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Fire severity shapes plant colonization effects on bacterial community structure, microbial biomass, and soil enzyme activity in secondary succession of a burned forest

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ABSTRACT

The increasing frequency and severity of wildfires has led to growing attention to the effects of fire disturbance on soil microbial communities and biogeochemical cycling. While many studies have examined fire impacts on plant communities, and a growing body of research is detailing the effects of fire on soil microbial communities, little attention has been paid to the interaction between plant recolonization and shifts in soil properties and microbial community structure and function. In this study, we examined the effect of a common post-fire colonizer plant species, *Corydalis aurea*, on soil chemistry, microbial biomass, soil enzyme activity and bacterial community structure one year after a major forest wildfire in Colorado, USA, in severely burned and lightly burned soils. Consistent with past research, we find significant differences in soil edaphic and biotic properties between severe and light burn soils. Further, our work suggests an important interaction between fire severity and plant effects by demonstrating that the recolonization of soils by *C. aurea* plants only has a significant effect on soil bacterial communities and biogeochemistry in severely burned soils, resulting in increases in percent nitrogen, extractable organic carbon, microbial biomass, β-glucosidase enzyme activity and shifts in bacterial community diversity. This work propounds the important role of plant colonization in succession by demonstrating a clear connection between plant colonization and bacterial community structure as well as the cycling of carbon in a post-fire landscape. This study conveys how the strength of plant–microbe interactions in secondary succession may shift based on an abiotic context, where plant effects are accentuated in harsher abiotic conditions of severe burn soils, with implications for bacterial community structure and enzyme activity.

1. Introduction

As patterns of fire severity and frequency shift amidst climate change, understanding how fires may influence ecosystem structure and function is of growing importance (Niboyet et al., 2011; Reichstein et al., 2013). In particular, the western U.S. and Rocky Mountain regions are expected to face more frequent and severe fires (Westerling, 2006; Miller et al., 2009; Rocca et al., 2014). The details of ecological succession, including revegetation, can strongly influence ecosystem structure and function after such fire disturbance (Scheiner and Willig, 2011). Revegetation is a vital process for recovery of ecosystem function due to both direct and indirect effects on soil physical, chemical, and biological properties. While a vast body of research has described ecological consequences of fire to aboveground (DeBano et al., 1998; Bond et al., 2005) and belowground communities (Dooley and Treseder, 2012; Ferrenberg et al., 2013; Pourreza et al., 2014), a dearth of research has addressed the effect of colonizer plants on belowground soil microbial communities after fire disturbance (Hart

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et al., 2003; López-Poma and Bautista, 2014), or explicitly evaluated such interactions in the field. Nonetheless, interactions between soil bacteria and plants may themselves be drivers of ecosystem succession (Reynolds et al., 2003; Knelman et al., 2012; Jangid et al., 2013) and overall microbial communities (bacteria and fungi) are central to fundamental ecosystem soil processes including carbon (C) flux, nutrient (nitrogen (N) and phosphorous (P)) cycling, and soil fertility (Van Der Heijden et al., 2008; Schmidt et al., 2014).

Extensive research has also established that fire severity strongly influences the degree to which soil physical, chemical, and biological properties are altered. For example, the severity of a fire can alter the magnitude of chemical and biological changes experienced by soils, such as changes in C, microbial biomass, enzyme activity, ammonium, and/or pH (Neary et al., 1999; Certini, 2005). As well, fire severity can also strongly influence carbon chemistry (Certini et al., 2011; Knicker et al., 2013) and microbial community structure/activity (Hamman et al., 2007; Weber et al., 2014), and because fire severity affects a multitude of soil properties so strongly, it may also modulate the relative importance of plant–microbe interactions. While research continues to uncover aspects of when and where changes in plant communities may affect microbial community structure and function, existing theory suggests that links between microbes and plants may be strongest amidst harsh abiotic conditions (Van Der Heijden et al., 2008). In this way, fire severity may prove important in understanding plant–microbe interactions in secondary succession. In particular, since early successional heterotrophic microbial communities often face C and nutrient constraints both after fire-disturbance and in general (Zak et al., 1990; Treseder et al., 2004; Fierer et al., 2010), we expect plant colonization to alter microbial community structure and function via alterations in soil edaphic properties. Given existing research and theory that suggests the importance of plant–microbe interactions varies across different abiotic conditions, we hypothesize that fire intensity will modulate plant effects, in which soils experiencing severe burns will exhibit a greater response to plant colonization than soils experiencing light burn.

Thus, in order to generate a better understanding of plant–microbe interactions following fire disturbances of varying severity, we examined patterns of soil chemistry, bacterial communities, microbial biomass, and overall extracellular enzyme activity of C, N, and P-acquiring enzymes, in four soil categories: severe burn unvegetated soils, severe burn revegetated soils, light burn unvegetated soils, and light burn revegetated soils from approximately one year after a major wildfire. We examined bacteria to assess a higher resolution picture of soil biotic responses to plant colonization during secondary succession revegetation processes, but we also assayed changes to total microbial biomass and enzyme activity – metrics that also integrate over the fungal community which may play a prominent role in post fire soil dynamics (Treseder et al., 2004; Gartner et al., 2012; Holden et al., 2012). Here, we investigated how plant recolonization after fire may vary in its impact on soil biogeochemistry under conditions of varying fire severity.

2. Materials and methods

2.1. Site description and sampling

We sampled soils from the High Park fire burns outside of Ft. Collins, CO, in July 2013. Soils were collected at the Buckhorn Camp property on the Colorado Front Range, which sits at ~2377 m above sea level. Samples were collected approximately one year after the fire, which occurred in June 2012 and is the 3rd largest fire in Colorado's recorded history to date based on area burned. The site included both severely and lightly burn areas within a continuous area of forest dominated by Ponderosa Pine *(Pinus ponderosa)* on similar slope and aspect, and with similar tree cover (in the area of latitude: 40.59 N; longitude: 105.32 W). General characteristics of these Front Range ponderosa pine forests are described by Veblen et al. (2000). Severe burn was defined as areas with no soil litter layer and trees that were fully scorched to the crown (Fig. S1). Light burn areas had a litter layer of 0.5–3.5 cm and partially scorched trees (55–286 cm scorch height) (Fig. S1). All trees were dead in the severe burn areas while live and dead trees were mixed in light burn areas. Eight replicate revegetated and unvegetated soil samples were collected from both severe and light burn areas at least 5 m apart across transects spanning 50 m in both light and severe burn landscapes. Revegetated soil samples were collected under *Corydalis aurea* plants, a native plant that is a common member of communities after fire disturbance and was dominant in both light and severe burn areas. Vegetated soil samples were taken from under *C. aurea* plants that were free of any other vegetation within a radius of at least 32 cm from the sampled soil. Unvegetated samples were free of vegetation within a 1.5 m radius. Soils were collected using a 5 cm diameter coring device to 5 cm depth and, for light burn soils, pine litter was removed prior to sampling. Thus, all samples included the top 5 cm of soil. Soils were immediately transported to labs at the University of Colorado at Boulder, passed through 2 mm mesh size sieves, and subsampled to be stored at 4 °C for soil chemical and enzyme analysis. A subsample was stored at −70 °C for molecular analysis.

2.2. Soil properties

Soils were dried at 100 °C for 48 h to determine gravimetric soil moisture, and soil pH was determined on fresh soils using a ratio of 2 g soil to 4 mL DI H_2O. Thirty milligrams of dried, ground soils were packed in tin capsules and then run on a Thermo Finnigan EA 1112 Series Flash Elemental Analyzer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) to determine % C and % N of samples (Matejovic, 1997). Within a day of collection, ~8 g fresh soil were extracted in 40 mL 0.5 M K_2SO_4 to determine NH_4^+ and NO_3^-/NO_2^- extractable N, total extractable non-purgeable organic carbon (extractable carbon excluding carbonates) (NPOC), and total dissolved nitrogen (TDN). Microbial biomass C was ascertained through a paired set of extractions on 72-h chloroform-fumigated samples as per standard methods (Brookes et al., 1985). Microbial biomass-C as reported was adjusted for extraction efficiency based on the literature correction value of 0.45 (Beck et al., 1997). All extractions included shaking for 1 h and filtering with Whatman no.1 paper (Whatman Incorporated, Florham Park, NJ, USA). Extracts were frozen until chemical analysis. NH_4^+ was measured on a BioTek Synergy 2 Multidetection Microplate Reader (BioTek, Winooski, VT, USA) and NO_3^-/NO_2^- were measured on a Lachat QuikChem 8500 Flow Injection Analyzer (Lachat Instruments, Loveland, CO, USA) from pre-fumigation extracts. NPOC in pre and post fumigation soils were measured on a Shimadzu TOC-V CSN Total Organic Carbon Analyzer (Shimadzu TOCvcpn, Kyoto, Japan).

To determine changes in carbon chemistry via humification, fluorescence spectroscopy was employed (Fellman et al., 2010). For each sample, 5 g fresh soil were extracted in nanopure H_2O, shaken for 1 h at 250 rpm and filtered through combusted (4 h at 450 °C) Whatman GF/F filters (Whatman Incorporated, Florham Park, NJ, USA) into combusted amber viles. First, UV–vis analysis was performed using an Agilent 8453 spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). For fluorescence analysis, a 1:10 dilution of extract:water was completed so that UV absorbance at 254 nm fell between 0.1 and 0.2 cm^{-1}. These diluted samples were then used for fluorescence analysis on a Fluoromax-3 spectrofluorometer (Horiba Jobin Yvon, Kyoto, Japan). A three dimensional

excitation-emission matrix (EEM) was collected for each sample at excitation wavelengths of 245–450 in increments of 10 nm, and at emission wavelengths of 300–600 nm in increments of 2 nm (Cory et al., 2010). EEMs were then corrected for instrument biases and processed according to Gabor et al. (2014) using MATLAB software (The MathWorks, Inc., 2014). In order to examine the degree to which organic mater was humified in each sample, the humification index (HIX) was calculated from collected EEMs (Zsolnay et al., 1999; Ohno, 2002).

2.3. Enzyme analysis

Enzyme activities for β-1,4-glucosidase (BG), β -1,4-N-acetylglucosaminidase (NAG), and acid phosphatase (aP) were determined; these enzymes are commonly used to assess the investment of the overall microbial community (fungi and bacteria) in the acquisition of the limiting elements C, N, and P (Sinsabaugh et al., 2008). Enzyme activity was measured via fluorometric microplate methods (Sinsabaugh et al., 2002; Weintraub et al., 2012). Enzyme analyses were completed using a 96-well assay plate method using ~1 g of refrigerated soil, 1 M sodium acetate buffer titrated to a pH of 7.3, and 4-methylumbelliferone standards, with the protocol detailed by Weintraub et al. (2012). The setup included 16 analytical replicates, quench corrections, standards, and negative controls for each sample. Fluorescence was measured using a microplate reader (Thermo Labsystems, Franklin, MA, USA) at 365 nm excitation and 460 nm emission to determine nmol activity h^{-1} g $soil^{-1}$.

2.4. DNA extractions and Illumina sequencing

DNA was extracted using MoBio's PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA), according the manufacturer's protocol. DNA samples were eluted in TE buffer. Samples were amplified with barcoded PCR using 515F/806R primers. The forward primer included the 5′ Illumina adaptor, a forward primer pad, and forward primer linker followed by the forward 515 primer. The reverse primer contained the reverse complement of the 3′ adaptor, golay barcode, reverse primer pad, reverse primer linker, and the reverse 806 primer. PCR was run with a reaction mixture of 25.0 μL including 8.6 μL PCR Grade H_2O, 12.5 μL FideliTaq Master Mix, 1.0 μL Primer 515F (10 μM), 1.0 μL Primer 806R (10 μM), 0.11 μL $MgCl_2$ (25 mM) in order to quench EDTA, and 2.79 μL template DNA (all samples normalized to 3.583 ng/μL). Negative controls were included. Samples were denatured at 94 °C for 2 min and then amplified in 25 cycles at 94 °C for 45 s, 50 °C for 60 s, and 68 °C for 45 s. A final extension was included of 5 min at 68 °C. All PCR products were run in triplicate and then combined into single samples. To eliminate primer dimer contamination, barcoded PCR product was purified using the QIAquick Gel Extraction Kit, according to the manufacturer's protocol. Samples were multiplexed according to DNA concentration as per quantification using the PicoGreen method on a microplate reader according to the manufacturer's protocol. Pooled DNA purity and quality was determined on a NanoDrop800. Final pooled DNA was purified using the UltraClean PCR Clean-up Kit, according to the manufacturer's protocol. The final multiplexed DNA sample, including negative controls, was sequenced at CU Boulder (BioFrontiers Institute, Boulder, CO) on an Illumina MiSeq with the MiSeq Reagent Kit v2, 300 cycles.

2.5. Statistical and sequence analysis

Using the R statistical environment (R Development Core Team, 2013), Analysis of Variance (ANOVA) tests and Tukey's honestly significant difference (HSD) post-hoc tests were employed to evaluate differences in all soil parameters and taxon relative abundances across the four categories: light and severe burn and vegetated and unvegetated. All enzymes, biomass, moisture, pH, TDN, NH_4^+, NO_2^{3-}, NPOC, and taxon relative abundances were natural log transformed to achieve normal distribution as checked via a Shapiro–Wilk test before ANOVA and Tukey's HSD post hock tests. Pearson's product–moment correlation coefficients were calculated for relationships among specific enzyme activities.

We assessed bacterial community composition and diversity using a combination of UPARSE and QIIME software packages for quality checking/OTU picking at a 97% identity level and community level analysis (Caporaso et al., 2010; Edgar, 2013). Quality filtering, chimera checking, OTU picking, and constructing an OTU table was performed in UPARSE according to default parameters and workflow on joined, paired end reads. QIIME was employed for downstream community analysis. Sequencing yielded a total of 781,359 sequences for 32 samples with a median sequence length of 253 base pairs. After quality filtering, 669,405 sequences remained in total and 5662 OTUs across all samples resulted from UPARSE OTU clustering. All samples were rarefied to an even 11,700 sequences, and rarefaction plots were constructed based on the Chao1 estimator (Fig. S2). All samples showed Good's coverage of over 95% as calculated in QIIME. In QIIME, sequences were aligned and a tree was built using the Randomized accelerated maximum likelihood (RAxML v7.3.0) method for tree building (Stamatakis, 2006). This tree was used for downstream dissimilarity analysis, though results with the FastTree tree building method (Price et al., 2009) as described in Knelman et al. (2014) showed the same significant patterns in downstream analyses. Alpha diversity metrics were calculated in QIIME using the gini_index, Shannon, and PD_whole_tree methods. Community dissimilarity matrices were constructed using the unweighted UniFrac method (Lozupone et al., 2006). Permutational MANOVAs (PERMANOVA) were performed in PRIMER E on the UniFrac beta diversity matrix and Mantel-like RELATE tests were used to examine correlation between this dissimilarity matrix and edaphic properties for severe burn soils (Clarke and Gorley, 2006). We assessed the relationship between all measured edaphic properties and BG activity in severe burn soils using the adonis function in the vegan package in R (Oksanen et al., 2013). This analysis showed if factors explained significant variation in BG activity across samples, and then demonstrated how much of that described variation was non-independent of revegetation as a factor in the model. Based on the findings of relationships between BG activity and particular edaphic factors, structural equation modeling in R (lavaan package) was used to more specifically determine how vegetation effects impacted BG activity in severe burn soils out of a variety of edaphic factors that showed relationships with BG activity (Grace, 2006; Rosseel, 2012). An a-priori model, based on our variance partitioning and documented ecological relationships, included both direct effects of vegetation on BG activity as well indirect effects via soil N, C, pH, moisture, and biomass. All variables were natural log transformed. This model, significantly different from the data, was respecified through the elimination of insignificant paths, resulting in an overall model that did not significantly differ from the data ($P > 0.05$) and individual pathways, which were in themselves significant ($P < 0.05$) (Grace, 2006).

2.6. Data availability

Sequences and mapping file/metadata have been made available via FigShare with the DOIs 10.6084/m9.figshare.1295229 and 10.6084/m9.figshare.1400496 respectively.

3. Results

3.1. Effects of burn severity and C. aurea plant colonization on edaphic properties

Soil edaphic properties showed strong signatures of fire severity. Measures of C chemistry and quantity showed significant differences in light and severe burn unvegetated soils, with severe burn soils containing higher relative levels of humics and significantly lower percent carbon. Nitrogen pools also showed significant differences with burn intensity as evidenced by lower percent N and TDN in severe burn vs. light burn unvegetated soils: however, the inorganic nitrogen content (NO_3^- and NH_4^+) did not differ between burn intensities (Table 1). Finally, severe burn soils showed significantly higher pH and soil moisture content than light burn soils.

In severely burned soils, *C. aurea* acted to alter chemical properties of soils, whereas in light burn soils, *C. aurea* had no statistically significant effects on soil edaphic properties (Table 1). Plant colonization in severe burn soils corresponded with significant increases in NPOC and percent N, and significant decreases in percent moisture in contrast with severe burn unvegetated soils (Table 1). Ammonium and nitrate showed declines under revegetation of both fire severities relative to unvegetated soil, but differences were not significant (Table 1).

3.2. Effects of burn severity and C. aurea plant colonization on bacterial community structure, microbial biomass and soil enzyme activity

We constructed a principal coordinates ordination which illustrated phylogenetic dissimilarity among soil communities (Fig. 1). PERMANOVA analysis demonstrated that overall phylogenetic bacterial community composition was significantly different between unvegetated soils of severe and light burn (Table S1). In part, this difference was evidenced in significant shifts in dominant taxa between soil communities of severe and light burn soils. For example, the relative abundance of Firmicutes in severe burn soils was significantly higher than in light burn soils, whereas Alphaproteobacteria was significantly lower in severe burn soils (Table 2). Verrucomicrobia also showed a significant decline in severe burn unvegetated soils. Microbial biomass was also significantly different between light and severe burn unvegetated soils, showing significant decreases with increasing fire severity (Table 2). No significant differences were found among categories in terms of alpha diversity. Plant recolonization only significantly increased overall microbial biomass in severe burn soils (Table 2). PERMANOVA analysis similarly demonstrated that plant recolonization only affected bacterial phylogenetic community structure in severe burn soils (PERMANOVA $P < 0.05$, Table S1, Fig. 1).

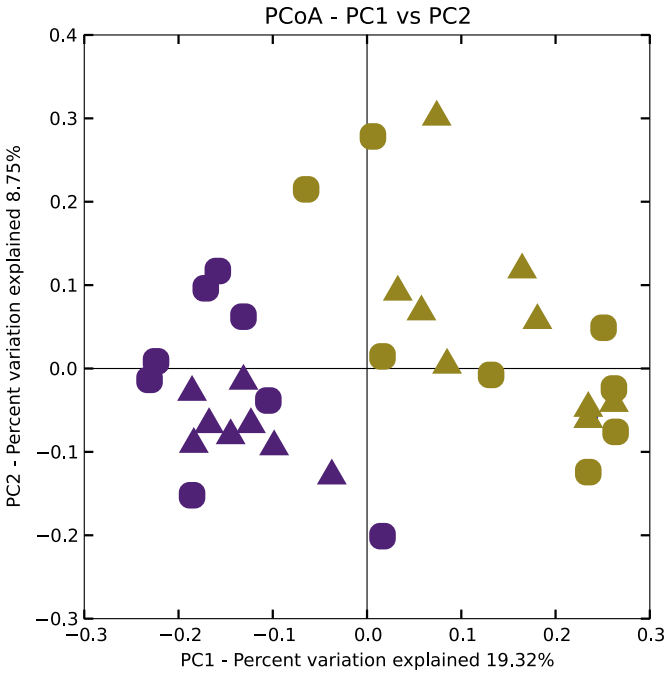

Fig. 1. This Principal Coordinates Analysis (PCoA) shows microbial community dissimilarity (UniFrac) among samples from the 4 different soil types: Purple = severe burn; Gold = light burn; Circles = unvegetated; Triangles = vegetated. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Exoenzyme activity showed significant differences between severe and light burn unvegetated soils, demonstrating effects of fire severity on microbial mediated enzyme activity related to C, N, and P cycling (Fig. 2). BG, NAG, and aP enzyme activity were significantly lower in severe burn soils than light burn soils (Fig. 2). Meanwhile the BG:NAG ratio was significantly higher in severe burn soils than light burn soils (Fig. 2). When enzyme activity was evaluated as specific enzyme activity per unit biomass C, NAG showed the same patterns across soil types as absolute activity, being significantly higher in light burn soils than severe burn soils (Tukey HSD, $P < 0.05$). BG and AP specific enzyme activity showed no significant differences in either comparisons of light and severe burn vegetated soils or light and severe burn unvegetated soils. Specific enzyme activities significantly correlated to varying degrees ($P < 0.05$, BG-NAG: $r = 0.83$; BG-aP: $r = 0.67$; NAG-aP: $r = 0.52$). Total BG enzyme activity was significantly greater in

Table 1
Means and standard deviation of edaphic properties across soil categories.

Category	Severe burn Unvegetated	Severe burn Revegetated	Light burn Unvegetated	Light burn Revegetated
TDN (mg N/kg soil)	1.36 (2.36)^{A}	6.18 (3.34)^{AB}	24.40 (20.15)^{B}	13.67 (13.03)^{B}
NH_4^+ (mg/kg soil)	27.49 (14.38)^{A}	10.75 (4.20)^{B}	27.92 (19.97)^{A}	14.93 (7.67)^{A}
NO_2^-/NO_3^-(mg/kg soil)	21.48 (17.43)^{A}	14.21 (7.87)^{A}	11.23 (14.98)^{AB}	3.68 (2.69)^{B}
Humification Index	5.03 (1.44)^{AD}	6.16 (0.61)^{A}	3.47 (0.81)^{BC}	4.19 (0.76)^{CD}
NPOC (mgC/kg soil)	153.35 (74.03)^{A}	280.95 (89.60)^{B}	157.97 (50.00)^{A}	195.04 (62.34)^{AB}
Percent ^{C}	2.96 (0.94)^{A}	5.50 (1.59)^{AB}	6.15 (2.40)^{B}	8.03 (2.30)^{B}
Percent N	0.15 (0.05)^{A}	0.26 (0.05)^{B}	0.27 (0.07)^{B}	0.32 (0.08)^{B}
C:N ratio	19.35 (2.64)^{A}	20.99 (2.91)^{AB}	22.42 (3.34)^{AB}	24.69 (3.78)^{B}
pH	6.41 (0.47)^{A}	6.86 (0.37)^{A}	5.62 (0.16)^{B}	5.70 (0.23)^{B}
Moisture (%)	2.41 (0.69)^{A}	1.36 (0.39)^{B}	1.02 (0.33)^{BC}	0.75 (0.24)^{C}

Letters denote significant differences as per Tukey HSD tests ($P < 0.05$).

Table 2
Means and standard deviation of microbial biomass C and bacterial community composition across soil categories.

Category	Severe burn	Severe burn	Light burn	Light burn
	Unvegetated	Revegetated	Unvegetated	Revegetated
Microbial biomass C (mg C/kg soil)	180.91 (46.18)^{A}	375.83 (104.36)^{B}	547.49 (315.39)^{B}	480.15 (196.86)^{B}
Acidobacteria (%)	6.83 (2.65)^{AB}	6.14 (1.71)^{A}	11.23 (5.37)^{B}	9.98 (2.96)^{AB}
Actinobacteria (%)	28.55 (4.29)^{AD}	31.20 (3.52)^{A}	20.85 (5.19)^{C}	20.55 (6.84)^{CD}
Bacteroidetes (%)	11.05 (3.12)	10.35 (2.24)	8.63 (4.74)	9.35 (2.91)
Firmicutes (%)	4.63 (3.52)^{A}	2.93 (1.04)^{A}	1.32 (1.22)^{B}	0.85 (0.78)^{B}
Alphaproteobacteria (%)	15.42 (1.38)^{A}	15.14 (1.88)^{A}	21.17 (3.73)^{B}	22.70 (2.18)^{B}
Betaproteobacteria (%)	16.41 (3.23)^{AB}	18.24 (3.03)^{A}	11.50 (6.90)^{B}	10.59 (3.92)^{B}
Verrucomicrobia (%)	4.30 (3.23)^{B}	4.65 (2.70)^{AB}	8.65 (3.80)^{A}	8.25 (3.10)^{A}

Letters denote significant differences as per Tukey HSD tests ($P < 0.05$).

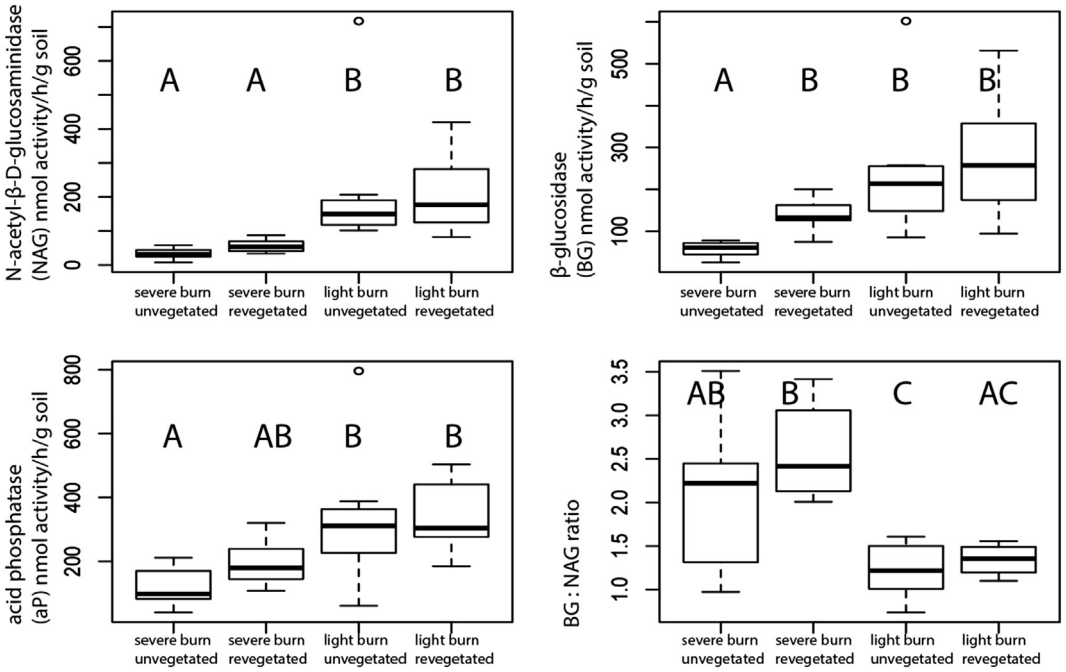

Fig. 2. BG, NAG, and aP activity (nmol activity h^{-1} g $soil^{-1}$) across different soil conditions. Letters denote significant differences (Tukey's HSD, $P < 0.05$).

plant-colonized soils than unvegetated ones in severe burn soils (Tukey's HSD $P < 0.05$); no other soil enzymes showed significant differences (Fig. 2).

In severe burn soils, plant recolonization showed significant effects on edaphic properties, bacterial community structure, and enzyme activity. Variance partitioning demonstrated that a variety of edaphic factors showed significant relationships with BG activity, but that the described variation was largely non-independent from revegetation, indicating the importance of indirect revegetation effects on BG activity (Table 3). Structural Equation Modeling, informed by the variance partitioning analysis, showed that vegetation indirectly drives changes in BG activity, primarily by impacting overall microbial biomass and TDN, which in turn drive variation in BG activity ($R^2 = 0.834$) (Fig. 3). This overall model fit the data (Chi-squared test, $P = 0.102$; Comparative Fit Index = 0.956) and all individual standardized path coefficients were significant ($P < 0.05$) (Fig. 3). In total, plant colonization described significant variation in BG activity via its impact on edaphic properties.

Table 3
Variance partitioning (adonis) of factors that explain BG activity.

Factor	R^2	P value	Percent of explained variation attributable to plant recolonization
Biomass	0.617	0.001	78.93
TDN	0.512	0.002	83.2
perC	0.562	0.001	80.96
perN	0.587	0.001	85.52
NPOC	0.337	0.008	95.85
Moisture	0.446	0.001	92.6

4. Discussion

Our study shows fire severity is a dominant factor in explaining the effects of fire on edaphic and microbial properties of soil. In agreement with past research, we show both decreases in C pools and shifts in C chemistry, humification, with increasing fire severity (Almendros and González-Vila, 1990; Neary et al., 1999; Certini, 2005; Neff et al., 2005) (Table 1). Indeed, severe burns may lead to decreases in soil organic nitrogen (Neary et al., 1999; Treseder et al., 2004; Neff et al., 2005), consistent with our results that show significant declines in TDN and percent N in severe vs. light burn soils (Table 1). In acidic soils, pH is also known to increase after fires, with more severe burns contributing more strongly to alkalization of soils as documented in our research (Certini, 2005) (Table 1).

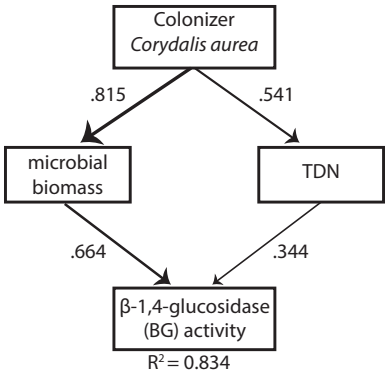

Fig. 3. This diagram of the Structural Equation Model for plant effects on BG exoenzyme activity shows that plant recolonization indirectly impacts BG activity via its influence on microbial biomass and TDN, for example. Standardized path coefficients are all significant ($P < 0.05$). Arrows are weighted in relation to path coefficients. Chi-squared $P = 0.102$ and $n = 16$.

We show that burn severity also impacts bacterial community structure and overall microbial biomass. Severe burn soils showed a significant reduction in overall microbial biomass and a significant change in the phylogenetic composition of bacterial communities in comparison with lightly burned soils, an effect which has been noted in previous work (Hamman et al., 2007; Pourreza et al., 2014; Weber et al., 2014). Indeed, post-fire changes to the soil environment, especially accentuated under severe burns, may create a unique environment that can influence microbial community assembly via both strong environmental filters and/or the opening of habitat to colonizer dispersal, which can therein influence ecosystem function as well (Ferrenberg et al., 2013; Nemergut et al., 2013; Knelman and Nemergut, 2014).

Although no changes were observed in alpha-diversity, changes in relative abundances of major bacterial taxa are coherent with ecological strategies of associated bacteria. For example, Firmicutes, with the capacity to form endospores and possibly withstand fire have been noted to burgeon after fire disturbances (Smith et al., 2008; Ferrenberg et al., 2013) (Table 2). Thus, in more severe fires, these organisms may have a comparative advantage, having withstood the severe burns, in immediately establishing in soils post disturbance. We also note significant decreases in Alphaproteobacteria relative abundance with increasing burn severity, which is consistent with research demonstrating greater Alphaproteobacteria abundances in higher nutrient soils (Knelman et al., 2012; Cederlund et al., 2014) (Table 2).

Importantly, our study is unique in exploring the effects of secondary plant succession via colonization by *C. aurea*, under the conditions of different burn severities, in regulating ecosystem function. Consistent with our hypotheses, we demonstrate an interaction between burn severity and revegetation, in which re-establishment of plant communities has a significant impact on soil edaphic and microbial properties in severe burn soils but not in light burn soils. In severely burned soils, plant colonization significantly increased extractable organic C and percent N, thereby having the effect of amending the soil nutrient status relative to unvegetated soils in severe burns. Litter and exudate inputs from newly established plants may alter carbon and nitrogen status of soils and feedback on microbial biomass and community structure (Van Der Heijden et al., 2008; Berg and Smalla, 2009). Although plant colonization correlated with significant shifts in microbial communities in severe burn soils (Fig. 1, Table S1), we were not able to attribute these observed overall community changes to shifts in measured edaphic properties, as Mantel-like RELATE tests showed no correlations among bacterial dissimilarity and edaphic factors. It is possible, that communities may be responding to unmeasured direct or indirect plant effects, such as more nuanced aspects of carbon chemistry (Meier and Bowman, 2008).

Plant-colonization only affected BG enzyme activity in severe burn soils. Our variance partitioning identified relationships between biomass, TDN, percent C, percent N, NPOC, and moisture with BG activity, that were, however, non independent of plant effects (Table 3). We then used SEM to generate a more mechanistic hypothesis of plant colonization driving shifts in BG activity via indirect effects on soil edaphic properties. In testing the indirect effect of plant recolonization via changes of these various edaphic properties, we found that plant driven changes in biomass and TDN fit the data to significantly describe variation in BG activity. Indeed, past research has shown that microbes may invest in C acquisition enzymes in response to the availability of resources, such as via plant inputs of carbon and nitrogen (Sinsabaugh and Moorhead, 1994; Sinsabaugh et al., 2008). Specific enzyme activity confirms that investment in NAG per unit biomass C is significantly higher in light burn versus severe burn soils. Further, BG:NAG ratios are also significantly higher in severe burn soils versus light burn unvegetated soils, indicating a change in the relative investment in C vs, N acquisition. As such, it appears that microbial communities in severe burn soils may be more limited by C while microbial communities in light burn soils may be more limited by N. These differences in BG:NAG ratios have been used as an indication of the relative allocation of resources toward C or N acquisition, demonstrating an overall shift in resource allocation strategy toward C acquisition in severe burn soils, not purely a change in enzyme potential as a function of microbial biomass (Sinsabaugh et al., 2009, 2008; Gartner et al., 2012) (Fig. 2).

Finally, while our research has focused on bacterial community structure, an important subset of the microbial community as a whole, for our higher resolution community analysis, our assessment of overall microbial biomass and soil enzyme activity may be driven by fungal components of the microbial community outside of the observed bacterial patterns, since fungi can play important roles in post fire landscapes, secondary succession, and biogeochemistry more generally (Reynolds et al., 2003; Treseder et al., 2004; Holden et al., 2012). Additionally, past research has demonstrated strong responses of mycorrhizal fungi and soil invertebrates to fire, which also may impact secondary succession in soils after fire due to their importance in ecosystem dynamics (Certini, 2005; Dooley and Treseder, 2012). In total, while biomass and enzyme activity measured in this study may suggest important changes in fungal communities with integral feedbacks on ecosystem development, further research is needed to better appreciate the relative contribution of bacterial and fungal responses to biomass and enzyme activity reported here.

5. Conclusion

As fires become more prevalent across the American West and the globe, it is vital to better constrain how affected ecosystems – and the important functions associated with them – will respond to such disturbance and recover. Beyond well known differences in the effect of fire severity, our work fills a gap in our understanding of post fire-disturbance ecosystem succession, demonstrating that the effects of plant colonization during secondary succession revegetation depend on fire severity, with the greatest impact on soil edaphic factors, bacterial community structure, overall microbial biomass, and soil enzyme activity in higher severity burns. Plant recolonization effects on BG enzyme potential is of interest, given the central role of exoenzymes in decomposition and nutrient

cycling, and suggests the importance of understanding plant effects on broader microbial community function associated with compositional changes as well (Uroz et al., 2010). Our work demonstrates that indirect soil effects of plant colonization drive increases in BG activity in severe burn soils. Further, our study indicates that plant colonization during revegetation may have stronger effects on belowground communities in harsher, more nutrient poor soil environments after severe burns, for example, building on a body of literature that is beginning to assess where and when aboveground communities will have effects on belowground microbial community structure and function in a rapidly changing world.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.08.004.

References

Almendros, G., González-Vila, F.J., 1990. Fire-induced transformation of soil organic matter from an oak forest. An experimental approach to the effects of fire on humic substances. Soil Science 149, 158–168.
Beck, T., Joergensen, R.G., Kandeler, E., Makeschin, F., Nuss, E., Oberholzer, H.R., Scheu, S., 1997. An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. Soil Biology and Biochemistry 29, 1023–1032.
Berg, G., Smalla, K., 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiology Ecology 68, 1–13.
Bond, W.J., Woodward, F.I., Midgley, G.F., 2005. The global distribution of ecosystems in a world without fire. New Phytologist 165, 525–538.
Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17, 837–842.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Meth 7, 335–336.
Cederlund, H., Wessén, E., Enwall, K., Jones, C.M., Juhanson, J., Pell, M., Philippot, L., Hallin, S., 2014. Soil carbon quality and nitrogen fertilization structure bacterial communities with predictable responses of major bacterial phyla. Applied Soil Ecology 84, 62–68.
Certini, G., 2005. Effects of fire on properties of forest soils: a review. Oecologia 143, 1–10.
Certini, G., Nocentini, C., Knicker, H., Arfaioli, P., Rumpel, C., 2011. Wildfire effects on soil organic matter quantity and quality in two fire-prone Mediterranean pine forests. Geoderma 167–168, 148–155.
Clarke, K.R., Gorley, R.N., 2006. PRIMER v6: User Manual/Tutorial. PRIMER–E, Plymouth, UK.
Cory, R.M., Miller, M.P., McKnight, D.M., Guerard, J.J., 2010. Effect of instrument-specific response on the analysis of fulvic acid fluorescence spectra. Limnology and Oceanography: Methods 8, 67–78.
DeBano, L.F., Neary, D.G., Ffolliott, P.F., 1998. Fire Effects on Ecosystems. John Wiley & Sons.
Dooley, S.R., Treseder, K.K., 2012. The effect of fire on microbial biomass: a meta-analysis of field studies. Biogeochemistry 109, 49–61.
Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods 10, 996–998.
Fellman, J.B., Hood, E., Spencer, R.G.M., 2010. Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: a review. Limnology and Oceanography 55, 2452–2462.
Ferrenberg, S., O'Neill, S.P., Knelman, J.E., Todd, B., Duggan, S., Bradley, D., Robinson, T., Schmidt, S.K., Townsend, A.R., Williams, M.W., Cleveland, C.C., Melbourne, B.A., Jiang, L., Nemergut, D.R., 2013. Changes in assembly processes in soil bacterial communities following a wildfire disturbance. The ISME Journal 7, 1102–1111.
Fierer, N., Nemergut, D., Knight, R., Craine, J.M., 2010. Changes through time: integrating microorganisms into the study of succession. Research in Microbiology 161, 635–642.
Gabor, R.S., Eilers, K., McKnight, D.M., Fierer, N., Anderson, S.P., 2014. From the litter layer to the saprolite: chemical changes in water-soluble soil organic matter and their correlation to microbial community composition. Soil Biology and Biochemistry 68, 166–176.
Gartner, T.B., Treseder, K.K., Malcolm, G.M., Sinsabaugh, R.L., 2012. Extracellular enzyme activity in the mycorrhizospheres of a boreal fire chronosequence. Pedobiologia 55, 121–127.
Grace, J.B., 2006. Structural Equation Modeling and Natural Systems. Cambridge University Press.
Hamman, S.T., Burke, I.C., Stromberger, M.E., 2007. Relationships between microbial community structure and soil environmental conditions in a recently burned system. Soil Biology and Biochemistry 39, 1703–1711.
Hart, S., DeLuca, T., Newman, G., MacKenzie, M.D., Boyle, S., 2003. Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. Forest Ecology and Management 220, 166–184.
Holden, S.R., Gutierrez, A., Treseder, K.K., 2012. Changes in soil fungal communities, extracellular enzyme activities, and litter decomposition across a fire chronosequence in Alaskan boreal forests. Ecosystems 16, 34–46.
Jangid, K., Whitman, W., Condron, L., Turner, B., Williams, M., 2013. Soil bacterial community succession during long-term ecosystem development. Molecular Ecology 22, 3415–3424.
Knelman, J.E., Legg, T.M., O'Neill, S.P., Washenberger, C.L., González, A., Cleveland, C.C., Nemergut, D.R., 2012. Bacterial community structure and function change in association with colonizer plants during early primary succession in a glacier forefield. Soil Biology and Biochemistry 46, 172–180.
Knelman, J.E., Nemergut, D.R., 2014. Changes in community assembly may shift the relationship between biodiversity and ecosystem function. Frontiers in Microbiology 5. http://dx.doi.org/10.3389/fmicb.2014.00424.
Knelman, J.E., Schmidt, S.K., Lynch, R.C., Darcy, J.L., Castle, S.C., Cleveland, C.C., Nemergut, D.R., 2014. Nutrient addition dramatically accelerates microbial community succession. PLoS ONE 9, e102609. http://dx.doi.org/10.1371/journal.pone.0102609.
Knicker, H., González-Vila, F.J., González-Vázquez, R., 2013. Biodegradability of organic matter in fire-affected mineral soils of Southern Spain. In: Soil Biology and Biochemistry, Special Issue: Interactions of Soil Minerals with Organic Components and Microorganisms VII and Enzymes in the Environment IV, vol. 56, pp. 31–39.
López-Poma, R., Bautista, S., 2014. Plant regeneration functional groups modulate the response to fire of soil enzyme activities in a Mediterranean shrubland. Soil Biology and Biochemistry 79, 5–13.
Lozupone, C., Hamady, M., Knight, R., 2006. UniFrac – an online tool for comparing microbial community diversity in a phylogenetic context. BMC Bioinformatics 7, 371.
Matejovic, I., 1997. Determination of carbon and nitrogen in samples of various soils by the dry combustion. Communications in Soil Science and Plant Analysis 28, 1499–1511.
Meier, C.L., Bowman, W.D., 2008. Links between plant litter chemistry, species diversity, and below-ground ecosystem function. Proceedings of the National Academy of Sciences 105, 19780.
Miller, J.D., Safford, H.D., Crimmins, M., Thode, A.E., 2009. Quantitative evidence for increasing Forest fire severity in the Sierra Nevada and Southern Cascade mountains, California and Nevada, USA. Ecosystems 12, 16–32.
Neary, D.G., Klopatek, C.C., DeBano, L.F., Ffolliott, P.F., 1999. Fire effects on belowground sustainability: a review and synthesis. Forest Ecology and Management 122, 51–71.
Neff, J.C., Harden, J.W., Gleixner, G., 2005. Fire effects on soil organic matter content, composition, and nutrients in boreal interior Alaska. Canadian Journal of Forest Research 35, 2178–2187.
Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F., Knelman, J.E., Darcy, J.L., Lynch, R.C., Wickey, P., Ferrenberg, S., 2013. Patterns and processes of microbial community assembly. Microbiology and Molecular Biology Reviews 77, 342–356.
Niboyet, A., Brown, J.R., Dijkstra, P., Blankinship, J.C., Leadley, P.W., Le Roux, X., Barthes, L., Barnard, R.L., Field, C.B., Hungate, B.A., 2011. Global change could amplify fire effects on soil greenhouse gas emissions. PLoS One 6, e20105.
Ohno, T., 2002. Fluorescence inner-filtering correction for determining the humification Index of dissolved organic matter. Environmental Science & Technology 36, 742–746.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P., O'Hara, R.B., Simpson, G., Solymos, P., Stevens, M.H., Wagner, H., 2013. Vegan: Community Ecology Package.
Pourreza, M., Hosseini, S.M., Safari Sinegani, A.A., Matinizadeh, M., Dick, W.A., 2014. Soil microbial activity in response to fire severity in Zagros oak (Quercus brantii Lindl.) forests, Iran, after one year. Geoderma 213, 95–102.
Price, M.N., Dehal, P.S., Arkin, A.P., 2009. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Molecular Biology and Evolution 26, 1641–1650.
R Development Core Team, 2013. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Reichstein, M., Bahn, M., Ciais, P., Frank, D., Mahecha, M.D., Seneviratne, S.I., Zscheischler, J., Beer, C., Buchmann, N., Frank, D.C., Papale, D., Rammig, A., Smith, P., Thonicke, K., van der Velde, M., Vicca, S., Walz, A., Wattenbach, M., 2013. Climate extremes and the carbon cycle. Nature 500, 287–295.
Reynolds, H.L., Packer, A., Bever, J.D., Clay, K., 2003. Grassroots ecology: plant-microbe-soil interactions as drivers of plant community structure and dynamics. Ecology 84, 2281–2291.
Rocca, M.E., Brown, P.M., MacDonald, L.H., Carrico, C.M., 2014. Climate change impacts on fire regimes and key ecosystem services in rocky mountain forests. Forest Ecology and Management 327, 290–305.
Rosseel, Y., 2012. lavaan: an R package for structural equation modeling. Journal of Statistical Software 48, 1–36.
Scheiner, S.M., Willig, M.R., 2011. The Theory of Ecology. University of Chicago Press.
Schmidt, S.K., King, A.J., Meier, C.L., Bowman, W.D., Farrer, E.C., Suding, K.N., Nemergut, D.R., 2014. Plant–microbe interactions at multiple scales across a high-elevation landscape. Plant Ecology & Diversity 0, 1–10.
Sinsabaugh, R., Carreiro, M., Repert, D., 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60, 1–24.
Sinsabaugh, R.L., Hill, B.H., Follstad Shah, J.J., 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462, 795–798.
Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11, 1252–1264.
Sinsabaugh, R., Moorhead, D., 1994. Resource-allocation to extracellular enzyme-production – a model for nitrogen and phosphorus control of litter decomposition. Soil Biology & Biochemistry 26, 1305–1311.
Smith, N.R., Kishchuk, B.E., Mohn, W.W., 2008. Effects of wildfire and harvest disturbances on forest soil bacterial communities. Applied and Environmental Microbiology 74, 216–224.
Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.
The MathWorks, Inc., 2014. MATLAB 8.0 and Statistics Toolbox 8.1. Natick, Massachusetts, United States.
Treseder, K.K., Mack, M.C., Cross, A., 2004. Relationships among fires, fungi, and soil dynamics in alaskan boreal forests. Ecological Applications 14, 1826–1838.
Uroz, S., Buée, M., Murat, C., Frey-Klett, P., Martin, F., 2010. Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. Environmental Microbiology Reports 2, 281–288.
Van Der Heijden, M.G., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11, 296–310.
Veblen, T.T., Kitzberger, T., Donnegan, J., 2000. Climatic and human influences on fire regimes in ponderosa pine forests in the colorado front range. Ecological Applications 10, 1178–1195.
Weber, C.F., Lockhart, J.S., Charaska, E., Aho, K., Lohse, K.A., 2014. Bacterial composition of soils in ponderosa pine and mixed conifer forests exposed to different wildfire burn severity. Soil Biology and Biochemistry 69, 242–250.
Weintraub, S.R., Wieder, W.R., Cleveland, C.C., Townsend, A.R., 2012. Organic matter inputs shift soil enzyme activity and allocation patterns in a wet tropical forest. Biogeochemistry 114, 313–326.
Westerling, A.L., 2006. Warming and earlier spring increase western U.S. forest wildfire activity. Science 313, 940–943.
Zak, D.R., Grigal, D.F., Gleeson, S., Tilman, D., 1990. Carbon and nitrogen cycling during old-field succession: constraints on plant and microbial biomass. Biogeochemistry 11, 111–129.
Zsolnay, A., Baigar, E., Jimenez, M., Steinweg, B., Saccomandi, F., 1999. Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. Chemosphere 38, 45–50.