason jars for up to 8 weeks. At weeks 0, 1, 2, 5 and 8, we removed 1 tube from each jar to destructively sample soil. To achieve approximate pH adjustments of -1, +1, and +2 pH units after 24 h, we added 10 μL 0.2 M hydrochloric acid (HCl), 50 μL 0.3 M sodium hydroxide (NaOH), or 100 μL 0.3 M NaOH per g soil, respectively. For the NH₄⁺ treatment, we used 5 mL of (NH₄)₂SO₄ solution containing 600 μg NH₄⁺ mL⁻¹ (which provided 75 μg NH₄⁺ g⁻¹ soil). This concentration was designed to mimic the amount of NH₄⁺ added to soil surfaces during fire (Christensen, 1973). Fresh char was obtained from sites where mountain residents burn brush around their homes to prevent fire spread. Thus char was freshly produced from mature chaparral vegetation growing near the 1.5- and 23-year old sampling sites. Total C and N concentrations in char were 526.64 and 5.08 mg g⁻¹, respectively (C:N was 104.54). For treated soils, we mixed 1 mg ground char g⁻¹ dry soil into each tube prior to adding liquid solutions. This concentration was calculated as a proportion of aboveground plant biomass estimated in nearby mature stands (Schlesinger and Gill, 1980). However, char deposits are highly heterogeneous, and while this concentration may be realistic averaged over a plot, it doesn't account for char hotspots that likely occur following wildfire. We also measured the total C and N content of char. Incubation units were stored in the dark at 20 °C.

At weeks 1, 2, 4, 5, and 8, soil respiration rates were determined by measuring CO₂ concentrations in the headspace of the sealed incubation jars. We used a glass syringe to draw samples through rubber septa on the lids of each jar, and CO₂ concentrations were analyzed using a LiCor infrared gas analyzer (Licor Model LI-6252) pumping at a speed of 40 mL min⁻¹. The jars were vented weekly (i.e., after each sampling, and at the beginning of week 6 and week 7) to prevent CO₂ concentrations from exceeding 2%. Then at weeks 1, 2, 5 and 8, we analyzed pH, nitrification potential, and exchangeable NH₄⁺ and NO₃⁻ concentrations (as above). Net mineralization and nitrification rates were calculated as differences between exchangeable total inorganic N (TIN) and NO₃⁻ concentrations between time points, respectively. We also measured TC,

TN, and microbial biomass C and N at the beginning and end of the incubation (as above). Results were normalized to $\mu\text{g g}^{-1}$ dry soil for nutrient and microbial biomass pools, $\mu\text{g g}^{-1}$ dry soil week^{-1} for respiration, net mineralization, and nitrification, and $\mu\text{g g}^{-1}$ dry soil hour^{-1} for nitrification potential.

2.5. Statistical analysis

To contrast soil physical and chemical properties between the more recently burned (1.5- and 4-year-old) and their adjacent mature (23- and 49-year-old) sites prior to incubation, we first used independent t-tests in SPSS version 22.0 within each watershed. Treatment effects on measured variables were then analyzed using linear mixed-effects models. Initial models were constructed with 4 fixed effects: stand age, pH, NH_4^+ , and char treatment. We compared all four age classes across watersheds, and inferred patterns corresponding to recently burned vs. mature sites within each watershed. However, it is important to note that replicating specific age classes was not possible across watersheds. Because there were potential substrate differences between watersheds, we could not completely rule out the confounding effects of spatial differences between recently burned (1.5- and 4-year-old) sites, or spatial differences between mature (23- and 49-year-old) sites. Fixed effects and interactions that were not statistically significant were then removed from the model iteratively based on their P-values, and then best-fit models were selected by minimizing Akaike's Information Criterion calculated using the restricted maximum likelihood method (REML-AIC). Thus, final models included only significant fixed effects or interactions, and exhibited REML-AIC values that were not statistically larger than those of the more complex models. Incubation jar was considered a random effect, and to account for autocorrelation in repeated measurements from within the same incubation jar, we used a first order autoregressive model structure. We also performed post-hoc analyses using the Bonferroni correction to compare treatment levels for fixed factors when their effects were significant. $P < 0.10$ was considered statistically significant since using a $P < 0.05$ would create a high risk of Type-II errors given our relatively small sampling size (Hurlbert and Lombardi, 2009).

3. Results

3.1. Soil physical and chemical properties prior to incubation

Soil moisture was low, and did not vary among sites, as soils were collected during the summer drought (Table 1). Bulk densities

were similar across sites (Table 1). Soils collected from recently burned (1.5-year-old) plots had higher pH ($P = 0.07$), more NH_4^+ ($P = 0.10$), and lower microbial N ($P = 0.04$) than soils from adjacent mature (23-year-old) sites (Table 1). None of the other measured properties were statistically different across sites ($P > 0.10$). In the Rattlesnake Canyon Watershed, where four years had passed since the most recent fire, no soil properties differed between 4-year-old and 49-year-old sites.

3.2. Respiration

Soil respiration rates were highest at the start of the 8-week incubation, and decreased over time in all treatments (Fig. 1). The average CO_2 flux varied significantly with age ($P < 0.01$; Appendix 1), being lowest in soils from 4-year-old sites (Bonferroni $P < 0.01$ for all comparisons). Respiration in the other age classes did not differ from one another. Respiration rates varied with pH treatment ($P < 0.01$; Appendix 1); rates were higher in the control and alkalized soils compared to the acidified treatment (Bonferroni $P < 0.10$ for all comparisons with the acidified treatment). Respiration did not differ with NH_4^+ or char treatments.

3.3. Extractable organic C and N

Extractable organic C (EOC) varied significantly among age classes ($P < 0.01$; Appendix 2) as follows: $1.5 > 23 > 49 > 4$ -year-old (Bonferroni $P < 0.02$ for all comparisons). EOC decreased over the course of incubation in soils from all age classes, but decreased most rapidly in soils from 1.5-year-old plots (Fig. 2). EOC did not however vary among pH or char treatments. Extractable organic N (EON) was often below detection. However, values did vary among age classes ($P < 0.01$; Appendix 2) as follows $49 \approx 23 > 4 > 1.5$ -year-old plots (Bonferroni $P < 0.01$ for all comparisons except between 23- and 49-year-old plots, which were not significantly different from one another). There was an interaction between age and NH_4^+ treatment, where in 1.5- and 4-year-old plots, EON remained higher in soils that were not fertilized than in soils that were fertilized ($P = 0.10$ and < 0.01 , respectively; Appendix 2). There was also an interaction between age and char, where in 4-year-old sites, EON remained higher in soils that were treated with char ($P < 0.01$; Appendix 3).

3.4. Microbial biomass C and N

Microbial biomass C decreased over time in control soils from all age classes except the most recently burned (1.5-year-old; Fig. 3a).

Table 1
Mean physical and chemical properties ± 1 SE prior to starting incubation averaged over plots in each age group.

Watershed:	Maria Ygnacio (Painted Cave)		Rattlesnake Canyon	
	1.5	23	4	49
Age (years since fire):				
Soil water content (g g^{-1})	0.06 \pm 0.01	0.06 \pm 0.01	0.04 \pm 0.01	0.05 \pm 0.01
Bulk density (g cm^{-3})	0.90 \pm 0.08	1.05 \pm 0.14	1.09 \pm 0.04	1.15 \pm 0.03
pH	6.02 \pm 0.21	4.56 \pm 0.31	5.75 \pm 0.12	5.61 \pm 0.03
TC (mg g^{-1})	41.38 \pm 8.09 ^a	30.11 \pm 13.57	23.69 \pm 6.53	13.92 \pm 2.29
TN (mg g^{-1})	2.91 \pm 0.64	1.68 \pm 0.67	1.58 \pm 0.60	0.78 \pm 0.09
$\text{NH}_4^+\text{-N}$ ($\mu\text{g g}^{-1}$)	41.27 \pm 11.47 ^a	10.08 \pm 4.41	3.59 \pm 1.37	1.50 \pm 0.71
$\text{NO}_3^-\text{-N}$ ($\mu\text{g g}^{-1}$)	21.61 \pm 10.63	11.24 \pm 7.29	1.36 \pm 0.70	0.21 \pm 0.08
TIN ($\mu\text{g g}^{-1}$)	62.89 \pm 14.51	21.32 \pm 11.71	4.95 \pm 1.88	1.71 \pm 0.68
Biomass C ($\mu\text{g g}^{-1}$)	107.29 \pm 17.48	213.04 \pm 106.34	251.04 \pm 66.35	325.17 \pm 58.29
Biomass N ($\mu\text{g g}^{-1}$)	3.42 \pm 3.42 ^a	17.63 \pm 2.12	13.35 \pm 6.82	20.16 \pm 1.88
EOC ($\mu\text{g g}^{-1}$)	238.73 \pm 65.89	146.21 \pm 13.88	58.06 \pm 15.83	103.75 \pm 36.10
EON ($\mu\text{g g}^{-1}$)	2.49 \pm 1.61	2.58 \pm 2.58	2.65 \pm 1.07	4.54 \pm 1.15
Nitrification potential ($\mu\text{g g}^{-1} \text{h}^{-1}$)	0.00 \pm 0.02	0.00 \pm 0.01	0.00 \pm 0.01	0.00 \pm 0.01

^a Indicates mean values for soils from recently burned sites were statistically different from those for soils in adjacent mature sites.

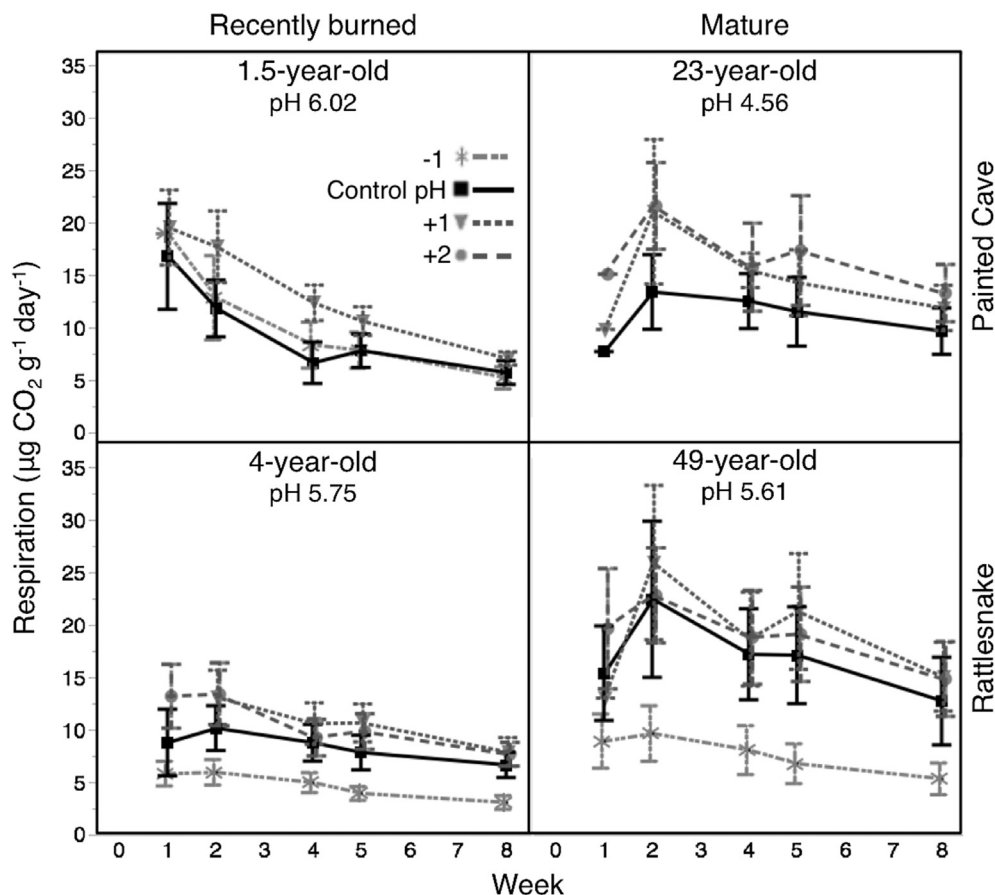


Fig. 1. Average daily respiration rates during the 8-week incubation for each pH treatment in each age class. Pre-incubation pH is noted at the top of panels for each age class. Measurements were taken during weeks 1, 2, 4, 5, and 8.

Also, biomass increased with age ($P < 0.01$), though it was not significantly different between 23- and 49-year-old plots (Bonferroni $P = 1.00$; Table 1). There was a significant interaction between age and char treatment, where microbial biomass C either remained constant, or decreased the least in soils from the 23- and 49-year-old sites that were treated with char ($P = 0.05$ and 0.00 , respectively), while in 4-year-old sites biomass C decreased more in soils that were treated with char ($P = 0.02$; Fig. 3a; Appendix 3).

Changes in microbial biomass N also varied among age classes ($P < 0.01$; Appendix 4). Changes were significantly lower in 4-year-old sites than in all other sites (Bonferroni $P < 0.01$, 0.01 and 0.10 , respectively; Fig. 3b). There was also a significant interaction between fertilization and pH: in unfertilized soils, acidified treatments accumulated more biomass N than alkalinized treatments ($P = 0.10$), while pH had no effect in fertilized soils (Appendix 4). By the end of the 8-week incubation, average microbial C:N ratios decreased from 13.8 to 5.4 in 1.5-year-old sites, from 25.4 to 18.8 in 4-year-old sites, and from 16.0 to 12.1 in 49-year-old sites. However in 23-year-old sites, average microbial C:N increased slightly from 11.5 to 12.7.

3.5. Inorganic N, net mineralization and nitrification

Total inorganic nitrogen (TIN) increased over the course of incubation in control soils from all sites (Appendix 5; Fig. 4). TIN accumulation varied among age classes as follows: $1.5 > 23 > 4 \approx 49$ -year-old plots (Bonferroni $P < 0.01$ for all comparisons except 4 vs. 49-year-old plots where $P = 0.16$). Thus inorganic N accumulated more in Painted Cave than in Rattlesnake

Canyon soils, and within each watershed, TIN was generally higher in sites that recently burned (though the difference was not significant in Rattlesnake Canyon). TIN was also higher in soils treated with NH_4^+ ($P < 0.01$; Appendix 5).

NO_3^- accumulation varied similarly among age classes, where $1.5 > 23 \approx 4 > 49$ -year-old plots (Bonferroni $P < 0.02$ for all comparisons except 23 vs. 4-year-old plots where $P = 0.87$). Also, similar to TIN, NO_3^- was accumulated more in soils treated with NH_4^+ ($P < 0.01$; Appendix 5). However, the proportion of TIN as NO_3^- was higher in control soils from recently burned (1.5- and 4-year-old), and 23-year-old plots than in control soils from mature (49-year-old) plots (Fig. 4), reflecting that in 1.5-, 4-, and 23-year-old plots, NO_3^- increased more rapidly than TIN, and over time, all mineralized N was nitrified. In 49-year-old plots on the other hand, mineralization and nitrification both occurred, but not all mineral N was nitrified (Fig. 4). Although char had no significant effects on N dynamics, it appears that in 1.5-year-old plots, it may have slightly enhanced TIN and NO_3^- pools, while slightly decreasing pools in older sites (Fig. 4e).

Net mineralization rates were higher in soils from 1-year-old plots than in soils from 49-year-old plots (Bonferroni $P = 0.02$), though there were no other differences among other age classes. Rates were also higher in soils treated with NH_4^+ ($P < 0.01$; Fig. 4a; Appendix 6), while pH and char treatments had no apparent effect (Fig. 4; Appendix 1). Net nitrification also varied among age classes ($P < 0.01$; Appendix 6); rates were significantly higher in 1.5-year-old plots, than in plots from other age classes (Bonferroni $P < 0.01$ for all comparisons with 1.5-year-old plots). Net nitrification was significantly higher in soils that were fertilized with NH_4^+ ($P < 0.01$;

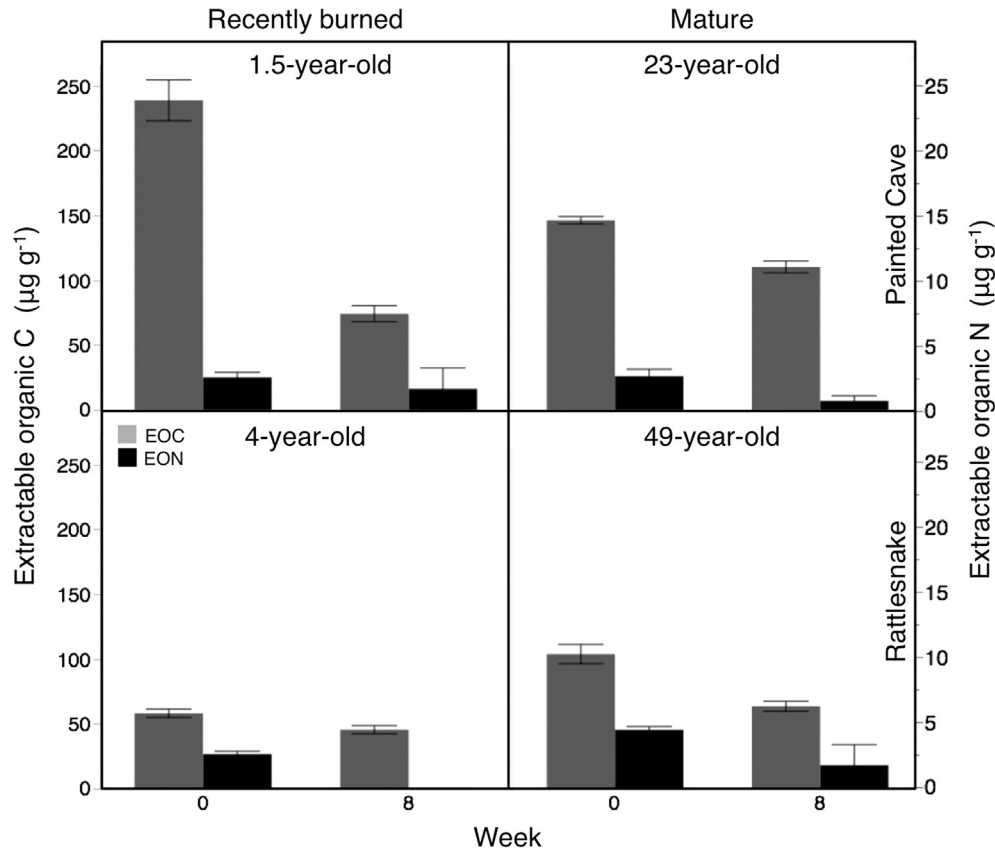


Fig. 2. Extractable organic C (EOC; left axis), and extractable organic N (EON; right axis) at the beginning and end of incubation for each age class.

Appendix 6; Fig. 4a). Net nitrification rates also increased slightly with alkalinization ($P = 0.09$; Appendix 6; Fig. 4b), and remained elevated even as alkalinized soils gradually returned towards their pre-incubation pH (Fig. 5). Similar to NO_3^- pools, nitrification rates increased more rapidly than mineralization in plots that recently burned, especially in 1.5-year-old plots, whereas in mature (23- and 49-year-old) plots, not all added NH_4^+ was nitrified.

3.6. Nitrification potential

Nitrification potential, which is a proxy for nitrifier abundance (Belser and Mays, 1980), varied among age classes ($P < 0.01$; Fig. 6; Appendix 7). It was significantly higher in 1.5-year-old plots than in plots from any other age class (Bonferroni $P < 0.01$ for all comparisons). Nitrification potential was also significantly higher in soils treated with NH_4^+ ($P < 0.01$; Appendix 7), and alkalinized treatments generated higher nitrification potential than acidified treatments ($P < 0.02$; Appendix 7). Fig. 6 shows nitrification potential over the 8-week incubation. Because initial (pre-incubation) nitrification potentials were low across all plots, those values are excluded from the figure. In recently burned (1.5- and 4-year-old) plots, nitrification potential peaked during the first week of incubation, whereas in mature (23- and 49-year-old) plots, potentials were generally lower, and did not peak until the second week (Fig. 6).

4. Discussion

We hypothesized that substrate supply, pH, and char content would be the core controls on nitrification following fire, and that the importance of these controls would decline over the course of

recovery. By manipulating these variables, we could, indeed, stimulate nitrification in mature soils and make them function more like recently burned soils, and vice versa. This suggests that we were capturing the major factors influencing nitrification patterns in these soils. However, nitrification rates still differed between the watersheds, presumably due to factors that either differed inherently between the sites or that accrued differently over time, such as mineralogy, native pH, soil C and N pools, and the composition of microbial communities.

Among the variables investigated, NH_4^+ supply and pH were important drivers of N cycling in chaparral, while char had no significant net effect (Fig. 4). However, char did help sustain microbial biomass in soils from mature (23- and 49-year-old) plots, suggesting that it may play a role in alleviating C limitation, and therefore in some circumstances may indirectly promote N-immobilization after fire.

4.1. Pre-incubation conditions along the age gradient

Although soil samples were collected in June, when biological activity was presumably minimal, NH_4^+ and NO_3^- concentrations were on average much higher in recently burned plots (1.5- and 4-year-old) than in neighboring mature plots (23- and 49-year-old; Table 1). In forested ecosystems, net nitrification rates are often negatively correlated with age and successional stage of the vegetation because disturbance uncouples N mobilization and uptake (Vitousek et al., 1982). Mineral N is usually more bioavailable during early succession, and therefore nitrification rates are also higher (Vitousek and Melillo, 1979; Kielland et al., 2006). On the other hand, a lack of NO_3^- in mature stands could reflect rapid immobilization of NO_3^- both by plants and microbes, and/or increased

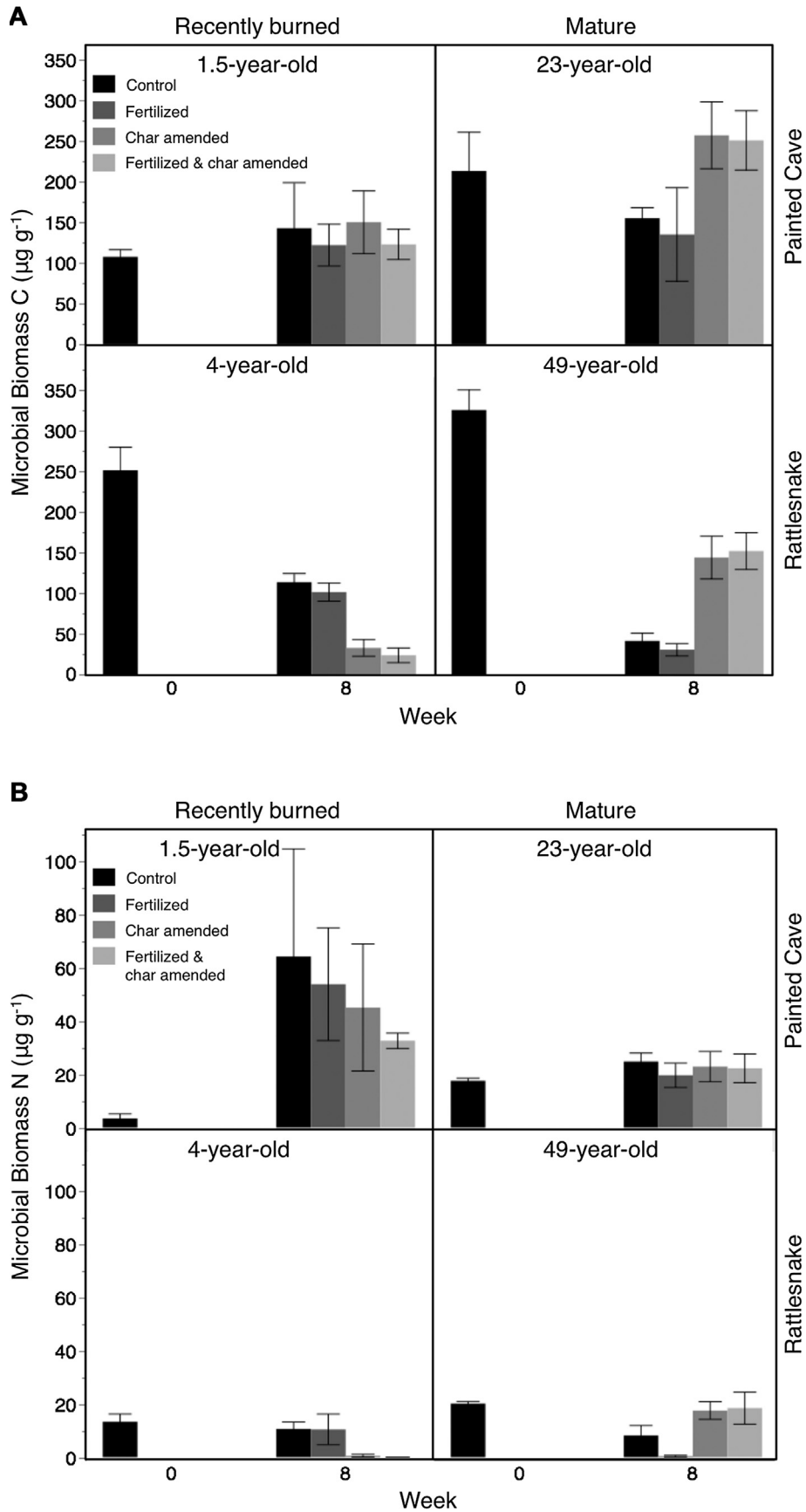


Fig. 3. (a) Microbial biomass C at beginning and end of incubation for control, NH_4^+ fertilized, and char amended soils, (b) microbial biomass N at beginning and end of incubation for control, NH_4^+ fertilized, and char amended soils.

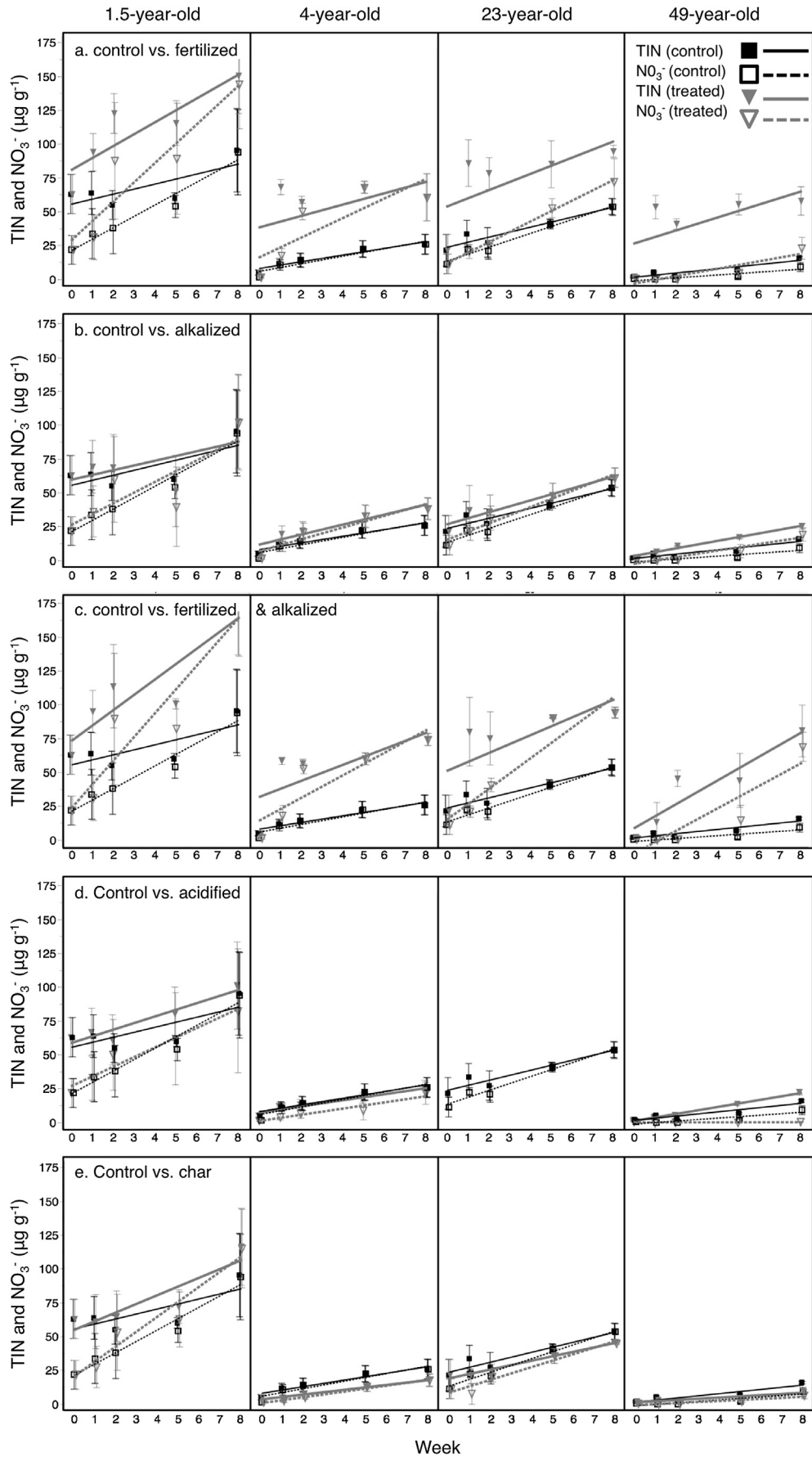


Fig. 4. Total inorganic N and nitrate in unburned and burned controls and alkalinized soils (top panel), unburned and burned controls, and fertilized soils (2nd panel), unburned and burned controls, and fertilized + alkalinized soils (3rd panel), unburned and burned control and acidified soils (4th panel), and unburned and burned controls + char amended soils (bottom panel). Inorganic N was measured in weeks 1, 2, 5, and 8.

leaching and denitrification should NO_3^- form (Belser, 1979; Stark and Hart, 1997; Neary et al., 1999). Low nitrification potentials in mature plots however support the notion that nitrifier population sizes decrease with stand age. Nonetheless, NH_4^+ and NO_3^- concentrations varied among plots within each of the recently burned and mature age classes (Table 1), suggesting that stand age is not the only factor driving variation in N-cycling rates.

4.2. Factors regulating N-cycling in chaparral

Substrate supply (i.e., NH_4^+) is considered a dominant driver of nitrification in chaparral and other ecosystems (Donaldson and Henderson, 1990; Homyak et al., 2014). Fires increase NH_4^+ supply to autotrophic nitrifiers by depositing N-rich ash on soil surfaces and reducing heterotrophic microbial biomass and plant cover (Raison, 1979). Not surprisingly then, NH_4^+ fertilization stimulated nitrification in soils from both recently burned (1.5- and 4-year-old) and mature (23- and 49-year-old) sites. However, in mature soils, nitrifiers did not consume all available substrate, while they did in recently burned control and recently burned soils fertilized with NH_4^+ (Fig. 4a). Therefore, substrate availability must interact with other drivers to constrain short-term nitrification dynamics in mature chaparral soils.

The NO_3^- that accumulates after fire is also thought to result from increases in soil pH (Christensen, 1973; Dunn et al., 1979; Fenn et al., 1993). Such increases can promote nitrification both indirectly, by increasing decomposition and mineralization, and directly, by stimulating the growth of nitrifier populations (Bååth et al., 1995; Ste-Marie and Paré, 1999). We found that respiration rates were highest in soils that were alkalized, and lowest in soils that were acidified, suggesting that decomposition can respond positively to increases in pH, yet, this did not translate to any significant increase in net mineralization rates. This may reflect changes in the composition of microbial communities, which became more N-rich (i.e., lower C:N) over the course of incubation in 1.5-, 4-, and 40-year-old age classes. We did however find that nitrification was higher in alkalized soils, though the effects were relatively small, perhaps because there was not sufficient NH_4^+ to stimulate nitrifier growth.

4.2.1. Interactions between substrate supply and pH

While increasing pH alone did not stimulate net-mineralization or strongly enhance nitrification, it did increase net nitrification rates in mature soils that were also fertilized (Fig. 4b,c), perhaps because nitrifiers are poor competitors for NH_4^+ when it is scarce. To increase nitrifier populations, NH_4^+ must be readily available. However, once nitrifier populations are established, they may be able to compete better for NH_4^+ , especially postfire when pH is elevated and heterotrophic microbial biomass is suppressed.

Although pH treatments did not significantly affect standing N pool sizes, when soils from mature (23- and 49-year-old) sites were alkalized, NO_3^- pools appeared to increase more rapidly than in control soils, reflecting that a greater proportion of mineral N was nitrified. Analogously, when acidified, soils from recently burned (1.5- and 4-year-old) sites responded more like soils from mature sites; net nitrification decelerated and NO_3^- accumulated at a similar rate to NH_4^+ . Note that when soils from 49-year-old sites were acidified, NO_3^- did not accumulate at all (Fig. 4c), suggesting that the apparent pH threshold for net nitrification is about 4.75 in these soils. The lowest limits for autotrophic nitrification are considered to be somewhere between pH 4.0 and 4.7 in soils (Persson and Wirén, 1995; Zhang et al., 2012; Shenoy et al., 2013). However, if NH_4^+ concentrations are high enough, soils will nitrify even when they are very acidic (De Boer and Kowalchuk, 2001). Indeed, when the 49-year-old acidified soils were also fertilized,

NO_3^- accumulated at a similar rate to that observed in the 49-year-old control soils. These results corroborate our first hypothesis that nitrification is regulated by synergy between NH_4^+ and pH, and these two drivers trade off with one another at different stages of recovery. Because NH_4^+ concentrations and pH are optimal after fire, enhanced nitrification frequently occurs.

Interestingly, in the already acidic soils from 23-year-old plots, some NO_3^- did accumulate in control soils, even when pH was below 4.7; a value that would constrain nitrification in most ecosystems (Motavalli et al., 1995). Perhaps other factors, such as substrate supply, stimulated nitrifier activity to the extent that it could overcome low pH. Although NH_4^+ concentrations in soils from 23-year-old sites were lower than those in 49-year-old sites (which did not accumulate NO_3^- at low pH), microbial biomass was also lower in 23-year-old sites, so perhaps nitrifiers had better access to NH_4^+ because competition was less intense. Alternatively, low levels of heterotrophic nitrification may be responsible for NO_3^- pools that accumulated in control soils from 23-year-old plots. Heterotrophs can contribute slightly to NO_3^- accumulation in acid soils (Schimel et al., 1984; De Boer and Kowalchuk, 2001), and heterotrophic nitrifiers often use organic N as substrate, which could explain the basal nitrification that occurred in 23-year-old plots despite relatively low NH_4^+ concentrations (Schimel et al., 1984; Stroo et al., 1986).

Overall, it appears that both fertilization and alkalization play an important role in stimulating rapid nitrification in chaparral. When soils from mature (23- and 49-year-old) sites were fertilized with NH_4^+ , inorganic N pools increased, but rates of mineralization did not change much and the increase in nitrification was relatively small (Fig. 4b). Similarly, when these soils were alkalized, little changed (Fig. 4d). However, when soils from mature sites were both fertilized and alkalized, nitrification rates increased most dramatically, and by the end of incubation most NH_4^+ was converted to NO_3^- , mirroring patterns in recently burned control soils—nitrate concentrations more than tripled in fertilized, alkalized soils compared with mature controls, and almost doubled compared with soils from mature sites that were only fertilized (Fig. 4a,b).

Once substrate additions have activated nitrifier populations, different combinations of substrate supply and pH may sustain their activity. Nitrification potential increased with NH_4^+ addition and pH, with the most dramatic effects occurring at the beginning of incubation in soils from 1.5-year-old plots (Fig. 6). However, soils have high buffering capacity, and field studies show that chaparral pH tends to return to its native state within a year after fire, while nitrification rates remain high (Hanan, unpublished). In this study, soils treated with HCl or NaOH gradually returned towards their pre-incubation pH (Fig. 5), however, nitrification rates remained elevated in soils that were both fertilized and alkalized (Fig. 4b).

Nitrification potential also appeared to ramp up more rapidly in soils from recently burned (1.5- and 4-year old) plots, whereas in soils from mature (23- and 49-year-old) plots nitrification potential took an extra week to reach peak rates (Fig. 6). Given favorable environmental conditions, nitrifiers in Mediterranean-type ecosystems can respond within hours to rewetting (Placella and Firestone, 2013). In this study, the delayed nitrifier response in mature plots likely occurred because nitrifier populations were initially small due to the combination of substrate-limitation, acidity, and drought conditions during the time of sampling.

4.2.2. Effects of char on microbial C-limitation and N-mineralization vs. immobilization

Char might also interact with NH_4^+ supply and pH to influence N-cycling and microbial dynamics (Pietikäinen et al., 2000). For example, char can stimulate nitrification by increasing soil pH (Yanai et al., 2007). We found that by the end of the incubation pH

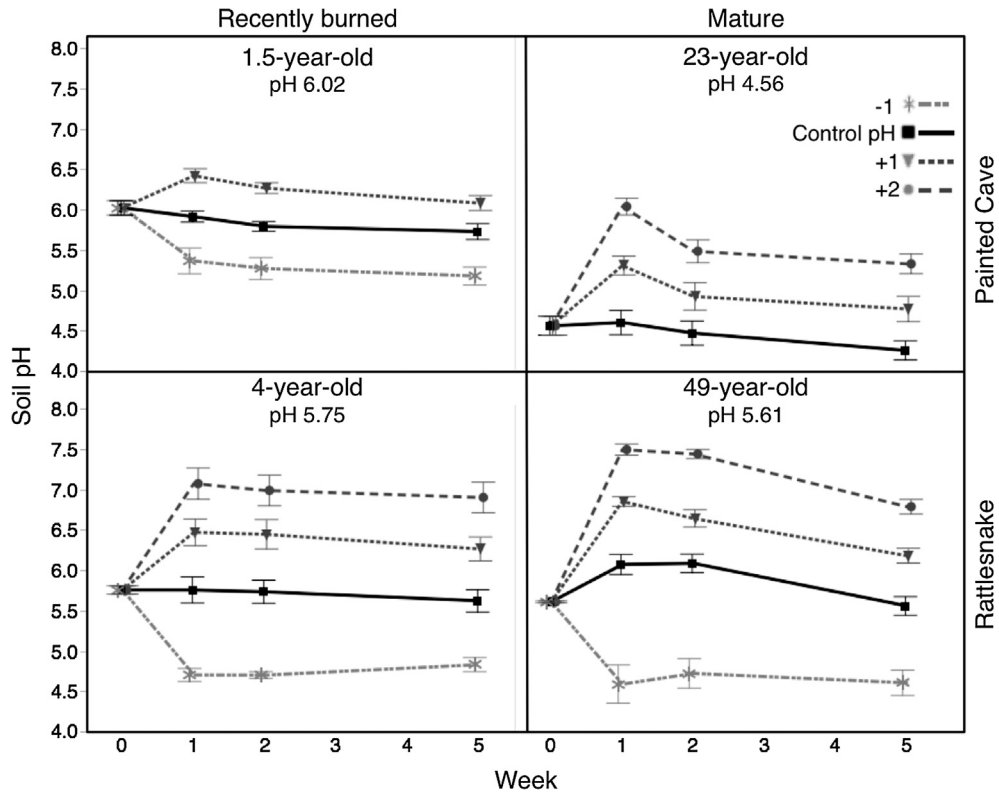


Fig. 5. Soil pH over time for each pH treatment in each age class. Pre-incubation pH is noted at the top of panels for each age class. Soil pH was measured in weeks 1, 2 and 5.

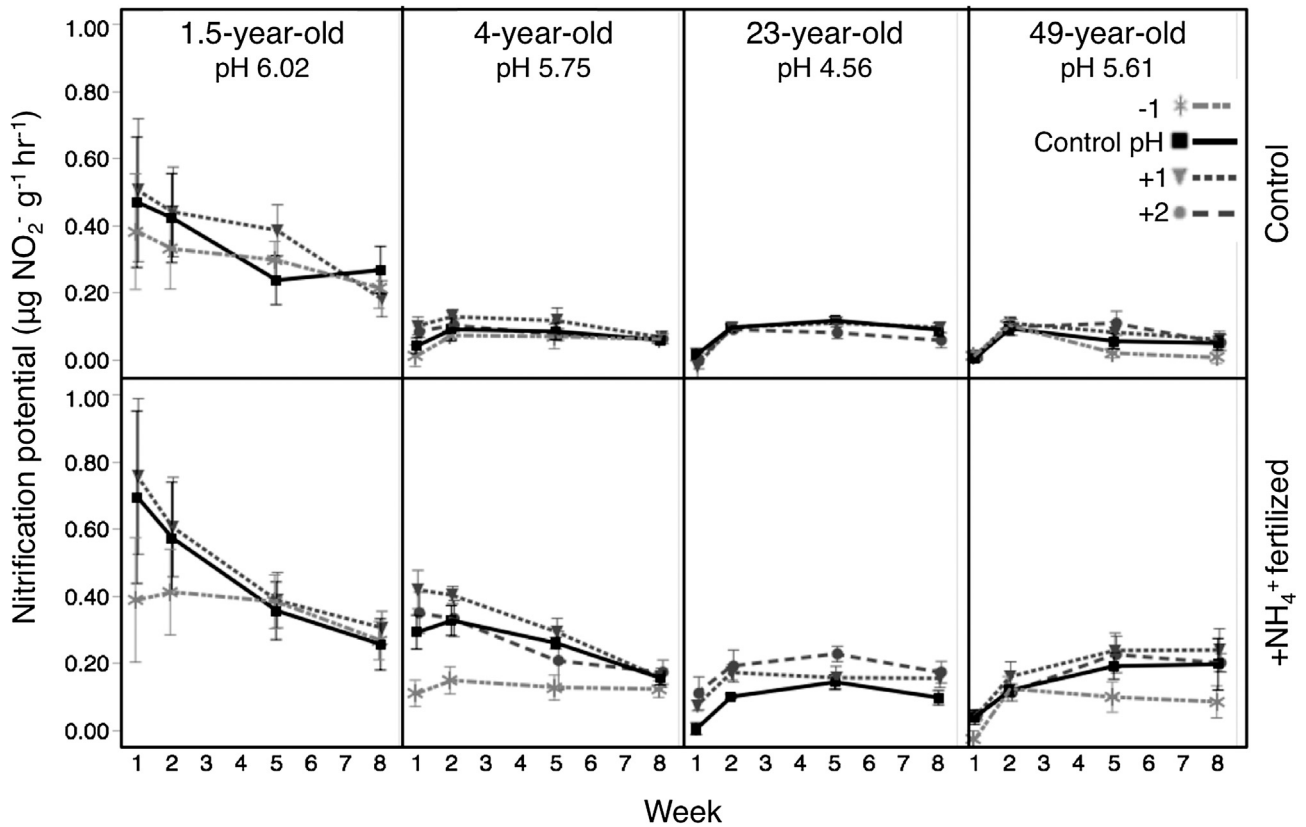


Fig. 6. Nitrification potential for all treatments over the course of incubation. Panels left to right represent each age class, while top and bottom panels separate N-fertilized treatments from controls. Native pH is noted at the top of panels for each age class. Nitrification potential was measured in weeks 1, 2, 5, and 8. pH treatments are displayed as separate lines in each panel. Char treatments are excluded from the figure since char had no significant effect on nitrification potential.

Table 2
Soil pH after 5 weeks of incubation with and without char addition.

Watershed	Age (years since fire)	Average pH in control soils	Average pH in control + char
Maria Ygnacio (Painted Cave)	1.5	6.00	5.98
	23	4.59	4.67
Rattlesnake	4	5.84	6.23
	49	5.98	6.14

was slightly higher in soils amended with char than in untreated controls, particularly in the 4- and 49-year old sites from Rattlesnake Canyon (Table 2). Char may also shield nitrifiers from toxins by adsorbing phenolic compounds such as terpenes, which might otherwise inhibit nitrifying bacteria (Thibault et al., 1982). However, it is more likely that polyphenols limit nitrification by providing C to heterotrophic microbes and/or binding substrates, thereby increasing N-immobilization (Sugai and Schimel, 1993; Souto et al., 2000; Fierer et al., 2001). Thus, char can stimulate nitrification by binding these polyphenolic compounds (DeLuca et al., 2002, 2006). Although we did not find strong evidence that char influenced nitrification in our eight-week incubation, there was some indication that it stimulated nitrification in the most recently burned (1.5-year-old) plots (Fig. 4e). In addition to char, the smoldering of organic matter can produce toxic substances that reduce microbial activity (Díaz-Raviña et al., 1996; Fritze et al., 1998), which might in turn reduce the extent to which heterotrophs compete for NH_4^+ , thereby stimulating nitrification.

On the other hand, nitrification can be inhibited if char supplies C to heterotrophic microbes, which might in turn compete with nitrifiers for NH_4^+ . Evidence for this mechanism is the sustained microbial biomass C relative to control and fertilized treatments in soils from mature (23- and 49-year-old) sites. In these soils, microbial biomass appeared to be C-limited. In more extreme cases, such as close to burned stumps or in localized char hotspots, heterotrophic biomass may increase to the extent that N-immobilization begins to inhibit nitrification. It should be noted that char was added to our incubations in low concentration to mimic realistic loads added from a single fire integrated over the entire plot. But perhaps in some places char might accumulate enough to affect C and N dynamics more substantially. There it might help sustain

microbial biomass while chaparral canopy and litter layers recover, given that it can persist in the soil for centuries (Seiler and Crutzen, 1980).

5. Conclusions

Overall, it appears that nitrification in chaparral is most powerfully constrained by substrate supply and pH. These factors may work independently, or amplify one another to stimulate and sustain nitrification through the early stages of recovery (Fig. 7). We found that when NH_4^+ was sufficiently high, pH determined the relative proportion of inorganic N that was nitrified. Although char did not influence inorganic N, it did appear to help sustain microbial populations in mature sites, suggesting that char may supply C to soil heterotrophs in some circumstances. In general however, fires substantially reduce heterotrophic microbial biomass (DeLuca et al., 2002; Hanan, unpublished), an effect which may be long-lasting because microbial populations can take many years or even decades to recover (Grasso et al., 1996; Choromanska and DeLuca, 2002). Suppressed heterotrophic biomass might allow nitrifiers to respond over longer timescales to short-term N-pulses and pH increases that occur immediately after fire. Because increases in substrate supply, elevated pH, and suppressed heterotrophic biomass interact and trade off to enhance nitrification postfire, N-uptake by recovering plants must play an important role in limiting leaching and gaseous N losses, and in maintaining long-term N capital in chaparral and other fire-prone ecosystems.

Acknowledgments

Thank you to Ken Marchus, Fred Navarro, Miguel Casermeiro, Sadie Iverson, Caryl Becerra, Joey Blankinship, Benjamin Boyce, Daniel Berg, Theo Tzeng, and Stephanie Ma for field and laboratory assistance. Jennifer King and Keri Opalk for laboratory instruments and instruction. We also thank the editor and anonymous reviewers for their constructive comments, which helped improve this manuscript. This project was supported by the Natural Hazards Fellowship from the California Institute for Research on Hazards, the Leal Anne Kerry Mertes Scholarship from the Department of Geography at UC Santa Barbara, and the Santa Barbara Coastal Long-Term Ecological Research project (OCE-0620276).

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2015.12.017>.

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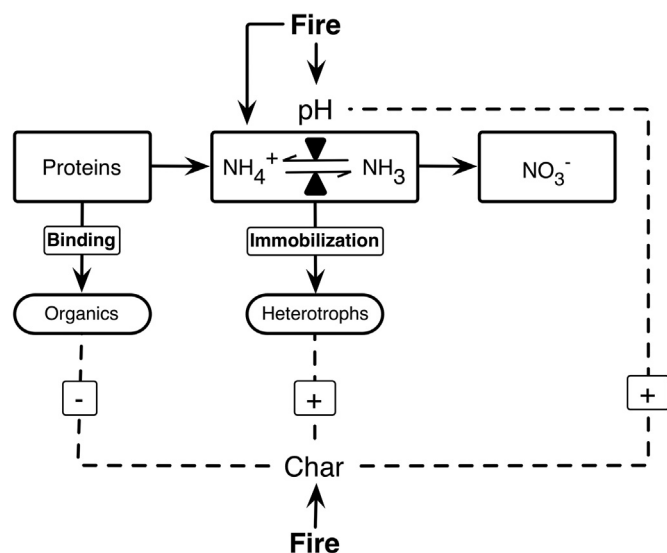


Fig. 7. Controls over nitrification following fire: enhanced nitrification post-fire may result from a combination of enhanced substrate supply, elevated pH, and/or char deposition.

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