UNIVERSITY OF CALIFORNIA, MERCED

Soil microbial ecology of the Sierra Nevada: Predictions for a warm and fiery future

A dissertation submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy

in

Environmental Systems

by

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Publications

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Dove, N.C., J.M Stark, G.S. Newman, and S.C. Hart (2019) Carbon control on terrestrial ecosystem function across contrasting site productivities: the carbon connection revisited. *Ecology* 100(7):e02695.

Cheng, H. **N.C. Dove**, J.M. Mena, T. Perez, S. Ul-Hasan. (2018) The Biota Project: A Case Study of a Multimedia, Grassroots Approach to Scientific Communication for Engaging Diverse Audiences. *Integrative and Comparative Biology*. DOI: 10.1093/icb/icy091

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Carey, C., **N.C. Dove**, J.M. Beman, S.C. Hart, E. L. Aronson. (2016) Meta-analysis reveals ammonia-oxidizing bacteria respond more strongly to nitrogen addition than ammonia-oxidizing archaea. *Soil Biology and Biochemistry*, 99, 158-166

Dove, N. C. and W.S. Keeton. (2015) Structural Complexity Enhancement increases fungal species richness in northern hardwood forests. *Fungal Ecology*, 13, 181-192.

Presentations

Dove, N.C., Taş, N., Hart, S.C. (Oral – *invited*) Soil microbial ecology of the Western US: Predications for a warm and fiery future. Yosemite Forum – Yosemite National Park April 10, 2018

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Dove, N.C., Hart, S.C. (Poster) Novel, high-severity fire influences microbial communities and biogeochemical processes: opening the "charcoal" box. Dept. of Energy Joint Genome Institute User Meeting. March 21, 2017

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University of Vermont, Carbon Dynamics Laboratory 2011-2013
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Professional Experience

Intern, Bureau of Land Management; Buffalo, WY May 2013-March 2014
Assessed the efficacy of wildfire restoration treatments using standard vegetation surveys. Also, designed and planned a native seed propagation facility, which is now in operation, to use for restoration practices.

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Outreach

Science Lead & Principal Writer, BIOTA 2015-present
BIOTA is a web documentary series aimed at communicating science to underrepresented demographics through film, art, and music. As Science Lead and Principal Writer, I am in charge of designing stories to document and managing a team of undergraduate students who identify and interview scientists for our stories.

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Community Initiatives for Collective Impact (CI4CI) is a non-profit started by myself and two others to fiscally sponsor non-profit activities in California's Central. While most major cities have resources for non-profit startups, rural, economically-depressed areas (where the need is greatest) are often overlooked. CI4CI aims to change this.

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Abstract of the Dissertation

Soil microbial ecology of the Sierra Nevada: Predictions for a warm and fiery future

by

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Doctor of Philosophy, Environmental Systems Program
University of California, Merced, 2019
Dr. Stephen C. Hart, Chair

Forest ecosystems of the Sierra Nevada are currently responding and are predicated to continue to respond to multiple global change stressors. In particular, increased temperatures and wildfire severity are likely to impact the health of these ecosystems and the services they provide, such as soil carbon (C) sequestration. However, the magnitude of these disturbances on microbial communities and their function, as well as their resilience, is still unclear. Using a combination of field, laboratory, and meta-analytical methods, I demonstrated that the soil environment, microbial communities, and their biogeochemical function are dramatically altered by these global change stressors and the resilience of these microbial communities is diminished compared to global averages. Specifically, I showed that soil fungal communities (fungal species richness and mycorrhizal colonization) respond negatively to fire, but the response is mediated by fungal guild, method of measurement, and time since fire (soil fungi recover from fire after one to two decades). I then compared this global baseline of fire recovery to soils recovering from an ecologically novel, high-severity fire in the Sierra Nevada. I found that the biogeochemistry of these soils (which fungi in part control) is still significantly altered 44 y post-fire, highlighting reduced resilience following ecologically novel disturbances. In particular, soil C in the mineral topsoil 44 y after fire was half that of the late successional control site. I also showed how another global change stressor, increase soil temperatures, affects microbial processes in a 4.5 y whole-profile warming experiment in the central Sierra Nevada. I found that 4.5 y of +4 °C warming affects microbial community composition, metabolism, and function throughout the soil profile, but that the response in the subsoils was somewhat muted. This suggests that subsoil microbial communities will take longer to acclimate to increase temperatures, possibly reducing their ability to efficiently assimilate and sequester C. Taken together, these findings have important implications for the microbial ecology and C cycling in Sierra Nevada soils such that future soil C sequestration will likely decrease if these disturbances continue to impact these ecosystems unabated.

1 Introduction

Forest soils provide numerous ecosystem services, such as nutrient retention and supply, carbon sequestration, and water storage (Binkley and Fisher 2012). Therefore, maintaining the health of forest ecosystems is imperative to human systems. However, these ecosystems are threatened by multiple global change stressors, including altered climate (Millar et al. 2016), increased fire frequency and severity (Westerling et al. 2006, Miller et al. 2009), and deforestation (Shukla et al. 1990), which have the potential to disrupt the biogeochemical cycling of soils and the health of forests. The mixed conifer-yellow pine forests of the Sierra Nevada of California, USA are no exception. These forests are particularly vulnerable to disturbance because of their Mediterranean-type climate, where hot, dry summers leave these forest susceptible to drought- and fire-induced mortality events (Crockett and Westerling 2017, Fettig et al. 2019). While the effects of global change stressors on the aboveground Sierra Nevada plant and animal communities have been well-documented (Steel et al. 2018), relatively less attention has been devoted to the belowground biotic communities (e.g., fungi, bacteria, and archaea), which underpin the biogeochemical cycles of earth and the health of forest ecosystems.

This dissertation focuses on two global change stressors of particular importance to the Sierra Nevada and, more broadly, the forests of western North America. Frequent, low-severity fire is an important process in many forest ecosystems (Safford and Stevens 2017, Miller and Safford 2017). However, due to the interaction between increasing forest fuels and the rapidly warming climate, many contemporary fires in western North America are currently burning at higher severities than their pre-1850 counterparts (Miller et al. 2009). Simultaneously, soils are predicted to warm 4 °C over the course of this century (IPCC 2014). A key question then is: how will the microbial communities and biogeochemical function respond to these disturbances?

Although fire effects on soil microbial communities have been reviewed qualitatively (Hart et al. 2005b, Cairney and Bastias 2007), patterns of the microbial response to fire have been idiosyncratic across studies, and general trends have been difficult to determine. To better understand general patterns of the microbial response to

fire across ecosystems, I conducted a quantitative meta-analysis focusing on the fungal component of microbial communities, namely the response of fungal species richness and mycorrhizal colonization (Chapter 2, published in *Fire Ecology* in 2017). Given that fungal species richness is an important predictor of decomposition rates (see review: van der Wal et al. 2013) and that mycorrhizae contribute large portions of plant nutrient and water acquisition (Smith and Read 2008), these two metrics have important ecosystem implications. Across all studies, I found that fire reduced fungal species richness by 28% and *in situ* mycorrhizal colonization by 21%. Furthermore, our meta-analysis revealed broad-scale patterns across modifying variables (e.g., biome, measurement method, etc.) that to-date have been hard to obtain with single-site, independent studies. Using continuous meta-regressions with time since fire as a predictor variable, I found that both fungal species richness and *in situ* mycorrhizal colonization approached zero (i.e., no effect) on decadal time-scales. I concluded that fire reduces fungal species richness and *in situ* mycorrhizal colonization, but if conditions allow communities to recover (e.g., without subsequent disturbance, favorable growing conditions), soil fungi are resilient to fire on decadal time scales, which likely contributes to overall ecosystem recovery.

With increasing fire severity, however, there is likely to be an exacerbated response and lengthened recovery of soil microbial communities and biogeochemical processes compared to historical (low-severity) fires, because of the altered selective pressures that soil microorganisms have been evolutionarily adapted to (e.g., heat resistance, carbon and nutrient availability). Therefore, I evaluated the long-term (40+ years) impact of ecologically novel, high-severity fire on soil carbon and nitrogen cycling using a wide array of biogeochemical assays and rate measurements using space for time substitution (Chapter 3, in revision at *Ecological Application*). Contrary to previous work studying fires within the natural range of variation (including many of the papers in the meta-analysis of Chapter 2), I showed that ecologically novel, high-severity fires have long-term (40+ years) effects on numerous biogeochemical properties and processes with consequences for stand recovery and carbon sequestration.

The impact of soil warming, while less of an acute disturbance, has the potential to dramatically alter soil carbon and nitrogen cycling through effects on the microbial community. Unlike increasing fire severity, the disturbance of increasing soil temperatures is expected to be ubiquitous across terrestrial landscapes, and has been studied extensively (Conant et al. 2011). However, even though warming increases nutrient availability (Rustad et al. 2001) and decreases carbon availability (Melillo et al. 2011), it is still unclear how this shift in resource stoichiometry interacts with warming to impact microbial communities. To address this question, I leveraged a 4.5 year whole-profile soil warming experiment (+4 °C) in the Sierra Nevada and conducted a series of resource amendment (carbon, nitrogen, and phosphorus) incubations (Chapter 4, in preparation for *Nature Microbiology*). I found that 4.5 years of warming significantly impacted the microbial community composition, particularly in topsoils. Furthermore, this change may in part be due to altered resource stoichiometry. Heated soils showed relatively greater carbon limitations, while unheated control soils had relatively greater nitrogen and phosphorus limitations. In the future, such limitations could dampen the increased respiration response in a warmer world.

The overall goal of this dissertation is to elucidate the impact of increasing fire severity and soil warming on microbial communities and facilitate the prediction of the microbial response. Through better understanding and prediction, forest managers of the Sierra Nevada, and similar forests elsewhere, will be better equipped to mitigate against and respond to such global change stressors and their impact on forest ecosystems.

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2 Fire reduces fungal species richness and mycorrhizal colonization: a meta-analysis

2.1 Abstract

Soil fungal communities perform many functions that help plants meet their nutritional demands. However, overall trends for fungal response to fire, which can be especially critical in a post-fire context, have been difficult to elucidate. We used meta-analytical techniques to investigate fungal response to fire across studies, ecosystems, and fire types. Change in fungal species richness and mycorrhizal colonization were used as the effect size metrics in random effects models. When different types of methods for assessing fungal species richness and mycorrhizal colonization were considered together, there was an average reduction of 28% in fungal species richness post-fire but no significant response in mycorrhizal colonization. In contrast, there was a 41% reduction in fungal species richness post-fire when assessed by sporocarp surveys, but fungal species richness was not significantly affected when assessed by molecular methods. Fire reduced mycorrhizal colonization measured *in situ* by 21%, yet no significant response occurred when assessed by *ex situ* bioassays. These findings suggest that the putative magnitude of fire effects on soil fungal communities may be dependent on the approach and assessment method used. Furthermore, biome, but not fire type (i.e., wildfire vs. prescribed fire) was a significant moderator of our categorical models, suggesting that biome might be a more useful predictor of fungal species richness response to fire than fire type. Reductions in fungal species richness and *in situ* mycorrhizal colonization post fire declined logarithmically and approached zero (i.e., no effect) at 22 and 11 years, respectively. We conclude that fire reduces fungal species richness and *in situ* mycorrhizal colonization, but if conditions allow communities to recover (e.g., without

subsequent disturbance, favorable growing conditions), soil fungi are resilient on decadal time scales; the resiliency of soil fungi likely contributes to the overall rapid ecosystem recovery following fire.

2.2 Introduction

A common goal of ecosystem management is the restoration and maintenance of critical ecological functions. Many of these processes, including decomposition, nutrient mineralization, and resource acquisition by plants, are moderated by soil fungi (Hobbie and Horton 2007, Baldrian et al. 2012, Phillips et al. 2013). Disturbance by wildfire is widespread globally among terrestrial ecosystems, affecting both above- and below-ground biotic communities, particularly soil fungi (Bond et al. 2005, Bond and Keeley 2005). The direct effects of extreme temperatures from fire in the upper soil horizons can cause drastic changes in the fungal community even though heat from fire generally only impacts surficial soil layers (DeBano 2000). This preferential sensitivity of fungi (compared to other soil microorganisms) to this form of ecosystem disturbance stems from both fungal intolerance to heat (Dunn et al. 1985, Izzo et al. 2006) and their greater abundance in surficial organic (O) horizons and the upper mineral soil (Baldrian et al. 2012). These direct and selective impacts of fire on soil fungi can potentially alter important ecosystem processes that fungi mediate.

Fungi are also impacted by wildfire through indirect effects on soil properties, which may permeate into deeper soil layers (Jones et al. 2003). For instance, fire generally: decreases canopy cover (due to tree mortality), thus increasing soil insolation (Ballard 2000); decreases surface albedo (by the blackening of soil), thus increasing the relative amount of absorbed shortwave radiation; and decreases the thickness, and thus insulation of the O horizon due to combustion (Hart et al. 2005b). These indirect effects of fire on the heat balance of soil can alter soil temperature regimes (Binkley and Fisher 2012). Additionally, hydrophobic soil layers are frequently formed by the partial combustion of organic matter (DeBano 2000), which leads to decreased water infiltration and altered soil hydrology. Changes in soil temperature and moisture may affect the phenology of fungal fruiting (Straatsma et al. 2001), mycorrhizal infectivity (Parke et al. 1983), and overall fungal activity (Hamman et al. 2007). Increases in nutrient availability post-fire are also likely a driver of fungal community dynamics (Anderson and Menges 1997, Treseder 2004, Bastias et al. 2006). These complex indirect effects of fire on soil physiochemical characteristics, combined with the direct heating effects, make it difficult to generalize about fire influences on soil fungal communities from individual studies.

Changes in aboveground vegetation may have the greatest impact on soil fungal communities in the later stages of ecosystem recovery (Hart et al. 2005b). Many ectomycorrhizal (ECM) species show some degree of host-specificity (Smith and Read 2008); thus, changes in the presence of certain host trees following fire may substantially affect the ECM community. Similarly, decreased woody canopy cover and increased graminoid density following fire can elevate arbuscular mycorrhizal (AM) fungal abundances compared to ECM fungi (Korb et al. 2003). Fire-induced changes to carbon (C) inputs could also alter soil saprobic fungal communities. For instance, increased

herbaceous C inputs and reduced lignin-rich woody litterfall post-fire (Kaye et al. 2005) could decrease the relative abundance of white-rot fungi (within the class *Agaricomycetes*) that uniquely produce lignin-degrading enzymes (Hanson et al. 2008, Floudas et al. 2012, Treseder and Lennon 2015). Clearly, above- and below-ground organismal communities are inextricably linked, such that the succession of fungal communities post-fire mimics, at least to some degree, that of plant communities (Frankland 1998). These changes may be long-lived, especially if fire induces significant plant mortality.

Inconsistencies in results from individual studies have hindered the ability to make general conclusions about possible linkages among fire, fungi, and ecosystem function. For example, studies have shown that wildfire can have negative (Visser 1995, Martín-Pinto et al. 2006, Hernández-Rodríguez et al. 2013, Motiejūnaitė et al. 2014), neutral (Jonsson et al. 1999, Mah et al. 2001, Chen and Cairney 2002), or positive (Hewitt et al. 2013) effects on fungal diversity. Similarly, many studies have found an overall decrease in mycorrhizal colonization post-fire (Dhillion et al. 1988, Rashid et al. 1997, Barker et al. 2013), while other studies have found no effect (Eom et al. 1999) or even increased colonization following fire (Herr et al. 1994, Rincón et al. 2014). Resolving these inconstancies in fungal response to fire should increase our understanding of decomposition, nutrient cycling, and productivity in post-fire landscapes because of the close coupling between fungi and ecosystem function. For instance, AM species richness strongly controls plant productivity in grassland ecosystems (Gange et al. 1993, van der Heijden et al. 1998, Vogelsang et al. 2006). In deciduous forests, *Betula* plant nutrient concentrations and productivity are positively correlated with increasing ECM species richness (Jonsson et al. 2001, Baxter and Dighton 2001). Furthermore, laboratory experiments show that species richness of saprobic fungi positively influence decomposition in species-poor environments or on recalcitrant organic substrates (both commonly created in post-fire environments; (Setälä and McLean 2004, van der Wal et al. 2013). Differences in mycorrhizal colonization can also have profound impacts on nutrient cycling by influencing nutrient acquisition by their plant hosts (Smith and Read 2008), and changes in the relative proportions between ECM and AM colonization following fire may impact the rates of cycling of these limiting nutrients (Phillips et al. 2013). Clearly, a more unified understanding of how fire influences soil fungal communities would help improve our ability to predict changes in ecosystem function following such disturbances.

These apparent idiosyncratic responses of soil fungi to fire are often attributed to differences in fire severity among studies (Dahlberg et al. 2001, Smith et al. 2004, Román and Miguel 2005, Cairney and Bastias 2007), and the degree to which the disturbance frequency and intensity are within the historic range of variability (Hart et al. 2005b). Fire intensity and severity are well-correlated to the amount of combustible fuels (Rothermel 1972, Binkley and Fisher 2012), which is influenced by a suite of factors, including: the ecosystem type or biome, land management practices, and fire type (i.e., wildfire vs. prescribed fire). These factors may covary with the response of soil fungal communities to disturbance by fire through their influences on fuel loading and possibly other mechanisms (e.g., fuel continuity, fuel combustibility, etc.; (Rothermel 1972, Scott and Burgan 2005).

Differences in fungal response to fire may be simply an artifact of the methods used to evaluate fungal communities. It is well-known that fungal communities assessed aboveground (i.e., sporocarps) rarely correspond to their belowground counterparts (Dahlberg et al. 1997, Jonsson et al. 1999, Horton and Bruns 2001, Fujimura et al. 2004). This is likely due in part to differences in sampling intensity (Horton and Bruns 2001), but may also reflect physiological and phenological differences in the fruiting frequencies of different fungal species. Similarly, the estimates of the response of mycorrhizal colonization of plant roots to fire may differ depending on methodology. For example, while fire probably reduces mycorrhizal inocula and host density (Hart et al. 2005b), mycorrhizal colonization assessed using *ex situ* bioassays, which measure colonization potential in the presence of suitable hosts, may not reflect *in situ* mycorrhizal colonization where host species or abundance may be limiting (Perry et al. 1987). Given the myriad of factors that can influence results from individual studies, the application of a robust, quantitative, and synthetic analysis of fire-fungal relationships may help identify characteristic fungal responses to fire and thus help predict associated changes in ecosystem function.

We used meta-analytical techniques to synthesize the saprobic and mycorrhizal fungal community response to fire across wildland ecosystems, fire types, and assessment methods. The lack of studies on the pathogenic or parasitic fungal community response to fire precluded the incorporation of these guilds in our analysis. Using this quantitative approach, we tested the following hypotheses: 1) fire causes an overall reduction in fungal species richness; 2) fire results in an overall reduction in mycorrhizal colonization of plant roots; 3) the apparent impact of fire on soil fungi is influenced by several moderating variables including the fungal guild studied (e.g., AM, ECM, wood-inhabiting fungi, and culturable microfungi), method of measurement, fire type (e.g., wildfire or prescribed fire, a single fire event or repeated fire), and biome; and 4) the impact of fire on fungal communities diminishes with time since fire. Our overarching goal was to elucidate previously unidentified trends and factors that contribute to post-fire ecosystem resilience by combining the results from all known studies of fire effects on fungal communities into a single set of statistical analyses.

2.3 Methods

2.3.1 Sources of data

Institute for Scientific Information (ISI) Web of Knowledge (now Clarivate Analytics Web of Science®) and Google Scholar databases were searched for field experiments studying the effects of fire on soil fungal communities using key words such as fung*, fire, wildfire, burn, diversity, richness, and colonization. We used "cited-by" functions from relevant studies to find related papers. Studies were collected for analysis until April 21, 2016. We focused on studies reporting fungal species richness and mycorrhizal colonization in burned versus unburned control treatments, rather than pre- versus post-fire to control for temporal variations in richness and colonization. Although this risks assigning treatment effects to spatial variation, many wildfire studies are

conducted post-hoc, without pre-fire samples. Therefore, we decided to standardize our data collection by using unburned sites as our control rather than a mix of unburned and pre-fire sites as controls. If an unburned control did not occur, then we used a pre-fire sample as a control, which occurred only in three cases (Olsson and Jonsson 2010, Goberna et al. 2012, Glassman et al. 2015). If a study used a chronosequence without a control, then the latest date of the chronosequence was used as the pre-fire sample. This situation also occurred only in three studies (Treseder et al. 2004, Holden et al. 2013, Sun et al. 2015), and the latest dates were all at least 100 year old boreal forests. Because our objective was to focus on field-relevant, ecosystem-level implications of fire alone, we excluded laboratory burning simulations and combination thin-burn treatments. Additionally, we limited our analysis to studies with reported replication ($n \geq 2$) and mean fungal species richness or mycorrhizal colonization. If studies reported Shannon's diversity index (H) and evenness (E), but did not report richness (S; e.g., (Martín-Pinto et al. 2006), we used the following equation to derive species richness:

$$S = \exp(H)/E$$

One of the assumptions of meta-analyses is that each study is independent of the others (Gurevitch and Hedges 1999). Therefore, we only used one data record (nearest to the conclusion of the fire; time = 0) for studies that employed repeated measures from the same experimental unit. However, we assumed independence between time points for studies that also assumed independence between time points (e.g., a fire chronosequence). Although this opens the potential to bias results towards individual studies, no study dominated the dataset (Tables 2-1 & 2-2), and relaxing the condition of one record per study allowed the dataset to double in size, increasing the robustness of the meta-analysis.

2.3.2 Data acquisition

The mean fungal species richness or mycorrhizal colonization and number of replicates for both burn and control treatments were recorded. Additionally, for the fungal species richness meta-analysis, we noted the fungal guild studied (e.g., ECM fungi, AM fungi, wood-inhabiting fungi, or culturable microfungi), method of measure (e.g., next-generation sequencing, sporocarp survey, spore morphology, hyphal morphology, and culture morphology), fire type (wildfire or prescribed fire; repeat [<15 y] or single fire event), biome, and time since fire. Only three biomes (e.g., temperate forest, boreal forest, or temperate shrubland/grassland) contained sufficient replication ($n \geq 2$) to be included in the meta-analysis (Table 2-1). We combined temperate shrublands and grasslands into the same biome category because of an insufficient number of studies in each of these biomes for robust inter-biome statistical comparisons (two for temperate shrubland and one for temperate grassland). We justify this grouping because both have relatively low aboveground biomass and a higher frequency of fire disturbance than boreal or temperate forests (Chapin et al. 2011). Only one biome classification was ambiguous (Xiang et al. 2015), but we used the reported dominant vegetation (*Larix* spp.) along with mean annual temperature (-4.7° C) and precipitation (500 mm) to classify the

study site as a boreal forest (Whittaker 1970). For the mycorrhizal colonization meta-analysis, we recorded the fungal guild studied (e.g., ECM fungi or AM fungi), whether the response was assessed *in situ* or using *ex situ* bioassays (i.e., method; *in situ* bioassays were excluded from analysis due to lack of studies), fire type (wildfire or prescribed fire; repeat [<15 y] or single fire event), biome (e.g., temperate forest, boreal forest, and temperate grassland), and time since fire. We included all measures of mycorrhizal colonization for our analysis including percent colonized seedlings, percent root tips colonized, and percent root length colonized. When means were presented in graphical format, we used Web Plot Digitizer 3.5 to extract data (Rohatgi 2014).

2.3.3 Statistical analysis

Random effects models were used to determine the significance of fungal species richness and mycorrhizal colonization response to fire. All cumulative and categorical analyses were conducted in MetaWin 2.1 (Rosenberg et al. 1997), and continuous analyses were conducted in R (R Development Core Team 2008) using the Metafor package (Viechtbauer 2010). We used R for the continuous analyses because MetaWin does not report R^2 or Akaike information criterion (AIC) statistics, which allows for the statistical comparison between models (i.e., linear versus logarithmic meta-regression).

The effect size was calculated as the natural log of the response ratio (ln[*R*]). The response ratio (*R*) is the mean of the treatment response divided by the mean of the control ($R = X_{treatment}/X_{control}$ (Hedges et al. 1999). For example, if ln[*R*] = 0, then there is no treatment effect. Post-analysis, effect sizes were converted to percent difference using the equation:

$$Difference\ (\%) = 100*(1-\exp(\ln(R)).$$

We weighted the effect sizes by the number of replicates (*n*) instead of the inverse variance (as is common in some meta-analyses) because many studies did not report standard deviation (SD) or standard error (SE) of the mean. We assumed that effect size records with higher replication were a stronger estimate of the population mean. Making this assumption allowed us to maximize sample size and improve the robustness of our analysis.

A random effects model was used to determine if $\ln[R] \neq 0$ (i.e., fire had a significant effect). We calculated bias-corrected bootstrap 95% confidence intervals (CIs) for each mean ln[*R*]. If CIs did not overlap with 0, then effects were considered significant at the $\alpha = 0.05$ level. Additionally, we used categorical random effects models to compare responses to fire among fungal guilds, methods of measurement, fire types, and biomes. If the categorical model showed significant differences among groups ($\alpha = 0.05$), then CIs were used to interpret multiple comparisons of group means; if CIs did not overlap, then groups were considered significantly different.

Continuous random effects models (meta-regressions) were conducted to determine if effect size varied with time since fire. Following (Aloe et al. 2010), we report R^2_{Meta} values rather than traditional R^2 based on ordinary least squares (OLS), because the assumption of equal variances needed for OLS does not hold in meta-regression (i.e., effect sizes are weighted). The statistic R^2_{Meta} describes the proportional

reduction in the amount of heterogeneity in the model after including moderators, and it is useful for interpreting the practical significance and comparing the fit of competing meta-regression models (López-López et al. 2014).

2.4 Results

2.4.1 Fire Effects on Fungal Species Richness

Overall, 68 records across 29 studies were considered suitable for meta-analysis (Table 2-1; Figure 2-1). Across studies, fire significantly reduced fungal species richness by an average of 28% (95% CI: -35 to -20%; Figure 2-2a). Additionally, heterogeneity within studies was not statistically significant ($Q_T = 68.3$, $P = 0.434$), indicating that fungal species richness responses to fire were consistent even though individual studies may not have had a significant effect (i.e., CIs encompassing 0). Our meta-analysis incorporated studies that investigated fire effects on different fungal guilds (Table 2-1). All guilds assessed except for wood-inhabiting fungi (WIF) showed significant negative response to fire (Figure 2-2a). The overall categorical model found marginally statistically significant differences among guilds ($P = 0.080$).

Six different methods of measuring fungal species richness were analyzed in the meta-analysis (Table 2-1). Negative response to fire was apparent for all measurement methods except next-generation sequencing (Figure 2-2b), and the model found significant differences among groups ($P = 0.002$). Richness assessed using culture morphology and sporocarp surveys showed the greatest response to fire, with average reductions of 66% (95% CI: -85 to -34%) and 41% (95% CI: -59 to -35%), respectively.

Repeat burning (within 15 years) reduced the negative effect on fungal species richness by almost half compared to single burns (average response of -18% and -30%, respectively), but this difference was not statistically significant ($P = 0.274$). This lack of statistical significance may be due to low statistical power given the few studies (n = 10) that have assessed the impacts of repeated burning on fungi (Figure 2-2c). Similarly, we were unable to detect a significant difference between wildfire and prescribed fire ($P = 0.603$). Nevertheless, we did find significant differences in fungal species richness response to fire across biomes ($P = 0.010$; Figure 2-2d), with temperate shrublands/grasslands showing the greatest mean reduction (95% CI: -80 to -23%) and boreal forests showing a non-significant reduction (95% CI: -35 to 2%).

We found a statistically significant and positive logarithmic correlation between the response ratio of fungal species richness and time since fire ($\ln[R] = 0.1976 \times \ln[\text{years since fire} + 1] - 0.62$, $R^2_{Meta} = 0.999$, $P < 0.001$; Figure 2-3). At time = 0, the mean reduction in fungal species richness was calculated as -46% (SE = 7%, $P < 0.001$), and the negative effect size was reduced logarithmically, crossing zero (i.e., no effect) at year 22.

2.4.2 Fire Effects on Mycorrhizal Colonization

Fifty-one records across 24 studies were used for our meta-analysis of the fire effects on mycorrhizal colonization (Table 2-2). Across all studies and records, mycorrhizal colonization post-fire was not significantly affected by fire (95% CI: -20 to 1%; Figure 2-4a). Heterogeneity was not statistically significant ($Q_T = 46.9$, $P = 0.600$), indicating that mycorrhizal colonization responses to fire were consistent even though individual studies may have had a significant effect. When analyzed separately by mycorrhizal type (i.e., ECM and AM), no significant post-fire effect was found for either type (Figure 2-4a). Additionally, the effect of fire on mycorrhizal colonization was not statistically significantly different among fire types or biomes (Supplementary Table 2-1).

There was a significant difference between fire effects on mycorrhizal colonization measured *in situ* and in *ex situ* bioassays ($P = 0.006$; Figure 2-4b). Mycorrhizal colonization was reduced on average by 21% following fire (95% CI: -36 to -2%) when assessed *in situ*, while a non-significant 11% increase (95% CI: -3 to 29%) in post-fire mycorrhizal colonization was observed when using *ex situ* bioassays. Due to this difference, categorical models (guild, unit of measurement, fire type, and biome) were re-analyzed using only *in situ* measured records to determine if categorical differences would then emerge; however, statistical significance for each categorical model remained unchanged (Supplementary Table 2-2).

Similar to our results for fungal species richness, we found a statistically significant and positive logarithmic correlation between the response ratio for mycorrhizal colonization and time since fire ($\ln[R] = 0.1588 \times \ln[\text{years since fire} + 1] - 0.2971$, $R^2_{Meta} = 0.31$, $P = 0.003$, Figure 2-5). At time = 0, mean reduction in mycorrhizal colonization was calculated as -26% (SE = 10%, $P = 0.003$), and the negative effect size was reduced logarithmically crossing zero (i.e., no effect) at year 5. Because mycorrhizal colonization assessed *in situ* showed a significantly greater negative response to fire than methods using *ex situ* bioassays, we ran a separate continuous model that included method as a predictor variable. In this model, both method ($P = 0.019$) and time ($P = 0.005$) were significant moderators of mycorhizal colonization response to fire ($R^2_{Meta} = 0.45$; $P = 0.001$). Furthermore, using data from only studies that measured mycorrhizal colonization response to fire *in situ*, we found that at time = 0, mean reduction in mycorrhizal colonization was calculated as -37% (SE = 12%, $P = 0.003$). The negative effect size was reduced logarithmically, crossing zero (i.e., no effect) at year 11 ($\ln[R] = 0.1870 \times \ln[\text{years since fire} + 1] - 0.4646$; $R^2_{Meta} = 0.32$; $P = 0.011$; Figure 2-5). Time was not a significant predictor of mycorrhizal colonization response to fire for studies that used *ex situ* bioassays ($P = 0.267$).

2.5 Discussion

2.5.1 Fire Effects on Fungal Species Richness

The meta-analytical model supported our hypothesis that fungal species richness is negatively impacted by fire. Fire likely eradicates fungal species that cannot withstand intense heat, reducing species richness to those species that have the ability to survive fire through fire-resistant propagules (Horton et al. 1998, Baar et al. 1999). Furthermore, physiochemical changes in the soil environment and shifts in vegetation composition following fire likely select for species able to best compete under fire-altered conditions (Hart et al. 2005b, Cairney and Bastias 2007). Given that fungal diversity generally is positively related to decomposition rates (Setälä and McLean 2004, van der Wal et al. 2013) and aboveground productivity (van der Heijden et al. 1998), reduced fungal species richness likely contributes to the decreases in these ecosystem processes commonly observed post-fire (Dore et al. 2010, Holden et al. 2013, Toberman et al. 2014). However, the magnitude of this response probably also depends on the functional redundancy of the soil microbial community, where functions that are performed by many species are not altered by differences in diversity (Nielsen et al. 2011).

The impact of fire on fungal species richness varied across fungal guilds, indicating that fire affects soil fungi differentially within terrestrial ecosystems. Although the overall categorical model did not suggest a difference in species richness among fungal guilds post-fire, the species richness in all individual guilds except for WIF was negatively impacted by fire (denoted by negative 95% CIs that did not overlap with 0). Wood-inhabiting fungi may have responded differently to fire because fire may have increased the variety of habitats (i.e., niches) available for this fungal guild compared to the other fungi. For instance, depending on the severity of fire, downed coarse woody debris (DCWD) may increase, and partial charring of wood may increase overall surface area for fungal colonization within this material (Pietikäinen et al. 2000). Many studies have found that DCWD availability positively correlates with WIF diversity (Nordén et al. 2004, Abrego and Salcedo 2013, Persiani et al. 2015), and experimentally enhanced DCWD has been shown to increase WIF species richness (Dove and Keeton 2015), while declines in DCWD have reduced WIF species richness (Bader et al. 1995). Hence, if fire maintains or increases DCWD available to pioneer fungal species, then WIF species richness will likely be resistant to fire disturbance (Berglund et al. 2011). As terrestrial ecosystem functioning is impacted by the activities of several different soil fungal guilds (e.g., mycorrhizal fungi increase plant nutrient acquisition, white-rot fungi regulate lignin degradation), understanding the disparate effects of fire on these guilds is essential for predicting how these various respective functions are affected by fire.

As expected, our meta-analyses demonstrated that the impact of fire on fungal richness changed depending on the method used to evaluate fungal species presence. These methods probably assess different fractions of the fungal community because they evaluate the presence of fungi on different temporal and spatial scales. For instance, sporocarp surveys oversample species that fruit more often than those with other life history strategies. Furthermore, because fungal fruiting is highly dependent on

temperature, moisture, and other chemical parameters (Fogel 1976, Zak and Wicklow 1978, Straatsma et al. 2001), most of which are modified by fire disturbance (Neary et al. 1999), treatment effects assessed by sporocarp surveys may be indicative of changes to fruiting conditions rather than the fungal communities themselves. Some fungal taxa, such as *Pezizales*, show strong phoenicoidy, increasing greatly in sporocarp abundance following fire (Petersen 1970). Yet, high numbers of sporocarps of *Pezizales* or other fungal taxa following fire may not be representative of their belowground abundance (Fujimura et al. 2004), and sporocarp surveys do not capture the non-fruiting diversity of fungi (Jonsson et al. 1999, Horton and Bruns 2001). Alternatively, belowground sampling and DNA sequencing techniques do not sample the same spatial extent as sporocarp surveys. (Horton and Bruns 2001) reviewed five studies that sampled above- and below-ground and found that < 0.1% of the area that was sampled visually for aboveground sporocarps was sampled belowground. Diminished sampling intensity through soil-coring and sequencing techniques may limit the ability to detect treatment effects such as fire. Given this apparent tradeoff (i.e., fruiting phenology effects vs. sampling intensity), we suggest multiple approaches be employed for evaluating the response of fungal species richness to disturbances such as fire (e.g., (Fujimura et al. 2004).

Our meta-analysis suggested that fungal richness was unaffected by fire type, a surprising result given numerous individual studies that found that higher severity burns diminish fungal diversity to a greater degree (Dahlberg et al. 2001, Smith et al. 2004, Rincón and Pueyo 2010, Persiani and Maggi 2013, Hewitt et al. 2013, Motiejūnaitė et al. 2014). The lack of significance in our meta-analytical study may be due to high variability in fire severity within each category (i.e., wildfire vs. prescribed fire). For example, prescribed burning in temperate climates during different seasons (e.g., spring compared to fall burns) can result in large differences in fire severity within the same terrestrial ecosystem, which may result in disparate fungal responses (Smith et al. 2004). Furthermore, wildfires vary greatly in severity both within a given fire and among different fires depending on fuel loading and weather conditions (Albini 1976). Therefore, simple generalizations of fungal response to fire based on fire type may be inadequate if the severity of the burn is not measured. We recommend that future studies measure burn severity (e.g., dNBR; (Miller and Thode 2007) as well as other fire characteristics (e.g., fire weather, fuel moisture, etc.) to more accurately compare the effects of fire on fungal communities across studies.

Our hypothesis that repeat fires (< 15 y) would influence fungal species richness response to fire was not supported by the meta-analysis. We speculate that heterogeneity within this category and low sample size of repeat fire (n = 12; 5 studies) likely contributed to this result. We expected that repeated fire would have a smaller effect on fungal species richness than single fire events because fuel loadings are generally lower after a single burn, and thus subsequent burns are generally lower in severity and higher in patchiness (Fernandes and Botelho 2003). Lower fire severity, in turn, should reduce heat-induced fungal mortality, and greater patchiness in fire effects should preserve post-fire refugia (Bastias et al. 2006). Furthermore, repeated fire best mimics natural processes in dry, fire-dependent ecosystems (commonly found in the western USA and globally); thus, it is likely that repeated fire in these ecosystems creates conditions that help

maintain fungal diversity (i.e., increased patchiness and plant diversity; (Bruns 1995, Buscardo et al. 2010, Oliver et al. 2015). However, the severity of repeat fires may not always be lower than single fire events. For instance, high severity fires in areas with long histories of fire suppression could result in increased shrub colonization with an increased likelihood of high-severity fire in subsequent burns (Coppoletta et al. 2016). Furthermore, our analysis combined repeat wildfires and prescribed fires into the same group across all biomes; separating this analysis by fire type or biome was not feasible given the lack of repeat fire studies. This amalgamation likely increased within-group heterogeneity, reducing our ability to distinguish differences between single and repeated fire. Frequent repeat fires may be necessary to maintain ecological benefits derived from prescribed fire at the individual ecosystem scale (Fernandes and Botelho 2003); yet, the global impact of repeated fire on soil fungal communities is still unclear. Future studies of fire impacts on soil fungal communities should include repeated fire in their experimental design.

We speculate that the significant difference in fungal species richness response among biomes is due to differences in the natural fire behavior within each biome rather than adaptations of the fungal community at the ecosystem scale. We expected that the smallest effects of fire on fungal species richness would be present in ecosystems with fungal species most adapted fire (i.e., frequent-fire biomes). However, the greatest effects were found in the temperate shrubland/grassland biome, which should include fungal species that are adapted to more frequent fire than those found in biomes that have infrequent fire, such as boreal forests. This unexpected result could be due to greater within-landscape variability in burn severity that occurs in wetter than in drier ecosystems (Turner and Romme 1994). Such mixed-severity fires have unburned patches that serve as refugia for plants and fungi during disturbance, and thus greater heterogeneity in burn severity could have maintained higher fungal species richness post-disturbance. Furthermore, the majority of studies in boreal forests took place in *Pinus sylvestris* forests of Fennoscandia, which burn historically at low severities.

Our results suggest that biome might be a more useful predictor of fungal response to fire than fire type, given that our meta-analysis found a significant difference in fire effects on fungal species richness among biomes but not among fire types (at least in broad categories such as wildfire vs. prescribed fire). However, while the temperate forest biome is well-represented in our analyses, few studies describe fungal species richness response to fire in other biomes. The combination of shrubland and grassland together into a single biome in our analysis also could have altered the significance of biome on fungal diversity response to fire. However, it is unlikely that combining these two groups contributed to a false-positive result because such a merger should only increase the variation within the group. This finding underpins the need to evaluate the effects of fire on fungi within each biome. Hence, future studies of fungal response to fire should prioritize underrepresented biomes such as temperate grassland, chaparral, boreal forest, and savanna.

2.5.2 Fire Effects on Mycorrhizal Colonization

Our meta-analysis showed that fire did not significantly affect mycorrhizal colonization when analyzed across a diverse array of studies. We expected fire to negatively influence overall mycorrhizal colonization through fire-induced mortality of mycorrhizal inocula, changes to soil physiochemical characteristics, or shifting plant species composition from mycorrhizal to non-mycorrhizal, ruderal post-fire plant communities (Hart et al. 2005b). Soil heating can drastically reduce active mycelium, especially in upper soil layers (Cairney and Bastias 2007). Additionally, host plant mortality eliminates the energy source (in the form of plant-derived photosynthates) for most mycorrhizal fungi. Thus, post-fire inoculum is derived primarily from the pre-fire sporebank rather than residual mycelia (Baar et al. 1999, Glassman et al. 2015). Although fungal spores show differential resistance to heat (Izzo et al. 2006), temperatures of 65 °C for extended periods of time (> 5 min.), which are commonly reached in the upper soil layers of both prescribed and wildfires (Neary et al. 1999), can completely denature even the most heat-resistant propagules (Peay et al. 2009). Furthermore, changes in soil chemistry (e.g., pH and nutrient availability) may influence belowground allocation of plant photosynthates to fungal symbionts. For example, increases in inorganic N availability through fire-induced mineralization (St. John and Rundel 1976) may reduce mycorrhizal colonization similarly to what occurs following N fertilization (Treseder 2004). However, given the diversity of biomes, methods, fire types, and guilds studied, the high variation in responses among studies (SD = 44%) may have led to a non-significant overall response.

We predicted that that fire would increase AM colonization relative to ECM colonization because increases in post-fire canopy openings would create new habitat for herbaceous AM plant hosts (MacKenzie et al. 2004). A shift in the host plants aboveground would thus drive a shift in the dominance of mycorrhizal symbionts belowground. Although this shift in dominance from ECM to AM colonization following fire has been shown in two individual studies (Korb et al. 2003, Treseder et al. 2004), the vast majority of studies used in this analysis only studied the effect of fire on one of these mycorrhizal guilds. Hence, we were unable to detect changes in the dominance of mycorrhizal guilds. We suggest that future studies assess both ECM and AM colonization because changes in the relative dominance of these guilds may have profound implications for post-fire ecosystem nutrient cycling (Phillips et al. 2013).

Where mycorrhizal colonization was assessed (using *in situ* indices or *ex situ* bioassays) significantly influenced the response of mycorrhizae to fire, suggesting that greenhouse- or growth chamber-based evaluations of mycorrhizal responses to disturbances may not be representative of in-field impacts. These differences likely reflect the discrepancy between actual colonization and inoculum potential post-fire. For instance, mycorrhizal colonization potential assessed by bioassay is conducted under near ideal environmental conditions. However, fire also affects soil abiotic conditions such as irradiance, temperature, and moisture (Neary et al. 1999), which also may indirectly affect mycorrhizal colonization (Parke et al. 1983, Perry et al. 1987). Additionally, the use of "bait" plants typically employed in *ex situ* bioassays may not accurately reflect the colonization potential by the native plants on site (Sýkorová et al. 2007). Fine root

density may also be reduced post-fire (Smith et al. 2004, Hart et al. 2005a); thus, discrepancies in root density between *in situ* and *ex situ* approaches may also explain observed differences in mycorrhizal colonization response to fire among studies. Because there was no effect of fire on mycorrhizal colonization via *ex situ* bioassay, but fire significantly reduced mycorrhizal colonization when assessed *in situ*, we speculate that fire reduces mycorrhizal colonization primarily because of unfavorable changes in environmental and host-density conditions in the field rather than due to direct reductions in fungal inocula. Regardless of the mechanism, our results suggest that the evaluation of the response of the mycorrhizal community to fire may be strongly dependent on the assay used.

2.5.3 Fungal Resilience to Fire

Our hypothesis that fire effects on fungal species richness and mycorrhizal colonization would diminish over time was supported by the meta-analysis, suggesting that the fungal community is relatively resilient to disturbances such as fire. For fungal species richness and *in situ* mycorrhizal colonization, a continuous logarithmic model fit the data, where effect sizes were most negative soon after fire and approached zero after 22 and 11 years, respectively. Nevertheless, results from individual studies that followed fungal species richness or mycorrhizal colonization post-fire over time showed a wide range in temporal responses. For instance, using a fire chronosequence of stand-replacing fires in southwestern US *Pinus ponderosa* forests, (Kurth et al. 2013) found that WIF species richness recovered to unburned richness values after about nine years. However, the recovery in both mycorrhizal and saprobic fungal species richness took 41 years after a stand-replacing wildfire in a *Pinus banksiana* chronosequence (Visser 1995). Such discrepancies in fungal species richness recovery rates to disturbance among studies are likely a combination of differences in the direct effects of fire severity on fungal mortality and differences in the complex suite of indirect effects of fire on soil fungi (Hart et al. 2005b, Cairney and Bastias 2007). Similarly, while studies that used fire chronosequences (3 - 46 y) found an increase in mycorrhizal colonization with time since fire (Treseder et al. 2004, Rincón et al. 2014), those that followed a site for < 1 year after a fire showed no change (Hartnett et al. 1994) or a continued decline in mycorrhizal colonization over time (Bentivenga and Hetrick 1991, Bellgard et al. 1994, Anderson and Menges 1997). Changes in mycorrhizal colonization measured within a year following fire may represent seasonal variation in mycorrhizal colonization rather than a fire effect, as suggested by corresponding temporal changes in mycorrhizal colonization in unburned control plots (Anderson and Menges 1997). Our meta-analyses used only the first sample date post-fire when studies sampled the same site repeatedly over time to prevent violation of data independence (Gurevitch and Hedges 1999). Therefore, it is not surprising that our temporal meta-regressions of fungal species richness or mycorrhizal colonization following fire across different studies may not be representative of results from individual studies that followed these changes over time from the same site, or from space-for-time substitutions (i.e., fire chronosequences).

2.6 Conclusion

By aggregating data from multiple ecosystems, fire types, and sampling methods, we showed that soil fungal communities (species richness and mycorrhizal colonization) are adversely affected by fire. However, short-term negative effects diminish quickly over time, returning to pre-fire levels within one to two decades. This finding has major implications for ecosystem recovery post-disturbance (Perry et al. 1989), as soil fungal communities are drivers of aboveground diversity (van der Heijden et al. 1998) and other important ecological functions and services (Ingham et al. 1985, Finlay 2008, van der Wal et al. 2013). Future studies should investigate linkages between post-fire fungal communities and ecosystem function to develop a mechanistic understanding of ecosystem processes in post-fire environments.

Our meta-analyses also revealed significant moderators of fungal species richness response to fire. Although quantifying disturbance-driven changes in fungal species richness are valuable from a biodiversity perspective, it is perhaps more important to quantify specifically how the structure of soil fungal communities change in response to disturbance. For instance, although Chen and Cairney (2002) found that overall fungal species richness did not change post-fire, they found that species assemblages changed from mainly ECM to AM fungi, with possible commensurate changes in nutrient cycling processes (Phillips et al. 2013). Furthermore, our analyses suggest that mycorrhizal colonization following fire is driven mainly by indirect, post-fire environmental changes rather than the direct effects of fire-induced fungal mortality. This finding suggests that resource managers may be able to manipulate site conditions post-fire to make them more conducive to post-fire mycorrhizal colonization of new plant propagules. Nevertheless, small sample sizes and underrepresented taxa, biomes, and fire types limit the inferential power of our conclusions from both meta-analyses. Further studies of fire effects on fungi in novel environments, targeting underrepresented fungal guilds and life stages, are needed to comprehensively assess the role of fire in shaping soil fungal communities. Additionally, we recommend that future studies document burn severity (e.g., dNBR; (Miller and Thode 2007), as well as other important fire characteristics (e.g., fire weather, fuel moisture), to provide the necessary context for improving our mechanistic understanding of the role of fire in sustaining soil fungal communities and the ecosystems they support.

2.7 References

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2.8 Tables

Table 2-1: Data used in meta-analysis of fungal species richness response to fire, including control replicates (n_c), control mean (x_c), experimental replicates (n_e), experimental mean (x_e), the natural log of the response ratio (ln[R]), and change in richness (%). [a]AM, arbuscular mycorrhizal fungi; ECM, ectomycorrhizal fungi; WIF, wood-inhabiting fungi [b]NGS, next-generation sequencing; DGGE, Denaturing gradient gel electrophoresis [c]P, prescribed fire; W, wildfire [†]Fire-resistant propagules (e.g., spores, residual hyphae)

Reference	Guild studied[a]	Unit of measurement[b]	Fire type[c]	Repeat burn?	Years since fire	Biome	Location	Change in richness (%)
(Barker et al. 2013)	ECM	morphotyping + molecular ID	W		3	Temperate Forest	BC, Can.	-21.3
	ECM	morphotyping + molecular ID	W		3	Temperate Forest	BC, Can.	-50.0
(Bartoli et al. 1991)	micro-fungi	culture morphology	W		0.01644	Temperate Forest	Italy	-84.9
(Buscardo et al. 2010)	ECM[†]	morphotyping + molecular ID	W		5	Temperate Forest	Portugal	23.6
	ECM[†]	morphotyping + molecular ID	W	x	5	Temperate Forest	Portugal	2.8
	ECM[†]	morphotyping + molecular ID	W	x	5	Temperate Forest	Portugal	-11.2
	ECM[†]	morphotyping + molecular ID	W		5	Temperate Forest	Portugal	-26.2
	ECM[†]	morphotyping + molecular ID	W	x	5	Temperate Forest	Portugal	-23.1
	ECM[†]	morphotyping + molecular ID	W	x	5	Temperate Forest	Portugal	-36.9
(Dahlberg et al. 2001)	ECM	morphotyping + molecular ID	P		0.3	Boreal Forest	Sweden	-41.0
	ECM	morphotyping + molecular ID	P		0.3	Boreal Forest	Sweden	NA
(Eom et al. 1999)	AM (spore)	spore morphology	P		1	Temperate Grassland	KS, USA	-28.6
(Glassman et al. 2015)	ECM[†]	morphotyping + molecular ID	W		1	Temperate Forest	CA, USA	-34.4
(Goberna et al. 2012)	All fungi	DGGE	P		0.00274	Temperate Shrubland	Spain	12.8
(Grishkan 2016)	micro-fungi	culture morphology	W		1.5	Temperate Forest	Israel	-36.1
	micro-fungi	culture morphology	W		0.5	Temperate Forest	Israel	-33.3
(Hernández-Rodríguez et al. 2013)	All fungi	sporocarp survey	W		1	Temperate Shrubland	Spain	-72.3
(Holden et al. 2013)	All fungi	NGS	W		0	Boreal Forest	AK, USA	-0.90
	All fungi	NGS	W		6	Boreal Forest	AK, USA	16.2
	All fungi	NGS	W		11	Boreal Forest	AK, USA	9.9
	All fungi	NGS	W		23	Boreal Forest	AK, USA	-3.8
	All fungi	NGS	W		54	Boreal Forest	AK, USA	-11.5
	All fungi	NGS	W		90	Boreal Forest	AK, USA	-18.4
(Jonsson et al. 1999)	ECM	morphotyping + molecular ID	W		62	Boreal Forest	Sweden	-11.1
(Junninen et al. 2008)	WIF	sporocarp survey	P		1	Boreal Forest	Finland	1.4

(Kipfer et al. 2011)	ECM	morphotyping + molecular ID	W		3.5	Temperate Forest	Switzerl and	-45.3
	ECM	morphotyping + molecular ID	W		15.5	Temperate Forest	Switzerl and	-8.0
(Kurth et al. 2013)	WIF	NGS	W		4	Temperate Forest	AZ, USA	-48.4
	WIF	NGS	W		9	Temperate Forest	AZ, USA	16.5
	WIF	NGS	W		13	Temperate Forest	AZ, USA	-7.0
	WIF	NGS	W		25	Temperate Forest	AZ, USA	97.0
	WIF	NGS	W		32	Temperate Forest	AZ, USA	0.0
(Longo et al. 2014)	AM (spore)	spore morphology	W		0.5	Temperate Forest	Argentin a	-38.4
	AM (spore)	spore morphology	W		0.5	Temperate Forest	Argentin a	-47.7
	AM (spore)	spore morphology	W		0.5	Temperate Forest	Argentin a	-37.7
	AM (spore)	spore morphology	W		0.5	Temperate Forest	Argentin a	-41.6
	AM (spore)	spore morphology	W		0.5	Temperate Forest	Argentin a	-38.6
(Mardji 2014)	ECM	sporocarp survey	W	x	14	Tropical Forest	India	-62.9
(Martín-Pinto et al. 2006)	All fungi	sporocarp survey	W		1	Temperate Forest	Spain	-60.4
	All fungi	sporocarp survey	W		1	Temperate Forest	Spain	-62.5
	All fungi	sporocarp survey	W		1	Temperate Shrubland	Spain	-55.0
	All fungi	sporocarp survey	W		1	Temperate Shrubland	Spain	-85.3
(Motiejūnaitė et al. 2014)	All fungi	sporocarp survey	W		1	Temperate Forest	Lithuani a	-66.5
	All fungi	sporocarp survey	W		1	Temperate Forest	Lithuani a	-40.3
(Oliver et al. 2015)	All fungi	NGS	P	x	3	Temperate Forest	GA, USA	-1.7
	All fungi	NGS	P	x	3	Temperate Forest	GA, USA	1.9
	All fungi	NGS	P	x	7	Temperate Forest	GA, USA	-1.2
	All fungi	NGS	P	x	10	Temperate Forest	GA, USA	2.9
(Olsson and Jonsson 2010)	WIF	sporocarp survey	P		1	Boreal Forest	Sweden	-39.4
(Rincón et al. 2014)	ECM	morphotyping + molecular ID	W		14	Temperate Forest	Spain	-16.4
	ECM	morphotyping + molecular ID	W		3	Temperate Forest	Spain	-24.7
(Robinson et al. 2008)	All fungi	sporocarp survey	W		1	Temperate Forest	Australia	-29.1
(Román and Miguel 2005)	ECM	hyphal morphotype	P		1	Temperate Forest	Spain	-13.3
(Smith et al. 2004)	ECM	morphotyping + molecular ID	P		1	Temperate Forest	OR, USA	-2.4
	ECM	morphotyping + molecular ID	P		1	Temperate Forest	OR, USA	-81.6
(Sun et al. 2015)	All fungi	NGS	W		2	Boreal Forest	Finland	49.2
	All fungi	NGS	W		42	Boreal Forest	Finland	18.4
	All fungi	NGS	W		60	Boreal Forest	Finland	6.2
	All fungi	NGS	W		60	Boreal Forest	Finland	7.7

(Trappe et al. 2009)	ECM	sporocarp survey	P		3	Temperate Forest	OR, USA	-20.3
	ECM	sporocarp survey	P		3	Temperate Forest	OR, USA	17.4
	ECM	sporocarp survey	P		3	Temperate Forest	OR, USA	17.4
(Trusty 2009)	ECM	morphotyping + molecular ID	W		5	Temperate Forest	MT, USA	-27.2
(Tuininga and Dighton 2004)	ECM	hyphal morphotype	P	x	0.3	Temperate Forest	NJ, USA	-3.8
	ECM	hyphal morphotype	P	x	0.3	Temperate Forest	NJ, USA	-19.5
(Xiang et al. 2015)	AM	NGS	W		1	Boreal Forest	China	-44.7
	AM	NGS	W		1	Boreal Forest	China	-53.4
	AM	NGS	W		11	Boreal Forest	China	-13.0
	AM	NGS	W		11	Boreal Forest	China	-39.0

Table 2-2: Data used in meta-analysis of mycorrhizal colonization response to fire including control replicates (n_c), control mean (x_c), experimental replicates (n_e), experimental mean (x_e), the natural log of the response ratio (ln[R]), and change in colonization (%). [a]AM = arbuscular mycorrhizal fungi and ECM = ectomycorrhizal fungi. [b]P = prescribed fire and W = wildfire.

Reference	Mycorrhizal type[a]	Unit of measurement	*in situ* or *ex situ*	Fire type[b]	Repeat burn?	Years since fire	Biome	Location	Change in coloniza-tion (%)
(Anderson and Menges 1997)	AM	% colonized seedlings	*ex situ*	P		0.083	Temperate Forest	FL, USA	72.7
(Bacon et al. 2009)	AM	% colonized seedlings	*ex situ*	W		33.000	Temperate Forest	AZ, USA	105
	AM	% colonized seedlings	*ex situ*	W		5.000	Temperate Forest	AZ, USA	113.3
	ECM	% colonized seedlings	*ex situ*	W		33.000	Temperate Forest	AZ, USA	25
	ECM	% colonized seedlings	*ex situ*	W		5.000	Temperate Forest	AZ, USA	-1.4
(Barker et al. 2013)	ECM	% colonized seedlings	*in situ*	W		0.330	Temperate Forest	BC, Can.	-49.0
	ECM	% colonized seedlings	*in situ*	P		0.330	Temperate Forest	BC, Can.	-40.8
(Bellgard et al. 1994)	AM	% root length	*ex situ*	W		0.083	Temperate Grassland	Australia	-2.4
(Bentivenga and Hetrick 1991)	AM	% root length	*in situ*	P		0.011	Temperate Grassland	KS, USA	-12.9
(Buscardo et al. 2010)	ECM	% root tips	*ex situ*	W		5.000	Temperate Forest	Portugal	7.5
	ECM	% root tips	*ex situ*	W	x	5.000	Temperate Forest	Portugal	9.1
	ECM	% root tips	*ex situ*	W	x	5.000	Temperate Forest	Portugal	16.7
	ECM	% root tips	*ex situ*	W		5.000	Temperate Forest	Portugal	-10.4
	ECM	% root tips	*ex situ*	W	x	5.000	Temperate Forest	Portugal	39.1
	ECM	% root tips	*ex situ*	W	x	5.000	Temperate Forest	Portugal	-40.0
(Dhillion et al. 1988)	AM	% root length	*in situ*	P	x	0.133	Temperate Grassland	IL, USA	-48.0
(Glassman et al. 2015)	ECM	% colonized seedlings	*ex situ*	W		1.000	Temperate Forest	CA, USA	-15.0
(Hartnett et al. 1994)	AM	% root length	*in situ*	P		0.250	Temperate Grassland	KS, USA	20.0
(Medve 1985)	AM	% root tips	*in situ*	P		0.330	Temperate Grassland	PA, USA	-0.4
(Miller et al. 1998)	ECM	% colonized seedlings	*ex situ*	W		1.000	Temperate Forest	WY, USA	62.3
	ECM	% colonized seedlings	*ex situ*	W		1.000	Temperate Forest	WY, USA	91.7
(Milne 2002)	ECM	% root tips	*ex situ*	W		0.250	Temperate Forest	Greece	-43.3
(RAMAN and NAGARAJAN 1996)	AM	% root tips	*in situ*	P		0.055	Tropical Forest	India	-33.3
(Rashid et al. 1997)	AM	% root length	*in situ*	W		0.417	Temperate Grassland	Pakistan	-59.7
	AM	% root length	*in situ*	W		0.417	Temperate Grassland	Pakistan	-51.9
(Rincón et al. 2014)	ECM	% root tips	*in situ*	W		14.00	Temperate Forest	Spain	16.5
	ECM	% root tips	*in situ*	W		3.000	Temperate Forest	Spain	-12.9
(Román and Miguel 2005)	ECM	% root length	*in situ*	P		1.000	Temperate Forest	Spain	-31.3

(Schoenberger and Perry 1982)	ECM	% root tips	*ex situ*	W	38.000	Temperate Forest	OR, USA	-22.5
	ECM	% root tips	*ex situ*	W	38.000	Temperate Forest	OR, USA	6.9
(Senthilkumar et al. 1995)	AM	% root tips	*in situ*	P	0.083	Tropical Grassland	India	-12.2
(Tipton 2016)	AM	% root length	*in situ*	P	1.000	Temperate Grassland	MO, USA	78.8
	AM	% root length	*in situ*	P	2.000	Temperate Grassland	MO, USA	81.8
	AM	% root length	*in situ*	P	4.000	Temperate Grassland	MO, USA	78.8
	AM	% root length	*in situ*	P	5.000	Temperate Grassland	MO, USA	-45.5
(Torres and Honrubia 1997)	ECM	% root tips	*ex situ*	W	1.000	Temperate Forest	Spain	6.6
	ECM	% root tips	*ex situ*	W	1.000	Temperate Forest	Spain	-17.1
(Treseder et al. 2004)	ECM	% root length	*in situ*	W	3.000	Boreal Forest	AK, USA	-78.2
	ECM	% root length	*in situ*	W	35.000	Boreal Forest	AK, USA	72.5
	ECM	% root length	*in situ*	W	46.000	Boreal Forest	AK, USA	36.8
	AM	% root length	*in situ*	W	3.000	Boreal Forest	AK, USA	10.8
	AM	% root length	*in situ*	W	35.000	Boreal Forest	AK, USA	6.9
	AM	% root length	*in situ*	W	46.000	Boreal Forest	AK, USA	17.6
(Trusty 2009)	ECM	% colonized seedlings	*in situ*	W	5.000	Temperate Forest	MT, USA	-8.8
(Vásquez-Gassibe et al. 2016)	ECM	% root tips	*ex situ*	W	1.000	Temperate Forest	Spain	-5.9
	ECM	% root tips	*ex situ*	W	1.000	Temperate Forest	Spain	-8.5
(Vilariño and Arines 1991)	AM	% root length	*in situ*	W	1.000	Temperate Forest	Spain	-54.9
	AM	% root length	*in situ*	W	1.000	Temperate Forest	Spain	-35.3
	AM	% root length	*in situ*	W	1.000	Temperate Forest	Spain	-27.5
	AM	% root length	*in situ*	W	1.000	Temperate Forest	Spain	-58.8
	AM	% root length	*in situ*	W	1.000	Temperate Forest	Spain	-29.4

2.9 Figures

Figure 2-1: Locations of studies used in the meta-analyses. Circles show locations of studies which colonization data were extracted from and triangles show locations of studies which richness data were extracted from.

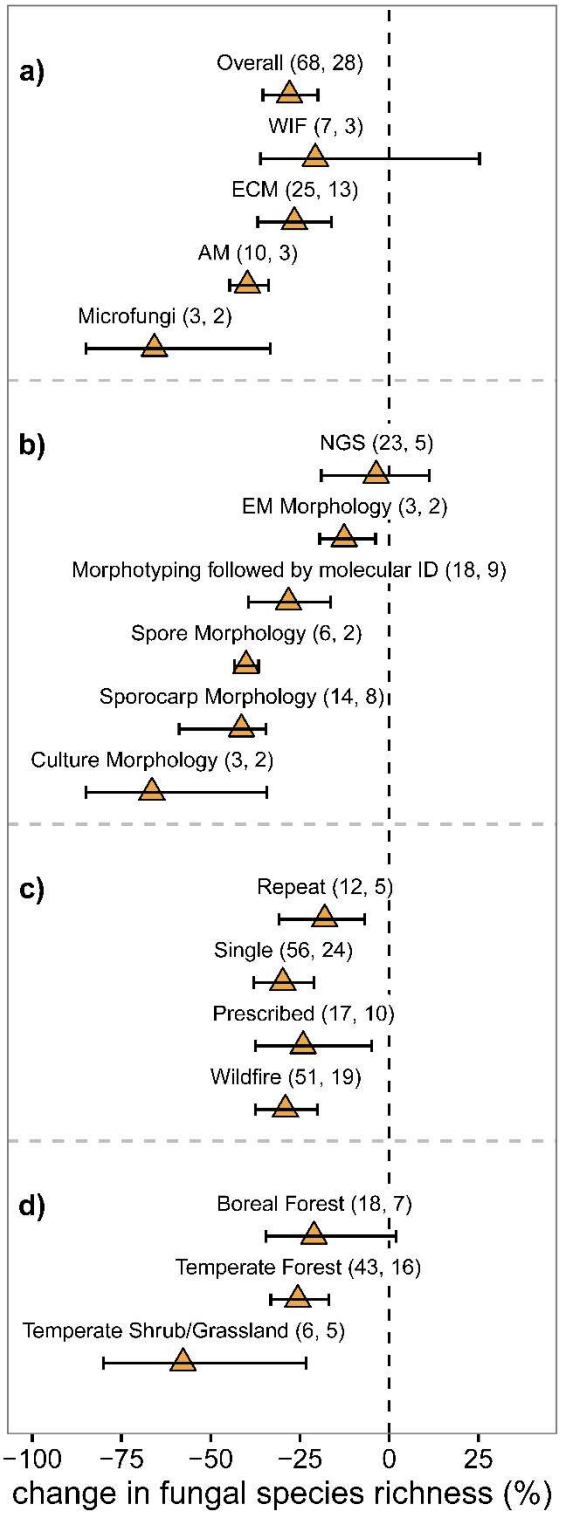


Figure 2-2: Mean response of fungal species richness to fire overall (groups combined) and among functional guilds (A), among methods of measurement (B), among fire types and frequencies (not mutually exclusive; C), and among biomes (D). Error bars show 95% bias-corrected bootstrap confidence intervals. Values show data records and number of studies, respectively. Eight studies were not included in the guild catagorical model because they studied the richness of all fungal species. Denaturing gradient gel electrophoresis (DGGE) was excluded in the measurement categorical model because only one study (Goberna et al. 2012) used this method. Tropical forest was excluded in the biome categorical model because only one study (Mardji 2014) occurred in this biome. Key: wood-inhabiting fungi (WIF), ectomycorrhizal fungi (ECM), and arbuscular mycorrhizal fungi (AM), microfungi represent the culturable fraction of soil fungi, next-generation sequencing (NGS), morphotyping followed by molecular identification (ID), and ectomycorrhizal (EM).

Figure 2-3: Mean responses of fungal species richness (ln[response ratio]) as a function of time since fire. Solid line represents best-fit meta-regression (ln[response ratio] = 0.1976 × ln[years since fire + 1] - 0.62), the shaded area shows the 95% bias-corrected bootstrap confidence interval around the line, and the dashed line shows no response (i.e., ln(response ratio) = 0).

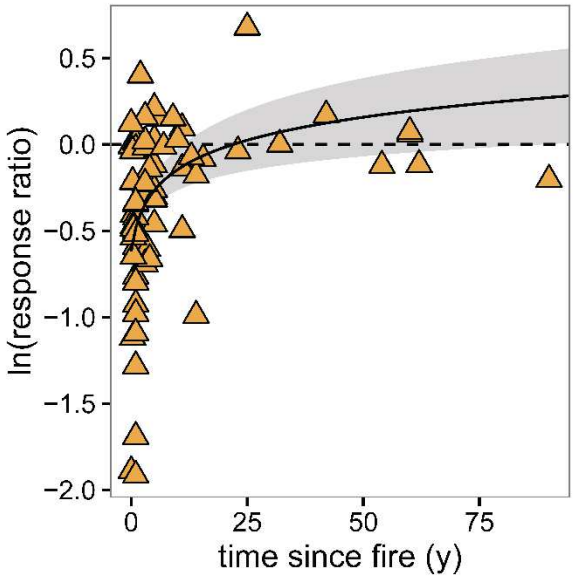


Figure 2-4: Mean response of mycorrhizal colonization to fire overall (groups combined) and between ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) fungi (A) and mean responses of mycorrhizal colonization to fire assessed *in situ* or by *ex situ* bioassays (B). Error bars show 95% bias-corrected bootstrap confidence intervals. Numbers show data records and number of studies, respectively.

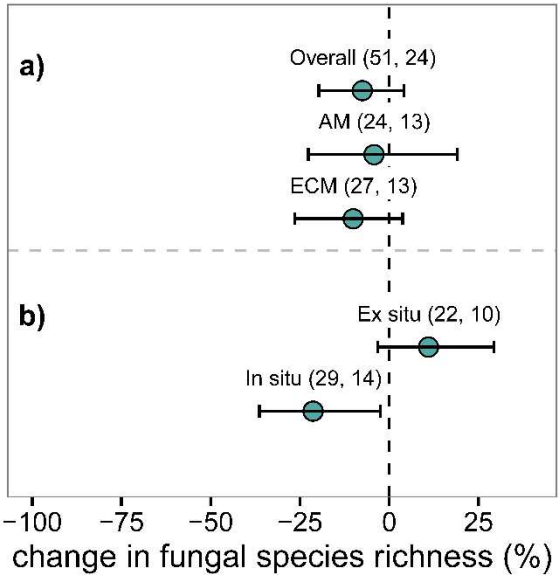
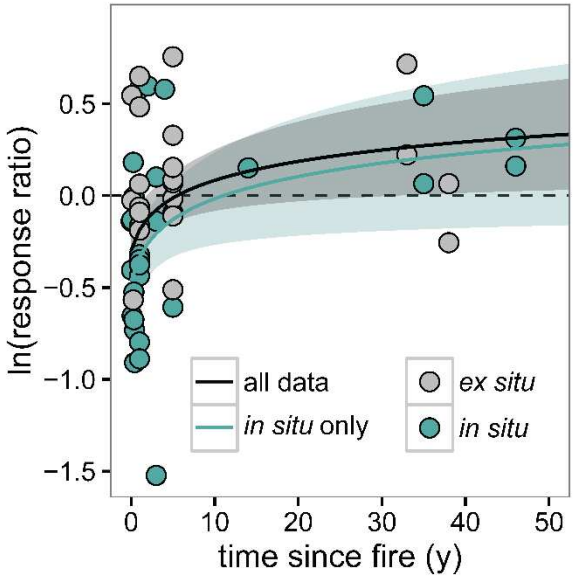


Figure 2-5: Mean responses of overall mycorrhizal colonization and *in situ* mycorrhizal colonization (ln[response ratio]) as a function of time since fire. Solid black line represents best-fit meta-regression of overall mycorrhizal colonization (gray and blue circles; ln[R] = 0.1588 × ln[years since fire + 1] - 0.2971) and gray shaded area shows the 95% bias-corrected bootstrap confidence interval around the line. Solid blue line represents best-fit meta-regression of *in situ* mycorrhizal colonization (blue circles; ln[R] = 0.1870 × ln[years since fire + 1] - 0.4646) and blue shaded area shows the 95% bias-corrected bootstrap confidence interval around the line. The dashed line shows no response (i.e., ln(response ratio) = 0).



2.10 Supplementary Tables

Supplementary Table 2-1: Results of statistical comparisons among groups for the response of overall mycorrhizal colonization to fire.

comparison	group	mean effect size (%)	95% CI	records[a]	number of studies	p-value
fire type						0.341
	prescribed fire	-0.50	-23.3 to 28.1	12	9	
	wildfire	-9.43	-23.8 to 5.1	39	15	
repeat fire						0.935
	repeated	-10.5	-44.9 to 20.8	5	2	
	single	-7.10	-20.2 to 8.6	46	22	
biome*						0.358
	temperate forest	-3.93	-17.6 to 10.9	32	14	
	boreal forest	-5.39	-50.1 to 39.1	6	1	
	temperate grassland	-14.7	-37.6 to 15.5	11	7	

[a]number of data records for each group
*Two studies were omitted because there were not enough records for tropical forest (Raman & Nagarajan 1996) and tropical grassland (Senthilkumar et al. 1995) for a statistical comparison.

Supplementary Table 2-2: Results of statistical comparisons among groups for the response of *in situ* mycorrhizal colonization to fire. Comparison between repeat fires and single fire events was not made due to insufficient studies.

comparison	group	mean effect size (%)	95% CI	records[a]	number of studies	p-value
guild						0.616
	ECM	-26.9	-54.5 to 9.2	9	5	
	AM	-17.8	-32.1 to 0.7	20	10	
fire type						0.219
	prescribed	-6.5	-25.7 to 17.9	11	8	
	wildfire	-28.5	-46.2 to -6.1	18	6	
biome*						0.419
	temperate forest	-35.2	-43.8 to -23.0	11	5	
	boreal forest	-5.4	-49.1 to 38.6	6	1	
	temperate grassland	-15.6	-40.7 to 19.5	10	6	

[a]number of data records for each group
*Two studies were omitted because there were not enough records for tropical forest (Raman & Nagarajan 1996) and tropical grassland (Senthilkumar et al. 1995) for a statistical comparison.

3 High-severity wildfire leads to multi-decadal impacts on soil biogeochemistry in mixed-conifer forests

3.1 Abstract

During the past century, systematic wildfire suppression has decreased fire frequency and increased fire severity in the western United States of America. While this has resulted in large ecological changes aboveground such as altered tree species composition and increased forest density, little is known about the long-term, belowground implications of altered, ecologically novel fire regimes, especially on soil biological processes. To better understand the long-term implications of ecologically novel, high-severity fire, we used a 44-y high-severity fire chronosequence in the Sierra Nevada where forests were historically adapted to frequent, low-severity fire, but were fire suppressed for at least 70 years. High-severity fire in the Sierra Nevada resulted in a long-term (44+ y) decrease (>50%, $p < 0.05$) in soil extracellular enzyme activities, basal microbial respiration (56-72%, $p < 0.05$), and organic carbon (>50%, $p < 0.05$) in the upper 5 cm compared to sites that had not been burned for at least 115 y. However, nitrogen (N) processes were only affected in the most-recent fire site (4 y post-fire). Net nitrification increased by over 600% in the most recent fire site ($p < 0.001$), but returned to similar levels as the unburned control in the 13-y site. Contrary to previous studies, we did not find a consistent effect of plant cover type on soil biogeochemical processes in mid-successional (10-50 y) forest soils. Rather, the 44-y reduction in soil organic carbon (C) quantity correlated positively with dampened C cycling processes. Our results show the drastic and long-term implication of ecologically novel, high-severity fire on soil biogeochemistry and underscore the need for long-term fire ecological experiment.

3.2 Introduction

Wildfire has historically regulated carbon (C) and nitrogen (N) cycling in yellow pine and mixed-conifer forests of the Sierra Nevada and other fire-adapted ecosystems commonly found throughout the western United States of America (Johnson et al. 2008). However, due to the interaction between increasing forest fuels (due to long-term fire suppression and early forest management practices) and the rapidly warming climate, contemporary fires in these ecosystems are outside their natural range of variation and are currently burning at higher severities than their pre-1850 counterparts (Miller et al. 2009b, Safford and Stevens 2017). This departure from historical conditions has the potential to affect the resistance and resilience of many soil processes to fire (Figure 3-1). The importance of fire effects on forest soil biogeochemistry has gained attention in recent years (e.g., Nave et al. 2011, Caon et al. 2014, Pellegrini et al. 2018, Butler et al. 2018); however, much of this work has focused on disturbances within the range of natural variation for that ecosystem. For example, many have studied stand-replacing fire in boreal or subalpine forests (Smithwick et al. 2005, Turner et al. 2007, Holden et al. 2016) or low-severity, prescribed fire in seasonally dry, temperate forests (Kaye and Hart 1998, Kaye et al. 2005, Grady and Hart 2006, Fultz et al. 2016). There has been relatively less focus on how C and N cycling processes respond to and recover from ecologically novel fire (i.e., fire severity outside the natural range of variation).

Before major Euro-American settlement began in the mid-1800s, the fire regime of yellow pine and mixed-conifer (YPMC) forests in California and the southwestern United States was dominated by frequent fires (fire return interval of one to two decades) of low to moderate vegetation burn severity, and high mortality of canopy trees was relatively rare (Fry et al. 2014, Safford and Stevens 2017). Trend assessments carried out since 1984 (the beginning of the LANDSAT dataset) show that the severity of wildfires in YPMC and similar forest types is increasing in California and the southwestern United States. Both the total area burned at high severity and the mean and maximum sizes of high-severity burn patches within fires are increasing (Miller et al. 2009b, Mallek et al. 2013, Dillon et al. 2016, Steel et al. 2018). Clearly, the modern fire regime is well outside the natural range of variation for fire severity, and the ecosystem-level effects of many modern fires can be reasonably characterized as "novel" (Safford and Stevens 2017, Miller and Safford 2017).

Direct and indirect impacts of fire on soil biogeochemistry and the relative magnitude of these effects vary with time since fire (Hart et al. 2005). On shorter time scales, wildfire has been shown to decrease soil extracellular enzyme activity (Boerner et al. 2008, Taş et al. 2014, Knelman et al. 2017) and microbial respiration (Grady and Hart 2006) due to the combustion of microbial biomass and soil organic C (SOC; Miesel et al. 2011, Knelman et al. 2015). Nitrogen availability within a few years after fire is generally high (Covington and Sackett 1992, DeLuca and Sala 2006), originating from fire-induced N mineralization (St. John and Rundel 1976), increased rates of organic matter decomposition (Kaye and Hart 1998), and increased N-fixing plant abundance (Johnson et al. 2005). Increases in N availability can result in lower N-limitation to soil microorganisms and increase nitrification (Kurth et al. 2014, Hanan et al. 2016). In the longer term, however, as plants recolonize post-fire, they begin to exert a greater

influence over soil microbial communities indirectly through root exudation, plant litter inputs, and increased nutrient uptake, or directly through formation of symbioses with soil microorganisms. Thus, in the longer term, plant communities have been suggested to be the primary drivers of soil biogeochemical processes (Hart et al. 2005).

Ecologically novel, high severity fire likely exacerbates the response and lengthens the recovery of soil biogeochemical processes compared to historical fires. Global change (including changing fire regimes) alters the selective pressures that organisms have been evolutionarily adapted to. Thus, the microbial communities, which control C and N cycling in soils, could be maladapted to these ecologically novel disturbances and post-fire conditions. This could reduce the resiliency of ecological functions that these organisms provide, hampering the recovery of the ecosystem. For example, under simulated drought conditions, plants responded positively when grown with drought-adapted microbial communities (Lau and Lennon 2012), showing that the adaption (or maladaptation) of microbial communities to global change can have ecosystem-level implications. However, the impact of ecologically novel fire on soil microbial communities and their function has yet to be fully elucidated.

We evaluated the long-term (40+ years) impact of ecologically novel, high-severity fire on soil C and N cycling using a wide array of biogeochemical assays and rate measurements using space for time substitution. We hypothesize that novel, high-severity fire will cause multi-decadal impacts on site and soil properties (e.g., vegetation, temperature and moisture dynamics, pH, SOC, nutrient availability, and microbial biomass), which will result in changes in the rates of biogeochemical processes. Specifically, we expect decreased soil extracellular enzyme activities and respiration and increased soil net N mineralization, net nitrification, and nitrifier activity (assessed by nitrification potential and N_2O fluxes) following high-severity fire in YPMC ecosystems. In the short-term (< 10 y), we hypothesize that impacts will be due to the *direct* impacts of fire: combustion of SOC and microbial biomass and mineralization of N. In the longer-term (> 10 y), we hypothesize that impacts will be due to the indirect effect of fire on soils through vegetation (i.e., plant cover type would have a greater effect with increased time since fire). Finally, we hypothesize that these effects will be more pronounced when soils are wetter and microbial activity is higher. Our overall goal is to determine the long-term impact of novel, high-severity fire on soil biogeochemical processes in order to quantify the resilience and identify the drivers of these processes at different stages of recovery. Developing a mechanistic understanding of the effects of high-severity fire and time since fire on soil biogeochemistry is critical for predicting how ecosystems respond to (and subsequently recover from) fire disturbances with severities outside their range of historic variability. Such information will be useful in management of fire-suppressed temperate forests and mitigation against the effects of these exceptional disturbance events (Adams 2013, Stephens et al. 2014).

3.3 Methods

3.3.1 Site description and experimental design

The study was conducted on the Eldorado National Forest, which is located in the Central Sierra Nevada of California, an area historically fire-suppressed like much of western North America (Figure 3-2, Safford and van de Water 2014). We sampled in areas of varying time since stand-replacing wildfire using a fire chronosequence in South Fork of the American River watershed. The fire sites are: the King Fire (4-y post-fire, 48% of fire area burned at high vegetation burn severity), the Freds Fire (13-y post-fire, 58% high severity), the Cleveland Fire (25-y post-fire, 64% high severity), and the Pilliken Fire (44-y post-fire, >50% high severity; Bohlman et al. 2016). The spatial extent of high severity burning in these fires is far outside the natural range of variation, which ranged from about 5-15% of the area of the average fire before Euro-American settlement of the region (Safford and Stevens 2017). We incorporated sites throughout the study area that had not burned since at least 1908 (Safford and van de Water 2014), which is the maximum period for which we know that no recorded burning occurred. We operationally defined this as our late successional site (> 115-y post-fire).

A fire chronosequence relies on the assumption that the variation in space (among sites) is equal to the variation in time since fire (Pickett 1989). This technique has been increasingly employed as a method for understanding the long-term trajectories of ecosystems post-fire (Kurth et al. 2013, Fest et al. 2015, Holden et al. 2016, Bohlman et al. 2016, Sun et al. 2016). Although chronosequences may not always be ideal for measuring temporal effects due to inherent differences in site properties and life histories, we minimized these limitations through careful plot selection. Late successional (i.e., pre-fire) vegetation at all sites is characterized as mixed-conifer forest. Dominant species include ponderosa pine (*Pinus ponderosa*), Jeffrey pine (*P. jeffreyi*), Douglas-fir (*Pseudotsuga menziesii*), white fir (*Abies concolor*), incense-cedar (*Calocedrus decurrens*), black oak (*Quercus kellogii*), and canyon live oak (*Q. chrysolepis*). Prior to fire, the 4-, 13-, 25-, and >115-y sites had similar Normalized Difference Vegetation Index values (repeated measures ANOVA: $p = 0.178$, data from Robinson et al. 2017), which correlates with the photosynthetic capacity of vegetation (the 44-y site burned before the data record of 1984). Soils are in the suborder Xerepts, with either an umbric or ochric epipedon (USDA-NRCS, 2015). Furthermore, all plots have a southern aspect (90-270°), are on moderate slopes (10-20%, characteristic of the fire affected areas), and are between 1100-1300 m elevation.

Because we were interested in the unassisted recovery after high-severity fire, we sampled only in areas that were not managed post-fire (e.g., salvage logging, herbicide application, planting) and were classified as high-severity burns (>75% basal area mortality of dominant and co-dominant canopy trees). All areas were salvage logged in the 44-y site, but the plots selected at this site were not manipulated further. While salvage logging may affect nutrient budgets and hydrologic regimes critical in early stand development (Johnson et al. 2005, Jennings et al. 2012), McGinnis et al. (2010) found no difference in shrub or grass cover between salvage logged and control sites in the Sierra Nevada. It is reasonable to conclude that salvage logging only minimally affected

microbial communities and their processes at this site because long-term impacts of fire on microbial communities are hypothesized to be mainly influenced by live aboveground vegetation (Hart et al. 2005). Burn severity was assessed using delta Normalized Burn Ratio (dNBR, Miller and Thode 2007) on Landsat imagery for the three most recent fires; for the 44-y site, dNBR was determined using a 1974 false-color composite Landsat Multispectral Scanner System (MSS) image because this fire occurred before Landsat was launched (Bohlman et al. 2016). Vegetation burn severity (as used in our study) does not always correlate well with soil burn severity (Safford et al. 2008, Miesel et al. 2018). However, comparisons among fires in different years are difficult due to changing methods in soil burn severity mapping. The California vegetation burn severity classification we used is standardized and objectively determined based on published relationships between the Landsat data and field conditions (e.g., Miller and Thode 2007, Miller et al. 2009a, 2009b). We were more comfortable using these more standardized products to map fire severity in our four chronologically disparate fires than the much more idiosyncratic soil burn severity maps (Safford et al. 2008).

Each site consisted of six to eight plots separated by at least 150 m (the 13 y and 44 y sites had only six and seven plots, respectively, due to sampling area constraints; all other sites had eight plots). Plot-centers were chosen randomly *a priori* using a GIS layer of appropriate site polygons (i.e., similar soils, elevation, aspect, burn severity, and management). Each plot was defined by a 5-m radius from plot-center. Within each plot, we sampled one point under each of the available lowest-stratum cover types. Cover types were tree (e.g., *Abies concolor*, *Pinus ponderosa*, *Quercus* spp.), seedling (e.g., *Pinus ponderosa*), shrub (*Arctostaphylos* spp., *Salix* spp.), nitrogen-fixing plant (*Ceonothus* spp., *Chamaebatia foliolosa*), herbaceous (e.g., *Carex* spp., Poaceae), and bare soil. When multiple representatives of the same cover type occurred within a plot (which occurred frequently), we sampled under the representative closest to plot-center.

3.3.2 Soil sampling and preparation

We sampled mineral soil at two periods during 2017 (June 8-15 and September 6-14), representing two extremes of seasonal soil moisture and microbial activity in the Sierra Nevada (Hart et al. 1992, Qi and Xu 2001). The organic horizon was removed with a sterile, gloved hand, and a 2-cm diameter soil corer sterilized with 10% bleach followed by 70% ethanol was used to sample the top 5 cm of mineral soil at each point. We did not sample the organic horizon, because many of the 4- and 13-y plots did not have one, and we decided to keep the sampling among sites consistent. To collect enough soil from each point (~100 g), we took and composited multiple (~10) cores within a 20-cm diameter area within a given cover type stratum. Soil samples were placed in a sterile bag and immediately placed on blue ice (4 °C). Upon returning to the laboratory, soils were sieved field-moist (<2 mm; sieve sterilized with 70% ethanol before and between samples), and were subsampled to be frozen (-20 °C), refrigerated (4 °C), or air-dried until further analysis.

3.3.3 Site physical, chemical, and vegetative characteristics

Hobo Onset temperature sensors (UA-002-08; Bourne, MA, USA) were deployed at the center of each plot buried to a depth of 5 cm into the mineral soil. They recorded temperature every 2 hours from December 3, 2016 until May 15, 2018 to determine potential differences in the soil thermal environment among cover-type strata and sites.

In addition to measuring gravimetric water content at each sample time, we measured the water holding capacity (WHC) of the soils to understand the longer-term soil moisture dynamics in the field. Briefly, 10 g of field-moist soil was saturated with 50 g of deionized water and drained for 24 hours using a funnel with Whatman 42 filter paper (> 2.5 μm retentiveness) into a 1 L Mason jar. Soils were loosely covered with the lid of the Mason jar to minimize evaporation. Water drained by gravity was weighed and WHC was calculated as the sum of the undrained water and field moist gravimetric water content of the soil.

Prior to C and N analysis, air-dried soils were ground to a fine powder using mortar and pestle. Approximately 10 mg of oven-dry, ground soil were weighed into tin capsules, and these samples were analyzed for total C and N by continuous-flow, direct combustion and mass spectrometry using the ECS 4010 CHNSO analyzer (Costech Analytical Technologies, Inc., Valencia, CA, USA). Air-dried soils were analyzed for pH in a $1:2_{w/v}$ ratio of soil to both deionized water and 0.01 M $CaCl_2$ solutions with an Orion DUAL STAR pH meter (Thermo Fisher Scientific, Waltham, MA, USA).

We measured available inorganic N and P using ion-exchange resin bags. Bags were buried at 5-cm depth in the mineral soil at each sampling point and deployed during two time periods: October 17-27, 2016 until June 8-15, 2017, and June 8-15, 2017 until September 6-14, 2017, which were operationally defined as “wet” and “dry” seasons, respectively. We extracted ion-exchange resin with 2 M KCl following Binkley and Matson (1983), and extracts were analyzed for ammonium, nitrate, and orthophosphate colormetrically using the Lachat AE Flow Injection Auto analyzer (Lachat Instruments, Inc., Milwaukee, WI, USA). Final concentrations were normalized by oven-dry resin weight and length of field incubation.

We used the line-point intercept method to estimate relative proportions of cover type within each plot (Parker 1951). We set up a 10-m transect, which was bisected by the plot-center. A random bearing was selected to determine the orientation of each transect. Starting at 1 m, we recorded the substratum cover type (i.e., tree, seedling, shrub, nitrogen-fixing plant, herbaceous, bare) along the transect at 1-m increments for nine total measurements per plot, which we translated into percent cover.

3.3.4 Microbial biomass

We measured microbial biomass C and N by chloroform fumigation extraction (Vance et al. 1987, Hart and Firestone 1991, Haubensak et al. 2002). Briefly, 20 g of previously frozen and thawed, field-moist mineral soil was split into two equal samples. The non-fumigated sample was immediately extracted with 0.5 M K_2SO_4, and the fumigated sample was incubated for seven days in a chloroform-filled desiccator. After fumigation, the fumigant was removed using repeated evacuations and the samples were

then extracted with 0.5 M K_2SO_4. Fumigated and non-fumigated extracts were analyzed on a total organic C and total N analyzer (Shimadzu TOC-Vcsh with TNM-1 Total Nitrogen Measuring Unit, Kyoto, Japan). The difference in C and N concentration between these extracts is the chloroform-labile C and N pools, respectively. We used an extraction efficiency factor (k_{eC}) of 0.45 to convert chloroform-labile C to microbial C (Beck et al. 1997) and an extraction efficiency factor (k_{eN}) of 0.54 to convert chloroform-labile N to microbial N (Brookes et al. 1985). The mass ratio of microbial C:N was calculated to evaluate changes in microbial biomass stoichiometry. Performing chloroform fumigation-extraction on frozen soils may slightly affect the absolute values, but the relatively differences among treatments are generally unaffected (Hart and Firestone 1991, Stenberg et al. 1998).

3.3.5 Extracellular Enzyme Assays

We measured potential extracellular enzyme activity of β-glucosidase (BG), N-acetylglucosaminidase (NAG), and acid phosphatase (AP) fluorometrically following Bell et al. (2013). Briefly, an 800 µl soil slurry consisting of 2.75 g of field-moist soil in 91 mL of 50 mM sodium acetate buffer (pH = 5.5) was incubated with 200 µl of each of the 100 µM 4-methylumbelliferone (MUB)-linked substrates. After a 3-h incubation at 20 °C, plates were centrifuged and supernatant was transferred to a black, flat-well plates. Fluorescence was measured on a Tecan M200 Pro (Tecan Group Ltd., Männedorf, Switzerland) using an excitation wavelength of 365 nm and an emission wavelength of 450 nm.

The enzyme BG catalyzes the hydrolysis of ether bonds in polysaccharides releasing glucose, and is thus involved in the degradation of organic C. The enzyme NAG is involved in releasing N-acetylglucosamine from oligosaccharides such as chitin and peptidoglycan, and thus is considered a N-acquiring enzyme. The enzyme AP is involved in releasing phosphate from ester bonds, representing a P-mineralizing enzyme (Burns et al. 2013). Extracellular enzyme activities were expressed on both oven-dry soil mass, SOC concentration, and microbial biomass-C bases. The first two bases reflect ecosystem-level properties, while the third represents a microbial community-level property. Expressing activity on a microbial biomass basis represents the nutritional status of the microbial community, which allows comparisons across vastly different ecosystems (Boerner et al. 2005).

3.3.6 Microbial Activity and Net N mineralization

We measured heterotrophic, methane-oxidizer, and nitrifier microbial activity by carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) efflux, respectively, without substrate additions *ex situ* following Zibilske (1994). Briefly, 20 g of refrigerated, field-moist soils were adjusted to WHC and incubated in the dark for 31 days at 21.5 °C (±1 °C) in 1 L Mason jars fitted with rubber septa. We sampled 16 ml of the headspace at day 3, 9, 17, and 31. After each sampling date, jars were opened and flushed with ambient air. Headspace samples were then analyzed for CO_2, CH_4, and N_2O by gas chromatography using Shimadzu GC-2014 fitted with thermal conductivity, flame

ionization, and electron capture detectors (Shimadzu Corporation, Columbia, MD, USA). Specific rates of net C mineralization were calculated by dividing the rates of CO_2–C efflux over the 31-day period by the soil total C concentrations.

Inorganic N pool sizes before and after the 31-day incubation were determined by extracting 10 g (WHC weight) subsamples with 50 mL of 2 M KCl, and analyzing the filtered extracts for NH_4^+ and NO_3^- as described previously. Net N transformation rates were determined by the net changes in inorganic N pools over the 31-day incubation period (Binkley and Hart 1989).

3.3.7 Nitrification Potential

We estimated potential rates of nitrification using the shaken soil-slurry method (Hart et al. 1994), which approximates the capacity of ammonia oxidation in soil. Briefly, we created a soil slurry of 15 g of field-moist soil in a 100 mL NH_4^+ and PO_4^{3-} solution (1.5 mM of NH_4^+ and 1 mM of PO_4^{3-}) in a 250-ml flask and capped with a rubber stopper. The high concentrations of NH_4^+ and PO_4^{3-} ensure that nitrification is not substrate limited. Flasks were shaken (180 rpm) for 24-h. At 2-, 4-, 22-, and 24-h, 10 ml of suspension was removed from each flask and centrifuged at 8000 ×g for 8 min. The supernatant (5 ml) was removed from the centrifuged soil slurry, placed into a disposable polypropylene tube, capped, and stored at −20 °C until analysis for NO_3^- as described above. Nitrification potential is calculated as the rate of NO_3^- accumulation over time.

3.3.8 Statistical Analyses

All statistical analyses were conducted in R (R Development Core Team 2008) using the car (Fox and Weisberg 2011), lme4 (Bates et al. 2015), and MuMIn (Barton 2018) packages. Significance was determined at the $\alpha = 0.05$ level for all statistical tests. Marginal statistical significance was determined at the $\alpha = 0.10$ level. The replicated regression experimental design (Cottingham et al. 2005) allowed us to analyze our chronosequence using both regression and Analysis of Variance (ANOVA). Hence, we were able to robustly determine biogeochemical relationships with time since fire using regression, while also allowing for ANOVA and Tukey's Test of Honest Significant Differences to determine differences among sites.

We assessed the recovery of biogeochemical properties and processes at the plot-scale. At the plot-scale, data were proportionally averaged by the relative abundance of cover types for each plot. These data were then analyzed using mixed-effects models and mixed-design ANOVAs with time since fire and sample date as fixed factors, and plot as a random factor. We assessed the importance of cover type in controlling soil biogeochemical processes (e.g., extracellular enzyme activity, microbial activity, and N transformations) at the sample-scale and during the June sampling date when the soils were wettest and the greatest differences among sites and cover types were expected. At the sample-scale, data were analyzed using ANOVA with fire site, substratum cover type, and their interaction as independent variables. We assessed differences in vegetative cover type compositions using a chi-squared independence test.

For all statistical analyses, we used QQ-plots and scale-location plots to inspect normality and homoscedasticity, respectively. If these assumptions were not met, data were natural log-transformed, verified for normality and homoscedasticity, and reanalyzed (as was the case for inorganic N assessed by resin bags, CH_4 and N_2O effluxes, extracellular enzyme activities, and N transformation processes including nitrification potential).

3.4 Results

3.4.1 Site and soil characteristics

Cover type composition changed significantly among chronosequence sites (χ^2: $p < 0.001$, Figure 3-3). Between the most-recent fire site (4-y) and latest successional site (>115-y post-fire), tree cover increased from 8.3% to 77.8%, while N-fixing plant cover decreased from 65.3% to 4.2%. Grass and shrub (non-N fixing) cover was highest in the 25- and 44-y fire sites, reaching maxima of 33.3% for both.

Soil characteristics (e.g., soil total C and N, WHC, resin-available N and P, and microbial biomass C and N) did not differ by or interact with cover type ($p > 0.100$; data not shown), but at the plot-scale, soil physical and chemical characteristics were significantly affected by time since fire (Table 3-1). Total C and N in the upper 5 cm of the mineral soil increased with time since fire (Soil C: $p < 0.001$, $R^2 = 0.33$, $n = 37$, Figure 3-4a; Soil N: $p = 0.014$, $R^2 = 0.14$, $n = 37$), and the values of these soil characteristics in the 4- through 44-y post-fire sites were about 50% of the values in the >115-y site (Table 3-1). During the wet season (Nov-June), inorganic N availability assessed by ion-exchange resin was elevated in the 4-y site (Table 3-1); NO_3^- availability was on average 73 times that of the other sites ($p < 0.001$), and NH_4^+ availability was over six times greater in the 4-y site than the 44-y site ($p = 0.015$), although statistically similar to the other sites. During the dry season (June-Sept.), only the NH_4^+ availability differed by site ($p = 0.014$), with the 4- and 13-y sites 2.8 and 4.7 times higher than the >115-y site, respectively (4-y: $p = 0.033$, 13-y: $p = 0.014$). During the wet season, the PO_4^{-3} availability was four orders of magnitude higher in the >115-y site compared to the 4-y site ($p = 0.001$), and increased logarithmically with time since fire ($p < 0.001$, $R^2 = 0.53$, $n = 15$, Table 3-1). The availability of PO_4^{-3} was not significantly different between the 13-y site and the >115-y sites. During the dry season, the relationship between time since fire and PO_4^{-3} availability was not significant ($p = 0.778$, $n = 36$). However, the PO_4^{-3} availability was 3.5 to 5.5 times higher in the 44-y site compared to the 4- and >115-y sites (4-y: $p = 0.026$, >115-y: $p = 0.024$). Soil pH was highest in the 4-y site and decreased logarithmically with time since fire ($p = 0.002$, $R^2 = 0.22$, $n = 37$).

Microbial biomass C and N increased with time since fire (C: $p = 0.008$, $R^2 = 0.16$, $n = 37$; N: $p = 0.016$, $R^2 = 0.13$, $n = 37$). However, we were only able to detect marginally significant differences in microbial biomass C and unable to detect significant differences in microbial biomass N among sites (ANOVA: $p = 0.079$ and $p = 0.101$, respectively; Table 3-1). Microbial biomass C to N mass (C:N) ratio also did not change across the sites ($p = 0.105$). Microbial biomass C normalized by SOC (specific biomass)

differed among sites ($p = 0.017$), with specific biomass 36% lower in the 4-y site compared to the 25-y site (the largest difference between sites, $p = 0.023$, Figure 3-4b).

3.4.2 Extracellular enzyme activity

For all extracellular enzymes assayed, activities normalized by soil mass at the plot-scale increased with time since fire (BG: $p < 0.001$, $R^2 = 0.62$, $n = 37$; NAG: $p < 0.001$, $R^2 = 0.55$, $n = 37$; AP: $p < 0.001$, $R^2 = 0.68$, $n = 37$; Figure 3-5a-c). However, there were significant interactions with sample date for BG and AP ($p < 0.001$), indicating that differences in activity among chronosequence sites were only apparent during the June (wet) sampling date. During the dry season sampling date, enzyme activities were consistently low. For the June sampling date, the 4-y site was, on average, 70-80% lower than the >115-y site for the assayed extracellular enzymes ($p < 0.001$). Even in the 44-y site, NAG and AP were still 69% and 58% lower than >115-y site, respectively (NAG: $p < 0.001$, AP: $p = 0.016$). Activity for BG in the 44-y site was only marginally lower than the >115-y site ($p = 0.077$, -39%). However, for BG and AP, the 25-y site was not significantly different from the >115-y site ($p = 0.933$ and $p = 0.279$, respectively).

Extracellular enzyme activities during the June sampling date normalized by microbial biomass increased logarithmically with time since fire (BG: $p = 0.032$, $R^2 = 0.10$, $n = 37$, Figure 3-4b; NAG: $p = 0.024$, $R^2 = 0.11$, $n = 37$, Figure 3-S1a; AP: $p = 0.001$, $R^2 = 0.24$, $n = 37$, Figure 3-S1b). The biomass-normalized enzyme activity for BG and AP was almost half in the 4-y site compared to all other sites ($p < 0.05$). However, for NAG, the 13- through 44-y sites were not significantly different from the 4-y site (13-y: $p = 0.130$, 25-y: $p = 0.977$, 44-y: $p = 0.781$), while the >115-y was significantly higher ($p = 0.033$).

Extracellular enzyme activity normalized by SOC showed similar patterns, increasing with time since fire for the June sampling date. However, while BG normalized by SOC increased logarithmically through the first 44 years ($p < 0.001$, $R^2 = 0.33$, $n = 37$), the 4 y site was not significantly different from the >115 y site ($p = 0.121$, Figure S2a). Both NAG and AP activities normalized by SOC increased logarithmically with time since fire (NAG: $p < 0.001$, $R^2 = 0.20$, $n = 37$, Figure 3-S2b; AP: $p < 0.001$, $R^2 = 0.27$, $n = 37$, Figure 3-S2c).

3.4.3 Microbial Activity

At the plot-scale, cumulative respiration increased with time since fire ($p < 0.001$, $R^2 = 0.34$, $n = 37$) but interacted significantly with sample date ($p < 0.001$; Figure 3-6a), indicating that differences in respiration among chronosequence sites were only apparent during the June sampling date. For the June sampling date, respiration was, on average, 65% lower for the 4 through 44-y sites compared to the >115-y site; however, the reduction was not significant in the 25-y site ($p = 0.158$, $p < 0.05$ for the other three sites). On average, the September sampling date had 25-63% lower respiration rates than the June sampling date ($p = 0.002$), with the greatest reductions in the >115-y site. June respiration normalized by SOC and MBC did not change with time since fire ($p = 0.778$

and p = 0.226, respectively, data not shown), resulting in respiration being positively correlated with SOC concentrations (r = 0.85, p < 0.001, n = 115, Figure 3-4c).

Net methane emissions were unaffected by time since fire (p = 0.201) and sample date (p = 0.806, Figure 3-6b). Nitrous oxide emissions increased logarithmically with time since fire (p < 0.001, R^2 = 0.29, n = 37) and were unaffected by sample date (p = 0.911, Figure 6c). Averaged for both sample dates, nitrous oxide emissions in the 4-y site were 10% of the >115-y site (p = 0.036), but the 13-y site was not significantly different from the >115-y site (p = 0.567).

3.4.4 Nitrogen transformations

At the plot-scale, net N mineralization and nitrification were highest in the 4-y fire site and decreased logarithmically with time since fire (net N mineralization: p < 0.001, R^2 = 0.30, n = 37; net nitrification: p < 0.001, R^2 = 0.22, n = 37; Figure 3-7a, b). However, there were significant interactions with sample date for both net N mineralization and net nitrification (p = 0.003 and p = 0.033, respectively). Differences in net N mineralization and net nitrification during the incubation were dampened for samples collected at the end of the dry season (i.e., September). At this sampling date, there were higher net rates of N transformations in the >115-y site compared to the June sampling date. During the wet season sampling date (i.e., June), net N mineralization decreased logarithmically from 0.96 (SE = 0.16) mg N kg^{-1} d^{-1} in the 4-y site to -0.10 (SE = 0.17) mg N kg^{-1} d^{-1} in the >115-y site, and net nitrification in the 4-y site was six times higher than the >115-y site (1.24 to 0.27 mg N kg^{-1} d^{-1}). For the June sampling date, both net N mineralization and nitrification were significantly higher in the 4-y site compared to the >115-y site (both: p < 0.001), but the 13-y through 44-y sites were not significantly different from the >115-y site (p > 0.100). Overall, net N mineralization and nitrification were highly correlated (Spearman's rho = 0.90, p < 0.001, n = 116).

At the plot-scale, soil nitrification potential showed a non-monotonic response to time since fire (p < 0.001; Figure 3-7c), with the highest potentials in the 44-y site. Nitrification potential at this site was about 3.5 times higher than the 13-y (p = 0.002) and >115-y sites (p < 0.001). The 4- and the 25-y sites were also 2 and 1.5 times higher than the >115-y site, respectively (both: p < 0.001). Nitrification potential was unaltered by the main effect of sample date (p = 0.109), and this trend was consistent across both sampling dates (i.e., no interaction; p = 0.300).

3.4.5 Influence of cover type

Cover type was not a consistent predictor of biogeochemical processes (Table 3-2, Figure S3-S5). However, microbial respiration and net nitrification significantly differed by cover type (p = 0.024 and p = 0.033, respectively). Upon further inspection using Tukey's HSD tests, microbial respiration under N-fixing plants was marginally higher than under the bare cover type (30%, p = 0.082), but we failed to detect even marginally significant differences among cover types for net nitrification due to the loss of degrees of freedom and inferential power when assessing multiple comparisons. There was also a significant cover type by time since fire interaction for N_2O emissions (p = 0.040). In the

44-y site, N_2O emissions were, on average, 10 times higher under N-fixing plants compared to trees, although this was only marginally significant ($p = 0.075$). There was a similar difference in the 25-y site with soils under N-fixing plants producing, on average, 10 times higher N_2O emissions than bare soils, but again this was only marginally different ($p = 0.058$).

3.5 Discussion

3.5.1 Multi-decadal impacts on C cycling

Consistent with our hypothesis, ecologically novel, high-severity fire resulted in multi-decadal alterations to the C cycle—specifically, decreased mineral SOC (Table 3-1), soil extracellular enzyme activity (Figure 3-5), and soil microbial respiration (Figure 3-6a), and the controls on C cycling (particularly soil microbial respiration) changed with time (Figure 3-4 a, b, c). Whereas combustion of the organic soil horizon is common, generally, losses of mineral SOC from fire are rare. In a global meta-analysis encompassing 57 publications across both prescribed fire and wildfire, post-fire mineral SOC concentrations decreased by 11% (Nave et al. 2011). Mineral SOC is more resistant to combustion compared to organic horizon C due to the high heat capacity of mineral soil (Neary et al. 1999). Hence, higher-severity fire patches have been shown to result in greater mineral soil C losses compared to low severity patches in northern mixed-hardwood forests (Kolka et al. 2014). Even under high-severity wildfire, other sites in the Sierra Nevada and similar dry coniferous forests did not have significant decreases in mineral SOC 3-30 y following fire compared to unburned sites (Johnson et al. 2005, Grady and Hart 2006, Kaye et al. 2010, Ross et al. 2012, Adkins et al. 2019). However, there were, on average, 20% decreases in soil C regardless of burn severity directly after fire across five mixed-conifer sites in California when the site was assessed pre/post fire (Miesel et al. 2018). This difference highlights the nuanced congruence between fire severity and mineral SOC loss, the importance of time since fire, and potential differences between experimental and retrospective studies. Our long-term (> 20 y) reduction in mineral SOC compared to unburned sites has, to the best of our knowledge, not been described by the literature. In another high-severity wildfire chronosequence in a Mediterranean *Pinus* forest mineral SOC recovered 12 y post-fire after an initial decrease (Kavdir et al. 2005). The scarcity of long-term high-severity fire chronosequences prevents a global interpretation of the long-term effects of high-severity fire on SOC pools and underscores the importance of long-term fire ecology research.

Surprisingly, wet season respiration normalized by SOC, which is an index of C quality and availability (Grady and Hart 2006, Rousk and Frey 2015), was unaffected by fire. Pyrolization of organic matter results in an increase in aromatic compounds that are generally more resistant to microbial decay (Keiluweit et al. 2010, Bird et al. 2015). Frequently, this results in decreased respiration (Grady and Hart 2006, Santos et al. 2012, Whitman et al. 2016). However, because wet season respiration in our study was positively correlated with SOC concentrations (Figure 3-4d), it is likely that long-term

decreased C quantity, not quality or accessibility, decreased rates of respiration throughout the first four decades post-fire (Figure 3-4a).

In the short-term (< 10 y), decreased soil microbial biomass and activity likely also decreased microbial respiration. While microbial biomass on a soil mass basis was only marginally significantly affected by fire, the fraction of SOC as microbial biomass C was lowest in the 4-y fire site (Figure 3-4b). This suggests that there were indirect effects of fire on biomass growth besides C, such as altered soil structure, decreases in the quality of litter inputs, or reductions in other limiting resources (e.g., PO_4^{3-} availability was lowest in the 4-y site, Table 3-1). Alternatively, fire-induced microbial mortality may still have had a lingering effect 4 y post-fire, as there was less microbial biomass C than would be expected per mass of total C. Additionally, wet season extracellular enzyme activity expressed on a microbial biomass basis and on a soil mass basis increased with time since fire (Figure 3-4a), suggesting that the microbial communities were less-active immediately post-fire. Enzyme activities generally are lower in burned sites than unburned controls (Guénon et al. 2013, Knelman et al. 2015, 2017). However, activities normalized by microbial biomass are often not reported. Thus, it is unclear if published patterns are in response to decreased microbial biomass or altered edaphic conditions that decrease activity. For instance, increased soil pH (seen in the 4-y site, Table 3-1) can have a negative effect on soil extracellular enzyme activity (Sinsabaugh et al. 2008, Kivlin and Treseder 2014) because the pH optima for many extracellular enzymes is ~5 (Leprince and Quiquampoix 1996, Turner 2010). Therefore, short-term changes in soil conditions along with decreased soil microbial biomass lowered soil enzyme activity and likely dampened microbial respiration (Figure 3-4 a, b, c).

Consistent methane efflux among time since fire sites is largely at odds with other studies that show that *in situ* methane uptake increases at least 10 y post-fire in semi-arid ecosystems (Gathany and Burke 2001, Sullivan et al. 2011). Whereas decreased plant cover altered soil moisture and temperature dynamics, which impact methane dynamics (Segers 1998), the presence of an O horizon and ability of methane to diffuse into the mineral soil is the greatest driver of methane uptake (Sullivan et al. 2011). However, unlike these previous studies, we measured methane efflux under laboratory conditions, controlling for water potential (i.e., soils were incubated at WHC) and temperature and disrupting the original soil structure. Our findings show that *potential* methane uptake slightly increases with time since fire, possibly due to the recovery of the methane-oxidizer community. There are relatively few studies describing methane dynamics post-fire in semi-arid ecosystems. Given that methane is an important greenhouse gas, which may change with wildfire, and that semi-arid ecosystems are projected to burn more frequently (Westerling et al. 2006, Restaino and Safford 2018), future studies of the C cycle in response to wildfire should include methane dynamics.

3.5.2 Short-term impacts on nitrogen cycling caused by increased nitrogen availability

Our hypothesis that N cycling processes would be increased by substrate availability shortly after fire (< 10 y) was supported by the data. The drastic increase in N availability after fire in fire-suppressed ecosystems is corroborated by numerous studies (St. John and Rundel 1976, Covington and Sackett 1992, Kaye and Hart 1998, DeLuca

and Sala 2006, Johnson et al. 2007, 2008, Kurth et al. 2014), and may be due to fire-induced N mineralization (St. John and Rundel 1976), wetter and warmer conditions that increase mineralization of organic matter (Kaye and Hart 1998), increased cover of N-fixing plants (Johnson et al. 2005), decreased C:N ratio of litter from post-fire vegetation (e.g., grasses, Boyle et al. 2005), or lower plant N uptake post-fire (Kurth et al. 2014). Combined with decreased C availability in the recent fire sites, post-fire N availability likely contributed to a relative decrease in microbial N demand and increased wet season net rates of N mineralization and nitrification possibly through decreased N immobilization (Kaye and Hart 1998).

Our hypothesis that fire-induced changes to N transformations would last longer than one decade was largely unsupported by the data. Whereas others have seen long-term increases in gross, net, and potential nitrification one to three decades post-wildfire in seasonally dry mixed-conifer forests similar to our sites and more globally (DeLuca and Sala 2006, Kurth et al. 2014, Wang et al. 2014), we only detected increased net nitrification and nitrate flux in the 4-y site and increased nitrification potential in the 44-y site compared to the >115-y site. The lack of correspondence between nitrification potential and net nitrification suggests that net nitrification was unaltered by changes to the nitrifier population size (which nitrification potential is directly related to, Belser 1979). Furthermore, N_2O efflux (which we used as a measure of nitrifier activity given soils were incubated under putative aerobic conditions) increased with time since fire and was also uncorrelated with both net nitrification and nitrification potential. This suggests that differences in the microsite heterogeneity of ammonium availability among sites caused variations in NO_3^- consumption further uncoupling gross and net rates of nitrification (Davidson et al. 1990). High rates of NO_3^- consumption in mature forests (with relatively high N-demand like our >115-y site) has been shown to mask gross nitrification in mature forest soils (Stark and Hart 1997), so it is not necessarily surprising that these three indices of the nitrifier community were uncorrelated among sites with vastly different C and N availabilities.

The relatively low rates of potential nitrification and N_2O efflux at the 4- through 25-y sites could be due to longer term decreases in population of ammonia oxidizers (Yeager et al. 2005, but see Long et al. 2014), which are more sensitive to heat compared to heterotrophic bacteria (Dunn et al. 1985). Differences in the recovery of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) could also drive the response of nitrification potential and nitrification-derived N_2O emissions, as AOB show a stronger relationship with nitrification potentials (Carey et al. 2016) and are relatively more "leaky" in terms of N_2O emissions during nitrification (Di et al. 2010). Therefore, further analysis of the microbial community might help elucidate different patterns of post-fire N-cycling among studies.

3.5.3 Influence of cover type

Our hypothesis that the long-term recovery of soil biogeochemical processes would be controlled by vegetative cover type was largely unsupported. In a few instances, rates of biogeochemical processes were greater under N-fixing plants (i.e., microbial respiration and N_2O emissions); however, we found no evidence of cover type

becoming more important with time since fire. This is largely at odds with the previous literature describing the recovery of biogeochemical processes following historical fires, which suggests that C and N inputs from recovering vegetation structure the microbial community composition and function (Hart et al. 2005). For instance, grass patches resulting from forest restoration treatments (which include prescribed burning) increase net rates of N transformations and enzyme activities and influence community-level physiological profiles likely through the supply of low C:N ratio plant litter (Kaye and Hart 1998, Boyle et al. 2005). Furthermore, the increase in N-fixing plant (e.g., *Ceanothus* spp.) abundance following wildfire has been linked with increased SOC and microbial activity (Johnson et al. 2005). These inputs may also interact with microbial processes, as increased shrub cover during secondary succession has been linked to greater concentrations of phenolic compounds which may inhibit nitrification (MacKenzie et al. 2004). Recovering vegetation may also affect N and water availability through plant demand for these soil resources. In the first year after high severity fire in a ponderosa pine (*Pinus ponderosa*) ecosystem, soils underneath the herb golden corydalis (*Corydalis aurea*) had higher levels of microbial biomass and soil N and lower levels of NH_4^+ and moisture than bare soils (Knelman et al. 2015). Our results demonstrate that plant cover type may not affect the recovery of biogeochemical processes after fire in all contexts.

It is possible that the effect of ecologically novel, high-severity fire was so great that it masked the influence of post-fire vegetation. Post-fire vegetation likely affects soil microorganisms and biogeochemical function through changes to the supply of C through litter and belowground inputs (e.g., net primary production), the quality of C (e.g., percent lignin, C:N ratio), competition for nutrients between the plant and microbial communities, and direct symbioses (e.g., mycorrhizae, Hart et al. 2005). However, mineral SOC concentrations only differed by fire not cover type, and C quality assessed by SOC-normalized respiration was similar regardless of fire and cover type. While post-fire plant C inputs may vary among cover types, these inputs are likely much smaller than the relatively large C pool combusted by these high-severity wildfires.

It is also possible that these vegetation effects emerge at different scales not sampled in our study. Rhizosphere microorganisms are often controlled by the species they have colonized (Bais et al. 2006); however, we did not differentiate between rhizosphere and bulk soil under each cover type. Also, the influence of vegetation can extend several meters into the surrounding bulk soil (Saetre 1999). Hence, patterns may emerge at the rhizosphere-, stand-, or plot-scale (Kaye and Hart 1998, Boyle et al. 2005, Hart et al. 2005) but not the soil-core scale (as in our study). Our plot-scale measurements were an integration of all cover types found in the plot, so we were not able to test a cover type effect at the plot-scale. The emergence of ecological phenomena at different scales is common and underscores the need for multi-scale approaches (Levin 1992). Whereas many biochemical transformations occur at microscopic scales, plants likely operate on scales orders of magnitude larger. Hence, understanding the interactions within the plant-soil-microbe system necessitates research at multiple scales, both spatial and temporal.

3.5.4 Seasonal dynamics of biogeochemical rates

Consistent with our hypothesis, differences among fire sites for a majority of the biogeochemical rates were only apparent in the wet season. The Sierra Nevada of California has a Mediterranean-type climate and is characterized by cool, wet winters and warm, relatively dry summers. During the dry late-summer months, soil microorganisms enter a state of relative dormancy (Lennon and Jones 2011). Hence, rates of extracellular enzyme activity measured at field-moisture and CO_2 efflux measured under non-water limiting conditions were consistently low across all sites. However, net rates of N mineralization and nitrification were consistently higher across all sites when sampled during the dry season. This indicates that soil microorganisms were still mineralizing organic matter, but likely with decreased N immobilization, suggesting that maintenance rather than growth was favored under these conditions. These findings further exemplify how microbial dormancy affects biogeochemical cycles (Hart et al. 1992, Stark and Firestone 1995, Schimel et al. 2007) and that the season during which soils are sampled can affect the ability to detect changes among treatments.

3.6 Conclusion and management implications

Previous studies suggest that soil biogeochemical processes are generally resilient to wildfire within the historical range of variation for that ecosystem. However, we show that impacts of fire can be long-lasting in instances of disturbances outside the historic range of variation (over twice the historical fire return interval for these forests, Fry et al. 2014). Our data suggest that high-severity wildfires have both short- and long-term implications. The transition from microbial biomass limitation of respiration at early stages of recovery to SOC limitation at later stages of recovery suggests the changing controls of biogeochemical processes post-fire, and underscores the importance of long-term fire ecology research. Whereas fire chronosequences may suffer from site-specific characteristics that cannot be controlled for (e.g., micro-scale variations in soil properties) or climatic events that are time-dependent (e.g., El Niño events), we show the utility of this approach when sites are carefully selected for common characteristics and when recovery from disturbances occur over long timescales.

At large spatial scales, high-severity fires in the mixed-conifer forests of the Sierra Nevada have numerous negative impacts on human health and ecosystem services. Prescribed burns, selective thinning, and fires managed for resource benefits can help prevent ecologically novel, predominantly high-severity fires (Safford et al. 2009). Hence, these management techniques could be useful in maintaining ecosystem services in the face of climate change and increasing fire severity in dry coniferous forests of the Sierra Nevada and elsewhere. For instance, little if any mineral SOC is combusted during prescribed or low-severity fire in coniferous forests (Boerner et al. 2009, Ryu et al. 2009, Nave et al. 2011, Pellegrini et al. 2018) in contrast to 20% loss during high-severity fire (Miesel et al. 2018). The long-term 45-69% decreases in mineral SOC, relative to the unburned site, found in our study are uncommon; however, areas burning at high-severity are proportionally increasing over time (Miller et al. 2009b, Steel et al. 2018) along with

fire frequency (Westerling et al. 2006). Therefore, these "rare" occurrences are becoming more common, putting large SOC pools found in temperate coniferous forests at risk. Our results suggest that sustainable forest management in the mixed-conifer forests of the Sierra Nevada should prioritize mitigation of ecologically novel, high-severity fire through prescribed burns, selective thinning, and wildland use fires.

3.7 References

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3.9 Tables

Table 3-1: Mean (standard error) plot-scale soil properties of the chronosequence sites and coefficients of determination (r^2) of the positive (+) or negative (-) correlation with time since fire (n = 37). Letters represent significant differences among sites (ANOVA; $\alpha = 0.05$).

	4-yr	13-yr	25-yr	44-yr	>115-yr	Time since fire (r^2)
Soil C (%)	7.81 (0.91)[a]	4.36 (0.50)[a]	6.42 (1.81)[a]	6.35 (0.72)[a]	14.25 (2.69)[b]	+ **0.32**
Soil N (%)	0.38 (0.05)[ab]	0.17 (0.02)[a]	0.29 (0.06)[ab]	0.30 (0.04)[ab]	0.56 (0.12)[b]	+ **0.14**[6]
Soil C:N	25.0 (0.9)[a]	30.0 (0.6)[bc]	27.6 (1.16)[abc]	25.2 (0.5)[ab]	31.3 (1.8)[c]	+ **0.13**[7]
DOC (K_2SO_4-extractable; mg kg^{-1})	205 (34)[ab]	91 (10)[a]	144 (27)[a]	135 (19)[a]	300 (48)[b]	+ **0.19**[7]
DON (Total – KCl-extractable; g kg^{-1})	3.76 (0.50)[b]	1.72 (0.21)[a]	2.87 (0.63)[ab]	3.03 (0.40)[ab]	5.56 (1.18)[b]	+ **0.14**[7]
Microbial biomass C (mg kg^{-1})	427 (71)	317 (44)	483 (66)	420 (75)	710 (156)	+ **0.16**
Microbial biomass N (mg kg^{-1})	54.2 (8.0)	50.6 (7.6)	85.9 (14.8)	71.3 (14.6)	104.0 (24.3)	+ **0.13**[8]
Microbial C:N	8.51 (0.91)	6.45 (0.19)	6.32 (0.68)	6.63 (0.73)	6.72 (0.56)	N.S.[9]
wet season[1] NH_4^+ (mg kg^{-1} resin d^{-1})	0.092 (0.037)[b]	0.031 (0.001)[ab]	0.051 (0.012)[ab]	0.015 (0.002)[a]	0.036 (0.011)[ab]	N.S.
dry season[2] NH_4^+ (mg kg^{-1} resin d^{-1})	0.165 (0.038)[b]	0.274 (0.128)[b]	0.142 (0.050)[ab]	0.107 (0.023)[ab]	0.058 (0.006)[a]	N.S.
wet season NO_3^- (mg kg^{-1} resin d^{-1})	4.002 (1.589)[b]	0.031 (0.001)[a]	0.051 (0.030)[a]	0.063 (0.030)[a]	0.248 (0.116)[a]	N.S.
dry season NO_3^- (mg kg^{-1} resin d^{-1})	0.102 (0.045)	0.040 (0.002)	0.040 (0.002)	0.052 (0.005)	0.040 (0.009)	N.S.
wet season PO_4^{3-} (µg kg^{-1} resin d^{-1})	0.2 (0.1)[a]	65.1 (25.2)[b]	11.8 (1.0)[b]	19.5 (1.2)[b]	182.0 (43.6)[b]	+ **0.53**[8]
dry season PO_4^{3-} (µg kg^{-1} resin d^{-1})	17.6 (2.9)[a]	45.1 (5.0)[ab]	21.9 (2.0)[ab]	78.0 (14.8)[b]	12.1 (1.28)[a]	N.S.
$pH_{0.01\ M\ CaCl2}$	5.57 (0.08)[b]	4.83 (0.14)[a]	4.73 (0.09)[a]	4.53 (0.09)[a]	4.97 (0.17)[a]	- **0.22**[6]
WHC[3] (kg kg^{-1})	0.81 (0.05)[a]	0.83 (0.10)[a]	0.83 (0.10)[a]	0.76 (0.09)[a]	1.38 (0.18)[b]	+ **0.31**[6]
Soil MAT[4] (°C)	12.6 (0.9)	12.7 (0.2)	11.3 (1.0)	10.6 (0.1)	9.4 (0.7)	- **0.26**
Growing season[5] mean temp. (°C)	17.33 (1.6)	18.5 (0.1)	16.7 (1.3)	15.0 (0.3)	12.7 (0.7)	- **0.37**

[1]Nov. – June [2]June – Sept. [3]Water Holding Capacity [4]Mean Annual Temperature [5]May – Dec. [6]natural log transformation of the independent variable [7]natural log transformation of the dependent variable [8]natural log transformation of the independent and dependent variable [9]Not significant ($p > 0.05$)

Table 3-2: Sample-scale summary statistics (p-values) for the influence of time since fire and substratum cover type on biogeochemical processes using analysis of variance.

	Time since fire	Cover type	Interaction
Microbial Activity			
CO_2 (mg C-CO_2 kg^{-1})	**< 0.001**	**0.024**	0.616
CH_4 (mg C-CH_4 kg^{-1})	**< 0.001**	0.652	0.775
N_2O (mg N-N_2O kg^{-1})	**0.001**	0.077	**0.040**
Exo-enzymes activities			
β-Glucosidase (μmol kg^{-1})	**< 0.001**	0.119	0.459
N-acetylglucosaminidase (μmol kg^{-1})	**< 0.001**	0.560	0.969
Acid Phosphatase (μmol kg^{-1})	**< 0.001**	0.213	0.072
N transformations			
Net N Mineralization (mg N kg^{-1})	**< 0.001**	0.056	0.288
Net Nitrification (mg N-NO_3^- kg^{-1})	**< 0.001**	**0.033**	0.494
Nitrification Potential (mg N-NO_3^- kg^{-1})	**< 0.001**	0.367	0.888

Key: Bolded values are considered significant ($\alpha = 0.05$)

3.10 Figures

Figure 3-1: Conceptual model of hypothesized recovery of ecosystem function after different disturbances. We hypothesize that whereas high-severity disturbances (gray line) may result in greater functional responses than low-severity disturbances (black line), resilience is most impacted by novel disturbances (orange line; e.g., wildfire with severity greater than the historic range of variation). The orange line is dashed at the tail end of recovery to indicate that ecosystems may not recover to pre-disturbance conditions after novel disturbances.

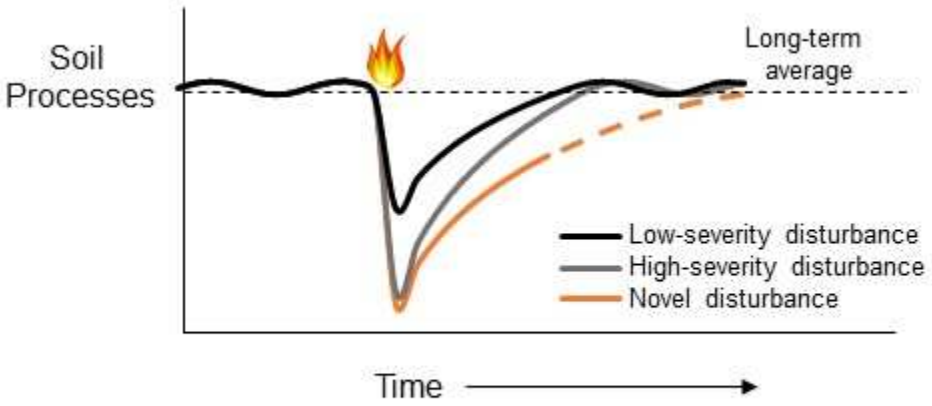


Figure 3-2: Map of the Central Sierra Nevada Fire Chronosequence. a) Symbols denote approximate locations of plots and polygons show fire perimeters (plots are at least 150 m apart and may not be visibly distinguishable at the spatial scale). b) Fire Return Interval Departure (Safford and van de Water 2014) is plotted for the Sierra Nevada with a red rectangle denoting the extent of the chronosequence area. Higher values represent greater fire suppression, and white lines show county boundaries for reference.

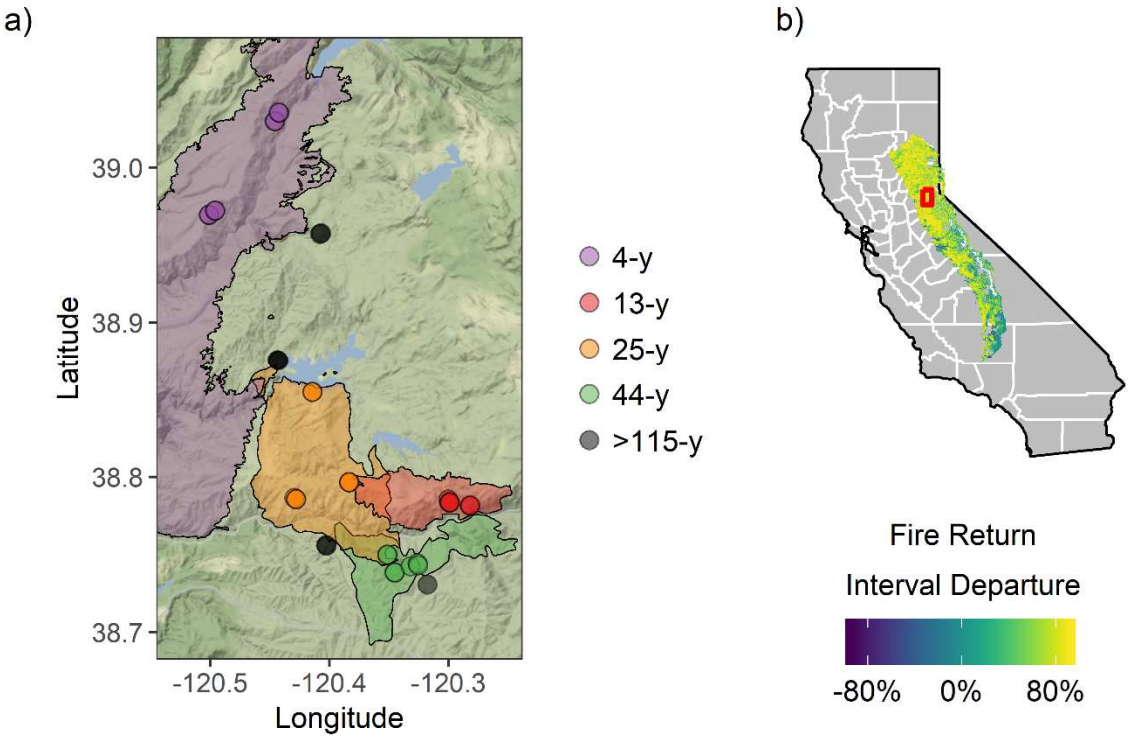


Figure 3-3: Plot-scale mean percent plant cover types for each chronosequence site (n = 8). Cover types are tree (e.g., *Abies concolor*, *Pinus ponderosa*, *Quercus* spp.,), tree seedling (e.g., *Pinus ponderosa*), shrub (*Arctostaphylos* spp., *Salix* spp.), nitrogen-fixing plant (*Ceanothus* spp., *Chamaebatia foliolosa*), herbaceous (e.g., *Carex* spp., Poaceae), and bare soil. Error bars show the standard error of the mean (13 y site: n = 6, 44 y site: n = 7, all other sites: n = 8).

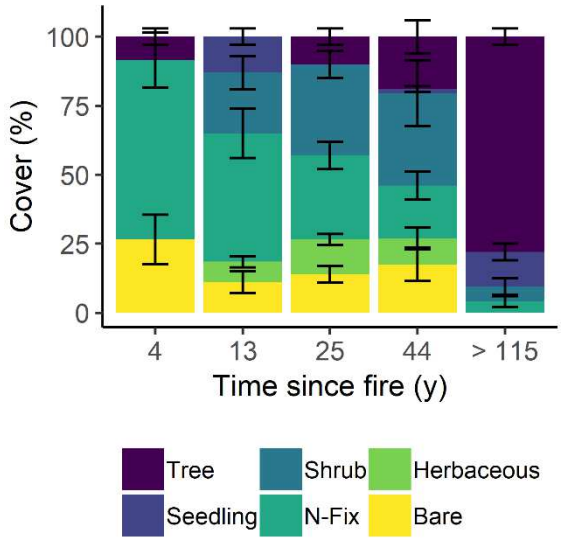


Figure 3-4: Control of carbon (C) cycling processes in the long-term recovery after fire. a) June plot-scale soil organic C (SOC; orange line represents third-order polynomial linear regression, $p < 0.001$, $R^2 = 0.35$) plotted against time since fire, b) MBC normalized by SOC against time since fire, c) β-glucosidase normalized by microbial biomass C (MBC; orange line represents second-order polynomial linear regression, $p = 0.012$, $R^2 = 0.18$) plotted against time since fire, and d) sample-scale respiration as a function of SOC quantity (orange line shows best-fit regression, $p < 0.001$, $R^2 = 0.56$, $n = 114$). For all panels, shaded region shows the 95% confidence interval of the best-fit regression. For plot-scale measurements (a, b, and c), n = 6 in the 13 y site, n = 7 in the 44 y site, and n = 8 in all other sites.

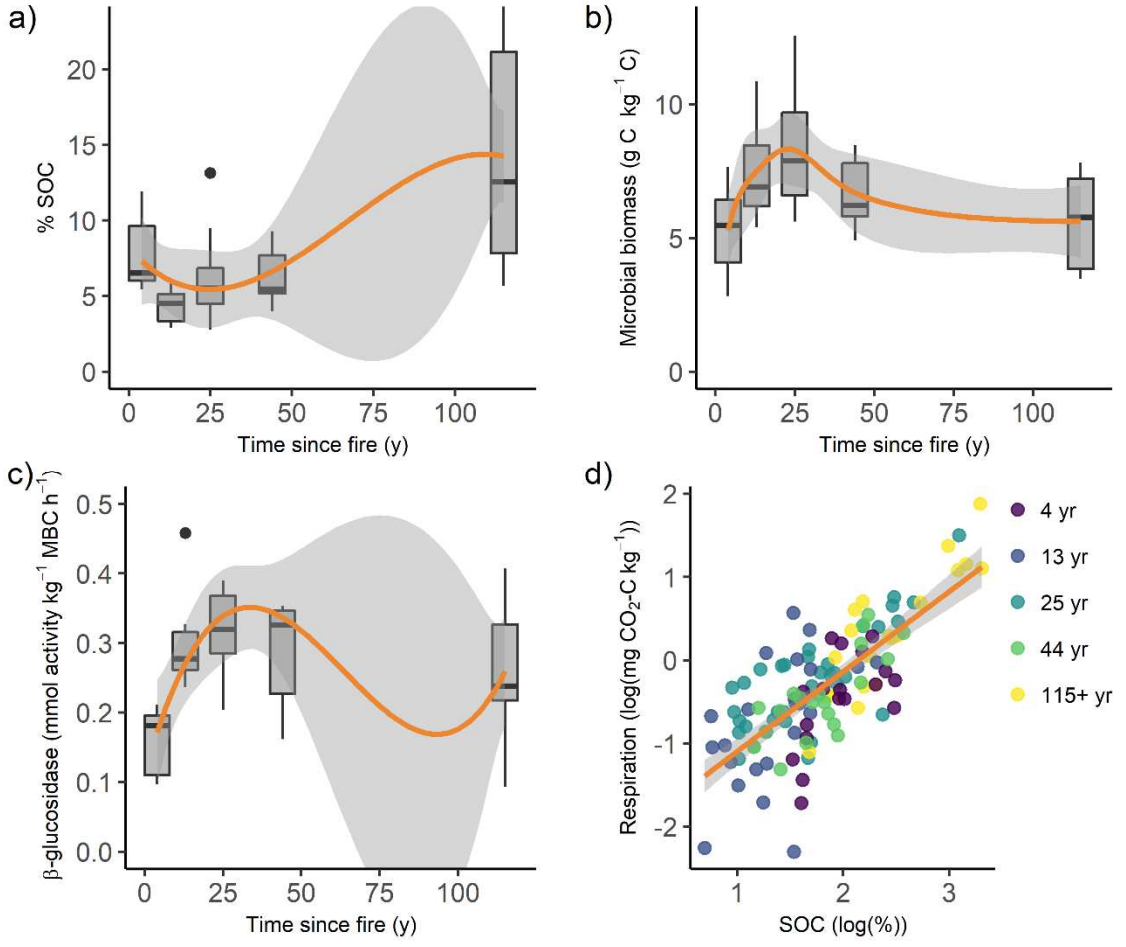


Figure 3-5: Plot-scale extracellular enzyme activity for (a) β-glucosidase (BG), (b) N-acetylglucosaminidase (NAG), and (c) acid phosphatase (AP) with time since fire (13 y site: n = 6, 44 y site: n = 7, all other sites: n = 8). Gray and white boxplots representing the median, interquartile range (IQR), and 1.5 x IQR for enzyme activities at each chronosequence site during June and September, respectively. Orange lines show the best-fit regression of extracellular enzyme activity with time since fire, and shaded area shows the 95% confidence interval of the regression.

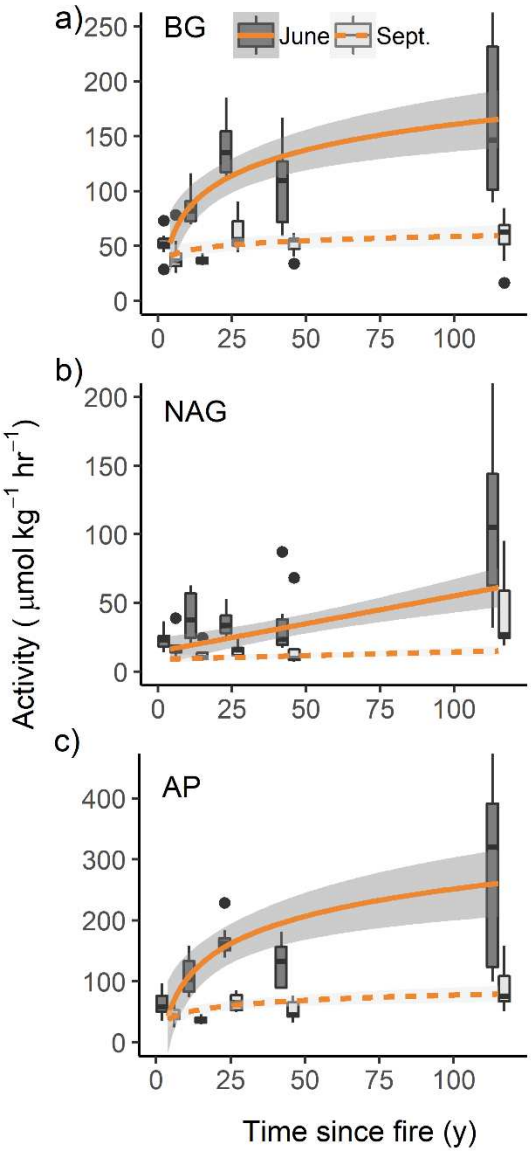


Figure 3-6: Plot-scale cumulative gas fluxes for (a) carbon dioxide (CO_2), (b) methane (CH_4), and (c) nitrous oxide (N_2O) from 31-day laboratory incubations (13 y site: n = 6, 44 y site: n = 7, all other sites: n = 8). Gray and white boxplots representing the median, interquartile range (IQR), and 1.5 x IQR for gas fluxes at each chronosequence site during June and September, respectively. Orange lines show the best-fit regression of gas emissions with time since fire, and shaded area shows the 95% confidence interval of the regression. There was no statistically significant interaction between sample date and time since fire for CH_4 and N_2O fluxes, so for these response variables only one regression line is shown.

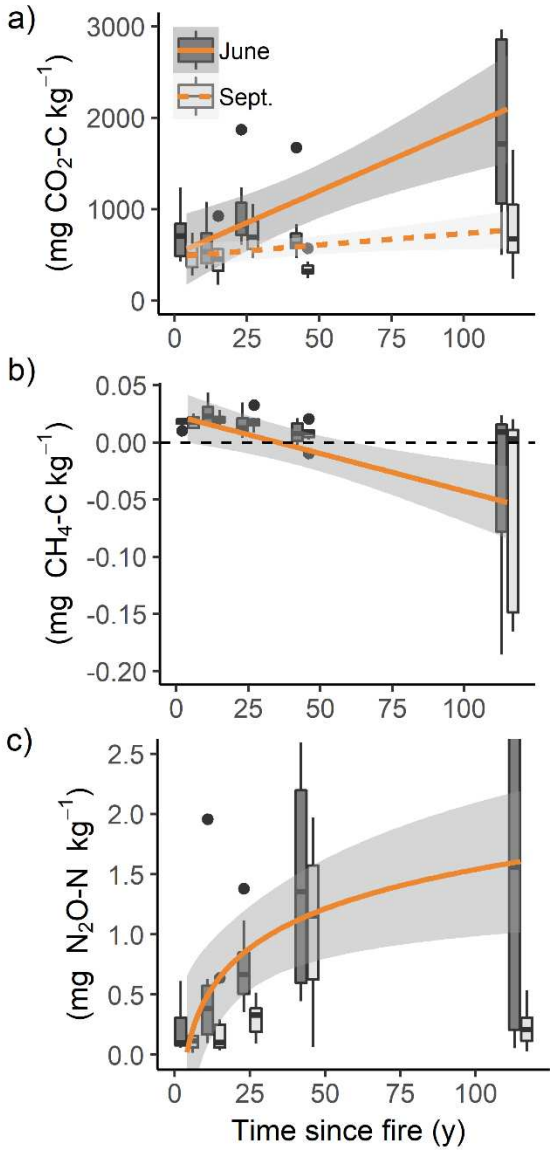
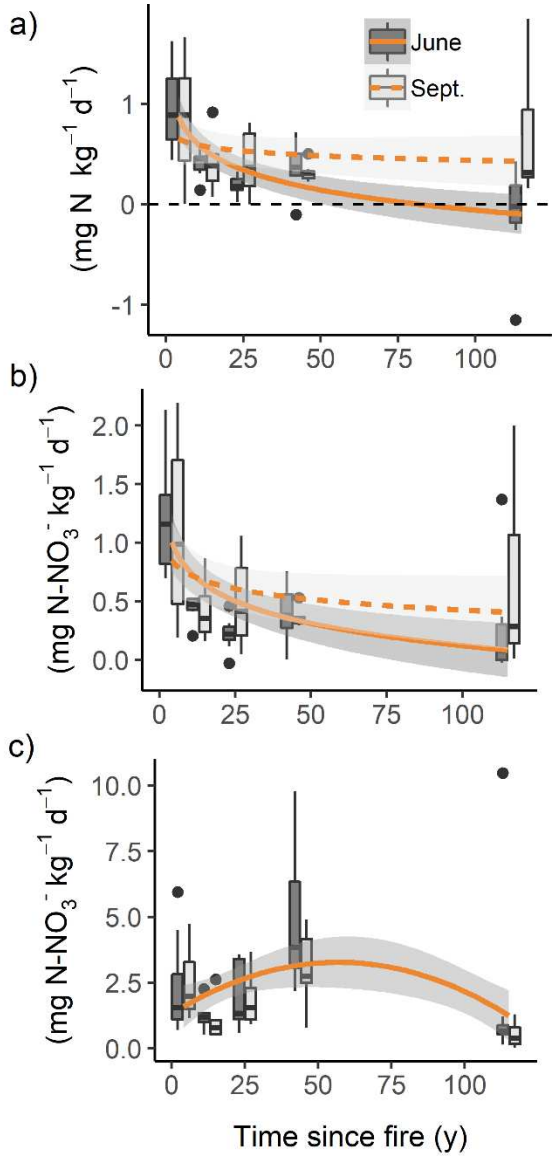


Figure 3-7: Plot scale net nitrogen (N) mineralization (a) and net nitrification (b) during a 31-day laboratory incubation, and potential nitrification (c; 13 y site: n = 6, 44 y site: n = 7, all other sites: n = 8). Gray and white boxplots representing the median, interquartile range (IQR), and 1.5 x IQR for N transformations at each chronosequence site during June and September, respectively. Orange lines show the best-fit regression of N transformations with time since fire, and shaded area shows the 95% confidence interval of the regression. There was no statistically significant interaction between sample date and time since fire for nitrification potential, so for this response variable only one regression line is shown.



3.10 Supplementary Figures

Figure 3-S1: Boxplots of June plot-scale (a) N-acetlyglucosaminidase and (b) acid phosphatase activity normalized by microbial biomass carbon (MBC) plotted against time since fire.

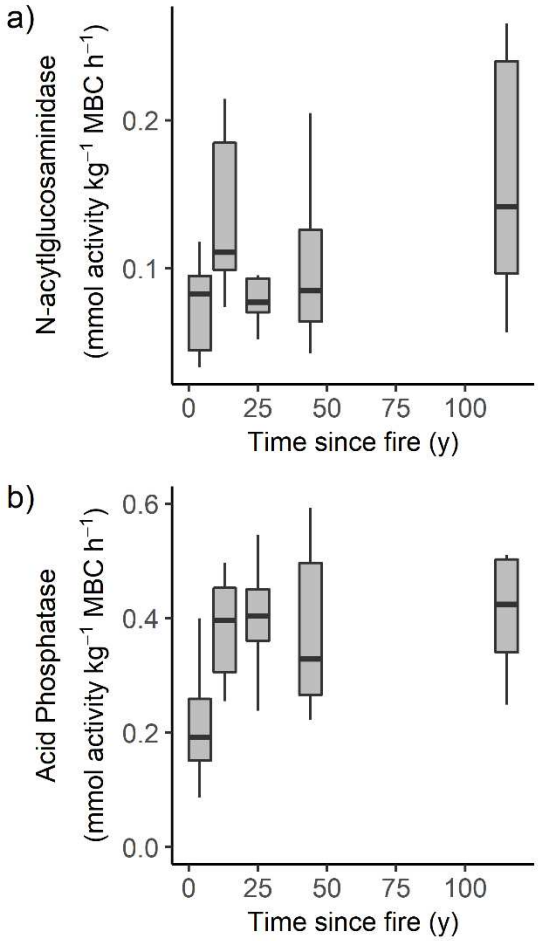


Figure 3-S2: Boxplots of June plot-scale (a) β-glucosidase, (b) N-acetlyglucosaminidase, and (c) acid phosphatase activity normalized by soil organic carbon (SOC) plotted against time since fire.

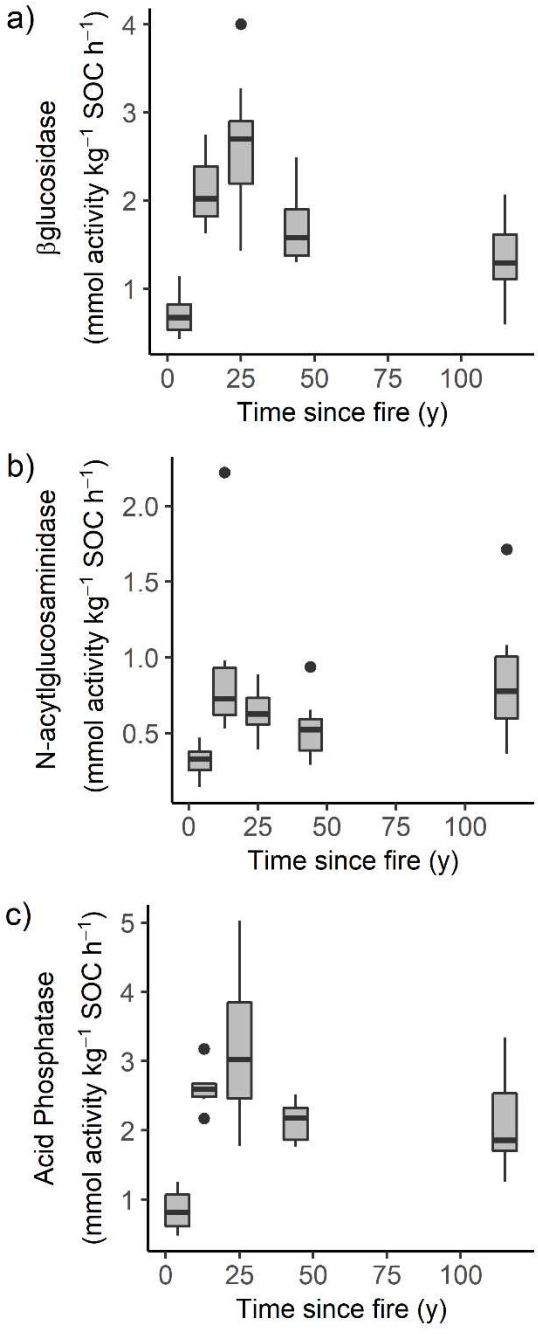


Figure 3-S3: Boxplots representing sample-scale extracellular enzyme activity for (a) β-glucosidase (BG), (b) N-acetylglucosaminidase (NAG), and (c) acid phosphatase (AP) by cover type against time since fire during the June sampling date.

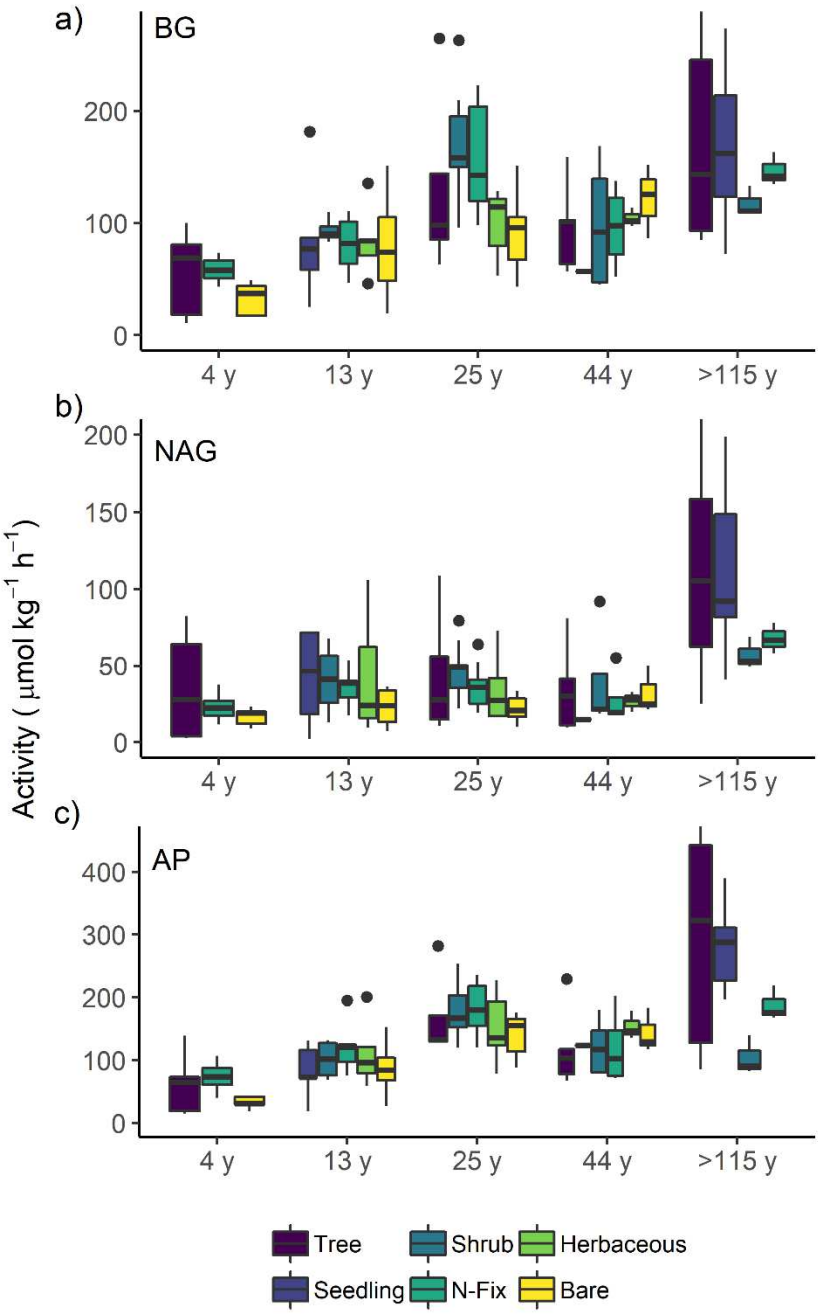


Figure 3-S4: Boxplots representing sample-scale cumulative gas fluxes for (a) carbon dioxide (CO_2), (b) methane (CH_4), and (c) nitrous oxide (N_2O) from 31-day laboratory incubations by cover type against time since fire during the June sampling date.

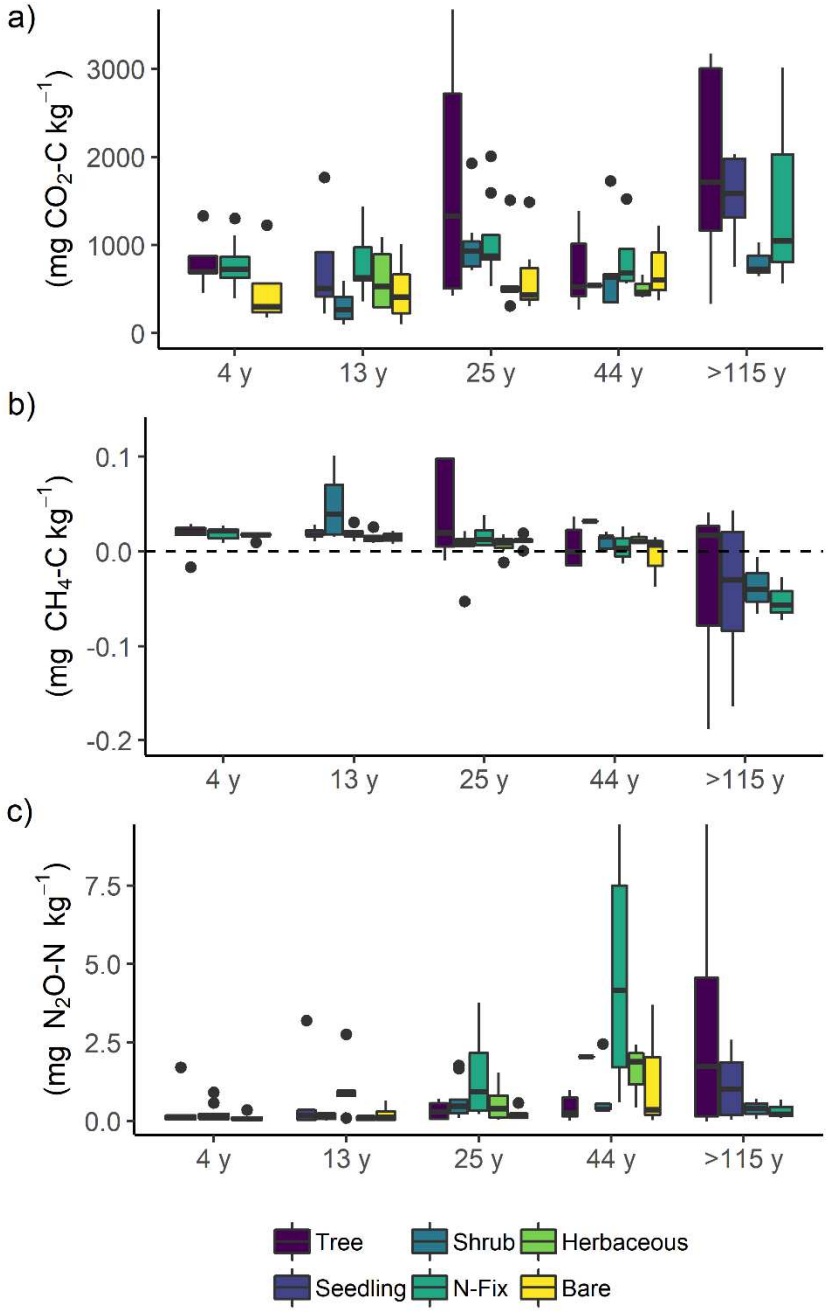
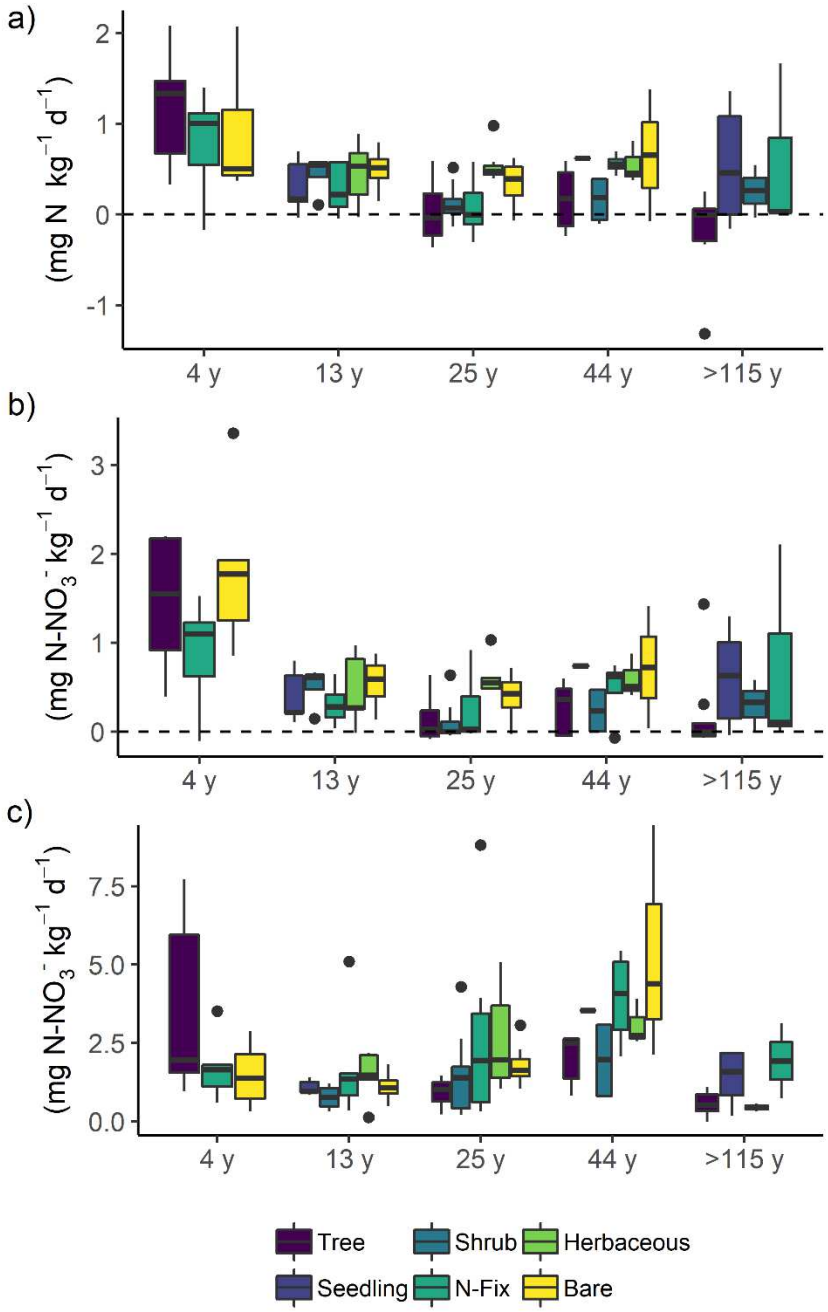


Figure 3-S5: Boxplots representing sample-scale (a) net nitrogen (N) mineralization and (b) net nitrification (b) during a 31-day laboratory incubation and (c) 24-h potential nitrification for the June sampling date. Data are grouped by cover type and are plotted against time since fire.



4 Thermal acclimation of soil microbial communities declines with depth

4.1 Abstract

Recent forecasts of global temperature increase are as high as 4 °C above pre-industrial temperatures by 2100 (RCP 8.5 IPCC 2014). As soils hold 1,500 – 2,400 Pg of soil organic carbon (SOC, Le Quéré et al. 2018), understanding the interactions among temperature, soils, and microorganisms is critical in predicting the stability of this carbon (C) pool, the C exchange between the atmosphere and the terrestrial landscape, and feedbacks on Earth's climate. Temperature acclimation is predicted to dampen the response of increased respiration in warmed soils (Bradford et al. 2008). However, mechanisms for microbial temperature acclimation, especially at depth, are yet to be discovered. The Blodgett Experimental Forest warming experiment (Hicks Pries et al. 2017) aims to elucidate the depth response of C cycling to a warmer world by heating the top meter of soil 4 °C above ambient temperatures. We show changes in the microbial community composition and functional capacity in response to warming are controlled, in part, by altered substrate availability, but that these changes are muted at depths below 30 cm. Our results demonstrate the often overlooked indirect effects of soil warming on microbial communities and show the relative resistance of subsoil microbial communities to altered soil conditions. Our finding that subsoil microbial communities are less responsive to global change suggests that subsoils will lag in their temperature acclimation, potentially dampening this hypothesized negative feedback.

4.2 Main

The effect of increased temperatures on soil microbial communities and microbial respiration is still largely uncertain (Conant et al. 2011), making it difficult to predict future feedbacks and to constrain long-term models of soil C storage (Sulman et al. 2018). While extracellular enzyme activity, which is the rate-limiting step of decomposition, correlates positively with temperature (German et al. 2012), long-term (>10 y) *in situ* measurements of soil respiration are more nuanced (Romero-Olivares et al. 2017, Melillo et al. 2017). These context-dependent responses may result from temperature acclimation of soil microbial communities to a physiological state that would reduce catabolism and soil carbon dioxide (CO_2) emissions (Bradford et al. 2008, Allison et al. 2010). Understanding changes to microbial metabolism in response to soil warming may, in part, resolve some of these uncertainties, constraining model predictions (Wieder

et al. 2013), and explaining the disparate findings of long-term empirical observations related to soil warming (Bradford et al. 2008, Conant et al. 2011, Romero-Olivares et al. 2017, Melillo et al. 2017).

In situ soil warming decreases C availability (Melillo et al. 2011, Crowther et al. 2016), alters C chemical composition (Feng et al. 2008), and increases nutrient availability (Rustad et al. 2001, Melillo et al. 2011). These are likely to affect rates of respiration through indirect effects on the soil microbial community. For instance, decreased C availability and quality (i.e., number of enzymatic steps necessary to depolymerize C compounds) decreases microbial C use efficiency (CUE), alters microbial community composition, and decreases respiration (Hart et al. 1994a, Frey et al. 2013, Dove et al. 2019). Increased nutrient availability decreases extracellular enzyme production for nutrient-acquiring enzymes (Olander and Vitousek 2000), which could increase CUE (Manzoni et al. 2012), but it could also increase biomass growth and alter microbial community structure (Fierer et al. 2012). Indeed, long-term soil warming has been shown to alter surface soil microbial community structure and metabolism (DeAngelis et al. 2015, Cheng et al. 2017), but it is unclear if this is caused solely by increased temperatures or in combination with altered resource availability (Billings and Ballantyne 2013).

A critical piece of missing information is the impact of warming on C cycling and microbial responses in deep soils (> 30 cm), as many soil warming experiments focus warming on only the upper 5 to 30 cm of soil. The microbial response at depth is non-negligible. For instance, Hicks Pries et al. (2017) recently showed 40% of the increased CO_2 emissions occurred below 15 cm when warming was extended throughout the upper meter of soil, and the apparent Q_{10} (i.e., temperature sensitivity) was similar throughout the soil profile. Soil microbial community structure in the subsoils is vastly different than its topsoil counterpart (Eilers et al. 2012, Taş et al. 2014). Along with slower turnover times at depth, different initial microbial community composition could lead to vastly disparate trajectories of community reorganization in response to long-term warming, affecting ecosystem processes (Glassman et al. 2018).

Warming-induced changes to resource availability could have an exacerbated effect on the subsoil microbial community where resource demand is the strongest. Resource availability at depth differs from the topsoil, where both C and nitrogen (N) availabilities decrease with depth (Jobbágy and Jackson 2000) because of smaller pool sizes and increased mineral protection of organic matter (Rasmussen et al. 2005). However, laboratory rates of C and N mineralization of added substrates were as strong in the subsoils as topsoils, suggesting that microbial competition and demand for C and N resources does not decrease with depth (Jones et al. 2018). Hence, subsoil microbial communities have as strong of substrate demands as topsoils, but mineralization in subsoil is substrate-limited. Because over half of soil organic C (SOC) is found below 20 cm (Jobbágy and Jackson 2000), incorporating subsoil responses to warming is critical in constraining predictions for long-term soil C storage.

In order to determine the impact of altered resource availability (e.g., C, N, and phosphorus [P]) on soil microbial community composition, physiology, and metabolism under a warming climate scenario, we analyzed samples from a 4.5 y soil warming (+4 °C above ambient) field experiment at the Blodgett Experimental Forest in the central

Sierra Nevada, CA. We used amplicon (16S and ITS) and shotgun metagenomic sequencing to define microbial community structure and metabolism, and coupled these analyses with a series of laboratory incubations to investigate relative resource limitations. We hypothesize that: 1) soil warming has reduced C availability and increased nutrient availability, resulting in increased C-limitation and decreased nutrient-limitation of the soil microbial community; 2) warming-altered resource stoichiometry has shifted microbial community composition, physiology, and metabolism towards more oligotrophic traits (e.g., higher microbial CUE, increase in genes encoding for enzyme degrading complex C compounds); and 3) these changes to the microbial community will be strongest in the subsoils where resource demand is greatest. We found the microbial communities in warmed sites shifted in their composition and metabolism towards more oligotrophic states, indicating increased C limitation. Yet, contrary to our hypothesis, subsoil microbial communities were *less* responsive to the direct and indirect effects of soil warming, which could diminish their ability to adapt to these altered conditions and maintain efficient growth.

4.3 Results

4.3.1 Soil chemical characteristics

After 4.5 y, +4 °C warming (Figure 4-1a) resulted in a 26% decrease in SOC ($p = 0.038$) in the 0-10 cm depth (no changes at lower soil depths, $p > 0.05$) and no changes to soil total N at any depth ($p > 0.05$, Table 4-1). This is consistent with *in situ* soil respiration data from these plots that show differences in the magnitude of the warming response with depth. During the first 22 months, respiration increased by 44% due to warming in the upper 30 cm compared to only a 17% increase in the 30-90 cm horizons (Hicks Pries et al. 2017). While soil total N was resistant to the warming treatments, inorganic N availability, assessed by laboratory net N mineralization (Binkley and Hart 1989), increased by 2- to 7-fold throughout the soil profile in response to warming ($p = 0.038$, Table 4-1).

4.3.2 Warming shifts microbial community composition and metabolism

We found weak but significant shifts in archaeal/bacterial ($p = 0.010$, $R^2 = 0.076$, Figure 4-2a) and fungal ($p < 0.001$, $R^2 = 0.094$, Figure 2b) community composition along the soil depth profile in response to 4.5 y continuous +4 °C warming. Overall, Blodgett Forest soils were dominated by *Acidobacteria*, *Proteobacteria*, and *Basidiomycota*. The heating increased the relative abundance *Actinobacteria* and *Planctomycetes*, and decreased the relative abundance of *Acidobacteria* and *Basidiomycota* throughout the soil profile (Supplemental Figure 4-S1). Responses between topsoil (0-30 cm) and subsoil (>30 cm) archaeal/bacterial communities differed. For example, heating in the topsoil significantly changed microbial populations within the same phylum. However, heating in the subsoil resulted in mostly decreased abundances of multiple operational taxonomic units (OTUs, Figure 4-S2a), particularly within *Bacteroidetes*, *Gemmatimonadetes*, and

Verrucomicrobia; nevertheless, overall alpha diversity was unchanged (Faith's phylogenetic diversity: $p = 0.934$). Similarly, we only detected a significant change in the ratio of *Ascomycota*:*Basidiomycota* read abundances in the 0-10 cm horizon ($p < 0.001$, Figure 4-S2b), highlighting the lack of fungal community composition response at depth.

Warming is altering microbial metabolism in topsoils. Using shotgun metagenomics from top (10-30 cm) and subsoils (60-80 cm), we investigated the abundance of genes encoding for carbohydrate-active enzymes (CAZy) based on the CAZy database (Cantarel et al. 2009, Lombard et al. 2014). While the overall composition of these enzymes did not differ by heating treatment ($p = 0.727$, Figure 4-S3), glycosyl transferases, polysaccharide lysases, carbohydrate esterases, and carbohydrate binding modules increased with heating in topsoils. Glycoside hydrolases gene families, on the other hand, both increased and decreased with warming ($p < 0.05$, Figure 4-3a) across both depths. Overall, topsoils were more responsive to warming than subsoils (52 vs. 8 differentially abundant gene families). However, the relative abundance of CAZy functional groups was not impacted by the heating treatment. We classified gene families into functional groups based on C substrates according to Benoit et al. (2012), Levasseur et al. (2013), and Tveit et al. (2014). Across both depths, abundance of genes encoding for cellulolytic, hemicellulolytic, liginolytic, and pectinolytic enzymes did not significantly differ with warming for both top- and subsoils ($p > 0.1$, Figure 4-3b).

4.3.3 Resource stoichiometry is a key factor affecting soil respiration under warming

We evaluated the impact of resource stoichiometry on microbial respiration by incubating heated and unheated soils with C and nutrient amendments for 30 days (Figure 4-1b). We found that respiration in heated soils was mostly constrained by a C-limitation, while unheated soils were relatively more nutrient-limited. These differences in limitations were only apparent in topsoils (10-30 cm) and not in subsoils (60-80 cm). Using the response ratio of C-amended to unamended soils and the ratio of C and nutrient-amended (CNP) soils to C-amended soils, we measured the relative C and nutrient limitation, respectively, of microbial respiration. Cumulative respiration was highest in the CNP-amended soils across depths and heating treatments ($p < 0.05$, Figure 4-S4a). Cumulative respiration was significantly more nutrient-limited in unheated topsoils than in heated topsoils ($p = 0.036$, Figure 4-S4b), but this difference was not apparent in the subsoils ($p = 0.957$). In contrast, the C-limitation of cumulative respiration was not significantly different among heating treatments ($p = 0.357$) and depths ($p = 0.120$). Because resource limitations may be most apparent during maximal rates of activity (Hart et al. 1992, Schimel et al. 2007), we also analyzed resource limitations of respiration during days 3 and 8, when the rate of respiration was near its maximum (Figure 4-S5). During this early period, there was a day × depth × heating interaction for both C and nutrient limitation (both: $p < 0.001$). Using multiple comparisons testing, we found that during the eighth day of the incubation, C limitation was 81% higher in heated topsoils than the unheated counterparts ($p = 0.033$, Figure 4-4). Also during the eighth day of the incubation, unheated topsoils had a 265% higher nutrient limitation than heated topsoils ($p < 0.001$, Figure 4-4). Differences in resource

limitations between heating treatments across all other date and depth combinations were not significant ($p > 0.1$), suggesting that differences in the relative resource limitations were only apparent during maximum activity and in topsoils.

4.3.4 Microbial community composition and growth are resistant to altered resource stoichiometry

The archaeal/bacterial but not fungal community composition was significantly altered by the resource amendments (archaea/bacteria: $p = 0.046$, $R^2 = 0.18$; fungi: $p = 0.832$, $R^2 = 0.06$; Figure 4-5). Changes in bacterial community composition were most pronounced in the CNP amendments across depths and heating treatments, with increases in the relative abundance of *Proteobacteria*, *Bacteriodetes*, and *Actinobacteria* and decreases in the relative abundance of *Verrucomicrobia* and *Planctomycetes* (Figure 4-S6).

In order to create a parallel comparison to respiration responses, we determined a response ratio for the archaeal/bacterial community shifts by calculating the Bray distance between the beginning and end of the incubation. We did not find a significant difference in C or nutrient limitation between the heating treatments across both depths (C limitation: $p = 0.765$, nutrient limitation: $p = 0.381$, Figure 4-6a). However, across depths and heating treatments, there was a greater nutrient than C limitation in the archaeal/bacterial community composition shift (paired t-test: $p < 0.001$). Across depth and heating treatments, the average C response was 0.97 (SE = 0.06, 1 = no effect), while the average nutrient response was 1.40 (SE = 0.05), suggesting that increasing nutrient availability results in more dissimilar communities than increases in C availability.

We also measured the change in microbial biomass (assessed by chloroform fumigation-extraction; Vance et al. 1987, Haubensak et al. 2002) during the 30-d incubation (i.e., biomass growth) among amendments to determine resource limitations. In contrast to our hypothesis, C and nutrient limitations of microbial biomass growth in the topsoils did not differ by heating treatments (C limitation: $p = 0.917$, nutrient limitation: $p = 0.117$; Figure 4-6b). Unfortunately, microbial biomass C assessed by chloroform fumigation-extraction can be difficult to detect in the presence of high background dissolved organic C (Geyer et al. 2018), as in the case of the C amended soils); this especially was true at greater soil depths, where microbial biomass is one to two orders of magnitude lower than surface soils (Fierer et al. 2003). Hence, we only analyzed the microbial biomass in the top soils.

4.3.5 Microbial carbon use efficiency declines with depth

We measured microbial CUE at the end of the 30-d resource amendment incubation using the isotopologue-metabolic modelling approach (Dijkstra et al. 2011) and found that CUE was 20% lower in the subsoils (0.12 – 0.59) than in the topsoils (0.39 – 0.63, $p < 0.001$; Figure 4-7). This represents a novel finding as microbial CUE is rarely measured in soils below 30 cm depth, and confirms predictions of declining CUE with decreased C concentrations due to the greater C cost of resource acquisition at depth

(Don et al. 2013). In contrast, changes in resource stoichiometry ($p = 0.582$, Figure 4-7) or heat treatment ($p = 0.879$) did not change CUE.

4.4 Discussion

Limited understanding of how changing environmental conditions affect microbial metabolism, especially at depth, leads to uncertainties in determining climate–carbon feedbacks and predictions for soil C sequestration (Todd-Brown et al. 2018). Here, we provide a depth-informed outlook to consequences of increasing soil temperature and resource stoichiometry on microbial metabolism.

Consistent with our hypothesis, 4.5 y of warming significantly shifted microbial community composition throughout the soil profile. However, responses between top- and sub-soils were fundamentally different. Topsoil changes were characterized by community reorganization across multiple phyla at the OTU scale, where overall diversity remained the same between control and heated plots. We also observed a general increase in *Actinobacteria* and *Ascomycota* at the phylum scale. This is similar to other studies that have found phylotypes that respond positively to heat are spread across multiple phyla (DeAngelis et al. 2015, Oliverio et al. 2017) and a near global increase in *Actinobacteria* with warming (Xiong et al. 2014, Pold et al. 2016, Liu et al. 2017, Koyama et al. 2018). However, our observed increase in *Ascomycota*:*Basidiomycota* with warming is largely uncorroborated. In fact, *Basidiomycota* has been found to increase in relative abundance with soil warming (Morrison et al. 2019), highlighting potential differences in fungal responses among ecosystems and experiments. In contrast, heating subsoil microbial communities resulted in a loss in bacterial diversity, suggesting that subsoil microbial communities were unable to capitalize on the new, warmed conditions. Laboratory warming (+ 10 °C) of subsoils showed that these microbial communities are in general less responsive to altered temperatures, at least in the short-term (30 days; Bai et al. 2019). We know of no other study to investigate microbial communities in response to *field* warming at depths greater than 40 cm; hence our results are the first to demonstrate that topsoil and subsoil microbial communities respond differently to increases in soil temperatures.

Differences in the response of top- and sub-soils are likely due, in part, to the metabolic differences between microbial communities in these horizons. As expected, topsoil and subsoil archaeal/bacterial communities were significantly different. Similar to other studies, we found an overall increase in *Acidiobacteria* and *Chlorofelxi* relative abundance at depth, which is consistent across numerous ecosystems (Will et al. 2010, Taş et al. 2014). Also, subsoil microbial communities had relatively lower CUE, which would favor respiration over biomass growth, so shifts in microbial community composition would occur more slowly. Decreased microbial CUE at depths below 60 cm represents a unique finding because microbial CUE is rarely measured at depth. In fact, we know of only one study to have measured microbial CUE below the topsoil. In two temperate forest soils, microbial CUE (assessed by ^{18}O incorporation and microbial respiration) was found to be stable with depth up to 40 cm (Spohn et al. 2016). However, numerous soil conditions continue to change with depth such that differences in

biogeochemical conditions and microbial communities at 60 cm could represent a significant threshold for changes in microbial CUE. These differences in the initial state of the microbial communities likely plays a role in the trajectory in response to warming, underscoring the need for investigating the response of subsoil microbial communities to global change.

Contrary to our hypothesis, altered topsoil substrate availability likely only explained a small portion of the change in the microbial community composition and metabolism due to warming. Altered microbial community composition in response to warming is often attributed, in part, to selection from shifting substrate availabilities (Zhou et al. 2017, Pold et al. 2017). In contrast to our expectations and other field warming studies (from 2-20 y in duration; Yue et al. 2015, Xue et al. 2016, Cheng et al. 2017), we found that the altered microbial metabolism with warming was not specific to any substrate class (cellulose, hemicellulose, lignin, or pectin). Similarly, results from our amendment-incubation experiment did not support the hypothesis that the heating treatment would interact with resource-induced changes to microbial community composition (although there was an overall amendment effect on archaeal/bacterial community composition). This suggests that warming had a substrate-independent effect (i.e., direct effect) on microbial community composition. For instance, warming likely increases cellular maintenance costs due partially to increase intracellular enzyme degradation (Manzoni et al. 2012). This would select for microorganisms that could minimize this cost, possibly through reduced cell sizes. Indeed, warming has been shown to select for smaller genomes (Sorensen et al. 2019), which has small cell sizes (Sabath et al. 2013). Another possible reason for altered microbial community structure with warming could be the indirect effect of soil moisture. At the time of sampling, gravimetric water content (kg H_2O/kg oven-dry soil) was about 20% lower in heated plots (unheated topsoil: 0.21 ± 0.01 [standard error of the mean]; heated topsoil: 0.17 ± 0.03; unheated subsoil: 0.16 ± 0.01, heated subsoil: 0.13 ± 0.01), which is consistent with long-term differences in volumetric water content at our site (Hicks Pries et al. 2017). In seasonally dry ecosystems such as this site, soil moisture can strongly effect microbial community structure and function throughout the soil profile (Qi et al. 2018); however, it is difficult to quantify the impact of soil moisture because we did not manipulate soil moisture independently of temperature. This emphasizes the several interacting direct and indirect effects of warming on microbial communities and the need of multi-factorial experiments that can elucidate these interactions (Blankinship et al. 2010, Brown et al. 2012, Zhou et al. 2017).

The nuanced measurements from our amendment-incubation experiment are likely attributed to the transience of the amendment effect, but suggest that altered resource stoichiometry from 4.5 y of warming impacts heterotrophic microbial activity. Consistent with our hypothesis, the maximal respiration was more C-limited in the heated plots than in the unheated plots, while the maximal respiration in the soils taken from the unheated plots were more nutrient-limited than the heated plots. This is consistent with field manipulations of enhanced carbon dioxide concentrations (eCO_2) and warming that show greatest respiration in warmed plots with increase plant C additions from eCO_2 (Carrillo et al. 2018). However, our one-time spike of C and nutrients did not result in a detectable effect on microbial characteristics measured at day 30 (fungal community

composition, net biomass growth, CUE). Chronic substrate additions may have influenced the more stable microbial properties in our experiment, but they would have further altered the microbial community structure (Isobe et al. 2019), making it difficult to attribute changes in heterotrophic activity to altered substrate availability or microbial community composition. Therefore, we show that there is a differential response of unheated/heated microbial communities to resource stoichiometry. Yet the ephemerality of the response mimics the ephemerality of the stimulus. Nevertheless, these findings suggest that when C becomes further limited, microbial activity and respiration will decline.

We show that 4.5 y of +4 °C warming moderately affects soil microbial community composition and metabolism throughout the soil profile. Resource availability and stoichiometry were important factors affecting topsoil heterotrophic microbial activity under warming, where C-limitation was a key constraint at high activity periods (i.e., hot-moments). Yet, microbial community composition, CUE, and growth were resistant to altered resource stoichiometry, and changes in metabolism were unrelated to predicted substrate classes. In contrast, subsoil microbial communities were less responsive to the direct and indirect effects of soil warming. At these depths, inherently oligotrophic conditions enable life of slow growing, low activity microorganisms that do not rapidly change in response to warming. Collectively, our results demonstrate a restructuring in topsoils and a relative resistance in subsoil microbial communities to altered soil conditions under a warming climate. Our finding that subsoil microbial communities may lack a response to increasing temperatures suggests that deep soils will lag in their temperature acclimation, potentially dampening this predicted negative feedback to global warming.

4.5 Methods

4.5.1 Study site, field experiment, and soil sampling

The University of California Blodgett Experimental Forest is located in the foothills of the central Sierra Nevada near Georgetown, CA at 1370 m above sea level. Mean annual precipitation is 1774 mm, with most of the precipitation occurring as snow from November through April. Mean annual air temperature is about 12.5 °C (Bird and Torn 2006). The experiment is in a thinned, 80-year-old even-aged mixed conifer forest. Dominant overstory species include ponderosa pine (*Pinus ponderosa*), sugar pine (*Pinus lambertiana*), incense cedar (*Calocedrus decurrens*), white fir (*Abies concolor*), and Douglas-fir (*Pseudotsuga menziesii*). The soils are Alfisols of granitic origin with a developed O horizon as part of the Holland-Bighill complex (Rasmussen et al. 2005).

The field experiment is explained in detail in Hicks Pries et al. (2017). Briefly, the experimental design consisted of three warmed and three control 3-m diameter circular plots. The warming treatment warmed the soil 4 °C above ambient temperatures to 1 m depth while maintaining the natural temperature gradient with depth (following the design of Hanson et al. 2011).

In June 2018, we sampled one soil core from each plot using a 5-cm diameter AMS corer (AMS Inc. Hayward Falls, ID, USA) and extracted the following depths: 0-10 cm, 10-30 cm, 30-45 cm, 45-60 cm, and 60-80 cm. Polycarbonate core sleeves were sterilized prior to sampling and were used only once. Samples were homogenized under sterile conditions in the field and a subsample was immediate placed on dry ice for DNA extraction. The remaining sample was placed on "blue" ice (4 °C) and refrigerated in the laboratory for 2 days until the incubation experiment.

4.5.2 Laboratory experiment

Upon returning to the laboratory, soils from the 10-30 cm depth (representing topsoils, mix of A and AB genetic horizons) and soils from the 60-80 cm depth (representing subsoils, Bt genetic horizon) were subsampled into 5-g aliquots and were given amendments in 0.4 ml solutions, representing ~10 times the microbial C and N and 20 times the microbial P found in these soils (*unpublished data*). We doubled the amount of P to account for P sorption onto mineral surfaces. Microbial N and P were calculated using a stoichiometric ratio of 60:7:1 C:N:P (Cleveland and Liptzin 2007). Topsoil aliquots were placed in 35 ml centrifuge tubes and given one of three amendments: 15 mg cellobiose-C and 4.82 mg NaCl (+C); 1.75 mg NH_4Cl-N, 0.5 mg NaH_2PO_4-P, and 15 mg cellobiose-C (+CNP); or 4.82 mg NaCl (control). Sodium chloride was given to the +C and control treatments to account for the mass of Na^+ and Cl^- ions in the +CNP treatment and to create osmotically similar solutions. Subsoil aliquots were given the same amendments but at levels 23% of the topsoil, commensurate the microbial biomass at this depth. We did not include a +NP treatment in this study because a pilot study using similar soils showed a minimal response to this amendment (*unpublished data*). Because we were interested in the *relative* resource demand, not *absolute*, we used the ratio of +CNP to +C to determine nutrient demand and +C to control to determine C demand.

The N addition in the CNP treatment in our study is much larger than many global change field experiments, which aim to mimic N deposition. However, our goal was to elucidate differences in N demand, which requires larger additions to detect (Sullivan et al. 2014). Our N addition, roughly equivalent to 35 g N m^{-2}, falls within the range of other studies with this goal (Table 4-S1).

Aliquots were covered loosely with Parafilm to minimize moisture loss, but allowing for some gas exchange. Soils from heated and control plots were separated and placed into two different incubators (Series KB 240, Binder Group LP, Camarillo, CA, USA) set to 14 and 10 °C, respectively, corresponding to the average temperatures of the field experiment at the time of sampling.

4.5.3 Soil total C and N

Air-dried soils were ground to a fine powder using mortar and pestle. Approximately 10 mg of oven-dry, ground soil were weighed into tin capsules, and these samples were analyzed for total C and N by continuous-flow, direct combustion and mass

spectrometry using the ECS 4010 CHNSO analyzer (Costech Analytical Technologies, Inc., Valencia, CA, USA).

4.5.4 Microbial respiration

We measured microbial respiration at 1, 3, 8, 16, and 28 days after the amendment addition. Four aliquots from each amendment × temperature × depth × replicate combination were uncovered and placed in a 500 ml wide-mouth Mason jar fitted with a butyl rubber septum. We immediately sampled 16 ml of headspace and placed the jars in their respective incubators. We again sampled 16 ml of headspace after 6 h to determine the hourly respiration rate, and aliquots were removed from each jar and re-covered with Parafilm. We used the same aliquots for respiration measurements throughout the incubation. Headspace samples were then analyzed for carbon dioxide by gas chromatography using a Shimadzu GC-2014 equipped with a thermal conductivity detector (Shimadzu Corporation, Columbia, MD, USA). Cumulative gas fluxes were determined by integrating the rates at different times throughout the incubation over the course of 28 days.

4.5.5 Biomass growth

We determined microbial biomass growth by the difference between microbial biomass at the beginning and end of the incubation. At the beginning and at 30 days into the incubation, we removed and froze a pair of 5-g aliquots from each amendment × temperature × depth × replicate combination for microbial biomass determination by chloroform fumigation extraction (Vance et al. 1987, Haubensak et al. 2002). Briefly, one paired aliquot, considered the non-fumigated sample, was immediately extracted with 0.5 M K_2SO_4, and the other paired aliquot, considered the fumigated sample, was incubated for five days in a chloroform-filled desiccator. After fumigation, the samples were also extracted with 0.5 M K_2SO_4. Fumigated and non-fumigated extracts were analyzed on a total organic C (Shimadzu TOC-Vcsh, Kyoto, Japan). The difference in C concentration between these extracts is the chloroform-labile C pool. We used an extraction efficiency factor (k_{eC}) of 0.45 to convert chloroform-labile C to microbial C (Beck et al. 1997).

4.5.6 Nitrogen availability

Inorganic N pool sizes before and after the 30-day incubation were determined by extracting a 5-g aliquot with 25 mL of 2 M KCl. Extracts were analyzed for ammonium (Lachat method: 12-107-06-1-B) and nitrate (Lachat method: 10-107-04-1-A) colormetrically using the Lachat AE Flow Injection Auto analyzer (Lachat Instruments, Inc., Milwaukee, WI, USA). Net N mineralization rates were determined by the net changes in inorganic N pools over the 30-day incubation period (Binkley and Hart 1989).

4.5.7 Microbial carbon use efficiency

We used the ^{13}C isotopologue-metabolic modeling approach following Dijkstra et al. (2011) to measure CUE at the end of the incubation. Briefly, four aliquots per replicate of each treatment were given one of four 2.5 mM, 0.5 ml amendments: 1-^{13}C pyruvate; 2,3-^{13}C pyruvate; 1-^{13}C glucose; or U-^{13}C glucose as metabolic tracers. Each aliquot was placed in a 500 ml wide-mouth Mason jar fitted with a butyl rubber septum. After 2 hours for topsoils and 12 hours for subsoils (because of slower metabolic rates at depth), we sampled 25 ml of headspace into an evacuated 20 ml vial. These samples were then analyzed for $^{13}CO_2$ concentrations using a G2131-i Cavity Ring Down Spectrometer CO_2 Isotope and Concentration Analyzer equipped with a Small Sample Isotope Module (Picarro Inc., Santa Clara, CA, USA). The ratios between $^{13}CO_2$ production rates from glucose and pyruvate isotopologues were calculated and used to model C allocated for energy production or biosynthesis.

4.5.8 DNA extraction, amplicon sequencing, and shotgun metagenomics

Total DNA was extracted from each sample by using 0.25-0.5 g of field moist soil as input (0.25 g for 0-10, 10-30, and 30-45 cm depths and 0.5 g for 45-60 and 60-80 cm depths) into the DNeasy PowerSoil Kit (Qiagen, Germantown, MD, USA) with minor modifications. Prior to bead-beating, the samples were incubated in bead-solution at 65 °C for 5 min. Cells in the samples were disrupted by bead beating with a 1600 MiniG (SPEX Sample Prep, Metuchen, NJ, USA) at a setting of 1500 rpm for 60 s and the DNA was further purified according to the kit protocol. DNA amounts were quantified using the Qubit dsDNA HS assay (Invitrogen, Carlsbad, CA, USA).

16S rRNA genes were amplified in PCR reactions using primers (F515/R806) that target the V4 region of the 16S rRNA gene, and ITS genes were amplified in PCR reactions using ITS1f/ITS2 primers (Walters et al. 2016). The reverse PCR primer was barcoded with a 12-base Golay code (Caporaso et al. 2010). The PCR reactions contained 2.5 µl Takara Ex Taq PCR buffer, 2 µl Takara dNTP mix, 0.7 µl Roche BSA (20 mg/ml), 0.5 µl each of the forward and reverse primers (10 µM final concentration), 0.125 µl Takara Ex Taq Hot Start DNA Polymerase (TaKaRa, Shiga, Japan), 1.0 µl genomic DNA (10 ng/reaction), and nuclease-free water in total volume of 25 µl. Reactions were held at 98 °C for 3 min. to denature the DNA, followed by amplification for 25 cycles at 98 °C for 30 s, 52 °C for 30 s, and 72 °C for 60 s; a final extension of 12 min. at 72 °C was added to ensure complete amplification.

Each sample was amplified in triplicate, combined, and purified using the Agencourt AMPure XP PCR purification system (Beckman Coulter, Brea, CA). The purified amplicons were quantified using the Qubit dsDNA HS assay and the size of the amplicons was determined using a Bioanalyzer with Agilent DNA 1000 chips (Agilent Technologies, Santa Clara, CA). Amplicons were pooled (10 ng/sample) and sequenced on one lane of the Illumina Miseq platform (Illumina Inc, San Diego, CA), resulting in 300 bp paired-end reads.

Paired-end sequences were overlapped and merged using FLASH (Magoč and Salzberg 2011). Quality filtering and demultiplexing were performed as described

previously (Bokulich et al. 2013). Sequences were grouped into operational taxonomic units (OTUs) based on 97% sequence identity, and chimeric sequences were removed using UPARSE (Edgar 2013). For 16S rRNA gene analysis, OTUs were given taxonomic assignments in QIIME (Caporaso et al. 2010) version 1.7.0 using the RDP classifier (Wang et al. 2007) and the SILVA database 132 (Quast et al. 2013). Phylogenetic trees were created using FastTree (Price et al. 2010) under QIIME's default parameters. For ITS genes, representative sequences from UPARSE were blasted against NCBI Nucleotide database using MegaBLAST under default parameters (Zhang et al. 2000). Resulting reports were manually curated and OTU tables were generated for further statistical analysis

We generated 12 metagenomes from the heated and control sites (10-30 and 60-80 cm depths) using a Kapa Biosystems LTP Library Preparation Kit for Illumina Platforms (Wilmington, DE, USA). We sheared 500 ng of genomic DNA using a Covaris S220 (Covaris, Woburn, MA, USA) with settings 140 PIP, 10.0 duty factor, 200 cycles/burst, and 65 seconds. Library preparation was conducted as described in the Kapa Biosystems protocol. Samples which has less than 70 ng DNA after library preparation were amplified using the reagents and recipe described in the Kapa protocol. PCR reactions were purified using Agencourt AmPure XP Beads (Beckman Coulter, Indianapolis, IN, USA). Final libraries were analyzed for size using a Bioanalyzer High Sensitivity kit (Agilent, Santa Clara, CA), and had a final product size ranging from ~200 bp to ~1500 bp. Libraries were pooled and sequenced using the Illumina NovaSeq paired end-read technology (at the UCSF Center for Advanced Technology, CA, USA).

Samples from each depth were co-assembled between control and heated treatments (n = 6 samples per depth) using MEGAHIT (Li et al. 2016) with a minimum contig length of 1000 bp. Then, each individual sample was mapped back to the MEGAHIT contigs with Bbmap (Bushnell 2014), and we extracted unmapped reads. Next, unmapped reads concatenated and re-assembled using SPAdes in the "meta" setting (Nurk et al. 2017). The newly assembled contigs folds were merged with the MEGAHIT contigs. Resulting assembled contigs were annotated for carbohydrate active enzymes using the CAZy database (Cantarel et al. 2009, Lombard et al. 2014), and taxonomy was classified using Kaiju (Menzel et al. 2016).

4.5.9 Statistical analysis

All statistical analyses were conducted in R (R Development Core Team 2008) using the car (Fox and Weisberg 2011), lme4 (Bates et al. 2015), phyloseq (McMurdie and Holmes 2013), DESeq2 (Love et al. 2014 p. 2), and vegan (Oksanen et al. 2013) packages. Significance was determined at the $\alpha = 0.05$ level for all statistical tests. Univariate biogeochemical data, including response ratios, were assessed by mixed design ANOVA, with depth, heating treatment, or amendment as fixed effects and plot as a random effect. We used QQ-plots and scale-location plots to inspect normality and homoscedasticity, respectively. If these assumptions were not met, data were natural log-transformed, verified for normality and homoscedasticity, and reanalyzed (as was the case for net N mineralization and cumulative respiration).

Amplicon data were proportionally normalized, and β-diversity was assessed by perMANOVA (Anderson and Walsh 2013), using weighted UniFrac distance (Lozupone and Knight) for 16S and Bray-Curtis distance (Bray and Curtis 1957) for ITS. We used different distance metrics between these two marker genes because polyphyly is widespread within the fungal kingdom (Bruns et al. 1991), and Bray distances do not incorporate phylogeny. We visualized differences using principal coordinate analysis for 16S and non-metric multidimensional scaling for ITS. These ordination methods were chosen because they best represented the clustering of the data as explained by the perMANOVA. The estimated fold change of microbial OTU and functional gene count abundance was assessed using Wald tests and shrinkage estimation for dispersions (Love et al. 2014).

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4.7 Tables

Table 4-1: Average total carbon and nitrogen (N) concentrations and net N mineralization (assessed during a 30-d laboratory incubation) across heating treatments throughout the soil profile, with standard error of the mean (n = 3) in parentheses. Net N mineralization was assayed only in the incubated soil (10-30 and 60-80 cm horizons).

	Carbon (%)		Nitrogen (%)		net N mineralization (mg kg^{-1} 30-d^{-1})	
Depth	unheated	heated	unheated	heated	unheated	heated
0-10 cm	10.9 (0.893)	8.06 (0.23)	0.251 (0.031)	0.191 (0.006)	-	-
10-30 cm	2.65 (0.563)	3.15 (0.312)	0.101 (0.021)	0.087 (0.004)	0.72 (0.21)	5.73 (2.41)
30-45 cm	1.07 (0.070)	1.46 (0.321)	0.034 (0.002)	0.054 (0.007)	-	-
45-60 cm	0.620 (0.073)	0.605 (0.009)	0.023 (0.001)	0.023 (0.003)	-	-
60-80 cm	0.458 (0.146)	0.396 (0.005)	0.016 (0.003)	0.013 (0.001)	0.33 (0.28)	0.97 (0.47)

4.8 Figures

Figure 4-1: Field (A) and laboratory (B) experimental design. To test for the effect of warming in the field (a), we sampled five soil horizons (0-10, 10-30, 30-45, 45-60, and 60-80 cm) from unheated and heated (4 °C) plots (n = 3). The 10-30 and 60-80 cm horizons from each plot were incubated for 30-d with no amendment (Cont.), cellobiose (+C), or cellobiose with inorganic nitrogen and phosphorus (+CNP) to assess the relative resource limitation of each soil (B). The response ratio of +C/Cont. and +CNP/+C was used to determine the relative C- and nutrient-limitation, respectively.

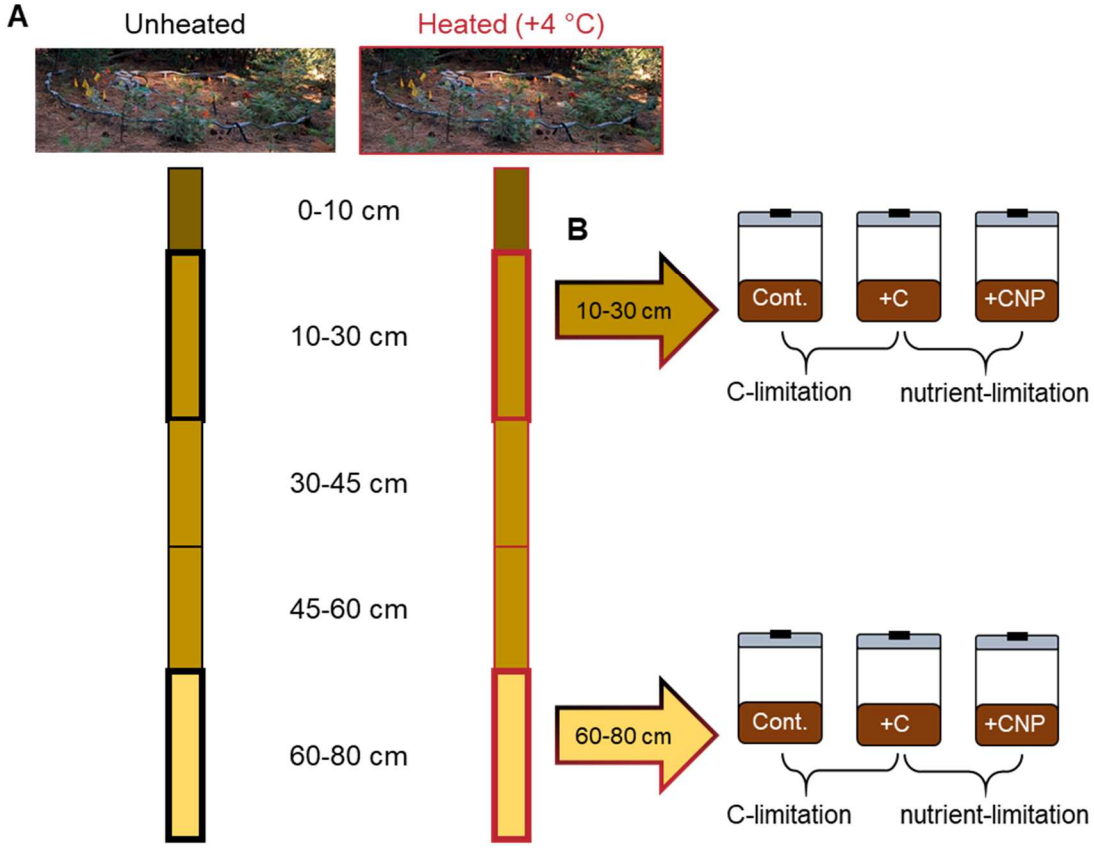


Figure 4-2: Ordinations of microbial communities using Principal Coordinates Analysis (PCoA) for archaea/bacteria (A) and non-metric multidimensional scaling (NMDS) for fungi (B). Red colors show heated plots and black colors show unheated control plots. Darker colors show upper depths and lighter colors show deeper depths.

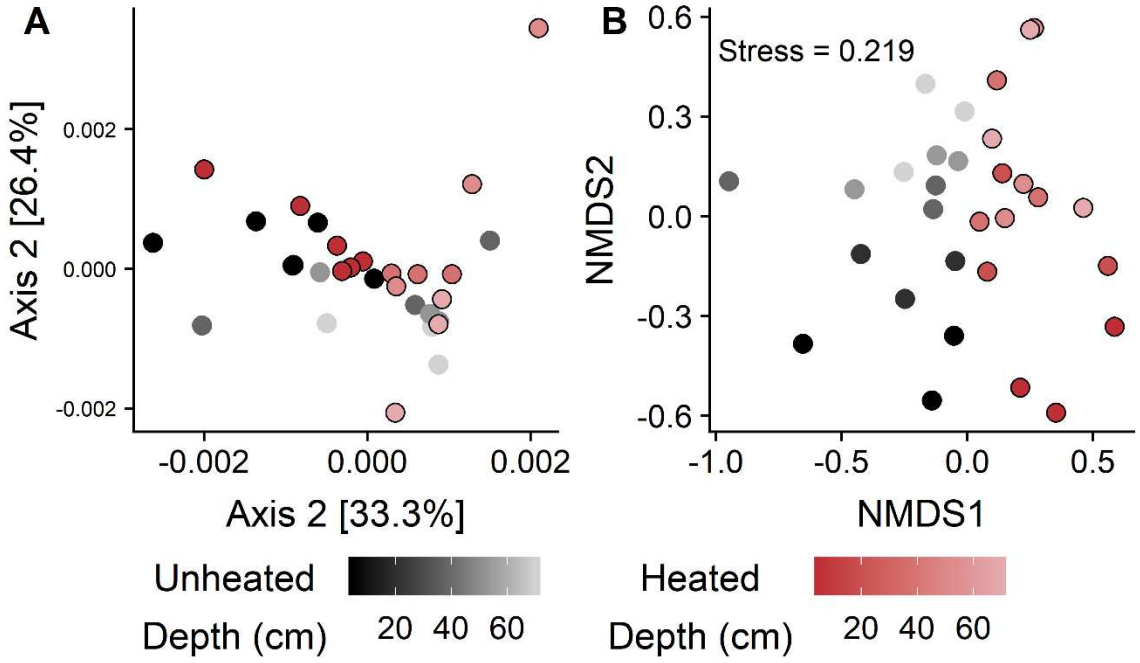


Figure 4-3: Changes in genes encoding for carbohydrate-active enzymes (CAZymes). Differentially abundant ($p < 0.05$) CAZyme gene families in heated compared to unheated soils with colors are coded by different gene functional groups (A). Dashed line at zero represents no effect. Boxplots representing the sum of read abundances for functionally classified CAZyme genes across heating treatments and depths (B).

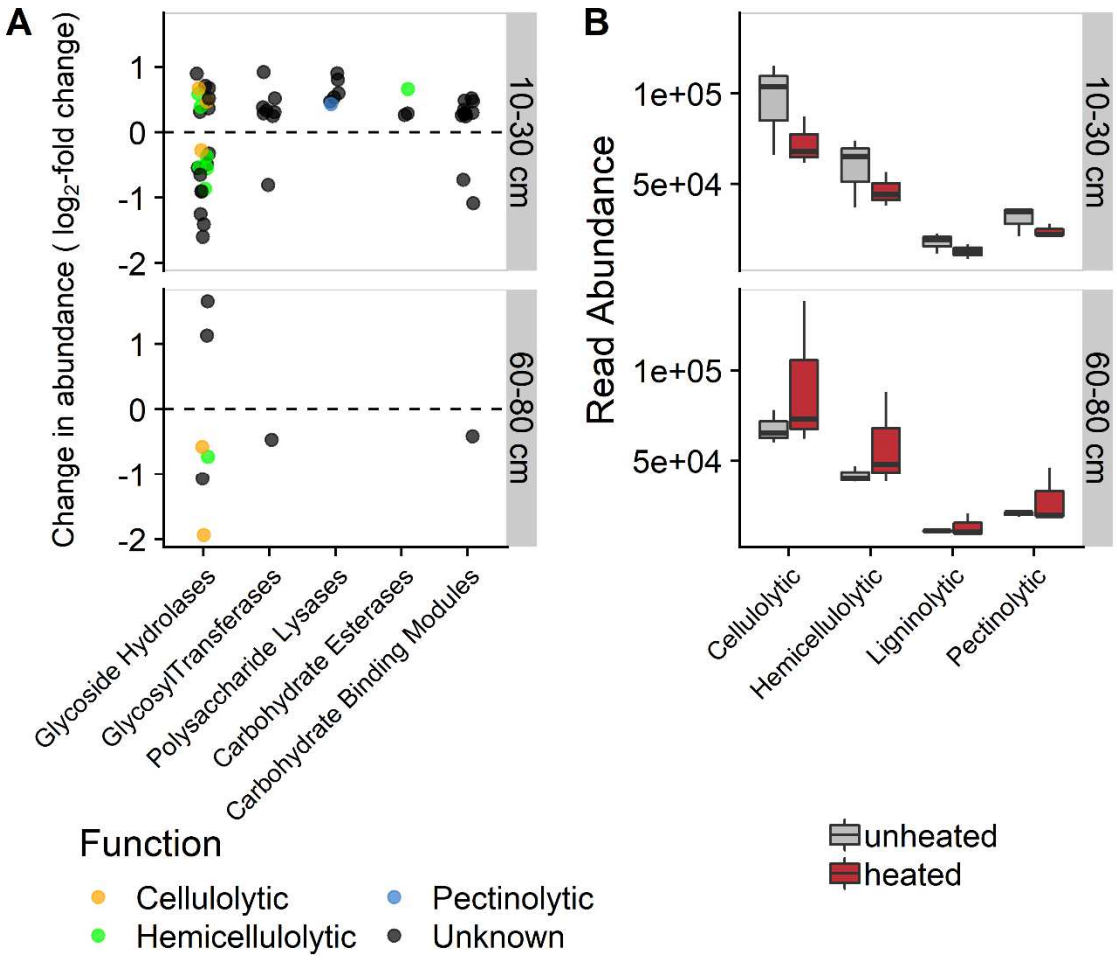


Figure 4-4: Boxplots showing resource limitations of microbial respiration at 3 and 8 days of the incubation across both depths and heating treatments. Carbon (C) limitation is assessed by the response of the C-amended soils divided by the control soil, and nutrient limitation is assessed by the response of the C- and nutrient-amended soil divided by the C-amended soil. Dashed line at 1.0 indicates no limitation.

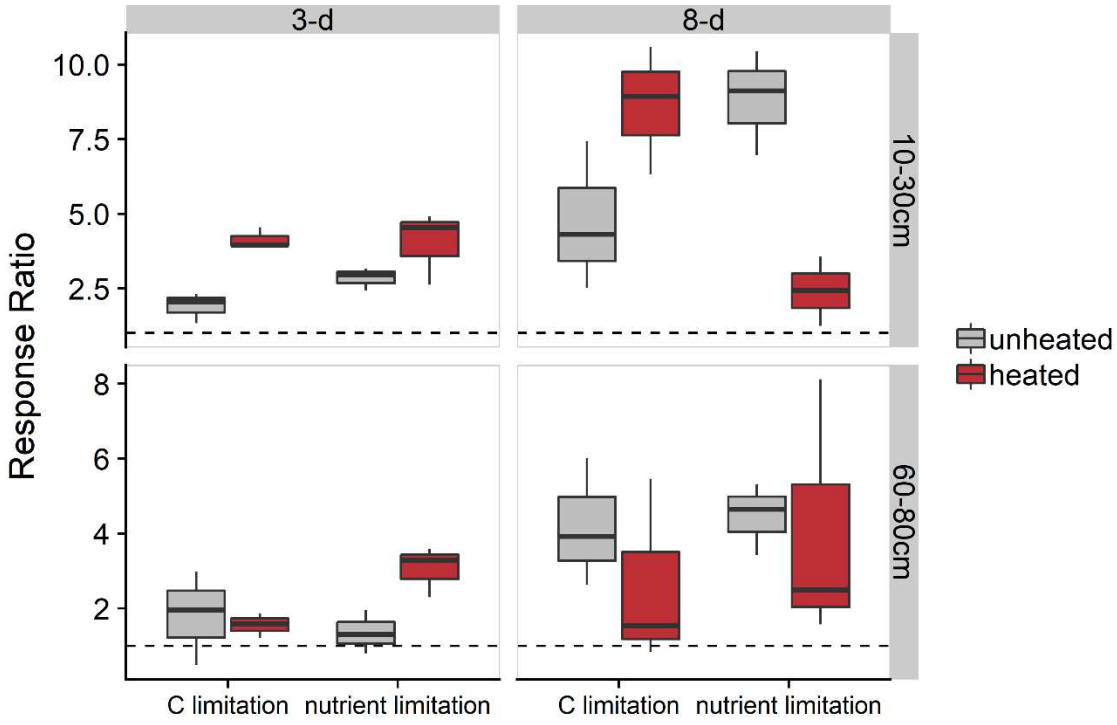


Figure 4-5: Change in community composition during the 30-d incubation amended with carbon (C) or carbon, nitrogen, and phosphorus (CNP) compared to the starting conditions and unamended control for archaea/bacteria (A) and fungi (B) across the heating treatments and depths. Principal coordinates analysis (PCoA) was used for archaea/bacteria and non-metric multidimensional scaling (NMDS) was used for fungi (stress = 0.233).

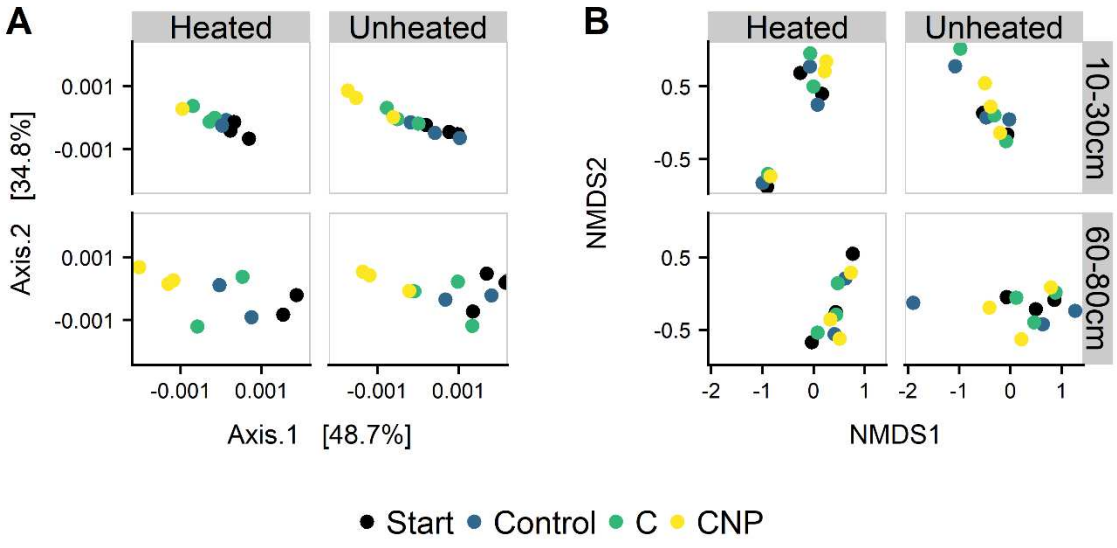


Figure 4-6: Boxplots showing resource limitations of archaeal/bacterial community composition shift (assessed by Bray-Curtis distance, A) and topsoil (10-30 cm) biomass growth (B) across heating treatments. Carbon (C) limitation is assessed by the response of the C-amended soils divided by the control soil, and nutrient limitation is assessed by the response of the C- and nutrient-amended soil divided by the C-amended soil. Dashed line at 1.0 indicates no limitation.

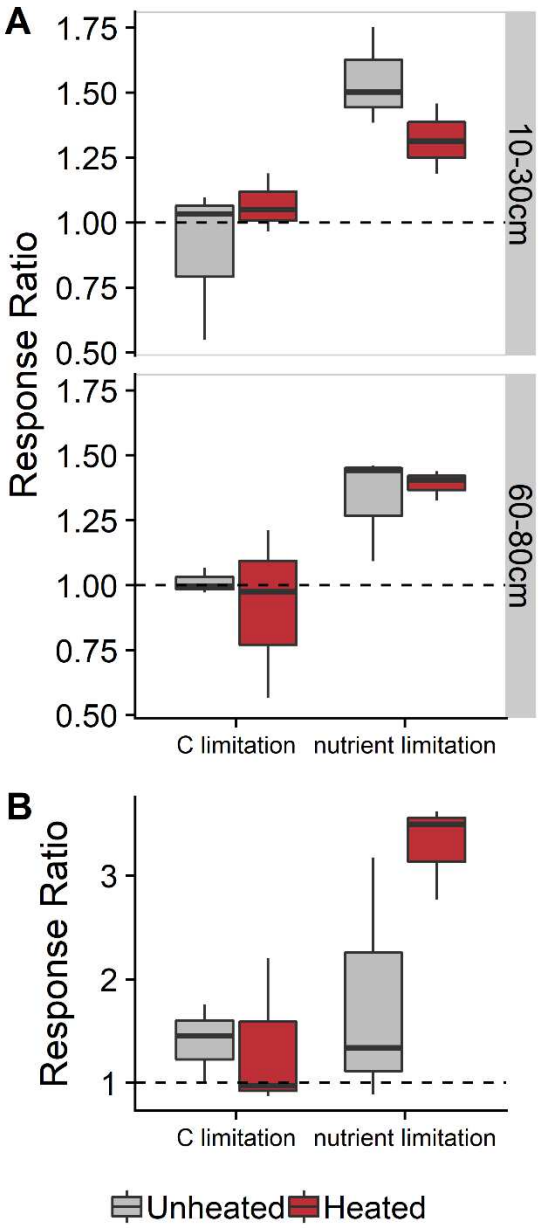
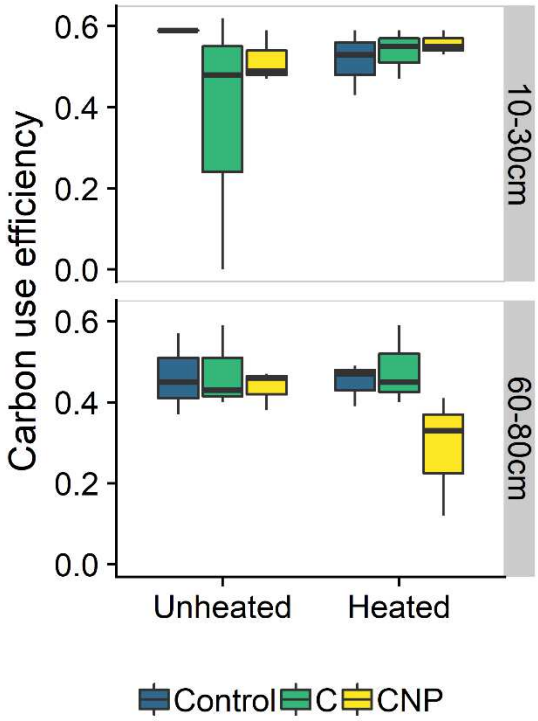


Figure 4-7: Boxplots showing carbon use efficiency across heating treatments, depths, and resource amendments. Key: carbon (C) and carbon, nitrogen, and phosphorus (CNP).



4.9 Supplementary Tables

Table 4-S1: Comparison of carbon (C), nitrogen, (N), and phosphorus (P) additions in multiple resource amendment incubations. Dashes represent no addition of elelment.

Reference	C (g C kg^{-1} soil)	N (g N kg^{-1} soil)	P (g P kg^{-1} soil)	ecosystem
This Study (topsoils)	**3**	**0.35**	**0.10**	**Temperate forest**
This Study (subsoils)	**0.7**	**0.081**	**0.023**	**Temperate forest**
Reed et al. 2011	4	0.8	0.8	Tropical forest
Marañon-Jimenez et al. 2018	0.69	0.035	0.018	Temperate grassland
Soong et al. 2018	-	0.37	0.195	Tropical forest
Spiers and McGill 1979	10	0.88	0.5	Agricultural
Waldrop and Firestone 2004	-	0.03	-	Tropical forest
Ouyang et al. 2008	-	1.00-0.04	0.1	Temperate steppe

4.10 Supplementary Figures

Figure 4-S1: Relative abundance of major bacterial phyla across heating treatments throughout the soil profile by 16S (A) and shotgun metagenomics (B).

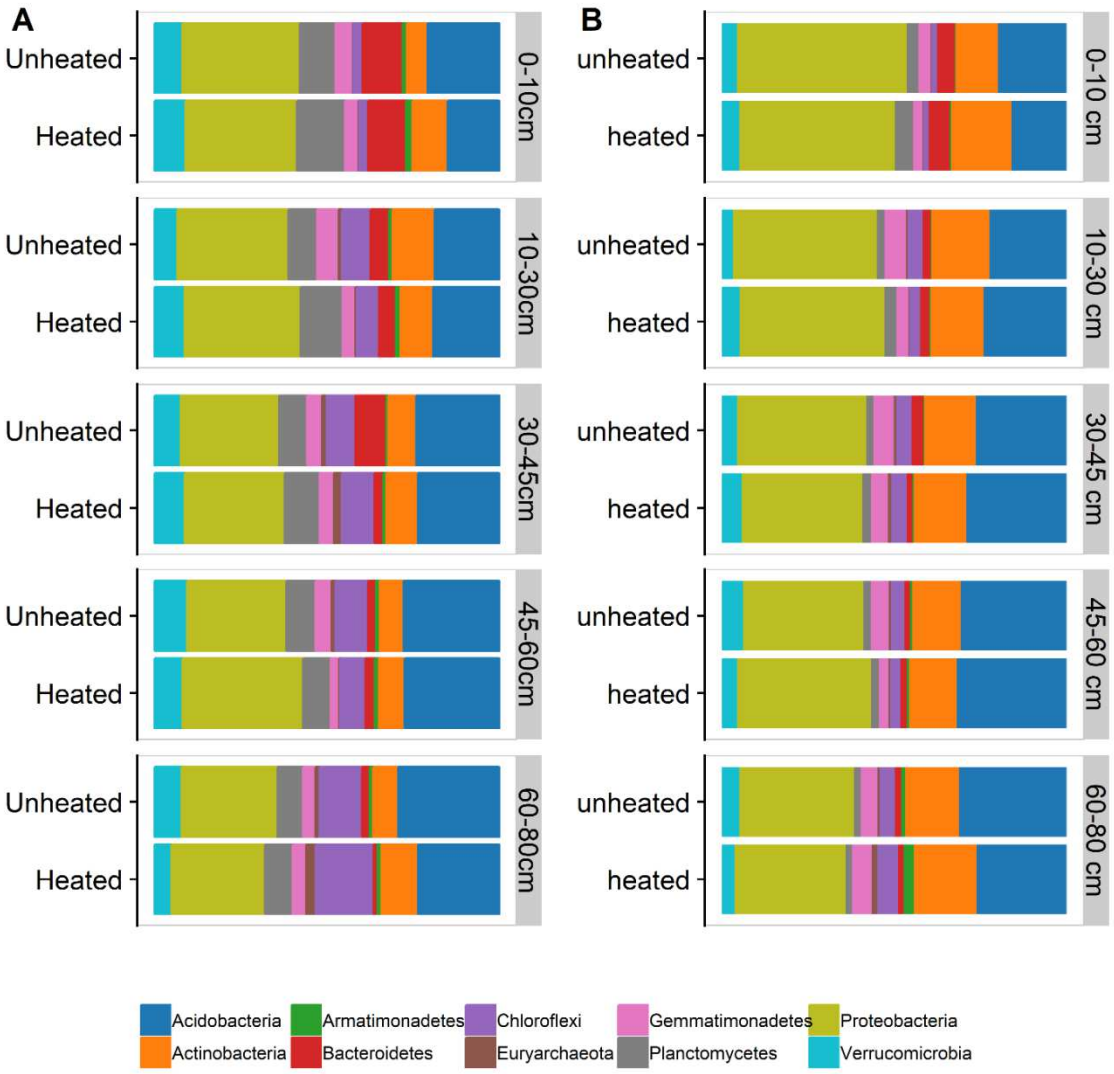


Figure 4-S2: Change in abundance of the microbial community with soil heating. Archaeal/bacterial operational taxonomic units with significant changes in abundance by soil heating ($p < 0.05$) are shown by phylum and across depths (A). Relative abundances of fungal phyla are shown for heated (red) and unheated (gray) soils B). Error bars show ± one standard error (n = 3).

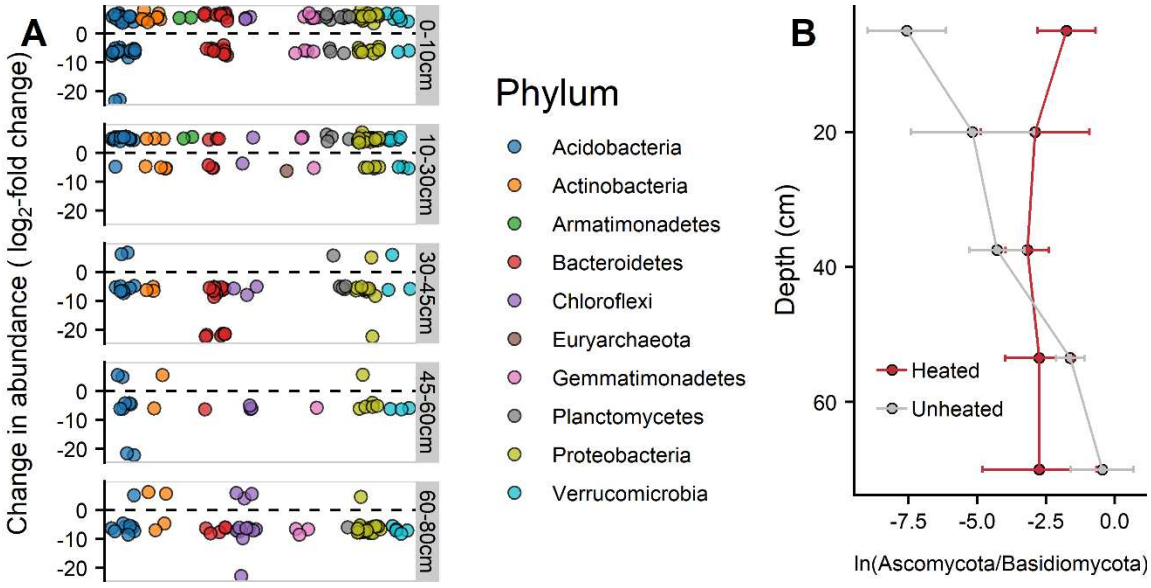


Figure 4-S3: Ordination of carbohydrate active functional gene families (Bray-Curtis distance) across heated (red) and unheated (gray) topsoils (10-30 cm, circles) and subsoils (60-80 cm, triangles). Stress = 0.060.

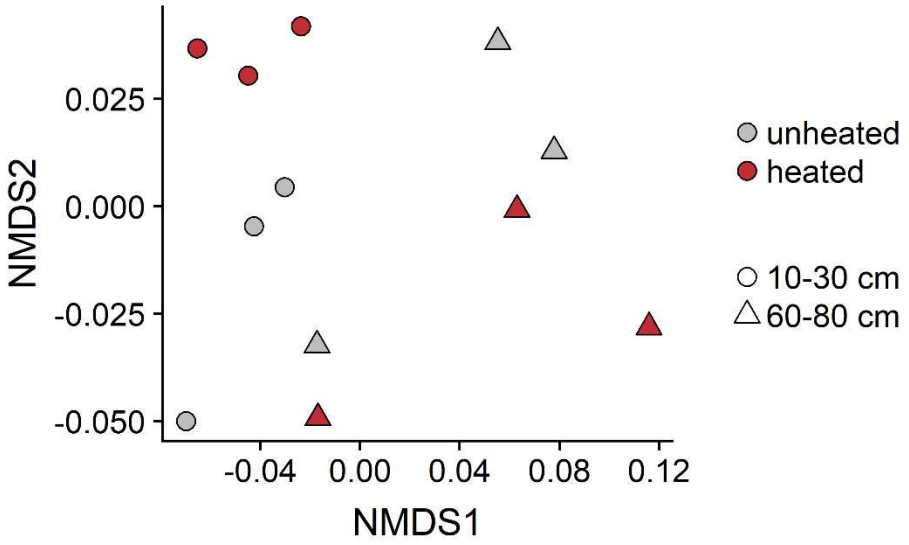


Figure 4-S4: Cumulative microbial respiration (with best-fit regression) measured over the 30-d incubation for each amended soil (A) and boxplots representing resource limitation of total respiration (B) across both depths and heating treatments. Key: carbon (C) and carbon, nitrogen, and phosphorus (CNP). Carbon (C) limitation is assessed by the response of the C-amended soils divided by the control soil, and nutrient limitation is assessed by the response of the C- and nutrient-amended soil divided by the C-amended soil. Dashed line at 1.0 indicates no limitation.

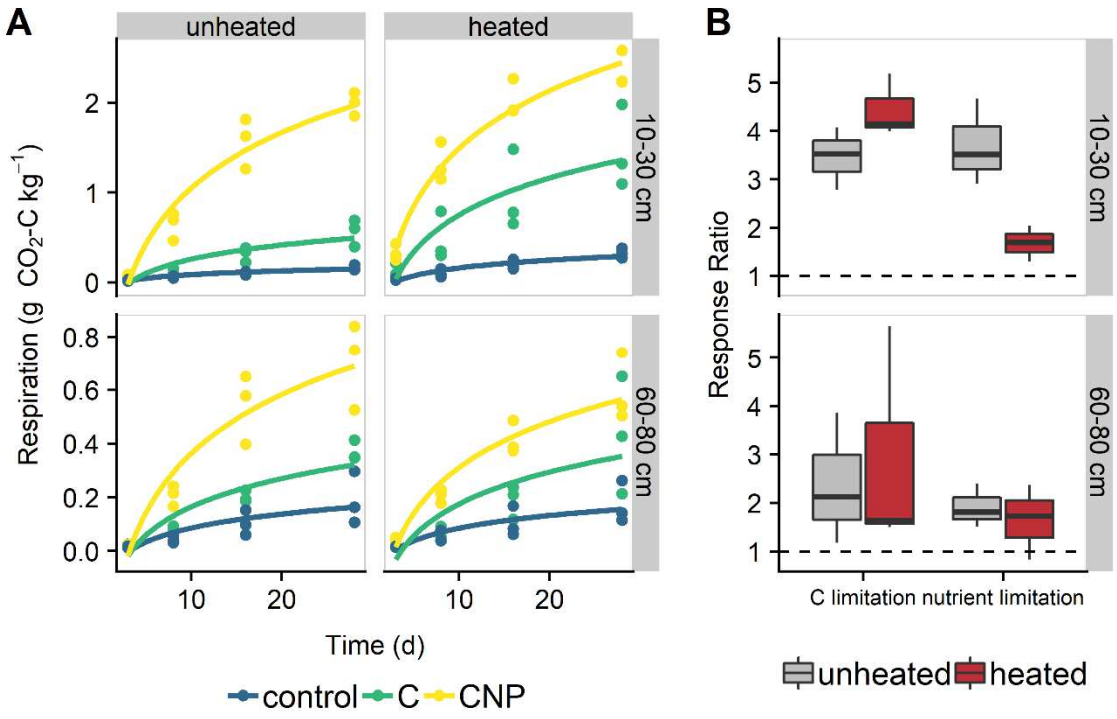


Figure 4-S5: Boxplots representing microbial respiration periodically measured across two depths amended with cellobiose (C) and cellobiose, nitrogen, and phosphorus (CNP) over a 30-d incubation.

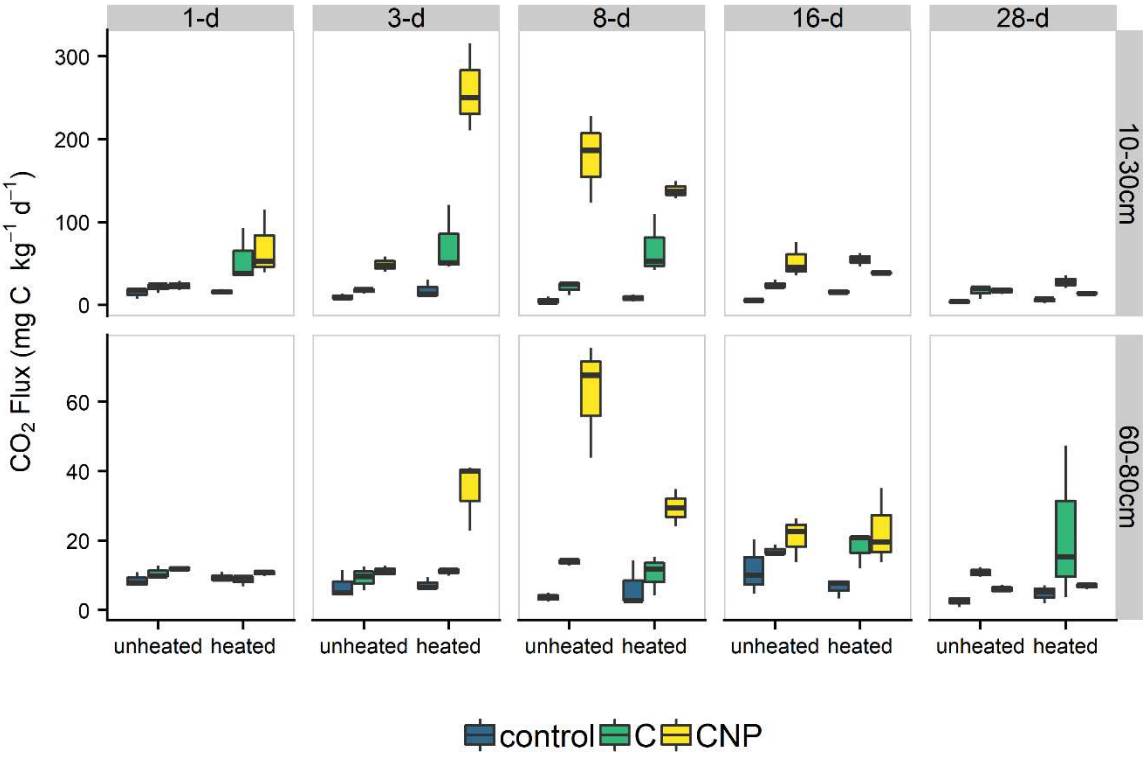
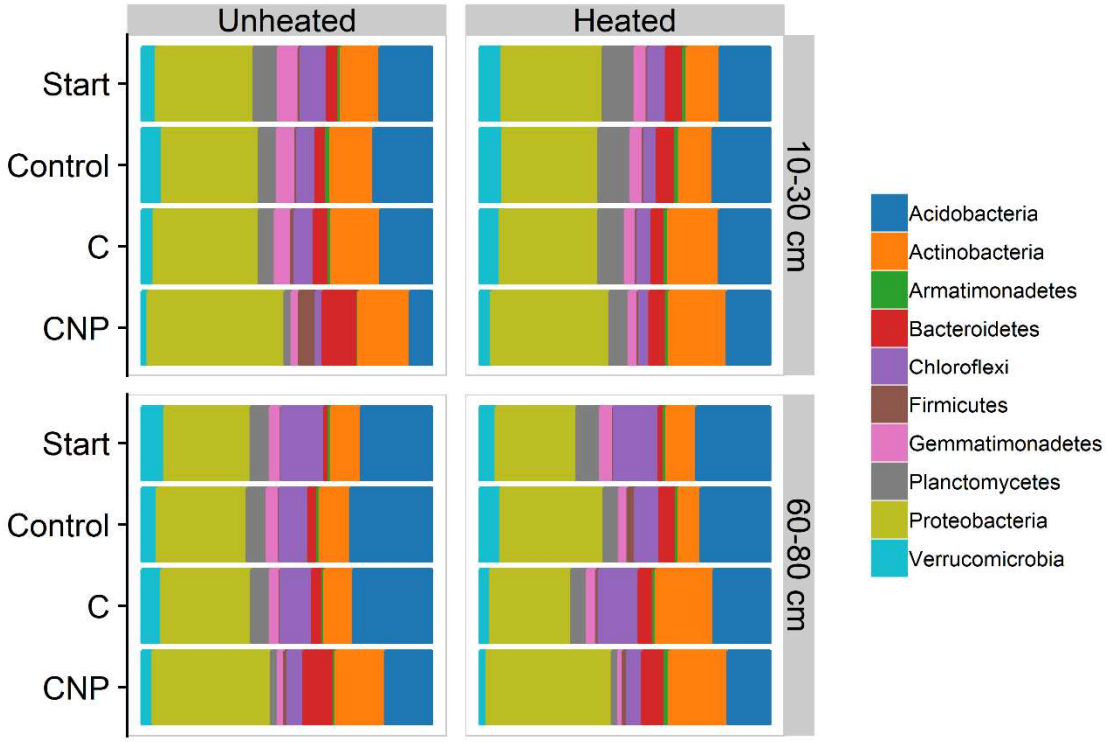


Figure 4-S6: Relative abundance of major bacterial phyla across heating treatments throughout the soil profile over a 30-d incubation amended with carbon (C) or carbon, nitrogen, and phosphorus (CNP) compared to the starting conditions and unamended control.



5 Conclusion

Understanding the microbial response to altered environmental conditions can help to elucidate important mechanisms that underlie microbial community structure and function. This dissertation spans a broad spectrum of ecological disturbances, ranging from chronic, low intensity alterations in environmental drivers (e.g., increased temperatures, Chapter 4), to one-time pulses of high intensity disruption (e.g., wildfire, Chapters 2 & 3). I found that both types of disturbances result in altered microbial states that impact ecosystem conditions affecting microbial resilience.

Increased fire severity (pulse-type disturbance) has the potential to radically alter soil microbial communities and biogeochemical function, possibly resulting in novel ecosystem states. In these states, microbial resilience is unclear. While soil fungi may recover from fire within one to two decades globally (Chapter 2), it is unlikely that soil fungi recovered at the same rates after high-severity fire in the Sierra Nevada. Rates of soil carbon (C) cycling, of which soil fungi are important contributors, were still dampened 44 y since high-severity fire (Chapter 3). These results demonstrate the vulnerability of Sierra Nevada microbial communities and their biogeochemical function to high-severity fire, and show dampened resilience compared to global averages.

This dissertation demonstrates that soil microbial communities are also sensitive to increased temperatures (press-type disturbance *sensu* Bender et al. 1984) and resulting soil chemical conditions (e.g., increased C limitation, Chapter 4). However, resiliency may differ between the top- and sub-soil microbial communities. Where certain topsoil (0-30 cm) microbial taxa are able to capitalize on new environmental conditions, most subsoil (>30 cm) microbial taxa decrease in abundance with soil warming. Similarly, these subsoil microbial communities are less sensitive to altered substrate availability. This suggests that subsoil microbial communities may be less responsive to increased soil temperatures. Because community reorganization is necessary for thermal acclimation (dampened respiration response to increased temperature; Bradford et al. 2008), subsoil microbial communities will likely be maladapted to increased temperatures, possibly hampering thermal acclimation and reducing soil C storage. Reduced C sequestration potential as a result of these disturbances would negatively impact California's climate goals (California Air Resources Board 2017).

However, forest management strategies such as prescribed burns, selective thinning, and fires managed for resource benefits could be used to prevent high-severity fire and increase ecosystem resilience and function (such as C sequestration). As the frequency of high-severity fire in the Sierra Nevada and western US more broadly is continuing to increase, these management strategies will become an increasingly important tool in mitigating against these disturbances. While solutions for preventing increased soil C losses with increased temperatures are less tractable, my dissertation shows that these feedbacks, especially at depth should be incorporated into future climate models, so we may be better prepared for future climate scenarios.

Overall, my dissertation shows the susceptibility of Sierra Nevada forest soils to global change, in particular their ability to store C throughout the soil profile. In the topsoil, soil organic C recovery following high-severity fire is significantly dampened and, in the subsoil, lack of thermal acclimation by the microbial community could reduce the efficiency of decomposition. Together, these findings highlight the issues associated with C sequestration in the Sierra Nevada. These findings should be useful for the development of California policies that prioritize soil C sequestration, including forest restoration treatments such as prescribed fire and tree thinning.

5.1 References

Bender, E. A., T. J. Case, and M. E.Gilpin. 1984. Perturbation experiments in community ecology: Theory and practice. Ecology 65:1-13.

Bradford, M. A., C. A. Davies, S. D. Frey, T. R. Maddox, J. M. Melillo, J. E. Mohan, J. F. Reynolds, K. K. Treseder, and M. D. Wallenstein. 2008. Thermal adaptation of soil microbial respiration to elevated temperature. Ecology Letters 11:1316–1327.