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

Joseph E. Knelman a, b, *, Emily B. Graham a, c, Nicole A. Trahan d, Steven K. Schmidt b, Diana R. Nemergut a, e

a Institute for Arctic and Alpine Research, University of Colorado at Boulder, Boulder, CO 80309, USA
b Department of Ecology and Evolutionary Biology, University of Colorado at Boulder, Boulder, CO 80309, USA
c Environmental Studies Program, University of Colorado at Boulder, Boulder, CO 80309, USA
d School of Natural Resources and the Environment, University of Arizona, Tucson, AZ 85721, USA
e Biology Department, Duke University, Durham, NC 27708, USA

**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.

© 2015 Elsevier Ltd. All rights reserved.

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
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 a 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 H2O. 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 K2SO4 to determine NH4 and NO3 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 no1. paper (Whatman Incorporated, Florham Park, NJ, USA). Extracts were frozen until chemical analysis. NH4 was measured on a BioTek Synergy 2 Multidetection Microplate Reader (BioTek, Winooski, VT, USA) and NO3/NO2 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 H2O, 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 vials. 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 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 matter 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-β-glucosidase (BG), β -1,4-N-acetyl-glucosaminidase (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 fluorimetric 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 H2O, 12.5 μL FideliTaq Master Mix, 1.0 μL Primer 515F (10 μM), 1.0 μL Primer 806R (10 μM), 0.11 μL MgCl2 (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. Puriﬁed DNA quality and purity 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 burnt and vegetated and unvegetated. All enzymes, biomass, moisture, pH, TDN, NH4, NO3, 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 hoc 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 MANOVA (PERMANOVA) were performed in PRIMER E on the UniFrac beta diversity matrix and Mantel-like 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 (NO₃ and NH₄) 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 recolonization 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).

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

<table>
<thead>
<tr>
<th>Category</th>
<th>Severe burn</th>
<th>Light burn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unvegetated</td>
<td>Revegetated</td>
</tr>
<tr>
<td></td>
<td>Unvegetated</td>
<td>Revegetated</td>
</tr>
<tr>
<td>TDN (mg N/kg soil)</td>
<td>1.36 (2.36)A</td>
<td>6.18 (3.34)AB</td>
</tr>
<tr>
<td>NH₄ (mg/kg soil)</td>
<td>27.49 (14.38)A</td>
<td>10.75 (4.20)B</td>
</tr>
<tr>
<td>NO₂ (mg/kg soil)</td>
<td>21.48 (17.43)A</td>
<td>14.21 (7.87)A</td>
</tr>
<tr>
<td>Humification Index</td>
<td>5.03 (1.44)A</td>
<td>6.16 (0.61)A</td>
</tr>
<tr>
<td>NPOC (mgC/kg soil)</td>
<td>153.35 (74.03)A</td>
<td>280.95 (89.60)B</td>
</tr>
<tr>
<td>Percent C</td>
<td>2.96 (0.94)A</td>
<td>5.50 (1.59)AB</td>
</tr>
<tr>
<td>Percent N</td>
<td>0.15 (0.05)A</td>
<td>0.26 (0.05)B</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>19.35 (2.64)A</td>
<td>20.99 (2.91)B</td>
</tr>
<tr>
<td>pH</td>
<td>6.41 (0.47)A</td>
<td>6.85 (0.37)A</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>2.41 (0.69)A</td>
<td>1.36 (0.39)B</td>
</tr>
</tbody>
</table>

Letters denote significant differences as per Tukey HSD tests (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.

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 alkalinization of soils as documented in our research (Certini, 2005) (Table 1).

### Table 3

<table>
<thead>
<tr>
<th>Factor</th>
<th>R^2</th>
<th>P value</th>
<th>Percent of explained variation attributable to plant recolonization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass</td>
<td>0.617</td>
<td>0.001</td>
<td>78.93</td>
</tr>
<tr>
<td>TDN</td>
<td>0.512</td>
<td>0.002</td>
<td>83.2</td>
</tr>
<tr>
<td>perC</td>
<td>0.562</td>
<td>0.001</td>
<td>80.96</td>
</tr>
<tr>
<td>perN</td>
<td>0.587</td>
<td>0.001</td>
<td>85.52</td>
</tr>
<tr>
<td>NPOC</td>
<td>0.337</td>
<td>0.008</td>
<td>95.85</td>
</tr>
<tr>
<td>Moisture</td>
<td>0.446</td>
<td>0.001</td>
<td>92.6</td>
</tr>
</tbody>
</table>

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

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

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.
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.

 Acknowledgments

This work was supported by the National Science Foundation of the USA through grant DEB-1258160 to DRN and SKS and a graduate research fellowship to JKE (DGE 1144083). Additional funding to JKE through the University of Colorado’s Beverly Sears Graduate Student Research Grant assisted with laboratory analysis. We thank Emily Dynes for help in the field and the Buckhorn United Methodist Camp for access to the research sites. We also appreciate the advice of Janet Prevey and Holly Hughes on botany details and analytical chemistry related to the study. We thank two anonymous reviewers for constructive comments on the analysis and communication of the content presented, which led to substantial improvement of the manuscript via the review process.

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


