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Effects of Traditional and Microbially-Focused Restoration Techniques on Soil Communities In Tallgrass Prairies

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EFFECTS OF TRADITIONAL AND MICROBIALLY-FOCUSED RESTORATION TECHNIQUES ON SOIL COMMUNITIES IN TALLGRASS PRAIRIES

by

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Tallgrass prairies have virtually disappeared in many parts of their former range due to the conversion of this ecosystem to farmland. In more recent years there have been efforts to restore these prairies on reclaimed agricultural land. However, these restored prairies do not resemble their remnant counterparts in many ways, such as in soil microbial community composition and metrics related to carbon storage. In Chapter 1, I show that bacterial communities in a restored prairie and an adjacent remnant prairie in southwest Michigan differ in their immediate and longer-term responses to prescribed fire, a commonly used prairie restoration and maintenance technique. Overall, results show that bacterial communities in the remnant prairie were more resilient to the prescribed fire event than the bacterial communities in the restored prairie. In Chapter 2, I explore the effects of carbon addition in the form of pure cellulose and plant biomass as well as the effects of plants and soil type on soil microbial communities and metrics related to carbon storage and in two new prairie restorations, one in southwest Michigan and one in eastern Minnesota. We found that through biomass addition there were increases in metrics related to carbon storage in both prairies when plants were present. Conversely, the response of the soil microbial communities differed in these two restorations in response to carbon addition and the presence of plants suggesting that differences in soil type can set restorations of different trajectories.

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CHAPTER I

DIFFERENTIAL RESPONSES OF SOIL BACTERIAL COMMUNITIES TO A PRESCIBED FIRE IN A PAIRED RESTORED AND REMNANT PRAIRIE SYSTEM

Restoration of prairie and oak savanna systems on former agricultural land is vital for improving soil health and ecosystem services. Restored and remnant systems are often managed using the same techniques, including implementation of prescribed fire. Yet, soil microbial communities and functions in these two types of systems typically differ, due to past land-use history and current plant community composition. In this study, we investigated the responses of soil bacterial communities, enzyme activities and putative functional pathways to the effects of prescribed fire in a paired remnant and restored prairie system located in southwest Michigan, USA. We examined the immediate effects of fire one day after the fire as well as the longer-term effects in a time series extending to 11-months after the fire. Our results indicate that the soil bacterial communities in the remnant were immediately responsive in composition but not in predicted function. Additionally, remnant community composition in burned plots returned to the composition in the control within one month, indicating resiliency in this community. In contrast, soil bacterial communities in the restoration shifted both compositionally and functionally one day after the fire, and continued to differ after 11-months. Past land-use effects on bacterial community composition and site-level heterogeneity, coupled with present-day differences in plant communities and litter quantity, mediated the different responses in the two systems. Our results suggest that land management plans aimed at increasing soil functional resiliency may require different management strategies for restored and remnant prairies.

Introduction

Tallgrass prairie and oak savanna ecosystems are some of the most endangered ecosystems in North America, with less than 10 % of original prairies and less than 1 % of original savannas remaining (Nuzzo, 1985; Samson & Knopf, 1994, Hoekstra et al. 2005). After European expansion into the Midwestern and Great Plains regions in the United States in the 1800s, these ecosystems were largely converted to farmland and fire was suppressed in many of the remaining fragments of these ecosystems (Bragg and Hulbert, 1976; Chapman and Brewer, 2008; Koper et al., 2010; Allen & Palmer, 2011; Dey & Kabrick, 2015). The conversion of these grasslands to agricultural fields has resulted in the loss of one of the largest carbon sinks in North America (Guzman & Al-Kaisi, 2010). Intense cultivation altered the carbon stores in the soil by reducing carbon protected in soil aggregates, removing deep-rooted native plants, and disrupting plant-associated soil microbial communities, while long-term fertilization continued to change both plant-associated and free living soil microbial consortia (West & Six, 2007; Fierer et al., 2013; Dai et al., 2018; House & Bever, 2018). Combined, these losses in biotic and abiotic carbon stores have resulted in an estimated loss of 60 % of soil carbon stocks from this North American ecosystem (Conant et al., 2017).

There have been widespread efforts to restore farmland and other degraded ecosystems back to native grasslands, carbon-sequestering vegetation for grazing, or bioenergy crops (Field et al. 2020). However, these restored grassland communities differ from the communities in remnant (undisturbed) grasslands. Remnants have more diverse native plant communities with higher abundances of native forbs and woody shrubs that have high conservation values, as well as lower abundances of C_4 grasses that dominate many restorations (Martin et al., 2005;

McLachlan & Knispel, 2005; Polley et al., 2005; Ladwig et al., 2018; Newbold et al., 2019). Importantly, plant communities with higher diversity have been shown to influence soil microbial communities by increasing microbial biomass and activity, and increasing litter decomposition and carbon storage (Lange et al., 2015; Santonja et al., 2017; Chen et al., 2018). Restored grassland systems also harbor shifted soil fungal and bacterial community composition and functions from those found in remnants (Jangid et al., 2010; Mackelprang et al., 2018; Docherty & Gutknecht, 2019). Many studies that examine restoration and management practices on soil microbes focus on soil fungi (e.g., Koziol & Bever, 2017; House & Bever, 2018; House & Bever, 2019; Bauer et al., 2020), while few have examined the effect of restoration on soil bacterial communities. Soil bacterial communities are an important component of ecosystems and mediate rate-limiting steps in soil biogeochemical cycles, which can influence overall ecosystem functioning and stability (Schimel & Schaeffer, 2012; Pérez-Valera et al., 2019). Multiple studies suggest that there is a long term legacy effect of agriculture on bacterial community structure and function in restored prairies (Jangid et al., 2010; Fierer et al., 2013; Barber et al., 2017; Mackelprang et al., 2018), and that it takes approximately three decades of restoration for bacterial communities in a restored prairie to approach convergence with communities found in remnants (e.g. Herzberger et al., 2014; Duncan et al., 2016; Barber et al. 2017). In some cases convergence may never occur, and restored soil communities are placed on a different trajectory from remnants after decades of agricultural manipulation (Jangid et al., 2010). The repercussions for function include lower carbon storage capacity in restored systems, which is important considering that carbon storage is a major incentive for restoring croplands back to grasslands. For example, in a paired restored and remnant experiment in Wisconsin, soils in the restored prairies stored 37 % less soil carbon and emitted more CO_2, indicating that the

functions of the microbial communities in the restored prairie may have never converged to those in the remnant (Kucharik et al., 2006). A major compositional difference between remnant and restored systems is that remnants have a higher relative abundance of Verrucomicrobia that are positively correlated with genes related to complex carbohydrate metabolism, while restored prairies have a greater relative abundance of Betaproteobacteria which have a faster growth rate and metabolize more labile carbon substrates (e.g. Jangid et al. 2010; Fierer et al. 2013). Despite these taxonomic and functional differences, restored and remnant grasslands are generally managed in similar ways, despite the potential for differential responses.

Prescribed fire is one of the most important tools in a land manager's toolbox for preventing invasive or woody species encroachment and maintaining native plant diversity. Ecological disturbances, such as prescribed fire, can exert positive, negative, or neutral effects on communities (Coyle, 2017; Cohen et al. 2021) depending on whether the communities are resistant or resilient to the fire disturbance (Holling, 1973). Fire has a positive effect on plant communities in grasslands as it reduces the establishment of woody species and maintains plant community structure and is commonly used by managers to upkeep and restore these ecosystems (Bragg & Hulbert, 1976; Collins, 1987; Gibson & Hulbert, 1987; Tester, 1989; Anderson, 2006; Collins & Calabrese, 2012; Bowles & Jones, 2013). Fire increases plant productivity by reducing litter buildup (Knapp & Seastedt, 1986; Hulbert, 1988; Ojima et al., 1994), increases solar radiation reaching the soil, improves nutrient availability and increases pH (Raison, 1979; Hulbert, 1988; Ojima et al., 1994; Docherty et al., 2012; Alcañiz et al., 2018). Fires in grasslands also stimulate root production, increasing the amount of plant-associated carbon entering the soil (Johnson & Matchett, 2001). By influencing soil nitrogen input, soil pH, increasing root biomass, and increasing the soil temperature, soil bacterial communities are indirectly influenced by

prescribed fires (Johnson & Matchett, 2001; Hart et al., 2005; Kitchen et al., 2009; Wang et al., 2016; Strong et al., 2017). These effects can alter bacterial community functions, such as extracellular enzyme activities. Activities of organic carbon-degrading and phosphorus-mobilizing extracellular enzymes decrease after a burn (Eivazi & Bayan 1996; Ajwa et al., 1999; Gutknecht et al., 2010; Fultz et al., 2016), while responses of nitrogen-mobilizing enzymes can vary (Ajwa et al. 1999; Gutknecht et al., 2010). While high-intensity fires can have direct effects on bacterial mortality in the top few centimeters of soil (Raison 1979, Dunn et al. 1985, Hart et al. 2005, Wang et al. 2012), most prescribed fires in grasslands do not reach high temperatures, so the effects on bacterial communities are through indirect mechanisms (Valette et al., 1994; Dooley & Treseder 2012; Vega et al., 2013; Akburak et al., 2018). Yet, because of long-term legacy effects of agricultural disturbance, both plant and soil bacterial communities in restored systems may not respond to prescribed fire in the same way as communities in remnant prairies.

Current restoration and prescribed fire practices do not appear to restore grasslands that fully resemble remnants in both plant and bacterial community composition, possibly resulting in different community responses to prescribed fire disturbance (Newbold et al., 2019; Ladwig et al., 2020). From a plant perspective, many restorations result in a dominance of C_4 grasses over other native species, reducing plant species richness, floristic quality and habitat for specialist pollinators (Collins, 1987; Gibson & Hulbert, 1987; Baer et al. 2002; Camill et al. 2004; McLachlan & Knispel 2005; Kwaiser & Hendrix, 2008; Collins & Calabrese, 2012). When burned, ash from combusted biomass in a restored prairie may contain a more homogenous chemical composition than a remnant due to differences in plant community structure, potentially facilitating different indirect effects on soil bacterial communities (Bodí, 2014; Quigley et al., 2019) .

While we know that prairie remnants harbor different bacterial communities than those found in restored prairies, especially in younger restorations, we do not know if these communities respond to fire disturbance in similar ways. In particular, differences in resistance and resilience to fire between restored and remnant systems could influence the overall trajectory of long-term management goals. To address this question, we conducted a field experiment in partnership with the Kalamazoo Nature Center (KNC) in Southwest Michigan, USA to determine the effect of a prescribed fire on soil bacterial community structure and function in a restored prairie and an adjacent remnant prairie over time. This region of Michigan is in an important ecotone between tallgrass prairies, oak savannas and deciduous forests, where species diversity is higher than within any one system, so it is particularly critical to manage systems in this region with practices that protect this transitional status. The partnership with the KNC allowed us to explore the effects of a typical prescribed fire conducted by a land management organization, which are inherently variable due to personal safety, coordination of the burn, weather conditions, and topography. Our objectives were to: 1) Determine whether soil bacterial community composition and function responded immediately (within one day) to prescribed fire and exhibited differential responses in the remnant and restored prairies; 2) Identify positive (resistant) fire-responding bacterial taxa in both prairie types immediately after a prescribed fire; 3) If immediate effects of fire were observed, determine whether bacterial community composition and function were resilient to fire disturbance longer term (over one year) and whether resilience differed by prairie type. We hypothesized that the soil bacterial communities in the remnant prairie would exhibit less of an immediate response to fire (resistance), with more positive fire responding taxa. We also hypothesized that the remnant communities would recover more quickly after fire (resilience) than in the restored prairie.

Methods

Site Description

We conducted this study from September 2013 to August 2014, in an agricultural field that had been restored to a tallgrass prairie and an adjacent remnant. Both sites are located in Kalamazoo County, Michigan, USA. Mean annual precipitation (based on 1981-2010; http://www.ncdc.noaa.gov) for Kalamazoo County is 907.8 mm with an average minimum temperature of 4.1^{o}C and an average maximum temperature of 14.67^{o}C. The total precipitation from September 2013-September 2014 was 758.70 mm with an average minimum temperature of 3.28^{o}C and an average maximum temperature of 14.06^{o}C (Kalamazoo/Battle Creek Int. Airport).

According to historical records, ca. 1800, the land where the agricultural field was located was characterized as black oak barrens (MNFI). From the early 1900's onward, this field was cultivated with rotations of corn and soybean crops. In 1993 KNC began restoring this property (~ 6.5 hectares) back to native tallgrass prairie using a Conservation Reserve Program (CRP) approved prairie seed mix that was skewed toward grasses (Bosse et al., 2016). The restored prairie was then managed with a prescribed fire return interval of every two to four years. The adjacent remnant prairie (~ 0.4 hectares) has never been tilled, however before acquisition of the prairie by the KNC it was unmanaged, allowing for the encroachment of woody vegetation (Bosse et al., 2016). After acquisition by the KNC the remnant prairie was also managed with prescribed fire every two to four years since the late 1970s (Bosse et al., 2016). Plant communities in both the restored and remnant sites were predominated by *Andropogon gerardii*

(Big Bluestem), which comprised 56 % of the plant community in the restored prairie and 23 % in the remnant.

The soils in both the restored and remnant prairies are Alfisols. The dominant soil series located in the restored prairie is Kalamazoo loam (KaC) while the dominant soil series in the remnant is Oshtemo sandy loam (OsD). Both soil series are well drained, however KaC is more suitable to crop production than OsD due to shallower slopes and better water retention (NCSS, 1979) which may the reason why the remnant prairie was never used for crop production. As is common for remnants, the remnant prairie is adjacent to an old railway line which existed from 1870-1970, which also may have deterred agricultural use on that parcel of land.

Experimental Design and Soil Sampling

Both the remnant and restored prairies were subjected to a prescribed burn on September 25, 2013, facilitated by KNC. Burn breaks were established so that only a portion of each prairie was burned leaving a control (unburned) portion in each prairie that was separated from the burned portion with a burn break (Figure S1). We created a grid system overlaid on a map of the two prairies, assigned each grid numbers and randomly chose the locations of five 1 x 1 m plots in the areas to be burned in each field. Similarly, we randomly chose the locations of five 1 x 1 m plots in the unburned and burned areas of the remnant. Due to prescribed fire safety and logistical considerations, we were required to choose unburned plot locations evenly spaced along the east edge of the restored prairie. GPS coordinates for all plot locations are shown in (Table S1). Between 1-4 days before the burn was implemented, we collected triplicate 7-cm deep soil cores from each plot and homogenized soils from the three cores into one sample per plot. We cleaned and sterilized corers between plots. We placed the soils from each core into a

quart-sized zip-sealing plastic bag and placed the bag in a cooler on ice until returning to the laboratory and completing soil analyses, as described below. We collected soil samples in the same way from all plots the day after the prescribed burn on September 26, 2013 (1-day post-fire), October 2013 (1 month post-fire), April 2014 (7-months post-fire) and August 2014 (11-months post-fire). At every time point all soil cores from all plots were collected within a 1-2 day period, avoiding locations of previous core collection. This yielded 30 soil cores for each prairie type at each time point, with 15 cores from the control (unburned) plots and 15 cores from the experimental (burned) plots.

Vegetation Sampling

To determine the effect of the prescribed burn on the plant communities we measured aboveground plant biomass, litter mass, and belowground root biomass pre-fire in September 2013, in October 2013 (1-month post-fire) and in August 2014 (11-months post-fire). We measured plant aboveground biomass by clipping the live shoot biomass from each 1 x 1 m plot and then placing the biomass into lawn bags. We also collected senesced litter biomass from each plot. We transported the biomass and litter to WMU's Finch Greenhouse, where we air dried it for two weeks before weighing it. We collected belowground plant biomass from each plot using one 20 cm soil core per plot, which was placed into a paper bag. The bags were transported to WMU and dried before root biomass was manually separated from the soil and weighed. We assessed plant community composition in August 2014 in four transects in the burned and unburned portions of the remnant and restored prairies. Plants were identified to the species level and enumerated using a point-intercept method, as detailed in Bonham (1989).

Soil Abiotic Measurements

On the day of each soil collection, we removed a 50-g subset of soil which was frozen at -80^{o}C until DNA extraction could be completed. We then sieved remaining field soil through a 2 mm sieve and measured soil pH, soil water content (SWC), soil organic matter (SOM) and conducted 0.5 M $NaHCO_3$ extractions for total soil phosphorus (TP) analysis as described in Docherty and Gutknecht (2019). We analyzed filtered $NaHCO_3$ extracts using molybdate-blue colorimetric analysis with a Brinkmann PC 900D probe colorimeter at 900 nm wavelength (Frank et al. 1998). To assess soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations, we conducted 2M KCl extractions using 10 g of fresh soil and 50 mL of 2M KCl. Extraction tubes were shaken on a platform shaker for 1 hour at 5000 rpm. We used vacuum filtration through a GF/F (Whatman) filter to collect the filtrate and stored it in a 20 mL plastic bottle at -20 C prior to analysis. We analyzed filtered KCl extracts for NH_4^+ and NO_3^- concentrations using colorimetric assays measured at 540 nm and 650 nm respectively using an Epoch BioTek 96-well plate reader (Rhine et al., 1998).

Extracellular Enzyme Activities

We assessed the extracellular enzyme activity (EEA) potential of four hydrolytic enzymes using fluorescent-linked substrates to determine how the prescribed burn impacted enzyme activity. Details of these methods have been described previously (Sinsabaugh et al., 2005; Gutknecht et al., 2010; German et al., 2011). We tested for activities of cellobiohydrolase using 4-MUB-cellobioside, β-glucosidase using 4-MUB-β-glucopyranoside, N-acetylglucosaminidase using 4-MUB-N-acetyl-β-glucosaminide, and phosphatase using 4-MUB-phosphate. Briefly, we added 1 g of soil to 100 mL of 50 mM Tris buffer (pH 7) in a 100 mL centrifuge bottle and

added a stir bar to the bottle and shook the mixture for 1 hour at 5000 rpm. We then added the soil slurry to black 96-well plates containing 50 mL of 200 mM 4-MUB-linked enzyme substrates. We included eight replicate wells for blanks (buffer alone), negative controls (only substrate solution or soil slurry with buffer), quench standards (4-MUB + soil slurry), and reference standards (4-MUB with buffer), as described in Gutknecht et al. (2010). After 50 minutes of reaction time in the dark at room temperature, we added 10 mL of 0.5 M NaOH and then read the plates 10 minutes after the NaOH addition. We measured fluorescence using a microplate fluorometer (Cary Eclipse) with 260 nm excitation and 465 emission filters. We calculated EEA potential as a rate expressed as (nmol substrate cleaved) x (g dry soil equivalent)$^{-1}$ h $^{-1}$.

DNA Extraction and Sequencing

We extracted genomic DNA with a PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA, USA) using 0.25 g of soil, according to the manufacturer's instructions. Amplicon preparation and Mi-Seq (Illumina, San Diego, CA, USA) sequencing were conducted at the Michigan State University Genomics Core – Research Technology Support Facility. Samples were sequenced in two Mi-Seq runs: the pre-fire, September, and October time points were run together, and the April and August time points were run together. The V4 hypervariable region of the 16S rRNA gene in Bacteria was amplified using primers 515F/806R (Kozich et al., 2013). DNA libraries were normalized using the Invitrogen SequalPrep Normalization Plate Kit -96 well, and samples from each replicate plate were pooled into single wells (Thermo Fisher Scientific, Waltham, MA, A1051001). Pooled samples were quantified using a Kapa Biosystems qPCR kit and samples were normalized to an equal concentration (Kapa Biosystems, Inc., Wilmington, MA, KK4824). Each sample pool was loaded on an Illumina Mi-Seq flow cell v2 and sequenced using a 500

(PE250) cycle reagent cartridge. Bases were called using Real Time Analysis (RTA) software v1.18.54, and RTA output was demultiplexed and converted to FASTQ files using Illumina Bc12Fastq v1.8.4. Sequence files for triplicate samples per plot at each time point are publicly available in the National Center for Biotechnology Information Sequence Read Archive (NCBI-SRA) under accession numbers PRJNA706578 (pre-fire files) and PRJNA706648 (post-fire time series). All datasets associated with this manuscript are publicly available through Mendeley Data (Docherty & Whitacre, 2021)

Bioinformatics Analysis and Putative Metabolic Pathway Prediction

After the raw sequence data was returned, we processed the sequences using the QIIME2 bioinformatics platform version 2019.7 (Bolyen et al., 2019) by time point and prairie type. We used Divisive Amplicon Denoising Algorithm (DADA2) to merge paired reads, filter by sequence quality, denoise, create an Amplicon Sequence Variant (ASV) table, and remove chimeras (Callahan et al., 2017). For DADA2, forward and reverse reads were truncated at the 15th base pair (bp) from the 5´ end to remove low quality regions of the sequences. From the 3´ end, the sequences were truncated at the 249th and 231st bp for the forward and reverse reads, respectively. Sequences from the three technical replicate cores per plot were merged into one analysis unit prior to further bioinformatics steps. Sequences that occurred less than two times in all samples were removed. ASVs can be considered as presumed error-free sequences for each sample and more accurately reconstruct amplicon-sequenced communities at the highest resolution as compared to OTU clustering methods (Callahan et al., 2016) ASVs were then taxonomically assigned using the Naïve Bayes Classifier trained on the SILVA (version 132) 99% OTU database (Quast et al., 2012). Sequences that could not be classified at the Phylum taxonomic level were discarded. The two separate sequencing runs differed in the number of

total sequences per sample, where the first run (pre-fire, September, and October time points) had fewer sequences per sample than the later sequencing run (April and August time points) (Table S2). We rarefied the final ASV table for each time point (pre-fire, 1-day post-fire, 1-month post-fire, 7-months post-fire, and 11-months post-fire) within each prairie. Because our experimental design includes one remnant and one restored prairie with replicates at the plot level within each prairie, we restricted our statistical analyses to compare experimental and control plots within each time point only, and differentially compare responses in the restored and remnant sites.

We used Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) to predict potential metagenomic pathways (Langille et al., 2013). PICRUSt requires an OTU table, so we conducted the same steps as above except that closed-reference OTUs were selected and taxonomic information was assigned using the Greengenes version 13.5 reference database (DeSantis et al. 2006). We then used the resulting OTU table to predict potential metagenomics pathways (KEGG 1-3) using PICRUSt implemented using Galaxy (Giardine, 2005; Goecks et al., 2010). We normalized data to account for multiple copies of 16S rRNA in bacteria. After normalization we used PICRUSt to predict the metagenomic content of each sample. We focused on predicted pathways that were related to carbon and nitrogen cycling, DNA repair, cell division, and stress responses such as endospore production because we expected that bacterial communities that are resistant to fire would contain more pathways related to stress responses.

Data Analysis and Statistics

We conducted statistical analyses using the R statistical environment (version 3.6.3) (R Core Team, 2020). Our experimental design consisted of five replicates for each burn treatment (control or burned) at each time point (pre-fire, 1-day post-fire, 1-month post-fire, 7-months post-fire, and 11-months post-fire) and for each prairie type (remnant or restored). We used this paired time-series approach so we could address our objectives concerning resistance and resiliency of the soil bacterial communities and functions to prescribed burning. Specifically, we used the pre-fire time point to determine that there we no major differences between experimental and control plots in each prairie pre-fire (see Supplementary Methods, Table S3, Table S4). We used the 1-day post-fire time point to address objectives 1 and 2 regarding resistance to the fire. The later time points (1, 7, and 11-months post-fire) were used to address objective 3 regarding longer-term resiliency after fire.

For univariate measurements, we split our analyses so significant differences were assessed between the burned and control treatments within the remnant and restored prairie at each time point using repeated measures ANOVA with treatment and time as fixed factors. These univariate measures included pH, SOM, soil temperature, SWC, TP, each of the four EEAs, and ASV diversity and richness. We conducted multivariate statistics using the vegan package in R, version 2.5-6 (Oksanen et al., 2019) and we visualized multivariate data using principal coordinates analyses (PCoA) for bacterial data and non-metric multidimensional scaling (NMDS) for plant data. We calculated weighted UniFrac distance matrices based on the 16S rRNA amplicon sequence data using QIIME2 (Lozupone & Knight, 2005). We then used QIIME2 to complete permutational multivariate analysis of variance tests (permanova) to

determine if there were significant differences between the two treatments in the restored and remnant prairies.

To address whether the bacterial communities in both prairies were resistant to the fire disturbance (objective 1), we determined whether there were differences between control and burned plots in bacterial community structure (β diversity), Shannon diversity (α diversity), and ASV richness 1-day post-fire, within each site. We used permanova analysis to determine whether there were significant differences in bacterial community structure. We also tested whether within-treatment variation (or distance from a centroid) differed between control and burned plots using the betadisper function in vegan at each time point (Anderson, 2006). We used the envfit function in vegan to determine which explanatory soil edaphic factors (pH, NO_3, NH_4, soil temperature, SWC) predicted significant variation soil bacterial communities in both prairie types. We calculated Shannon diversity and ASV richness using QIIME2 and used a Kruskal-Wallis test to determine whether there were significant differences between control and burned plots immediately post-fire. To address objective 2, we determined which bacterial families were differentially abundant between burned and control plots in both prairies 1-day post-fire, using Welch's *t*-test with Storey FDR (False Discovery Rate) correction in STAMP (Statistical analysis of taxonomic and functional profiles) (version: STAMP v2.1.3; Parks et al., 2014). We also used STAMP to test differences among putative metagenomics pathways predicted by PICRUSt between the burned and control plots using Welch's *t*-test with Storey FDR correction. Where appropriate we performed Pearson correlation analysis to examine the relationship between bacterial taxa that were significantly different between burn treatments and potential metagenomics pathways. To address whether bacterial communities in both prairie types were resilient to the fire disturbance (objective 3) we used the methods described above to

determine if the effect of fire on the soil bacteria community composition persisted at later time points (1-, 7-, and 11-months post-fire), or whether communities returned to their pre-fire state. We also determined which bacterial families were significantly different between burned treatments 11-months post-fire as described above. In addition, we performed Pearson correlation analysis to examine the relationship between bacterial taxa and soil edaphic factors that were significantly different between burn treatments 11-months post-fire.

Results

The overarching goal of this study was to determine whether there were differences in resistance and resilience to prescribed fire in soil bacterial communities in an adjacent remnant and restored prairies. Overall, our results suggest that bacterial communities in the remnant and restored prairies responded differently to prescribed fire, both immediately and over the longer-term. Below we describe in detail that bacterial community composition in both systems responded immediately to the prescribed fire, but functional responses were only observed in the restored prairie (objective 1). Fire-responsive taxa were present in both prairies, but the identities of these taxa differed (objective 2). Finally, bacterial communities in the two prairies exhibited differences in longer-term responses to recovery from prescribed fire (objective 3). Our results suggest that bacterial communities in the remnant prairie were more resilient to prescribed fire over the one-year time series, while communities in the restored prairie were less resilient. Conversely, the function of the bacterial communities in the restored prairie were resilient, and returned to their original levels, while functional parameters in the remnant did not change, and remained resistant longer-term.

Immediate Effects of Fire on Soil Properties, Community Composition and Function

The soil bacterial community composition in both the restored and remnant prairies was not resistant to the effects of the prescribed fire with changes occurring in community structure in both prairie types within 1-day post-fire (Fig. 1). Though the same phyla or sub-phyla predominated both prairies before the fire (Verrucomicrobia, Acidobacteria, Alphaproteobacteria, Bacteroidetes, Actinobacteria, Betaproteobacteria, Deltaproteobacteria, Planctomycetes, and Gammaproteobacteria), community structure differed between the two prairies (Fig. 2). Immediate responses to burning differed between the restored and remnant systems, both in abiotic factors and in bacterial community responses. While burning mediated significant immediate responses in bacterial community composition in both systems, different abiotic variables explained community variation in each prairie type (Fig 1). The prescribed burn increased soil pH in both prairie types but was related to an increase in soil NH_4^+ concentration only in the remnant (Tables S5 and S6). Below we describe immediate responses to prescribed fire in the restored prairie and remnant prairie in separate sections.

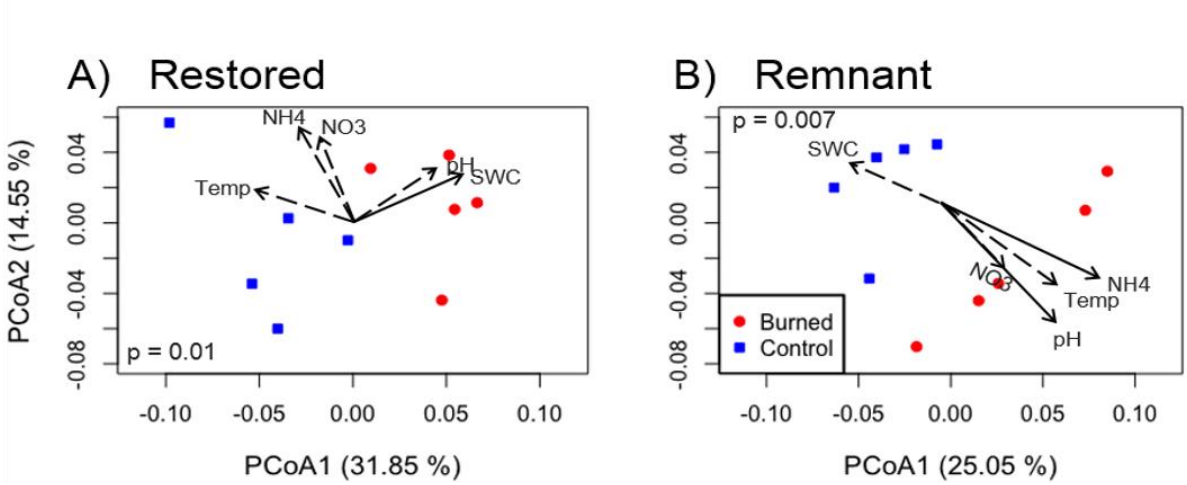

Figure 1. Ordinations of bacterial communities 1-day post-fire. Principal coordinates analysis (PCoA) ordinations based on weighted UniFrac distances between bacterial communities one-day post fire, identified using 16S rRNA amplicon sequencing for control (blue squares) and burned (red circles) plots in **A)** the restored prairie and **B)** the remnant prairie. Significance of permanova results was determined at $\alpha = 0.05$. In both the remnant and restored prairies, the bacterial communities differed significantly between the control and burned plots ($p = 0.01$ and $p = 0.007$, respectively). Edaphic factors are represented by arrows, and the length of each arrow is proportional to the explanatory power of each variable; solid arrows indicate variables with significant explanatory power and dashed arrows indicate variables that were not significantly explanatory. SWC explained a significant amount of variation in the restored prairie bacterial community structure ($R^2 = 0.71$, $p = 0.015$). pH and NH_4^+ concentration explained a significant amount of variation in the remnant prairie bacterial community structure ($R^2 = 0.63$, $p = 0.04$ and $R^2 = 0.75$, $p = 0.017$; respectively).

Immediate Effects of Fire in the Restored Prairie

In the restored prairie, burned plots had a lower ASV diversity than control plots 1-day post-fire. There was no significant difference in ASV richness between plots (Table S5). There were no differences in group dispersions (distance from centroid) in the bacterial communities between treatments (Table S7). Soil water content (SWC) explained a significant amount of variation in bacterial community structure ($R^2 = 0.71$, $p = 0.015$; Fig. 1A), and SWC was significantly higher in the burned plots than in the control plots. Specific taxonomic groups

displayed distinct positive or negative responses to fire. Specifically, taxa within the classes Betaproteobacteria, Gammaproteobactetria, and Deltaproteobacteria were negative fire responders and were significantly less abundant in the burned plots than in the control ($p = 0.0045$, $p = 0.0068$ and $p = 0.013$, respectively; Fig. 2). In contrast, the relative abundance of Verrucomicrobia was 1.36 x more abundant in the burned plots than in the control, though this was not significant ($p = 0.093$). Four bacterial families were significantly different in abundance between the burned and control plots of the restored prairie ($p < 0.05$, Fig. 3). Taxa that were negative fire responders were the families *Flavobacteriaceae* [Phylum: Bacteroidetes], *Micropepsaceae* [Sub-phylum: Alphaproteobacteria], and an unclassified family of the order *Desulfuromonadales* [Sub-phylum: Deltaproteobacteria]. Only one family was a positive fire responder; this was *Xiphinematobacteraceae* [Phylum: Verrucomicrobia].

There were immediate functional responses in the restored prairie 1-day post-fire. β-glucosidase and phosphatase enzyme activities were both lower in the burned plots (Table S5) and there was a strong effect of fire on predicted metabolic pathways (Fig. 4). Predicted pathways indicating simple carbohydrate metabolism such as fructose, mannose, and galactose metabolism were higher in the burned plots relative to the control (Fig. 4). Additionally, putative pathways indicating nitrogen mobilization such as alanine, aspartate, glutamate, and other amino acid metabolism pathways were also higher in the burned plots compared to the control, which could indicate nitrogen limitation after the prescribed fire. Pathways for DNA repair, such nucleotide excision repair and base excision repair, and sporulation were more abundant in the burned plots as well. Conversely, predicted pathways related to cell division, DNA replication, translation and plant-pathogen interaction were lower in the burned plots than in the control plots.

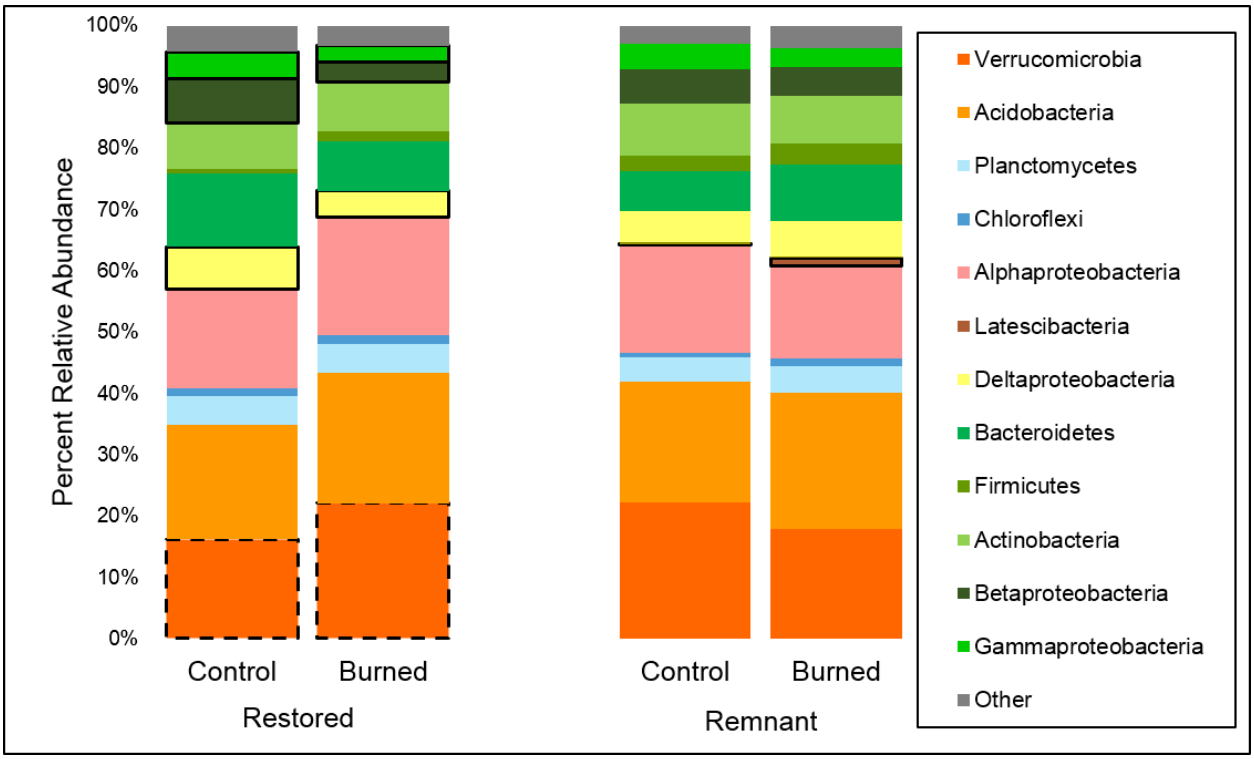

Figure 2. Stacked bar plots of bacterial phyla in the remnant and restored prairies. Percent relative abundance of bacterial phyla that comprise >1 % relative abundance in control and burned treatments in the remnant prairie and restored prairie 1-day post-fire. Presumed oligotrophic phyla are shown in shades of orange while presumed copiotrophic phyla are shown in shades of green. Phyla that differ significantly between treatments are indicated with bold lines and phyla that were marginally different were indicated with a dashed line. Significance was determined at $\alpha = 0.05$. The relative abundance of Latescibacteria was significantly higher in the burned treatment as compared to the control in the remnant prairie ($p = 0.03$). The relative abundances of Betaproteobacteria, Gammproteobacteria, and Deltaproteobacteria were significantly lower in the burned treatment as compared to the control in the restored prairie ($p = 0.0045$, $p = 0.0068$, and $p = 0.013$; respectively). The relative abundance of Verrucomicrobia 1.36 x higher in the restored prairie ($p = 0.093$) after burning.

Of the bacterial taxa that displayed distinct responses to the fire in the restored prairie, the relative abundances of positive fire responders correlated with predicted metabolic pathways for carbohydrate metabolism, while taxa that decreased in abundance were positively correlated with metabolic pathways for cell division and DNA replication. When examined at the phylum level,

Betaproteobacteria and Gammaproteobacteria were positively correlated with putative pathways for cell division ($r^2 = 0.83$ and 0.85, respectively; Table S8) while Verrucomicrobia were positively correlated with putative fructose and mannose metabolism pathways ($r^2 = 0.88$; Table S8). Betaproteobacteria were also positively correlated with the pathway for DNA replication while both Betaproteobacteria and Gammaproteobacteria were negatively correlated with pathways for carbohydrate metabolism and DNA repair (Table S8). At the family level all taxa that were negative fire responders (*Flavobacteriaceae*, *Micropepsaceae* and an unclassified family in the order *Desulfuromonadales*) were positively correlated with the pathway for cell division (Table S9). *Xiphinematobacteraceae* [Phylum: Verrucomicrobia], which was a positive fire responder, was negatively correlated with pathways involved in cell division and DNA replication and positively correlated for pathways for carbohydrate metabolism (Table S9).

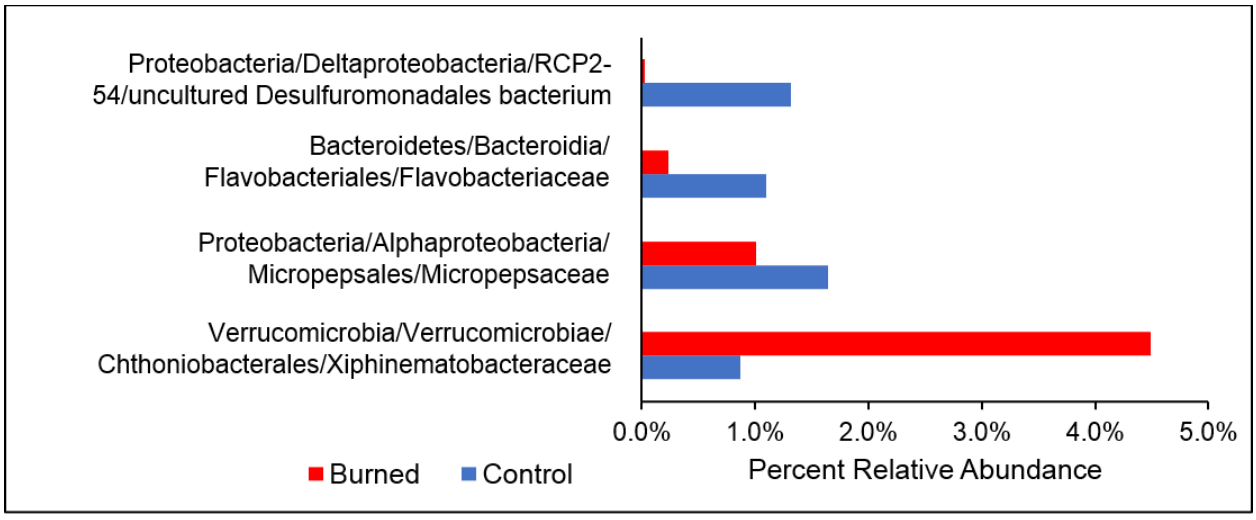

Figure 3. Abundance of bacterial families that were differentially abundant in the burned and control plots of the restored prairie. Significance was determined at $\alpha = 0.05$. P-values were corrected using Storey false discovery rate ($p < 0.05$).

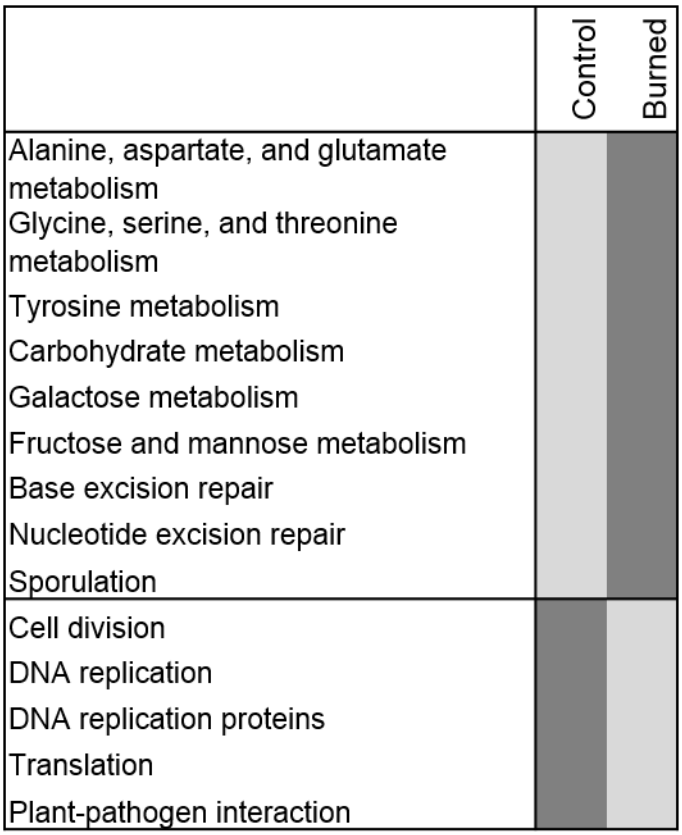

Figure 4. Heatmap of functional pathways in the restored prairie. Heatmap indicating significant differences in the proportion of functional gene categories at KEGG level 3 for control vs burned plots 1-day post-fire in the restored prairie. Significance was determined at $\alpha = 0.05$, dark shading indicates that the proportion of the functional gene category is higher. P-values were corrected using Storey false discovery rate ($p < 0.05$).

Immediate Responses to Fire in the Remnant Prairie

Immediate responses to fire in the remnant prairie differed from those we observed in the restored prairie in several key ways. In the remnant, ASV diversity and richness were higher in the burned plots than the control plots (Table S6). While there was an immediate response to burning in the remnant soil bacterial community (Fig. 1B), the edaphic factors that explained the variation in community structure differed from that in the restored prairie. In the remnant, pH and NH_4^+ concentration explained a significant amount of variation in the bacterial community structure in the burned and control plots ($R^2 = 0.63$, $p = 0.04$ and $R^2 = 0.75$, $p = 0.017$ respectively; Fig. 1B). Although there was a significant shift in bacterial community structure in the remnant immediately after the burn, relative abundances of all major phyla remained stable,

and only one low-abundance phylum (Latescibacteria*)* was positively responsive to fire ($p = 0.03$, Fig. 2). There were also no differences in group dispersions in the bacterial communities between treatments (Table S6). No bacterial families differed in abundance between the burned and control plots in the remnant. In addition, none of the functional responses we observed in the restored prairie were evident in the remnant. Extracellular enzyme activities and metabolic pathways did not differ between the control and burned plots 1-day post-fire (Table S6, Fig. S3).

Overall, our results show that bacterial community composition and function in the restored and remnant prairies we examined exhibited different immediate responses to prescribed fire. Bacterial communities in both prairies changed immediately post-fire, indicating that the communities were not resistant to fire. But, in the restored prairie the response was within major phyla within the community which was coupled with significant functional responses. In contrast, in the remnant, there were no changes in the relative abundances of the dominant soil phyla and there were no functional responses among the parameters we measured.

Longer-term Effects of Fire on Soil Properties and Community Composition

Differences in recovery from fire between the remnant and restored prairies were also evident over the longer-term. Overall, our results indicate that soil bacterial communities and edaphic properties in the restored prairie were not resilient to the effects of prescribed fire after one year post-fire (Fig. 5). In contrast the remnant soil bacterial communities were only moderately affected by the prescribed fire immediately, and were resilient to fire and the ensuing changes in soil edaphic factors throughout the year following the fire (Fig. 5). Below we describe year-long responses to prescribed fire in the restored prairie and remnant prairie in separate sections.

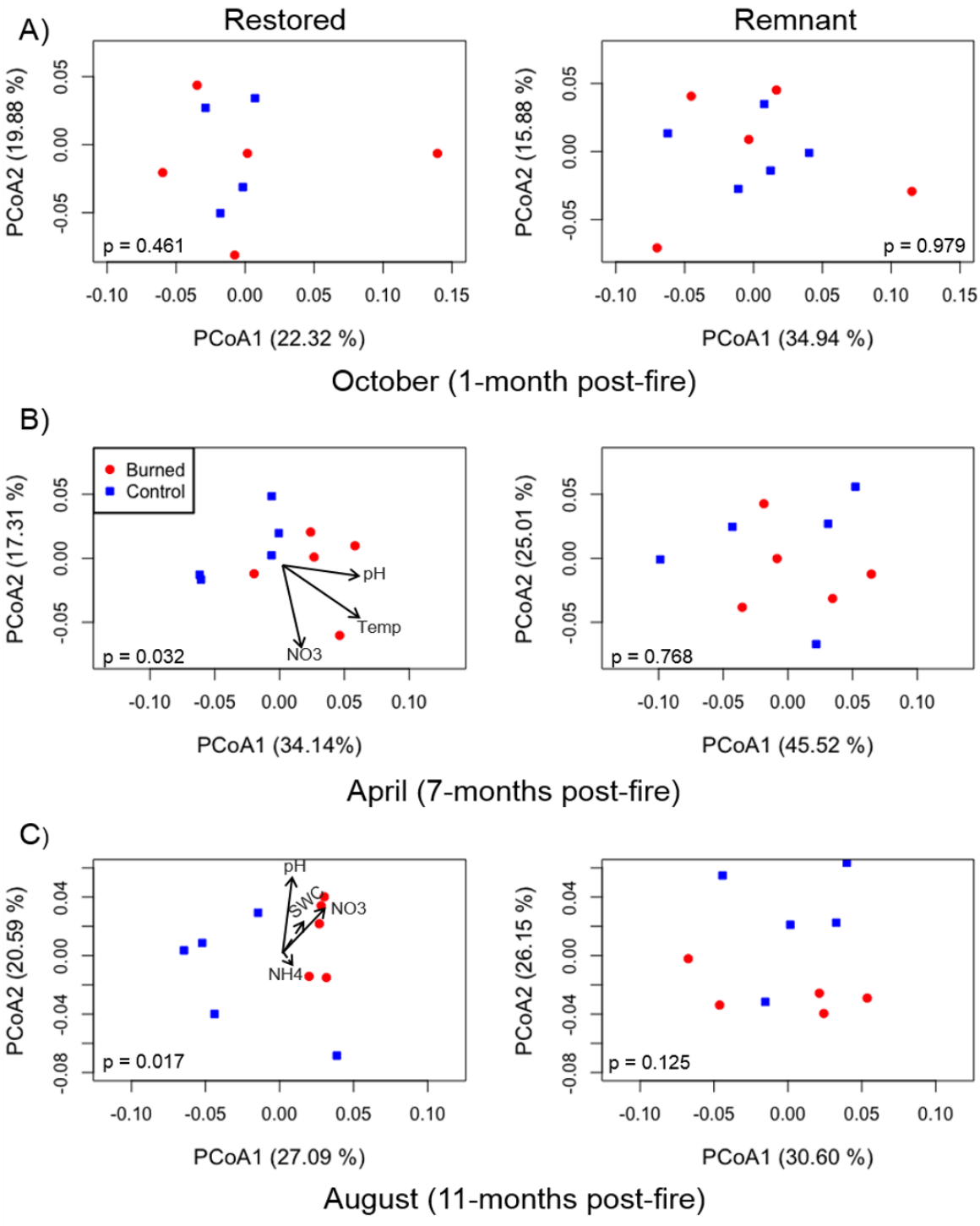
A)
Restored
Remnant
PCoA2 (19.88 %)
PCoA2 (15.88 %)
p = 0.461
p = 0.979
PCoA1 (22.32 %)
PCoA1 (34.94 %)
October (1-month post-fire)
B)
Burned
Control
PCoA2 (17.31 %)
PCoA2 (25.01 %)
pH
Temp
NO3
p = 0.032
p = 0.768
PCoA1 (34.14%)
PCoA1 (45.52 %)
April (7-months post-fire)
C)
PCoA2 (20.59 %)
PCoA2 (26.15 %)
pH
SWC
NO3
NH4
p = 0.017
p = 0.125
PCoA1 (27.09 %)
PCoA1 (30.60 %)
August (11-months post-fire)

Figure 5. Ordinations of the bacterial communities in the restored and remnant prairies longer term. Principal coordinates analysis ordinations based on weighted UniFrac distances between bacterial communities identified using 16S rRNA amplicon sequencing for control (blue squares) and burned (red circles) plots in the restored prairie (left) and the remnant prairie (right) at **A)** 1-month post-fire **B)** 7-months post-fire and **C)** 11-month post-fire. Significance was determined at $\alpha = 0.05$. In the restored prairie the bacterial communities did not significantly differ between treatments 1-month post-fire ($p = 0.461$). At 7- and 11-months post-fire the bacterial communities did differ significantly between treatments in the restored prairie ($p = 0.032$ and $p = 0.017$, respectively). Edaphic factors are represented by arrows, and the length of each arrow is proportional to the explanatory power of each variable. Solid arrows indicate variables with significant explanatory power and dashed arrows indicate variables that were not significantly explanatory. pH, soil temperature and NO_3^- concentration explained a significant amount of variation in the restored prairie bacterial community structure 7-months post-fire plots ($R^2 = 0.69$, $p = 0.014$; $R^2 = 0.95$, $p = 0.001$; $R^2 = 0.82$, $p = 0.003$, respectively). pH and NO_3^- concentration explained a significant amount of variation in the restored prairie bacterial community structure 11-months post-fire plots ($R^2 = 0.85$, $p = 0.001$ and $R^2 = 0.64$, $p = 0.022$, respectively). In the remnant prairie the bacterial communities did not significantly differ between treatments at any of the timepoints ($p = 0.979$, $p = 0.768$, and $p = 0.125$; respectively), and edaphic factors did not explain any significant variation.

Longer-term Effects of Fire on Soil Properties and Community Composition in the Restored Prairie

The soil bacterial communities in the restored prairie were not resilient to the prescribed fire and the corresponding changes in soil edaphic factors. While there were no differences in ASV richness and diversity in the restored prairie 1-, 7-, or 11-months post-fire (Table S5), there were longer-term effects of fire on bacterial community composition in the restored prairie 7- and 11-months post-fire (Fig. 5). Seven-months post-fire pH, soil temperature, and NO_3^- concentration explained a significant amount of variation in the bacterial community in the burned and control plots ($R^2 = 0.69$, $p = 0.014$; $R^2 = 0.95$, $p = 0.001$; $R^2 = 0.82$, $p = 0.003$, respectively: Fig. 5B). Eleven-months post-fire, pH and NO_3^- concentration explained a significant amount of variation in the bacterial community in the burned and control plots ($R^2 =$

0.85, p = 0.001 and R^2 = 0.64, p = 0.022, respectively; Fig. 5C). The burned plots had significantly higher β-glucosidase and phosphatase activities 7- and 11-months post-fire, respectively (Table S5). The burned plots also exhibited significantly less aboveground plant litter (AGL) for the remainder of the experiment (Table S6), while the aboveground plant biomass (AGB) recovered to levels found in the control plots 11-months post-fire (Table S5). The prescribed fire had no effect on plant community composition in the restored prairie (p = 0.34, Fig. S4, Table S10).

To examine the longer term effects of fire on fire-responsive bacterial taxa, we compared control and burned plots at the 11-month time point. Relative abundances of several taxonomic groups differed in between the burned and control plots in the restored prairie. Specifically, the relative abundance of Deltaproteobacteria was higher in the burned plots than in the control (p = 0.046) while Planctomycetes were less abundant (p < 0.001). Betaproteobacteria were also marginally more abundant (p = 0.086) in the burned plots while Verrucomicrobia were marginally less abundant (p = 0.088, Fig. S5). The relative abundance of Deltaproteobacteria was positively correlated with soil pH (r^2 = 0.66, Table S11) while the abundance of Betaproteobacteria was positively correlated with NO_3^- (r^2 = 0.70, Table S11). Planctomycetes relative abundance was positively correlated with AGL (r^2 = 0.80, Table S11) and negatively correlated with NO_3^- (r^2 = -0.77, Table S10). Four bacterial families were significantly different in abundance between the burned and control plots of the restored prairie (p < 0.05, Fig. 6). *Phaselicystidaceae* [Sub-phylum: Deltaproteobacteria] and an unclassified family in Gp5 of Acidobacteria were positively responsive in the burned plots. The families *Opitutaceae* [Phylum: Verrucomicrobia] and an unclassified family in *Planctomycetales* [Phylum: Planctomycetes] were less abundant in the burned plots. *Phaselicystidaceae* and Gp5 [Phylum: Acidobacteria]

were positively correlated with NO_3 concentration, pH, and SOM while *Opitutaceae* was positively correlated with AGL (Table S12). Both *Opitutaceae* and *Planctomycetales* were

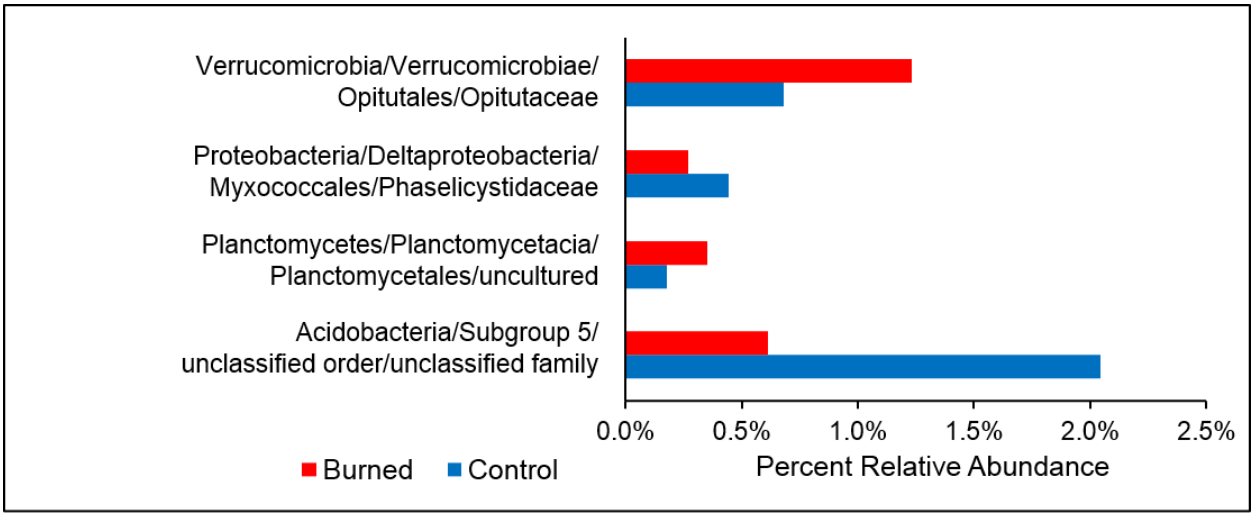

Figure 6. Abundance of bacterial families that were differentially abundant in the burned and control plots of the restored prairie 11-months post-fire. Significance was determined at $\alpha = 0.05$. P-values were corrected using Storey false discovery rate ($p < 0.05$).

negatively correlated with NO_3^- concentrations (Table S12).

Longer-term Effects of Fire on Soil Properties and Community Composition in the Remnant Prairie

In contrast to the bacterial communities in the restored prairie, the bacterial communities in the remnant prairie were resilient to fire with no lasting effects of the burn event on bacterial community composition (Fig. 5). There were no significant differences in ASV richness and diversity between treatments for the 1-month, 7-months and 11-months post-fire time points (Table S7). However, NAG activity was significantly higher in the burned plots of the remnant 1-month post-fire relative to the control (Table S6). The prescribed fire did not reduce the amount of AGL in the remnant prairie (Table S6) or shift plant community structure and

composition ($p = 0.434$, Fig. S4, Table S10). The prescribed fire led to a decrease in AGB after one month, but AGB recovered to levels in the control plots 11-months post-fire (Table S6).

Overall, our results indicate that bacterial communities in the restored and remnant prairies exhibited different longer-term responses to prescribed fire. Bacterial communities in the restored prairie were not resilient to the effects of prescribed fire, exhibiting both immediate and long-term changes after the fire. In contrast the bacterial communities in the remnant were mostly resistant to the immediate effects of prescribed fire, and recovered before 1-month post-fire.

Discussion

Influences of Spatial Heterogeneity on Soil Bacterial Community Responses in the Restored and Remnant Prairie

Our results indicate that bacterial communities in both the restored and remnant prairie immediately responded to prescribed fire and did not exhibit ecological resistance in community composition. However, the magnitude and types of responses exhibited by communities in each system were fundamentally different. In both prairies, bacterial communities experienced significant in structure 1-day post-fire. These changes in community structure could be due to direct effects of the fire event through soil heating and subsequent bacterial mortality, however this may be a less prominent mechanism due to the low burn intensity of prescribed fires (Valette et al., 1994; Kranz and Whitman, 2019). The change in bacterial community structure in such a short period of time may also be due to bacterial responses to changes in soil edaphic factors mediated by the fire. In both sites, we observed increases in soil pH in the burned plots 1-day post-fire, which is a well-known global driver of bacterial community structure in soils (Lauber

et al., 2009; Fultz et al., 2016). This acute response has also been observed in other studies that investigated responses to fire (Fultz et al., 2016; Pérez-Valera et al., 2019), even in the absence of changes in soil edaphic factors (Fultz et al. 2016). This finding lends support to the idea that fire-induced bacterial mortality could have caused the shift in bacterial community structure in both prairie types 1-day post-fire, despite the low intensity burn of the prescribed fire.

While both prairies exhibited immediate shifts in community composition, the results of these shifts were fundamentally different in each site. In the remnant prairie, ASV diversity was higher in burned plots 1-day post-fire, while ASV diversity was higher in the control plots in the restored prairie. The contrasting responses of alpha diversity to the prescribed may be due to differences in the spatial heterogeneity of the soil environment in each prairie. The remnant prairie was never subjected to agricultural practices such as tilling, monocropping, and a uniform application of nutrients, all of which tend to decrease spatial heterogeneity in the soil environment (Röver & Kaiser, 1999). Conversely, the restored prairie had been subjected to these practices for decades. Thus, spatial heterogeneity of the soil environment in the remnant is presumably higher than in the restored prairie due to the legacy of agriculture. The heterogeneity of soil edaphic factors and AGL in the remnant may also have increased in the burned plots. This is based first on our observation of larger confidence intervals for univariate measurements in these plots. The other line of evidence for this is that the higher plant diversity in those plots, likely resulted in more heterogeneous biochar addition across the burned remnant plots (Quigley et al. 2019). Differences in soil spatial heterogeneity in the two prairies are important because higher spatial heterogeneity in the soil environment can be positively correlated with higher bacterial alpha diversity (Curd et al., 2018). Therefore, the dissimilar impacts of fire on the

heterogeneity of the soil environment may have influenced the different responses of bacterial alpha diversity to fire.

How spatial heterogeneity influences microbial communities — and ultimately carbon, nitrogen and other types of nutrient cycling — are factors that land managers may not consider when developing management plans for properties with different land-use histories. Properties with low spatial heterogeneity, such as restored prairies on former agricultural land may produce a more homogenous burn when prescribed fire is applied. However, from a soil health perspective, these systems may benefit from intentionally patchier burns to increase spatial heterogeneity of the soil environment and maximize microbial diversity and function.

Differential Immediate Functional Responses to Prescribed Fire in the Restored and Remnant Prairies

While bacterial community structure in the remnant prairie immediately changed in response to the prescribed fire, the functional attributes we measured were resistant to the disturbance. In contrast, functional parameters in the restored prairie responded significantly to the fire. This is mostly likely because the prescribed burn significantly reduced AGL in the restored prairie but not in the remnant, resulting in differential indirect effects of fire in the two systems. Reducing AGL in the restored prairie would allow for more UV radiation to reach the soil surface which can result in higher soil temperatures, increasing rates of processes such as nitrogen mineralization and litter decomposition (Raison, 1979; Hulbert, 1988; Ojima et al., 1994; Wang et al., 2017). This likely explains why we observed higher predicted bacterial pathways for amino acid and carbohydrate metabolisms in the burned plots of the restored prairie. Also, many prairie plants increase carbon allocation to roots post-fire (Johnson &

Matchett, 2001; Kitchen et al., 2009) which may also have led to an increase in root exudate production that could stimulate the activity of bacteria in the rhizosphere. We also observed an increase in the abundance of the predicted pathway for nucleotide excision repair in the burned restored plots, which may also have resulted from increased UV radiation at the soil surface, perhaps triggering higher rates of DNA damage in soil bacteria (Goosen & Moolenaar, 2008; Kisker et al., 2013).

In addition to differential functional responses, we only identified positive and negative fire-responsive taxa in the restored prairie, and not in the remnant. For example, Betaproteobacteria responded negatively to fire disturbance, while Verrucomicrobia responded positively. In other systems, Proteobacteria have been shown to exhibit a delayed response to abrupt environmental changes, such as changes to water availability, or due to a fire (Placella et al., 2012; Jurburg et al., 2017) which could explain why we observed a decrease in their relative abundance post-fire in the burned plots. The delayed response to disturbance by Proteobacteria — specifically Beta- and Gammaproteobacteria — is likely due to a lower number of active ribosomes per cell, as compared to organisms that are more resilient to disturbance, such as Verrucomicrobia (Placella et al., 2012). Within the phylum Verrucomicrobia, members of the family *Xiphinematobacteraceae* exhibited a fourfold increase in relative abundance in the burned plots immediately after the fire, suggesting that this group may either be particularly resilient to fire disturbance and/or that they are capable of rapid responses to new environmental conditions. Little is known about this family, but *Xiphinematobacteraceae* relative abundances were negatively correlated with pathways related to cell division and DNA replication, and positively correlated with pathways for complex carbohydrate metabolism, suggesting traits suited for an oligotrophic life history. This supports findings by Fierer et al. (2013), where Verrucomicrobia

were more abundant in remnant prairie sites and were correlated with traits associated with oligotrophy. In contrast, we identified several negative fire-responders in the restored site, including the family *Flavobacteriaceae*. The decrease in this family is in contrast to other studies that have found this family to be a positive fire responder (Whitman et al., 2019; Adkins et al., 2020). While these studies were conducted in forest ecosystems and examined the longer-term effects of fire, our study provides additional insight into the immediate response of this family to fire in prairies. All bacterial families that were negative fire responders were also positively correlated with putative pathways for cell division. This may suggest that these less fire-tolerant bacterial families, such as *Flavobacteriaceae*, may be adapted for replicating quickly after a disturbance event (Fierer, 2017) and may provide some context into why Whitman et al. (2019) and Adkins et al. (2020) and observed *Flavobacteriaceae* to be a positive fire responder in the longer-term. Importantly, decreases in bacterial diversity due to prescribed fire may also impact soil carbon and nitrogen cycles by decreasing functional diversity (Wagg et al., 2019). For example, a metanalysis of studies that manipulated soil microbial diversity found that bacterial diversity is positively correlated with litter decomposition and negatively correlated with soil respiration (de Graaff et al., 2015). Shifts in how carbon is cycled in prairies due to fire may then impact the rate at which plant litter is degraded and incorporated.

Longer-term Resilience in the Remnant Prairie, but not in the Restored Prairie

Bacterial community structure recovered in the burned plots of the remnant prairie within 1-month post-fire, converging on the bacterial communities in the unburned control plots. This indicates that the remnant bacterial communities were resilient to both the direct and indirect effects of prescribed fire within a relatively rapid time period. In contrast, bacterial communities in the restored prairie were not resilient to the effects of fire and did not converge on the

community composition present in the control plots within the year-long time period of our study. Soil pH and NO_3^- increased in the burned plots in both prairie systems in our study, which is consistent with the finding that these variables increase after prescribed fires in grassland systems, due to the deposition of alkaline, nitrogen-rich ash from partially combusted plant materials (Picone et al., 2003; Docherty et al., 2012; Alcañiz et al., 2018). However, it was only in the restored prairie that these two variables explained a significant amount of variation in bacterial community composition 11-months post-fire (Fig. 5). At higher levels of taxonomic classification, we observed an increase in the relative abundance of Deltaproteobacteria and a decrease in Planctomycetes 11-months post-fire in the burned plots of the restored prairie. The increase in Deltaproteobacteria may have been related to increases in SOM in the burned plots, coupled with increases in NO_3^- as suggested by (Pérez-Valera et al., 2019). Conversely, we found that Planctomycetes were positively correlated with AGL and negatively correlated with NO_3^- concentrations. Nitrogen addition has related to decreased Planctomycetes abundances (Ramirez et al., 2012), potentially explaining why they were negatively correlated with NO_3^-. The correlation of Planctomycetes with AGL may also be explained by observations of increased N mineralization rates in prairie soils where plant litter has been removed (Raison, 1979; Hulbert, 1988; Ojima et al., 1994).

We also observed longer-term differences in the relative abundance of certain bacterial families in the restored prairie, but not in the remnant. Bacterial families that increased in abundance were positively correlated with nutrient and SOM levels while those that decreased were negatively correlated. For example, *Phaselicystidaceae* and Acidobacteria: Gp5 have been shown to increase in abundance with higher nutrient levels (Naether et al., 2012; Wang et al., 2020) which supports our finding that they were positively correlated with NO_3^-. Furthermore,

the order *Myxococcales*, which contains the family *Phaselicystidaceae*, are adapted to soils that have neutral pH levels (Wang et al., 2020; W. Wang et al., 2020) which may explain why this group was more abundant in the burned plots. *Myxococcales* are predatory bacterial group that feed on other bacteria through the secretion of bacteriolytic enzymes (Wang et al., 2020) and have the potential to shift soil bacterial community structure through predation (Morgan et al., 2010; Velicer et al., 2013). As a result, indirect effects of fire on community composition have the potential to create conditions that favor bacterial predation, which in turn directly affects bacterial community composition. However, further work is required in this area to examine the effects of prescribed fire on soil trophic interactions in different systems.

While we also observed similar increases in pH and NO_3^- in the remnant prairie as well, these changes did not have the long-term impacts on bacterial community structure. There has been some evidence to suggest that bacterial communities in remnant ecosystems, such as in eucalyptus forests which are adapted to fire disturbance, recover more quickly from fire disturbance than bacterial communities found in a highly disturbed community (Prendergast-Miller et al., 2017). Grassland ecosystems are also uniquely adapted to fire, with North American prairies historically burning every few years prior to human-induced fire suppression (Hulbert, 1988; Anderson, 2006). Our results demonstrate that bacterial communities in this paired restored and remnant prairie system do not respond in the same ways to fire, which is likely due to the history of agricultural disturbance in the restored prairie. This history of disturbance in the restoration has likely resulted in a soil bacterial community that is less fire-adapted than in the remnant.

Conducting prescribed burns at the scale used in this study is not without complexity. An alternative explanation of these results is that the restored and remnant prairies may not have

experienced the same level of fire intensity, as evidenced by the minimal litter reduction we observed in the remnant prairie post-fire compared to the restored. However, this difference is also a function of the plant communities present in each prairie. The restored prairie had a much higher abundance of *Andropogon gerardii* (Big Bluestem grass) than the remnant, which provides higher fuel loads (i.e., litter) than abundant forbs that were present in the remnant. Differences in plant community composition between co-located restored and remnant prairies is a common observation, with many restorations dominated by grasses, and less diverse than remnant neighbors (Ladwig et al., 2018; Newbold et al., 2019). Locating adjacent remnant and restored systems that experience the same weather systems, as well as coordinating a prescribed burn on the same day for both prairies, and implementing burn breaks for control plots, makes for a difficult scientific endeavor. Our study is unique in that it is the only one to our knowledge that examines the short term and longer-term effects of prescribed fire on bacterial community structure and function in a paired remnant and restored prairie. Due to the complexity of the experimental design, our study only represents one restored and one remnant located in southwest Michigan, so further research that includes additional prairies in different geographic location is required to verify these results in other systems. In particular, responses may vary depending on soil types, ecotypes, and for prairies of different restoration ages.

The burn we implemented, and the differences in plant communities and spatial heterogeneity in the remnant and restored prairies that we observed, are all typical of the complexities that land managers face when determining management practices. Our results demonstrate that restored and remnant bacterial communities in this system exhibited fundamentally different short-term and long-term responses to prescribed fire, driven at least in part by a plant-mediated mechanism resulting from past land use history. Land managers must

take many considerations into account when creating prescribed fire management plans, including ecosystem type, threatened and game species, and financial constraints (Cohen et al. 2021). Our results suggest that land managers seeking to improve soil health and soil microbial ecosystem resilience may wish to consider different management practices in restorations and remnants. This is particularly true for grasslands in the Great Plains region of North America, where grasslands are still being converted to farmland at a rate of 1-5% (Wright & Wimberly, 2013). Management decisions that improve soil health and increase ecosystem resilience in grasslands are more important than ever, due to the urgency of protecting these ecosystems from the effects of climate change.

Conclusions

We examined the responses of soil bacterial community composition and function to prescribed fire in a paired restored and remnant prairie system located in southwest Michigan, USA. Communities in the two prairies exhibited different responses to this common management practice. Bacterial communities in the remnant site shifted one day after the fire, but then returned to the original composition in the control within one month. Additionally, no microbial functional parameters shifted in response to the fire, suggesting community resilience and functional resistance in the remnant. In contrast, bacterial communities in restored soils also shifted one day after the fire, particularly affecting the relative abundances of several major bacterial phyla, which allowed us to identify several fire-responsive taxa. This was coupled with shifts in functional parameters, including increased putative pathways for carbohydrate metabolism and decreased pathways for cell division, DNA replication and translation, indicative of shifts toward organisms with oligotrophic life history characteristics. In the longer-term, shifts in the restored prairie continued to be observed 11-months after the prescribed fire, when the

study was concluded. We conclude the reasons for the differences in these responses is because the past land-use history of the restored prairie selects for different soil bacterial communities and also a predominance of grass species in the plant community. This grass litter increases fuel loads, enabling more biomass mineralization following a prescribed fire and allowing for more UV exposure and increased soil temperatures. In contrast, more diverse plant communities containing a greater abundance of forbs in the remnant yielded lower fuel loads, reducing the short- and long-term impacts of prescribed fire on soil bacterial communities and functions. Taken together, our results suggest that land managers wishing to restore for soil functional resilience in former agricultural systems will need to consider different land management strategies for restored and remnant systems. This could include different burn frequencies for different systems, or manually manipulating fuel loads in restorations.

CHAPTER II

EXPLORING THE EFFECTS OF CARBON ADDITON AND PLANT PRESENCE ON BELOWGROUND RESTORATION IN TWO RECLAIMED PRAIRIES ON DIFFERENT SOIL TYPES

The tallgrass prairie has been largely converted into farmland to support the growth of an ever-growing human population. The destruction of this ecosystem has meant not only the loss of habitat for hundreds of species but also the loss of a major soil carbon sink in North America. Although there have been efforts to restore tallgrass prairies, the focus has been almost exclusively on above-ground restoration. Restoration of the belowground ecosystem services such as carbon sequestration have been largely ignored. To address this, we added carbon addition treatments in the form of pure cellulose and prairie plant biomass as soil amendments with the goal of setting new prairie restorations on a trajectory towards increased carbon storage. We also wanted to determine how plants modulated the effect of the carbon addition treatments. In addition, to determine if these carbon treatments worked on multiple soil types, we set up two restorations on sites with different soil textures. We found that through biomass addition there were increases in metrics related to carbon storage in both prairies when plants were present. Conversely, the response of the soil microbial communities differed in these two restorations in response to carbon addition and the presence of plants suggesting that differences in soil type can set restorations of different trajectories.

Introduction

Tallgrass prairie restoration and maintenance is one of the major goals for many restoration practitioners in the Midwestern United States (Rowe, 2010). This is due to the almost complete disappearance of prairies from the landscape (Samson & Knopf, 1994) as prairies were converted to farmland following European expansion in the mid-1800's (Bragg & Hulbert, 1976; Telles et

al., 2013). As a result of this conversion and cultivation, an estimated 60 % of the soil carbon stocks from this ecosystem were released into the atmosphere (Conant et al., 2017). The ensuing cultivation of these prairie soils altered the soil carbon stores by reducing the amount of carbon protected in soil aggregates, removing deep-rooted native plants, and disrupting soil microbial communities (Dai et al., 2018; Fierer et al., 2013; House & Bever, 2018; West & Six, 2007).

While land managers strive to restore degraded land back to tallgrass prairie, they face an uphill battle because tallgrass prairies are still being converted to farmland with as much as 5% of the remaining prairie being converted to row crop every year (Wright & Wimberly, 2013). Given these daunting statistics, it is imperative to restore these remaining prairies as completely as possible, particularly with respect to soil health and carbon storage. Many prairie restoration efforts have almost exclusively focused on reestablishing visible metrics of restoration, such as the native plant community. While reestablishing these native prairie plant communities is crucial to prairie restoration, as they incur many benefits such as increasing ecological diversity, providing habitat for native pollinators and birds, reducing soil erosion, and many other benefits (e.g. Schulte et al., 2017.; Telles et al., 2013; Werling et al., 2014) belowground soil restoration of prairies should be considered as well. While soil carbon storage in reclaimed prairies is greater than in agricultural land, it falls well short of the carbon storage potential of remnant prairies (Camill et al., 2004). For example, differences in soil carbon storage were observed in the oldest known prairie restoration as compared to an adjacent remnant prairie in Wisconsin, USA where soils in the restored prairie stored 37 % less soil carbon and emitted more CO_2 (Kucharik et al., 2006). Furthermore, it has been shown that the rate of carbon accumulation in reclaimed prairies decreases over time (Bruce et al., 1999; Jastrow, 1996; Kucharik, 2007; Post et al., 2004), eventually leveling out so that no further accumulation occurs after 33 years (West & Six, 2007).

These differences in carbon storage between remnant and restored prairies may be due in part to differences in their microbial communities. Many studies suggest that there is a long-term legacy effect of agriculture on microbial community structure and function in restored prairies (Barber et al., 2017; Fierer et al., 2013; House & Bever, 2018; Jangid et al., 2010; Mackelprang et al., 2018), and that it takes approximately three decades of restoration for microbial communities in restored prairies to approach convergence with those in remnants (e.g. Barber et al., 2017; Duncan et al., 2016; Herzberger et al., 2015), if convergence ever occurs (Jangid et al., 2010). Specifically, prairie remnant bacterial communities tend to have higher abundances of Verrucomicrobia than restored prairies (Barber et al., 2017; Docherty & Gutknecht, 2019). Verrucomicrobia are thought to be a slower growing bacterial phylum and are positively correlated with functional pathways related to carbohydrate metabolism and negatively correlated with pathways for nitrogen metabolism (Fierer et al., 2013). Rhizobia are important symbionts with native legumes which depend on specific rhizobia in the soil to thrive (Poole et al., 2018). Soil fungi, which are significant contributors to soil carbon storage, due to their more efficient use of recalcitrant substrates are also lower in abundance in restored prairies (Treseder, 2016). In particular, mycorrhizal fungi are crucial symbionts with native plant species, so their low abundance can reduce plant restoration success, particularly for late-successional species (Bauer et al., 2020; House & Bever, 2018, 2018; Koziol & Bever, 2017; Middleton & Bever, 2012).

With the knowledge that soil microbial communities and carbon storage differ between remnant and restored prairies, multiple studies have sought to shift the microbial communities and increase carbon stores in restored prairies to benefit restoration success. Many of these studies involve the introduction of mycorrhizal fungi to increase the establishment of late

successional prairie plant species and increase plant species richness with great success (e.g. House and Bever, 2018; Koziol and Bever, 2017). Other studies have inoculated new restorations with soil or microorganisms derived from remnant prairies with varying degrees of success (e.g. Docherty & Gutknecht, 2019; Grman et al., 2020; Middleton & Bever, 2012). For example, Middleton and Bever (2012) found that the addition of remnant soil to new prairie restorations increased establishment of later successional plants. In contrast Docherty and Gutknecht (2019) found that extracts of remnant prairie soil did not lead to shifts in the microbial community or other metrics. However, even if successful, removing soil from the few remaining remnants to better restore reclaimed prairies is not feasible on a large scale. What may be a more promising approach is the addition of recalcitrant carbon substrates to the soil to facilitate community shifts by selecting for slow-growing microorganisms that are present in low abundances. For example, Blumenthal et al. (2003) found that soil carbon addition in the form of sawdust can increase biomass of desired prairie plant species while decreasing the biomass of 'weedy' undesirables in a field setting. In a greenhouse study conducted by our research group, cellulose addition to agricultural soil can increase total microbial biomass, fungal-to-bacterial lipid ratios, and shift bacterial communities towards those with higher abundances of slower-growing taxa (i.e. Verrucomicrobia) (Docherty & Gutknecht, 2019). However, carbon addition as pure cellulose microcrystalline is too expensive to implement on a large scale, so it is not a practical solution. These studies provide promising evidence that shifting soil microbial communities by substrate addition can be accomplished, but further research is necessary to determine whether this approach works on all soil types and to develop realistic management recommendations using this approach.

The development of microbial restoration methods that work in various soil types has not been a focus in previous studies (e.g. Docherty & Gutknecht, 2019; House & Bever, 2019; Koziol & Bever, 2017; Middleton & Bever, 2012). Differences in soil type can significantly impact the restoration of plant communities, soil microbial communities and overall restoration success (Davidson et al., 2019; Gornish & Santos, 2016), which already varies due to other factors, such as precipitation during the year of restoration, restoration method and plant seed mix applied. (Brudvig et al., 2017). For example, Jangid et al. (2010) found that in two prairie restorations that were established around the same time, were managed in similar ways, but were established on different soil types harbored significantly different microbial communities. Another study found similar results, in which microbial communities in paired prairie restorations were set on different trajectories due to soil type (Bach et al., 2010). Different soil types can have such a large impact because of their different physical and chemical properties (Bach et al., 2010; Singh et al., 2007). Soil clay content can have a large impact on the availability of organic carbon and microbial biomass (Franzluebbers et al., 1996; McLauchlan, 2006) and a higher soil clay content can provide microbes protection from desiccation, fluctuations in pH, and predation (Bushby & Marshall, 1977; Elliott et al., 1980; Stotzky & Rem, 2011). In contrast, coarser soils may have more active microbial communities due to larger pore spaces and increased access to soil organic carbon (Franzluebbers et al., 1996). Ultimately, all these different properties that vary by soil type can have a large impact on restoration in prairies and may influence any carbon-addition experimental results.

The overarching goal of our study is to test whether similar changes in microbial community composition and soil characteristics are observed with additives in newly established restorations on two different soil types. Since cellulose microcrystalline addition was a

promising treatment in a previous study, but unrealistic on a large scale, we compared this additive to the equivalent amount of cellulose added as prairie plant biomass. To address these goals, we conducted two field experiments on different soil types to examine the effects of each carbon addition strategy (cellulose or biomass) over time. Finally, we wanted to determine whether the presence of plants modulates any specific shifts in soil microbial composition, activity or metrics associated with carbon storage, such as respiration, soil organic matter and total carbon concentrations. To address this last goal, we planted the same seed mix at the two different sites, and then weeded one half of each experimental plot to compare results of each addition with and without plants present. We hypothesized (H1) that cellulose and biomass addition would result in similar shifts in both soil characteristics and microbial community composition with increases in metrics related to carbon storage with concurrent increases in fungal and microbial biomass and the relative abundance of Verrucomicrobia, but that the shifts would vary by soil type. We also hypothesized (H2) that the presence of plants would help to modulate shifts in soil characteristics and community composition with the presence of plants resulting in larger shifts. Lastly, we hypothesized (H3) that there would be more shifts in metrics related to carbon storage and the microbial community in the coarse loam than in the fine loam due to the more open pore spaces.

Methods

Site Description

This study was conducted in partnership with two land management organizations. We conducted the study from April 2019 to October 2020, with two sites: a prairie conservation corridor at the Edward Lowe Foundation in Cass County, Michigan, USA (Fig. S6) and a whole field prairie restoration in Washington County, Minnesota, USA (Fig. S7). The prairie restoration

in Michigan is located on a Schoolcraft loam (Mollisol) and the Minnesota restoration is located on an Antigo silt-loam (Alfisol). The two soil types differ in texture, where the Schoolcraft loam is a fine loam (i.e. more clay) while the Antigo silt-loam is a coarse loam (i.e. more sand). Throughout the rest of this paper, we will refer to the sites by their soil texture, where the Michigan site is called the "fine loam site" and the Minnesota site is called the "coarse loam site". Mean annual precipitation for Cass County, MI is 978 mm with an average minimum temperature of 4.0^{o}C and an average maximum temperature of 14.83^{o}C (based on 1985-2010; http://www.ncdc.noaa.gov). During the study period, mean annual precipitation was 1024 mm with an average minimum temperature of 4.6^{o}C and an average maximum temperature of 14.9^{o}C. Mean annual precipitation for Washington County, MN is 812 mm with an average minimum temperature of 1.9^{o}C and an average maximum temperature of 12.6^{o}C (based on 1985-2010; http://www.ncdc.noaa.gov). During the study period, mean annual precipitation was 868 mm with an average minimum temperature of 1.9^{o}C and an average maximum temperature of 12.0^{o}C.

Experimental Design

To examine the effects of carbon substrate additions, we applied carbon to plots in two forms: cellulose microcrystalline and dried *Schizachyrium scoparium* (little bluestem) grass biomass. We also set up control plots which received no carbon additions but were disturbed in the same way as the treatment plots. At each site we set up a split plot design which included 36 total plots (six replicates x three carbon addition treatments x two plant treatments). The main plots (carbon addition treatments) were 1x1 m and were separated by 0.5 m buffer strips within each super plot. Each main plot was divided into two 1 x 0.5 m subplots to which the plant treatment was applied: seeding of prairie seed mix (plants present) and no seeding (plants absent). Plastic garden liner was used to line each 1x1 m plot.

Carbon Addition

We applied carbon addition in two forms (cellulose microcrystalline and little bluestem biomass) as two treatments to their respective plots on April 28th, 2019 (fine loam) and May 15th, 2019 (coarse loam). For the cellulose microcrystalline treatment plots, we added 1.125 kg powdered cellulose mixed with 8 L of water to the top ~30 cm of soil. For the biomass treatment we added 2.8 kg of little bluestem biomass (seed heads removed) with 8 L to the top 30 cm of soil. We used 2.8 kg of little bluestem biomass for this treatment because it contains roughly the same amount of cellulose as the amount of cellulose microcrystalline that we added to the cellulose plots (Jefferson et al., 2004). The control treatment consisted of 8 L of water applied to the top 30 cm of soil. To apply each of these treatments to the top 30 cm of soil, we dug each plot to 30 cm and then layered each treatment between layers of soil, adding L of water with 25% of each treatment to each layer, resulting in a total of four layers of treatment mixed into the soil.

Plant Treatments

Directly after the carbon treatments were applied to the soil, we superimposed the plant treatments: plants-present and plants-absent. For the plants-present treatment, we randomized each half-plot and added 250 mL of a prairie seed mix, purchased from Minnesota Native Landscapes, Otsego, MN, USA. The species mix and proportions are described in Fig. S8. We did not seed the plants-absent treatment. After the plant treatments were applied, we added a layer of dried grass biomass as thatch to help retain moisture and protect the seeds. We then maintained the plants-absent treatment by removing seedlings by hand from those sub-plots every three weeks during the growing season to keep the soil bare. After treatments were added

to the plots, we installed PVC soil collars into each sub-pot to a height of 4.5 cm from the soil surface to the top of the collar, to be used for soil reparation measurements.

Soil Sampling

We collected three 10-cm deep x 5.5 cm diameter soil cores from each sub-plot each summer in 2019 and 2020. Specifically, we sampled the fine loam plots on July 8th and 10th 2019 and August 14, 2020 and the coarse loam plots on July 9th, 2019 and September 6th, 2020. We cleaned and sterilized corers between plots. We placed the soils from each sub-plot into a single quart-sized zip-sealing plastic bags to create composite cores for each sub-plot. We homogenized the soils and then placed the bags in a cooler on ice until returning to the laboratory at WMU. In total, this yielded 36 samples for each site (fine loam or coarse loam) at each time point.

Vegetation Sampling and Bulk Density

To determine the effect of the treatments on the plant communities we measured aboveground plant biomass (AGB) and belowground root biomass (BGB) in Summer 2020 only. We measured plant aboveground biomass by clipping shoot biomass from each 1 x 1 m plot and then placing the biomass into paper lawn bags. We air dried the biomass for two weeks in a greenhouse before weighing it. We collected belowground plant biomass from each plot with a 20 cm soil core which was then placed into a paper bag. We air dried soils in the bags and then separated the roots from the dried soils and weighed the roots. We measured soil bulk density of each planted plot by inserting a steel cylinder of known volume into each plot. These soils were then transferred to a paper bag and transported to WMU and dried before calculating the soil bulk density (dry weight • volume $^{-1}$).

Soil Respiration Measurements

We measured soil CO_2 flux at each site using a LI-COR 870-01 CO_2/H_2O gas analyzer (LI-COR, Lincoln, NE, USA). We measured in the mornings between 8:00 am and 10:00 am (Eastern Daylight Time) to avoid changes in flux as temperatures warmed. We also measured soil flux either before soil sampling or at least a week after soil sampling occurred to avoid changes in CO_2 flux due to soil disturbance.

Soil Abiotic Measurements

After collection, we froze all soils at -20^{o}C prior to analysis. On the day of analysis, we removed a 100 g subset of soil which we kept frozen until DNA extraction and phospholipid fatty acid (PLFA) analysis could be completed, as described below. We sieved the remaining field soil through a 2 mm sieve and measured soil pH, soil water content (SWC), and soil organic matter (SOM) as described in Docherty and Gutknecht (2019). For soil ammonium (NH_4^+) and nitrate (NO_3^-) analyses, we conducted 2M KCl extractions using 10 g of soil and 50 mL of 2M KCl. Extraction tubes were shaken on a platform shaker for 1 hour at 5000 rpm. We used vacuum filtration through a Whatman GF/F filter (Global Life Sciences Solutions, Marlborough, MA, USA) to collect the filtrate which was then stored in a 20 mL plastic bottle at -20 C prior to analysis. KCl extracts were analyzed for NH_4^+ and NO_3^- concentrations using colorimetric assays measured at 540 nm and 650 nm respectively using an Epoch 96-well plate reader (BioTek, Winooski, VT, USA) (Rhine et al., 1998). We measured potassium permanganate oxidizable carbon (POXc) as described by Weil et al. (2003) to determine the amount of labile (i.e. easily oxidizable) carbon in the soil. Total soil percent soil carbon (% C), total soil percent soil nitrogen (% N), and the ratio of C:N were determined on oven-dried, ground soil samples using combustion analysis (Elementar pyrocube; Elementar Americas, Ronkonkoma, NY, USA).

Extracellular Enzyme Activities

We assessed the activity potential of four hydrolytic enzymes using fluorescent-linked substrates to determine how the carbon addition treatments and the presence/absence of plants impacted the extracellular enzyme activities, using the techniques described previously (Docherty & Gutknecht, 2019; German et al., 2011; Gutknecht et al., 2010; Sinsabaugh et al., 2005). We tested for activities of cellobiohydrolase using 4-MUB-cellobioside, β-glucosidase using 4-MUB-β-glucopyranoside, N-acetylglucosaminidase using 4-MUB-N-acetyl-β-glucosaminide, and xylosidase using 4-MUB-β-D-xylopyranoside. All substrates for extracellular enzyme assays were purchased from Millipore-Sigma (St. Louis, MO, USA).

Phospholipid Fatty Acid (PLFA) Analysis

We determined total lipid biomass, bacterial lipid biomass, and fungal lipid biomass using PLFA. Analysis was conducted at Regen Ag Lab, LLC (Pleasanton, Nebraska, USA). Phospholipids were extracted from 2 g of freeze-dried soil using a chloroform–methanol–citrate buffer mixture (1:2.5:0.8, v/v/v). The phospholipid fatty acids were then separated by solid phase extraction and methylated. The resulting phospholipids were then identified by gas chromatography (Agilent ChemStation, Wilmington, DE, USA) with a Sherlock microbial identification system (MIDI, Newark, NJ, USA). Total microbial biomass (MB) was calculated as the sum of all lipids <20 carbon atoms in length. Fungal biomass (FB) was calculated as the sum of 18:2ω6,9c and 18:1ω9c lipid biomarkers, as described by Frostegard & Baath (1996). Bacterial biomass was calculated as the sum of 13:0 iso, 15:0 iso, 15:0 anteiso, 16:1 ω 7c, 16:1 ω 9c, 17:0 iso, 17:0 anteiso, and 18:1ω7c lipid biomarkers (Zelles, 1999).

DNA Extraction and Sequencing

We extracted genomic DNA with a DNeasy PowerSoil DNA Isolation Kit (Qiagen, Hilden Germany) using 0.25 g of soil according to the manufacturer's instructions. Amplicon preparation and Mi-Seq (Illumina, San Diego, CA, USA) sequencing was conducted at the Michigan State University Genomics Core Facility. We used two separate paired-end sequencing runs for samples from summer 2019 and summer 2020. The V4 hypervariable region of the 16S rRNA gene in Bacteria was amplified using primers 515F/806R (Kozich et al., 2013). DNA libraries were normalized using the Invitrogen SequalPrep Normalization Plate Kit -96 well (Thermo Fisher Scientific, Waltham, MA, USA), and samples from each replicate plate were pooled into single wells. Pooled samples were quantified using a Kapa Biosystems qPCR kit (Kapa Biosystems, Inc., Wilmington, MA, USA) and samples were normalized to an equal concentration. Each sample pool was loaded on an Illumina Mi-Seq flow cell v2 and sequenced using a 500 (PE250) cycle reagent cartridge. Bases were called using Real Time Analysis (RTA) software v1.18.54, and RTA output was demultiplexed and converted to FASTQ files using Illumina Bc12Fastq v1.8.4.

Sequence Processing and Analysis

After the raw sequence data was returned, we cleaned and processed the sequences using the QIIME2 bioinformatics platform version 2020.8 (Bolyen et al., 2019). We used Divisive Amplicon Denoising Algorithm (DADA2) to merge paired reads, filter by sequence quality, denoise, create an Amplicon Sequence Variant (ASV) table, and remove chimeras (Callahan et al., 2017). For DADA2 forward and reverse reads were truncated at the 15th base pair (bp) from the 5´ end to remove low quality regions of the sequences. From the 3´ end, the sequences were

truncated at the 248th and 200th bp for the forward and reverse reads, respectively. ASVs were taxonomically assigned using the Naïve Bayes Classifier trained on the SILVA version 138, 99% Operational Taxonomic Unit (OTU) database (Quast et al., 2013). ASVs that could not be classified at the Phylum taxonomic level or were associated with Archaea, Eukaryotes, mitochondria, or chloroplasts were discarded. After cleanup the number of sequences per sample ranged from 6,914 to 78,408 for 2019 and 16,452 to 45776 for 2020.

To determine if there was an effect of the carbon addition treatments and plant presence on the function of the soil bacterial community in summer 2020, we predicted the metagenomic functional potential of the communities using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUST2) (Douglas et al. 2019). Specifically, we used PICRUST2 to infer MetaCyc metabolic pathways which are hierarchically grouped based on metabolic function (Caspi et al. 2018). We focused our analysis on pathways related to C and N-compound degradation including: alcohol degradation, amine and polyamine degradation, amino acid degradation, aromatic compound degradation, carbohydrate degradation, carboxylate degradation, fatty acid and lipid degradation, nucleoside and nucleotide degradation, polymeric compound degradation, and secondary metabolite degradation pathways.

Data Analysis and Statistics

As is assumed by our experimental design, the bacterial communities in the fine loam and coarse loam sites differed from one another (Figs. S9A, S9B), so our results for soil amendments and plant treatments are presented separately for the two soil types. All analyses were conducted using R (version 3.6.3.). To address H1 and H2, we used linear mixed-effects models with the lmer function in in the lme4 package, version 1.1-21 (Bates et al., 2015) to determine if there

were any effects of carbon addition treatments, plant treatments, and if were interactions between treatments. We applied lme to all univariate characteristics that we measured, which included: SOM, SWC, pH, NH_4^+, NO_3^-, POXc, % C, % N, C:N ratio, CO_2 flux, AGB, BGB, soil bulk density, EEAs, Shannon diversity (ASVs), ASV richness, total microbial lipid biomass, fungal lipid biomass, bacterial lipid biomass, relative abundance of the six most abundant bacterial phyla, and functional pathways. Since our experiment includes a split-plot design, block was treated as a random factor in the lme models, while carbon addition treatments and plant treatments were treated as fixed effects. We tested the significance of each fixed effect and their interaction with analysis of variance (ANOVA) and we used the emmeans package, version 1.5.4 (Length, 2021) to determine the pairwise comparison for each treatment level with Tukey adjusted p-values. We qualitatively compared the results of these tests between the two soil types to address H3.

We conducted multivariate statistical analyses using the vegan package, version 2.5-6 (Oksanen et al., 2019) and we visualized multivariate data using principal coordinates analyses (PCoA) and nonmetric multidimensional scaling (NMDS). To further address H1-H2, we determined whether there were differences in bacterial communities due to the different treatments by calculating weighted UniFrac distance matrices (β diversity) (Lozupone & Knight, 2005; McMurdie & Holmes, 2013). We used the adonis function in vegan to determine if there were significant differences between treatments at each site. Again, we qualitatively compared the results from each soil type to address H3.

We used structural equation modeling (SEM) to determine paths underlying the observed effects of treatments on soil and microbial characteristics differed by soil type. We constructed SEMs by comparing biomass addition results to control results in one SEM, and cellulose

addition results to control results in a second SEM (H1). We then compared the two SEMs for when plants were either present or absent (H2) and for the two soil types (H3). Since the carbon addition treatments were categorical, we modeled them as binary, i.e., biomass treatment was modeled as 1 and control treatment modeled as 0. SEMs were built using the piecewiseSEM package, version 2.1.0 (Lefcheck, 2016) and the lme4 package. Piecewise SEM is a multivariate statistical technique which incorporates several explanatory and response variables into a single causal network, represented as a set of regression equations (Lefcheck, 2016). This differs from traditional SEMS in which relationships among variables are estimated simultaneously in a single variance-covariance matrix rather than locally as independent regression equations as done in piecewise SEMS. We evaluated the assumption of independence between explanatory variables in our SEMs with the test for directed separation provided in the output of the psem function in the piecewiseSEM package. If explanatory variables were highly correlated, we specified correlated errors between the variables. We then used the Fisher's C test to confirm the goodness of fit of the modelling results. We then modified our models according to the significance of each path, removing paths with $p > 0.15$ starting with paths with the largest p-values until all paths had a $p < 0.15$. Initially, we included the same predictors for the SEMs constructed for the biomass and cellulose treatments for each site.

Results

Overall, the goal of this study was to determine whether cellulose and biomass addition would shift both soil and microbial characteristics that could potentially set restorations on a path towards higher carbon storage and to determine how plants modulated these effects. Overall, our results demonstrate that cellulose and biomass addition elicited different responses, and that metrics related to carbon storage increased only with biomass addition in both soil types.

However, bacterial communities responded differently to the carbon addition treatments in the different soil types, and biomass addition effects were more pronounced in the coarse loam than in the fine loam. There were similar effects of the carbon addition treatments in 2019 and 2020, but they were more pronounced in 2020, while there were few effects of plant presence in 2019 (Tables S12 and S13). Therefore, we focus on the results from 2020 in the remainder of this investigation. We present the results of the overall carbon addition treatments and plant effects first and then in separate sections present the results for when plants were absent and then for when plants were present.

Fine Loam Soil

Fine Loam Overall Carbon Treatment and Plant Effects

There was no overall effect of the carbon addition treatments in the fine loam soil on bacterial community composition, but the presence of plants shifted bacterial communities significantly (Fig. 1A). There were no differences in most soil characteristics between plots with plants absent and plots with plants present except for SOM and POXc which were both higher in the sub-plots with plants present (Table 1). Conversely, there were more differences in soil characteristics between the carbon addition treatments overall. SOM, CO_2 flux, and the C:N ratio were all significantly higher in the biomass plots as compared to the control plots (Table 1). There was a marginal increase in % C in both the cellulose and biomass plots as compared to the control ($p = 0.054$) and a significant increase in % N in the cellulose plots (Table 1). While there was no overall effect of carbon addition treatment on soil pH and POXc there was a significant interaction between carbon addition and plant presence ($p = 0.022$ and $p = 0.018$, respectively). In terms of microbial community characteristics there were no differences in ASV richness,

Shannon diversity, total lipid biomass, bacterial lipid biomass, or any EEAs between plots with plants absent vs. present or with the carbon addition treatments (Table 2). However, we did observe significant shifts in community composition. There was 51 % more fungal lipid biomass in the plots with plants present as compared to plots with plants absent, however this was only a marginal increase ($p = 0.07$, Table 2). Within the bacterial community, the relative abundance of Acidobacteria was higher in plots with plants present (Fig. 2), but no other phyla differed significantly. There were no differences in the predicted functional pathways that we examined between the carbon addition treatments. The pathways involved in nucleotide and nucleoside degradation differed with the presence or absence of plants, where both pathways were more abundant when plants were absent ($p = 0.034$).

Carbon Treatment Effects with Plants Absent in the Fine Loam

When plants were absent, the bacterial community composition in the biomass plots differed significantly from both the communities in the control and cellulose plots ($p = 0.037$; Fig. 1B). There were no differences in the relative abundance of any major bacterial phyla associated with either the biomass or cellulose treatments (Fig. 2). The soil and microbial characteristics between the carbon addition treatments in the sub-plots with plants absent followed similar trends as with the overall treatment effect (Tables 1 and 2). While not significant at the overall treatment level, soil pH was significantly lower in the biomass plots as compared to the control plots (Table 1).

Piecewise structural equation models constructed for the biomass to control comparison (Fig. 3A) and the cellulose to control comparison (Fig. 3B) revealed different effects of the treatments on ASV diversity (Shannon). However, both models indicated that there was a

positive direct relationship between biomass and cellulose addition with soil NO_3^- and a direct negative relationship between NO_3^- and Shannon diversity. The biomass model (Fig. 3A) also showed that biomass addition had a direct negative impact on pH and SOM which then both had direct negative impacts on ASV diversity. Conversely, the cellulose model (Fig. 3B) revealed that cellulose addition only impacted ASV diversity through a direct effect on NO_3^- concentration while there was no direct effect of cellulose addition on any of the other soil characteristics.

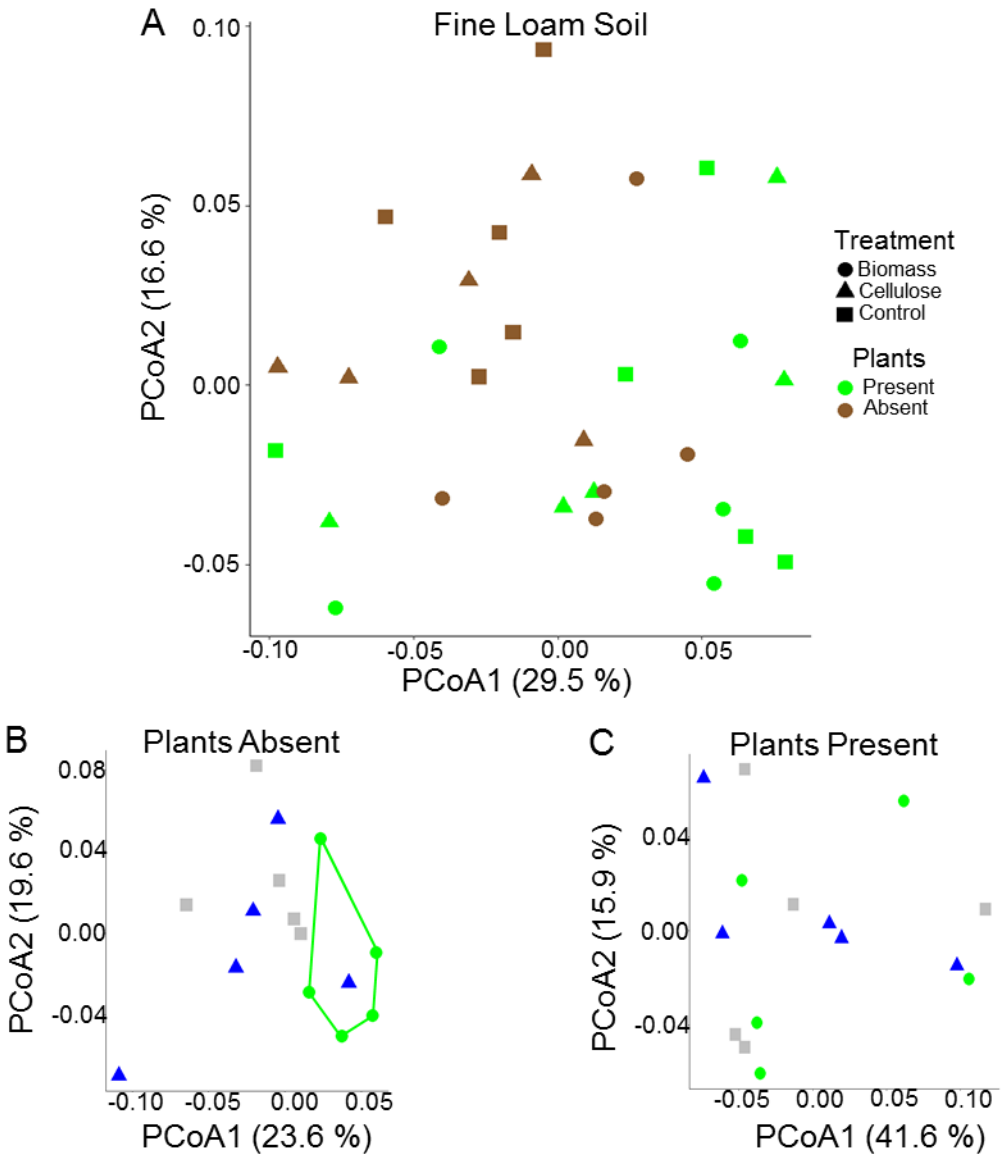

Figure 7. Ordinations of bacterial communities in the fine loam. Principal coordinates analysis (PCoA) ordinations based on weighted UniFrac distances between bacterial communities in the fine loam soil, identified using 16S rRNA amplicon sequencing. **A) Overall carbon addition treatment and plant effects.** There is a significant effect of the presence or absence of plants on the communities ($p = 0.012$) while there is no overall treatment effect ($p = 0.169$). **B) Bacterial communities in plots with plants absent.** There is a significant effect of biomass addition on the communities ($p = 0.037$). The green line represents the ordination space occupied by the biomass plots. **C) Bacterial communities in plots with plants present.** There is no treatment effect on the communities $p = 0.86$). Significance of adonis results was determined at $\alpha = 0.05$.

Table 1. Results of linear mixed models for soil characteristics in the fine loam. Results of linear-mixed effect models examining the effects of carbon addition (Treatment) and plant presence (Plants) on soil characteristics in the fine loam soil (± SE).

	SOM (%)	CO_2 Flux (μmol CO_2 $m^{-2}s^{-1}$)	pH	SWC (%)	NO_3^- (ug g $soil^{-1}$)	NH_4^+ (ug g $soil^{-1}$)	POXc (mg kg $soil^{-1}$)	% C	% N	C:N	Bulk density (g cm^{-3})
p value											
Treatment	**0.027**	**0.012**	0.42	0.23	0.42	0.85	0.12	0.054	**0.025**	**0.025**	0.088
Plants Present	**0.049**	0.41	0.38	0.56	0.78	0.11	**0.039**	0.49	0.7	0.57	NA
Treatment x Plants Present	0.53	0.54	**0.022**	0.45	0.4	0.35	**0.018**	0.79	0.65	0.99	NA
Overall Treatment											
Control	3.18±0.08**A**	3.80±0.056**A**	6.47±0.08	14.2±0.45	39.11±8.96	18.6±5.3	493±18.60	1.13±0.084	0.13±0.01**A**	8.81±0.22**A**	1.29±0.063
Cellulose	3.39±0.10**AB**	4.03±0.18**AB**	6.39±0.08	15.0±0.34	35.16±7.76	22.5±5.66	525±22.63	1.28±0.059	0.14±0.01**B**	8.9±0.14**AB**	1.31±0.035
Biomass	3.58±0.14**B**	4.43±0.14**B**	6.34±0.07	14.0±0.56	23.49±4.2	22.2±5.08	571 ± 26.92	1.29±0.078	0.14±0.01**AB**	9.46±0.20**B**	1.15±0.085
Plants											
Plants Absent	3.3±0.07**A**	4.02±0.12	6.37±0.07	14.3±0.74	34.5±6.72	159±68.21	518±17.65**A**	1.21±0.047	0.134±0.004	9.00±0.15	NA
Plants Present	3.48±0.11**B**	4.15±0.14	6.44±0.05	14.5±0.57	30.34±5.53	263±85.14	541±22.23**B**	1.25±0.075	0.136±0.006	9.11±0.19	NA
Plants Absent											
Control	3.16±0.08	NS	6.61+0.09**A**	NS	NS	NS	503±26.95	1.11±0.081	0.129±0.007	8.76±0.24 **A**	NA
Cellulose	3.25±0.13	NS	6.28±0.14**AB**	NS	NS	NS	495±32.75	1.26±0.053	0.143±0.005	8.85±0.21**AB**	NA
Biomass	3.49±0.14	NS	6.21+0.08**B**	NS	NS	NS	557±33.11	1.27±0.11	0.132±0.009	9.4±0.27**B**	NA
Plants Present											
Control	3.21±0.14**A**	NS	6.47+0.11	NS	NS	NS	483±25.85**A**	1.15±0.16	0.125±0.01	8.87±0.40**A**	NA
Cellulose	3.53±0.14**AB**	NS	6.39+0.07	NS	NS	NS	555±28.32**AB**	1.3±0.11	0.144±0.009	8.96±0.22**AB**	NA
Biomass	3.70±0.24**B**	NS	6.34+0.09	NS	NS	NS	584±47.39**B**	1.31±0.12	0.139±0.013	9.51±0.32 **B**	NA

SWC: Soil Water Content; SOM: Soil Organic Matter; % C: Percent Total Carbon; % N: Percent Total Nitrogen, C:N: Carbon to nitrogen ratio.; POXc: Permanganate Oxidizable Carbon

Bolded text indicates significance at $p < 0.05$.

Means with the same letters are not significantly different while those with different letter are significantly different ($p < 0.05$)

Table 2. Results of linear-mixed effect models for biotic characteristics in the fine loam. Results of linear-mixed effect models examining the effects of carbon addition (Treatment) and plant presence (Plant Present) on plant and microbial characteristics in the fine loam soil (± SE).

	BGB (g)	AGB (kg)	ASV Richness	ASV Diversity	Total MB (nmol g soil^{-1})	Fungal Biomass (nmol g soil^{-1})	Bacterial Biomass (nmol g soil^{-1})	β-glucosidase (nmol hr^{-1} g soil^{-1})	Cellobiohydrolase (nmol hr^{-1} g soil^{-1})	NAG (nmol hr^{-1} g soil^{-1})	Xylosidase (nmol hr^{-1} g soil^{-1})
p value											
Treatment	0.94	0.34	0.71	0.75	0.30	0.18	0.48	0.81	0.73	0.93	0.86
Plants Present	NA	NA	0.37	0.35	0.19	0.07	0.23	0.13	0.71	0.39	0.19
Treatment x Plants Present	NA	NA	0.51	0.49	0.90	0.76	0.92	0.76	0.13	0.14	0.36
Overall Treatment											
Control	0.153±0.051	372±26.64	658±37.8	6.09±0.08	628±58.8	41.0±9.2	283±33.9	74.1±12.8	49.3±13.8	65.4±20.3	96.7±22.2
Cellulose	0.141±0.035	472±42.98	656±50.9	6.05±0.12	600±86.5	55.1±14.6	306±52.0	83.5±24.9	58.3±15.4	72.0±16.3	93.8±18.3
Biomass	0.135±0.039	420±92.25	613±55.0	6.01±0.12	719±95.7	76.8±22.3	337±52.2	75.0±15.8	49.0±9.9	68.8±14.3	85.6±22.1
Plants											
Plants Absent	NA	NA	620±22.3	6.01±0.06	606±56.0	45.9±8.3	287±31.4	67.2±10.6	50.5±5.6	63.8±8.3	82.4±10.3
Plants Present	NA	NA	665±36.6	6.09±0.08	692±35.7	69.4±10.6	330±20.5	87.8±10.3	53.9±9.1	73.6±11.1	101.7±13.1

ASV: Amplicon sequence variant; MB: Microbial Biomass; NAG: N-acetylglucosaminidase
Bolded text indicates significance at $p < 0.05$.
Means with the same letters are not significantly different while those with different letter are significantly different ($p < 0.05$)

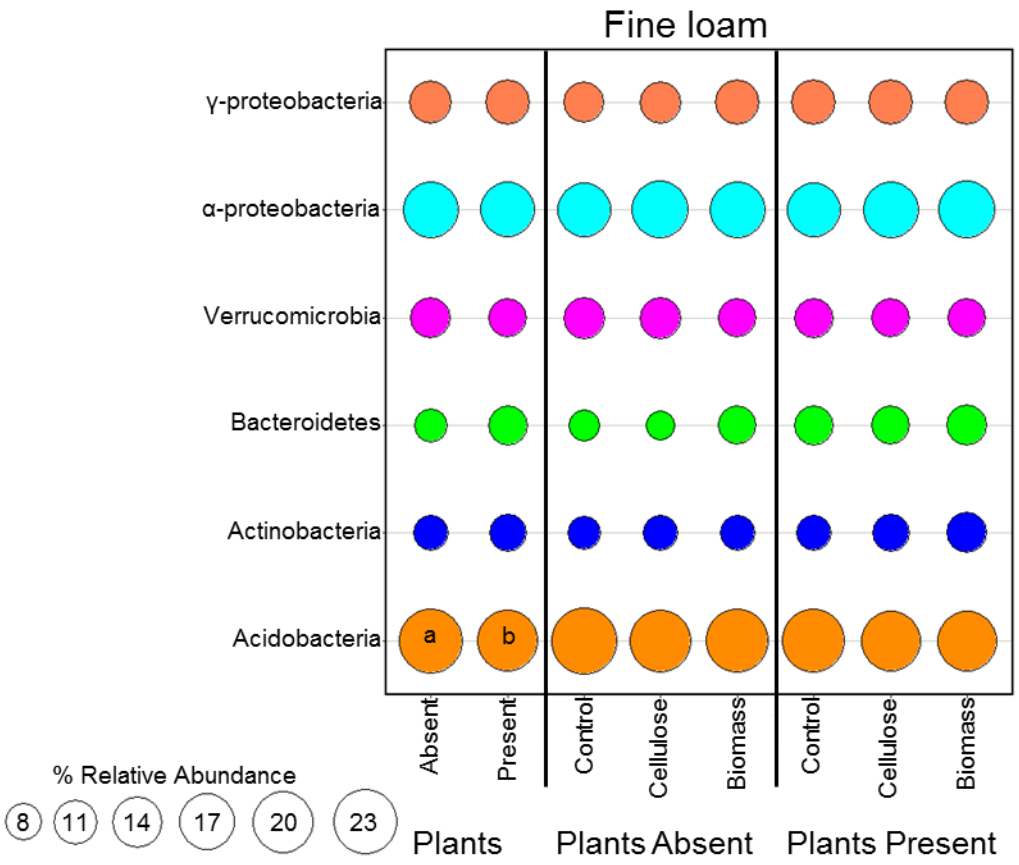

Figure 8. Bubble plot of bacterial relative abundance in the fine loam. Bubble plot depicting the percent relative abundance of the six most abundant bacterial phyla in the fine loam soil and Letters indicate significant differences between phyla in each treatment grouping. Significance was determined at $\alpha = 0.05$.

Carbon Treatment Effects with Plants Present in the Fine Loam

In contrast to the carbon addition treatments with plants absent there were no differences in the bacterial community structure between the carbon addition treatments with plants present ($p = 0.86$, Fig. 1C). There were also no phyla that significantly differed in relative abundance between the carbon addition treatments with plants present (Fig. 2). Soil and microbial characteristics between the carbon addition treatments in the sub-plots with plants present followed similar trends as with the overall treatment effect (Table 1), except for POXc. Biomass addition increased POXc by 21 % ($p = 0.04$) as compared to the control while there was no

significant effect on POXc from cellulose addition (Table 1) There were no differences in AGB, BGB, and soil bulk density with either carbon addition treatment (Table 2).

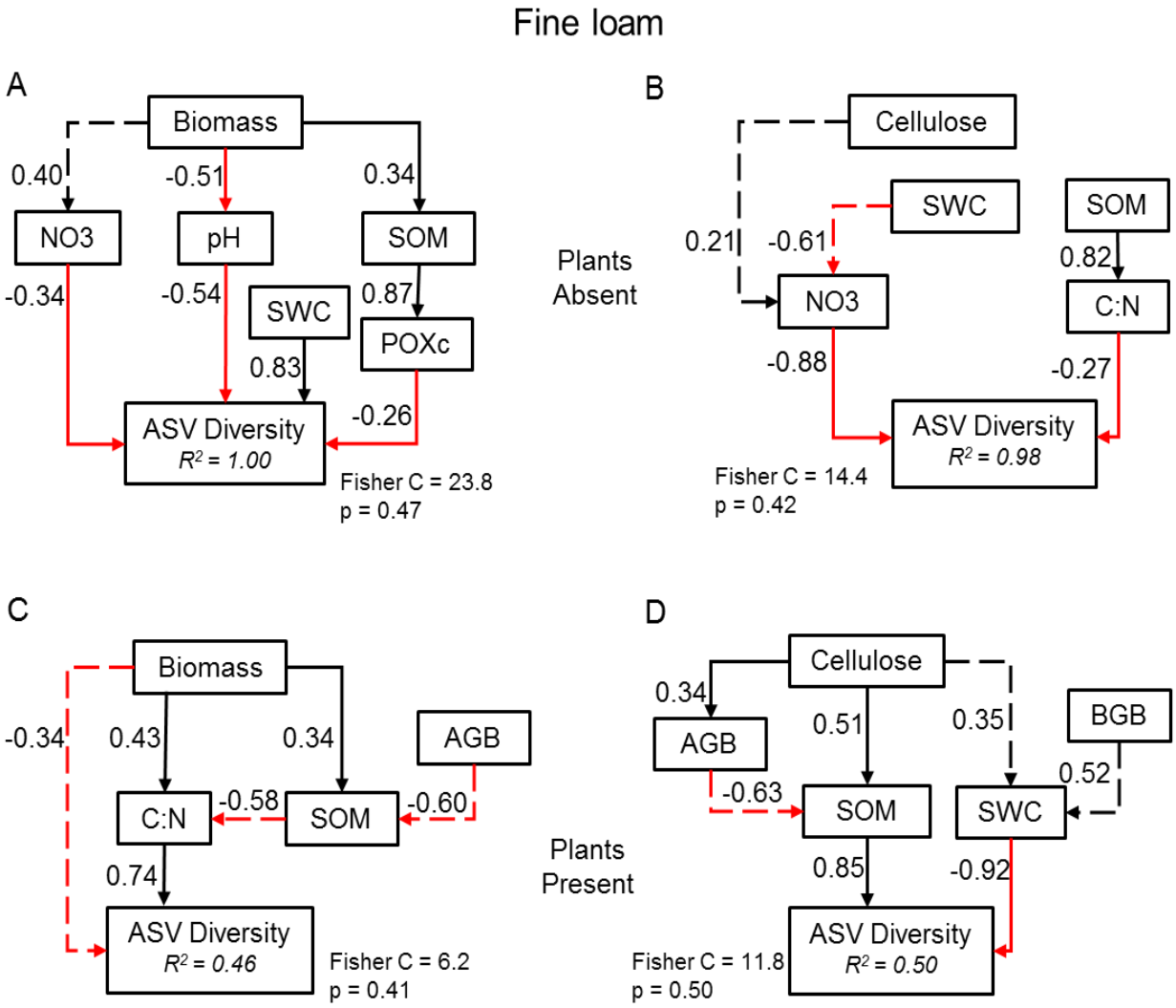

Figure 9. Structural equation models for the fine loam. Structural equation models exploring the effects of the carbon addition treatments on soil and plant characteristics and overall relationship with ASV diversity in the fine loam. Black arrows indicate positive effects and red arrows indicate negative effects. Paths that are significant ($p < 0.05$) are shown as solid lines while paths that are marginally significant ($0.05 < p < 0.15$) are shown as dashed lines. Numbers adjacent to lines are standardized path coefficients and conditional R^2 values (based on fixed and random effects) are reported for ASV diversity. All models are well supported by the data ($p > 0.05$) and the Fisher's C statistic and p value are shown next to each model.

Structural equation modeling revealed that biomass (Fig. 3C) and cellulose (Fig. 3D) explained similar amounts of variation in ASV diversity when plants were present ($R^2 = 0.46$ and $R^2 = 0.50$, respectively). In both models, treatment addition increased SOM, while plant AGB had negatively affected SOM. In the biomass model there were no direct linkages between plant characteristics and treatment while in the cellulose model, cellulose addition had a positive effect on AGB. The biomass model also showed that biomass addition increased the soil C:N ratio which led to an increase in ASV diversity while there was a direct negative effect of biomass addition on ASV diversity. In contrast, the cellulose model indicated that cellulose addition increased SWC which had a negative effect on ASV diversity.

Overall, our results indicate that the presence of plants was more important in structuring the bacterial community in the fine loam soil than either of the carbon addition treatments. However, both carbon addition treatments had a greater impact on soil characteristics, and biomass addition had larger impacts on soil characteristics related to carbon storage than cellulose addition when plants were present. Biomass and cellulose addition also differed in how they impacted ASV diversity which was largely due to different effects on soil characteristics.

Coarse Loam Soil

Coarse Loam Overall Treatment and Plant Effects

There was a significant effect of carbon addition in the form of cellulose on the bacterial communities in the coarse loam (Fig. 4A). There was also a significant effect of the presence of plants on the bacterial communities (Fig. 4A). Whether plants were present or absent in the coarse loam soil had a significant effect on most soil characteristics (Table 3). SOM, CO_2, pH, SWC, POXc, were all significantly higher in plots with plants present as compared to those with

plants absent, while NO_3^- was significantly lower in plots with plants present. There were also significant effects of the carbon addition treatments on the soil characteristics as well which will be elucidated below. In terms of microbial characteristics there were no differences between plots with plants absent vs. present for ASV richness, Shannon diversity, total microbial biomass, bacterial biomass, or activities of any EEAs other than xylosidase activity which was higher in plots with plants absent (Table 4). Conversely, fungal biomass was 81 % higher in the plots with plants present than plots with plants absent ($p = 0.002$, Table 4). In the bacterial community, relative abundances of Bacteroidetes increased when plants were present (Fig. 5). In terms of predicated functional pathways, the relative abundance of pathways related to alcohol and carbohydrate degradation were higher in plots with plants absent ($p = 0.029$ and $p = 0.041$, respectively) while carboxylate degradation was higher in plots with plants present ($p = 0.046$).

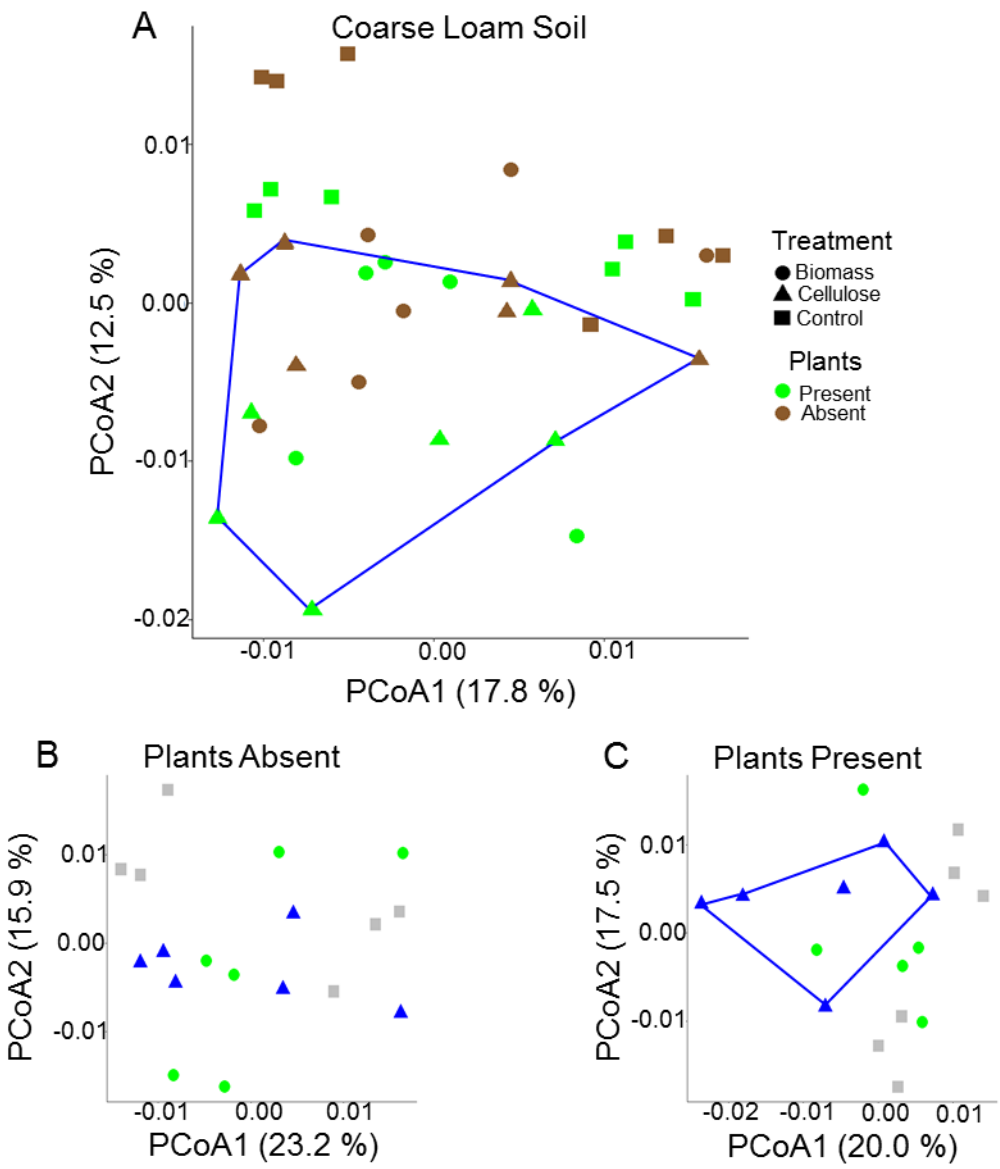

Figure 10. Ordinations of bacterial communities in the coarse loam. Principal coordinates analysis (PCoA) ordinations based on weighted UniFrac distances between bacterial communities in the coarse loam soil, identified using 16S rRNA amplicon sequencing. **A) Overall carbon addition treatment and plant effects.** There is a significant effect of the presence or absence of plants on the communities ($p = 0.001$) and a significant treatment effect ($p = 0.009$) driven by differences between the communities in the cellulose and control plots ($p = 0.002$). **B) Bacterial communities in plots with plants absent.** There is no treatment effect on the communities ($p = 0.37$). **C) Bacterial communities in plots with plants present.** There is a significant treatment effect on the communities ($p = 0.001$) driven by differences between the cellulose and control plots ($p = 0.042$). Significance of adonis results was determined at $\alpha = 0.05$. The solid blue line represents the ordination space occupied by the cellulose plots where significant.

Table 3. Results of linear-mixed effect models for soil characteristics in the coarse loam. Results of linear-mixed effect models examining the effects of carbon addition (Treatment) and plant presence (Plant Present) on soil characteristics in the coarse loam soil (± SE).

	SOM (%)	CO_2 Flux (μmol CO_2 $m^{-2}s^{-1}$)	pH	SWC (%)	NO_3^- (ug g $soil^{-1}$)	NH_4^+ (ug g $soil^{-1}$)	POXc (mg kg $soil^{-1}$)	% C	% N	C:N	Bulk density (g cm^{-3})
p value											
Treatment	**0.004**	**< 0.001**	**< 0.001**	**< 0.001**	**0.012**	**< 0.001**	**<0.001**	0.26	0.58	**0.01**	**0.015**
Plants Present	**< 0.001**	**< 0.001**	**0.036**	**0.023**	**0.002**	0.66	**<0.001**	0.85	0.35	0.26	NA
Treatment x Plants Present	0.12	0.67	0.7	**0.019**	0.83	0.71	0.5	0.99	0.75	0.73	NA
Overall Treatment											
Control	2.9±0.048**A**	1.77±0.12**A**	8.25±0.097**A**	16.7±0.18**A**	13.33±7.99**A**	38.5±1.40**A**	347±17.05**A**	1.01±0.02	0.118±0.002	8.58±0.17**A**	1.2±0.020**A**
Cellulose	2.83±0.049**A**	1.85±0.11**A**	7.49±0.056**B**	17.1±0.24**A**	72.66±40.24**B**	47.7±1.21**B**	328±12.42**A**	1.07±0.06	0.123±0.01	8.62±0.28**A**	1.2±0.02**A**
Biomass	3.08±0.084**B**	2.27±0.17**B**	7.19±0.11**B**	19.4±0.22**B**	30.87±8.53**AB**	53.2±1.49**B**	420±13.54**B**	1.16±0.09	0.122±0.01	9.52±0.49**B**	1.12±0.03**B**
Plants											
Plants Absent	2.83±0.032**A**	1.6±0.081**A**	7.67±0.13**A**	17.4±0.36**A**	62.00±26.57**A**	46.8±1.90	339±13.02**A**	1.07±0.03	0.123±0.002	8.71±0.12	NA
Plants Present	3.05±0.064**B**	2.32±0.085**B**	7.59±0.14**B**	17.9±0.29**B**	15.90±7.88**B**	46.1±1.77	391±14.39**B**	1.09±0.05	0.119±0.003	9.11±0.28	NA
Plants Absent											
Control	2.82 ± 0.047	1.41 ± 0.10**A**	8.27 ± 0.13**A**	16.4 ± 0.22**A**	24.59 ± 15.14	39.2 ± 2.39**A**	315 ± 15.93**A**	1.01±0.02	0.118±0.002	8.57±0.17	NA
Cellulose	2.76 ± 0.049	1.54 ± 0.079**AB**	7.53 ± 0.09**B**	16.7 ± 0.29**A**	117.25 ± 76.74	48.6 ± 1.95**B**	306 ± 11.53**B**	1.05±0.04	0.124±0.004	8.47±0.15	NA
Biomass	2.9 ± 0.061	1.85 ± 0.18**B**	7.21 ± 0.16**B**	19.5 ± 0.21**B**	44.16 ± 11.20	52.6 ± 2.90**B**	396 ± 19.09**B**	1.15±0.06	0.126±0.004	9.09±0.22	NA
Plants Present											
Control	2.97±0.075**A**	2.13±0.053**A**	8.23±0.15**A**	17.0±0.26**A**	2.08±1.06**A**	37.8±1.64**A**	380±24.54**A**	1.0±0.02	0.117±0.003	8.59±0.19**A**	NA
Cellulose	2.91±0.078**A**	2.16±0.11**A**	7.44±0.075**B**	17.6±0.30**A**	28.06±20.99**B**	46.8±1.51**B**	349±19.03**A**	1.08±0.08	0.123±0.01	8.77±0.15**A**	NA
Biomass	3.26±0.12**B**	2.68±0.15**B**	7.1±0.16**B**	19.2±0.39**B**	17.58±11.14**AB**	53.8±1.15**B**	444±14.57**B**	1.18±0.12	0.118±0.01	9.96±0.63**B**	NA

SWC: Soil Water Content; SOM: Soil Organic Matter; % C: Percent Total Carbon; % N: Percent Total Nitrogen, C:N: Carbon to nitrogen ratio; POXc: Permanganate oxidizable carbon.

Bolded text indicates significance at $p < 0.05$.

Means with the same letters are not significantly different while those with different letter are significantly different ($p < 0.05$)

Table 4. Results of linear mixed models biotic characteristics in the coarse loam. Results of linear-mixed effect models examining the effects of carbon addition (Treatment) and plant presence (Plant Present) on plant and microbial characteristics in the coarse loam soil (± SE).

	BGB (g)	AGB (kg)	ASV Richness	ASV Diversity	Total MB (nmol g soil^{-1})	Fungal Biomass (nmol g soil^{-1})	Bacterial Biomass (nmol g soil^{-1})	β-glucosidase (nmol hr^{-1} g soil^{-1})	Cellobiohydrolase (nmol hr^{-1} g soil^{-1})	NAG (nmol hr^{-1} g soil^{-1})	Xylosidase (nmol hr^{-1} g soil^{-1})
p value											
Treatment	**0.02**	**0.001**	**0.049**	0.012	0.16	**0.019**	0.26	0.76	0.12	0.23	0.14
Plants Present	NA	NA	0.48	0.39	0.69	**0.002**	0.77	0.14	1	0.9	**0.031**
Treatment x Plants Present	NA	NA	0.44	0.53	0.06	**0.001**	0.07	0.88	0.72	0.45	0.11
Overall Treatment											
Control	0.47±0.062**A**	0.348±0.027**A**	583±49.2	5.86±0.09**A**	343±88.5	26.6±9.2**AB**	142±42.0	34.1±9.4	47.4±11.5	37±15.1	62.1±10.8
Cellulose	0.50±0.09**A**	0.262±0.019**B**	696±37.2	6.05±0.06**AB**	290±66.7	24.1±14.3**B**	119±36.5	45.3±9.4	48±11.5	51.6±15.1	91.2±10.8
Biomass	0.94±0.17**B**	0.34±0.035**A**	701±44.9	6.07±0.07**B**	384±53.2	42.9±11.8**A**	146±22.6	58.9±9.4	79.3±11.5	82.6±15.1	88.2±10.8
Plants											
Plants Absent	NA	NA	648±24.9	5.97±0.05	332±42.7	22.2±5.4**A**	133±21.6	51.3±7.2	59.7±7.9	57.0±10.8	91.9±7.8**A**
Plants Present	NA	NA	672±31.8	6.02±0.05	346±40.0	40.2±7.6**B**	138±20.6	41.0±7.2	56.7±7.9	57.1±10.8	69.5±7.8**B**
Plants Absent											
Control	NA	NA	541±31.5**A**	5.79±0.07**A**	422±103.5	36.3±11.5**A**	179±51.9	37.8±12.2	45.9±13.4	41.8±18.8	67.2±13.6
Cellulose	NA	NA	705±19.5**B**	6.05±0.06**B**	229±45.5	5.3±0.73**B**	85.1±16.2	50.5±12.2	51.7±13.4	58.1±18.8	94.3±13.6
Biomass	NA	NA	697±34.0**B**	6.07±0.05**B**	345±45.2	25.3±8.4**A**	136±20.5	65.5±12.2	81.6±13.4	71.2±18.8	114.2±13.6
Plants Present											
Control	NA	NA	625±60.2	5.93±0.11	263±63.1	17.0±4.1**A**	104±25.4	30.4±12.2	49.0±13.4	32.1±18.8	57.1±13.6
Cellulose	NA	NA	686±51.2	6.05±0.06	351±78.9	42.9±17.6**AB**	152±47.1	40.1±12.2	44.3±13.4	45.2±18.8	89.3±13.6
Biomass	NA	NA	706±57.3	6.07±0.09	424±59.8	60.6±8.4**B**	156±25.7	52.4±12.2	77.0±13.4	94.1±18.8	62.1±13.6

ASV: Amplicon sequence variant; MB: Microbial Biomass; NAG: N-acetylglucosaminidase
Bolded text indicates significance at $p < 0.05$.
Means with the same letters are not significantly different while those with different letter are significantly different ($p < 0.05$)

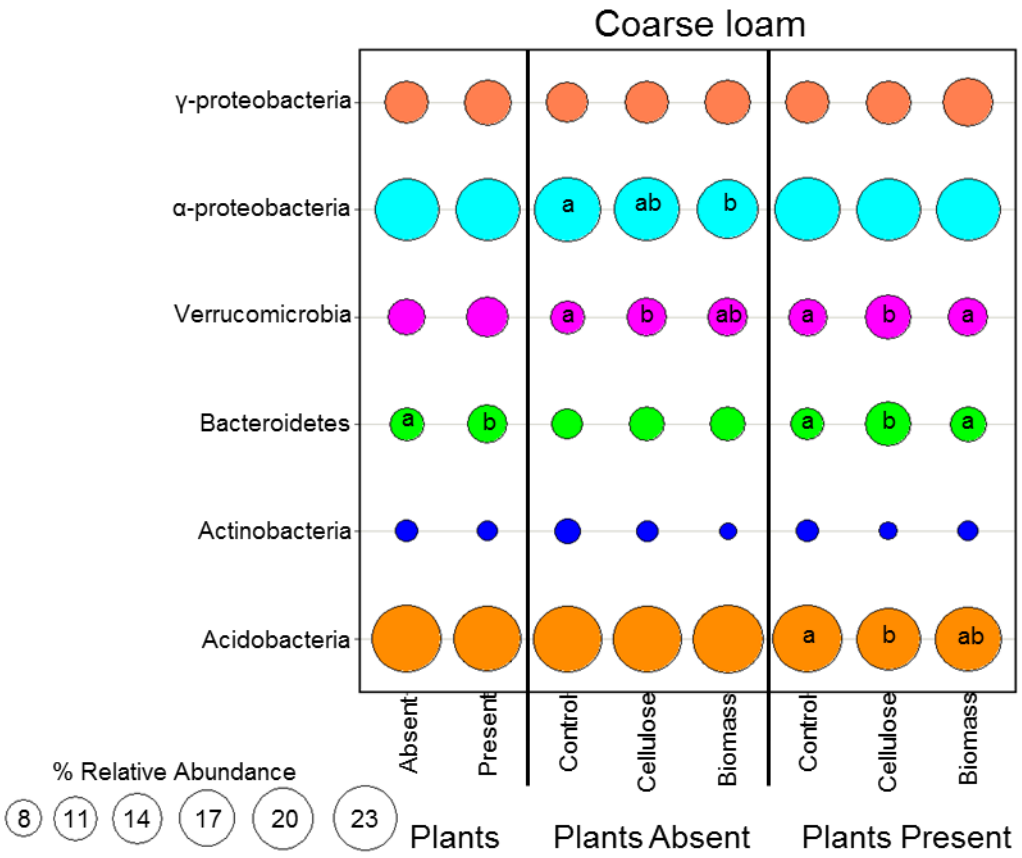

Figure 11. Bubble plot of bacterial relative abundance in the coarse loam. Bubble plot depicting the percent relative abundance of the six most abundant bacterial phyla in the coarse loam soil. Letters indicate significant differences between phyla in each treatment grouping. Significance was determined at $\alpha = 0.05$.

Carbon Treatment Effects with Plants Absent in the Coarse Loam

There were no differences in bacterial community structure between the carbon addition treatments with plants absent in the coarse loam soil ($p = 0.37$, Fig. 4B). While there were no differences in overall community structure there were differences in major bacterial phyla between the carbon addition treatments. Cellulose addition increased the relative abundance of Verrucomicrobia by 20 % (Fig. 5, $p = 0.04$) as compared to the control. In contrast biomass addition decreased the relative abundance of α-proteobacteria by 14 % ($p = 0.008$). Both cellulose and biomass addition increased ASV richness by 30 % and 28 %, respectively (Table 4,

p = 0.046 and p = 0.034) which led to higher ASV diversity as well with both treatments (Table 4). There was no effect of either treatment on total microbial or bacterial biomass while cellulose addition decreased fungal biomass by 85 % ($p < 0.001$) relative to the control and was also significantly lower as compared to the biomass treatment (Table 4). There was no effect of either treatment on EEAs (Table 4) and there were only small effects on predicted functional pathways with a lower relative abundance for alcohol degradation pathways in the biomass plots (p = 0.031) and a lower relative abundance of pathways for polymeric compound degradation in the cellulose plots (p = 0.026).

There was a significant effect of the carbon addition treatments on most soil characteristics with plants absent except for SOM, NO_3^-, and soil C:N ratio (Table 3). Both biomass and cellulose addition were associated with significant decreases in soil pH and a significant increase in NH_4^+ concentration while biomass addition was related to a significant increase in percent SWC (Table 3). Biomass addition increased POXc by 26 % ($p < 0.001$) and increased soil CO_2 flux by 30 % (p = 0.03) while there was no effect of cellulose on either of these metrics (Table 3).

Structural equation modeling revealed that there were different effects of soil characteristics on ASV diversity in the biomass and cellulose treatments when plants were absent (Fig. 6A and 6B). The cellulose model explained more variation in ASV diversity than the biomass model ($R^2 = 0.82$, $R^2 = 0.65$; respectively). Biomass addition had positive effects on soil NH_4^+ and SWC which had positive effects on ASV diversity. In contrast the cellulose model included more paths than the biomass model since microbial biomass (MB) significantly impacted ASV diversity which was not the case in the biomass model. Overall, in the cellulose

model MB was negatively affected by cellulose addition by both direct and indirect effects (Fig. 6B). Then ASV diversity was negatively affected by MB but positively affected by NH_4^+.

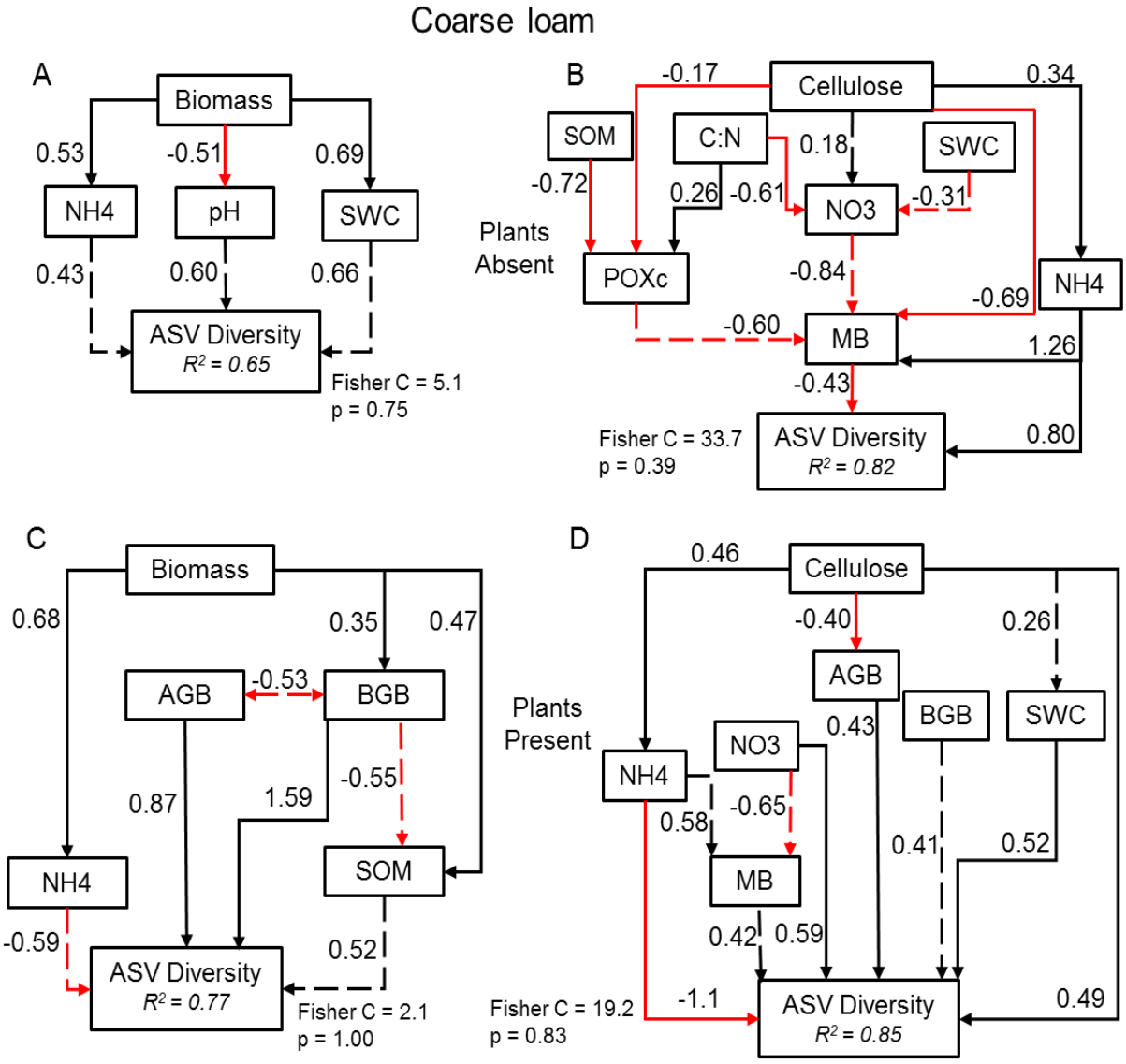

Figure 12. Structural equation models for the coarse loam soil. Structural equation models exploring the effects of the carbon addition treatments on soil and plant characteristics and overall relationship with ASV diversity in the coarse loam. Black arrows indicate positive effects and red arrows indicate negative effects. Paths that are significant ($p < 0.05$) are shown as solid lines while paths that are marginally significant ($0.05 < p < 0.15$) are shown as dashed lines. Numbers adjacent to lines are standardized path coefficients and conditional R^2 values (based on fixed and random effects) are reported for ASV diversity. All models are well supported by the data ($p > 0.05$) and the Fisher's C statistic and p value are shown next to each model.

Carbon Treatment Effects with Plants Present in the Coarse Loam

Bacterial community composition in the cellulose plots with plants present differed significantly from the communities in the control plots with plants present ($p = 0.042$) but not the biomass plots (Fig. 4A). Cellulose addition shifted the relative abundances of several bacterial phyla while there were no effects of biomass addition on these phyla. For example, in the cellulose amended plots the relative abundance of Acidobacteria decreased by 15 % (Fig 5, $p = 0.04$). Conversely, there was a 65 % increase in the relative abundance of Bacteroidetes ($p < 0.001$) and a 34 % increase in the relative abundance of Verrucomicrobia ($p < 0.001$). In contrast to when plants were absent there was no significant effect of either carbon addition treatment on ASV richness or Shannon diversity when plants were present (Table 4). Bacterial lipid biomass was not affected by either treatment, but biomass addition increased fungal lipid biomass by 256 % ($p = 0.009$) while there was no effect of cellulose addition on fungal lipid biomass (Table 4). Biomass addition also increased total lipid biomass with an increase of 61 % which was marginally significant ($p = 0.08$). There were no differences in EEAs between the carbon addition treatments with plants present (Table 4) while there were some differences in predicted functional pathways. Both biomass and cellulose treatments increased secondary metabolite ($p = 0.004$ and $p < 0.001$; respectively) and carboxylate degradation ($p = 0.047$ and $p = 0.021$; respectively) while only cellulose addition led to a decrease in nucleoside and nucleotide degradation ($p = 0.038$).

Similar to when plants were absent, when plants were present there was a significant effect of the carbon addition treatments on most soil characteristics (Table 3). Both biomass and cellulose addition increased NH_4^+ concentration and decreased soil pH, but SWC was only higher in the biomass amended plots while NO_3^- concentration was only higher in the cellulose

plots (Table 3). In contrast to when plants were absent, SOM increased by 10 % ($p = 0.004$) and the C:N ratio increased by 16 % ($p = 0.008$) in the biomass plots relative to the control while there was no effect of cellulose addition (Table 3). Biomass addition was associated with a 17% increase in POXc ($p = 0.007$) and a 26 % increase in CO_2 flux ($p = 0.01$) relative to the control, while there was no effect of cellulose addition on these metrics. Biomass addition was also associated with a 100% increase in BGB relative to the control ($p = 0.03$) and significantly decreased soil bulk density while cellulose addition decreased plant AGB by 25 % ($p = 0.002$; Table 4).

Structural equation models constructed for biomass and cellulose treatments when plants were present explained similar amounts of variation in ASV diversity ($R^2 = 0.77$ and $R^2 = 0.85$, Fig 6 C and D; respectively). In both models, treatment was associated with direct increases in NH_4^+ concentrations, which in turn had a direct negative effect on ASV diversity. Also, in both models plant AGB and BGB positively affected ASV diversity while biomass addition increased BGB and cellulose addition decreased AGB. In the cellulose model only, MB had a positive effect on ASV diversity. In the biomass model BGB had a positive effect on SOM which had a positive effect on ASV diversity (Fig. 6C). Cellulose addition had a directly increased ASV diversity while there was no direct effect of biomass addition on diversity (Fig 6D).

Overall, in the coarse loam soil the presence of plants had a large effect on the structure of the soil bacterial communities and had significant impacts on soil characteristics as well. Conversely, cellulose addition also had a significant impact on the composition of the bacterial communities while biomass addition had a lesser effect. However, biomass addition increased metrics related to carbon storage more than the cellulose addition which had little effect on these metrics. Biomass and cellulose addition also differed in how they impacted ASV diversity both

when plants were absent and when they were present mainly due to how they effected soil characteristics.

Discussion

There were similar but different responses in the two soil types to the carbon addition treatments and plant presence, suggesting that the same treatments may not be able to be universally applied on multiple soil types. In addition, cellulose and plant biomass application yielded different results for both soil and microbial characteristics where biomass addition increased metrics related to carbon storage and cellulose addition had more of an impact on the bacterial community. Conversely, plants modulated the effect of both of the carbon addition treatments on both soil and microbial characteristics with plants increasing carbon storage metrics and leading to shifts in the microbial community.

Biomass and Cellulose Addition are not Equivalent

Overall, our results suggest that plant biomass addition and cellulose addition are not equivalent soil amendments. We found that in both soil types biomass addition increased metrics related to carbon storage, such as POXc, and SOM, while there were no changes in these metrics with pure cellulose addition one year post restoration. Furthermore, these changes were most evident in the plots where plants were present. We also found that there were more shifts in soil and microbial characteristics in the coarse loam than in the fine loam, but that the shifts in metrics related to carbon storage were similar in both soils. We initially compared pure cellulose and plant biomass because while cellulose addition has been shown to increase SOM storage in an earlier study (Docherty & Gutknect, 2019) it is relatively expensive to implement on a large-scale costing at least 30 dollars and ranging upwards of 100 dollars per kilogram depending on

the supplier. In comparison mowed plant biomass is relatively cheap and easy for mangers to come by as they most likely are already mowing prairies and fields on their property for restoration and aesthetic reasons making biomass addition a much more practical approach. Also, it seems that biomass addition may be better, at least in the short-term, at increasing metrics related to carbon storage.

In both soil types, permanganate oxidizable carbon (POXc) significantly increased with biomass addition when plants were present and there were overall greater increases in the coarse loam than in the fine loam. POXc is a measure of the biologically active carbon pool in the soil and represents a more processed and stabilized pool of labile carbon (Culman et al., 2012). According to multiple studies (Culman et al., 2012; Hurisso et al., 2013; Lussier et al., 2020) this makes POXc a good indicator for practices that aim to increase carbon sequestration, such as ours, as POXc is generally more recalcitrant than other forms of labile carbon (Thoumazeau et al., 2020). In addition, the amount of SOM increased with biomass addition which in likely due to a combination of the addition of the biomass itself, but also degraded forms the added biomass and microbial biomass. Cellulose addition did not lead to increases in either POXc or SOM indicating that addition of pure cellulose into reclaimed prairie soils is not effective at increasing metrics related to carbon storage in the short term. Additionally, SEMs showed that biomass addition had a positive effect on SOM in all but one model (Fig. 6A), whereas there was only a single model in which cellulose addition impacted SOM (Fig. 3D) which again suggests that these additions do not have equivalent impacts on soil carbon metrics. The differences in these carbon metrics our two carbon addition treatments are likely due to the higher diversity of substrates produced from decomposing plant biomass vs. degradation products that can result from pure cellulose. For example, the degradation of cellulose is likely to lead to the production

of hemicelluloses and simple sugars such as glucose while plant biomass contains cellulose it also contains a variety of other compounds such as lignin, hemicelluloses, and simple sugars which may result in a more diverse variety of byproducts from their degradation. Another possibility is that the differences we observed are due to a larger amount of carbon added to the soil through the plant biomass addition, as we used 2.8 kg of *Schizachyrium scoparium* (little bluestem) biomass to reach similar concentrations of cellulose into the soil as the cellulose treatment, and not total carbon.

In addition to differences in soil characteristics in the cellulose and biomass treatments we also found that shifts in microbial characteristics varied between the two amendments as well, and that these shifts were more pronounced in the coarse loam. For example, both Verrucomicrobia and Bacteroidetes were more abundant in the cellulose treatment in the coarse loam as compared to the control but there were no changes in the abundance of these bacterial phyla with biomass addition. These observations are in agreement with other studies that have found both Bacteroidetes and Verrucomicobia increase in relative abundance with cellulose addition (Docherty & Gutknecht, 2019; Pepe-Ranney et al., 2016; Štursová et al., 2012). We expected that since the plant biomass that was added to the soil contained roughly equivalent amounts of cellulose as compared to the cellulose treatment that both Verrucomicrobia and Bacteroidetes would also increase in abundance, however that was not the case. Instead, biomass addition did not lead to any shifts in either of these phyla in both soil types. This may be explained by the higher diversity of substrates contained in plant biomass as compared to pure cellulose that may support the growth of more diverse groups of bacteria, but this effect may differ by soil type. Previous work provides evidence that straw addition does not stimulate increased bacterial diversity, but that it supports higher microbial biomass (Ahn et al., 2012; Sun

et al., 2015; Yuan et al., 2013). In agreement with this, our SEMs showed that in the fine loam, both cellulose and biomass addition generally had minimal or negative effects on bacterial diversity, through a variety impacts on soil characteristics. Conversely, in the coarse loam, our SEMs revealed that there were positive effects of biomass and cellulose addition on bacterial diversity, which suggests that the response of bacterial diversity to these additions may be modulated by soil type and the soils inherent characteristics. Even though there were shifts in bacterial community composition with the cellulose treatment, there were very few effects on predicted community functionality, suggesting that while cellulose addition may shift bacterial community composition that the changes does not affect the function of the community. Biomass addition also resulted in very few shifts in predicted community functionality.

In both the coarse and fine loam soils there was a trend towards increased total lipid biomass and fungal lipid biomass with biomass addition in plots where plants were present. However, cellulose addition did not have the same effect. In natural systems fungi dominate the decomposition of more recalcitrant organic matter, such as linin and cellulose (Boer et al., 2005; Güsewell & Gessner, 2009) which may explain why there was a trend towards increased fungal lipid biomass with the biomass addition treatment. This increase in fungal lipid biomass, which was more prominent in the coarse loam, may also have further implications for increased carbon storage, as increased fungal lipid biomass has been associated with increased soil carbon storage potential (Jastrow et al., 2007). The increase in fungal lipid biomass with biomass addition in the coarse loam may also be in part due to an increase in root biomass (BGB) which may have also led to increased associations between plant roots and arbuscular mycorrhizal fungi (AMF). While we did not explicitly examine the prominent lipid biomarker from AMF (16:1ω5c)

(Ngosong et al., 2012) some AMF also produce the biomarker 18:1 ω9c (Graham et al., 1995) which we included in calculating fungal biomass.

One concern for land managers who wish to implement these practices to create a climate-ready prairie restoration is the generation of a priming effect where more soil carbon is emitted as CO_2 than stored in the soil due to increased microbial activity (Fontaine et al., 2011; Fontaine et al., 2004; Fontaine et al., 2004; Wu et al., 2019). However, we did not observe this with the biomass addition treatment as there was an increase in both POXc and SOM both soils when plants were present. While biomass addition likely did lead to a priming effect, the amount of new carbon that was added to the soil may have offset this effect resulting in an increase in the amount of carbon stored in the soil. This has been indicated by other studies in which the addition of plant residues into the soil leads to a priming effect in which old soil organic carbon is mineralized to carbon dioxide, but that the addition of new carbon offsets the loss of older organic carbon (Liang et al., 2018; Xu et al., 2019). Specifically, more than 5 g of biomass input per 100 g of soil has been shown to offset the priming effect (Xu et al., 2019) and while we did not measure the specific biomass to soil ratio we likely added more biomass than needed to offset the PE effect.

Effects of Plants on Soil and Microbial Characteristics

In both the fine and coarse loam soils plants had an impact on soil characteristics, however the effect of plants on these characteristics were more prominent in the coarse loam soil. In both soils, plants increased metrics related to soil carbon storage with the most notable increases in POXc and SOM. POXc represents a labile carbon pool, so the increases in POXc with the presence of plants may be explained by the accumulation of fresh plant inputs, such as root exudates, and the accumulation of plant litter (Six et al., 2006). Increases in SOM with plants

present is also explained in a similar manner because inputs of fresh plant litter can also lead to increases in SOM (Six et al., 2006). Furthermore, plant roots are considered to be hotspots for organic matter formation, as plant roots transfer carbon to the soil through root exudates and dead root biomass (Vidal et al., 2018).

The presence of plants also had a significant effect on soil bacterial communities and other microbial characteristics. In both the fine and coarse loam, the presence of plants shifted the soil bacterial community structure as compared to the plots without plants which would be expected as plants select for communities that are distinct from those found in bulk soil (Turner et al., 2013). In both soil types, there was an increase in the relative abundance of Bacteroidetes and a decrease in the relative abundances of Acidobacteria when plants were present, though these trends were not always significant. Acidobacteria are a thought to be a slower growing bacteria phylum with low max growth rates but high growth yields and high substrate affinity that are more abundant in nutrient-poor bulk soil than in the plant rhizosphere (Fierer et al., 2007). Conversely, Bacteroidetes tend to be more abundant in the rhizosphere than in bulk soil which likely explains why there was an increase in this phyla when plants were present (Vieira, 2018). The presence of plants did not seem to modulate bacterial diversity in either soil type, though SEMs showed that that plant biomass had positive effects on bacterial diversity in the coarse loam with both the biomass and cellulose treatments. There are multiple studies that indicate that there are varying impacts of plants on bacterial diversity (e.g. Baruch et al., 2020; Prashar et al., 2014; Zul et al., 2007), so further research is needed into why these different effects occur but these different effects may be due to in part differences in soil type (Singh et al., 2007).

In addition to differences in the bacterial community, fungal lipid biomass increased in both the fine and coarse loams when plants were present. This increase in fungal biomass is

likely due to higher amounts of root exudates where plants were present as suggested by Eisenhauer et al. (2017) that found increased fungal biomass is linked to increases in root exudates. Also, increases in fugal biomass may be due to associations between AMF and plant roots as well. While we did not explicitly measure plant root biomass in plots where plants were absent, we expect that root biomass was low due to plant exclusion.

Soil Types

Overall, there were differences with both the carbon addition treatments and the presence or absence of plants in the different soil types. The coarse loam, which is an Alfisol, had greater shifts in both soil and microbial characteristics with the carbon addition treatments than in the fine loam which is a Mollisol. These differences may be partially attributed to differences in clay content in the soils with the fine loam generally having a higher clay content (~16 %) than the coarse loam (~12 %) as higher clay content can lead to slower rates of carbon accumulation (West & Six, 2007). Also, the fine loam soil had more SOM and active carbon (POXc) than the coarse loam soil overall which can also lead to slower rates of carbon accumulation, due to already higher carbon stores, and act as a buffer against changes in other soil characteristics such as pH (Jansen van Rensburg et al., 2009; West & Six, 2007).

The mitigation of the shifts in soil characteristics in the fine loam may explain why we observed fewer shifts in microbial characteristics compared to the coarse loam. For example, there was an overall shift in the bacterial community due to cellulose addition in the coarse loam while there was no overall shift in the bacterial community structure due to either of the carbon addition treatments in the fine loam. This may be because there were larger changes in soil pH, NH_4^+, NO_3^+, and SWC which are important factors that can lead to shifts in bacterial community composition (e.g. Fierer et al., 2012; Lauber et al., 2009). Additionally, higher soil clay content

can protect microbes from predation, changes in pH, and desiccation(Bushby & Marshall, 1977; Elliott et al., 1980; Stotzky & Rem, 2011) which may explain why there were more shifts in bacterial community structure in the coarse loam as well. Soils with higher clay contents can also support more microbial biomass (Franzluebbers et al., 1996) which we observed in our study with there being 83 % more microbial biomass in the fine loam than the coarse loam. This may be explained by differences in clay content but may also be due to different levels of soil carbon pools as well. Our study along with others (e.g. Bach et al., 2010; Jangid et al., 2010) highlights the importance of testing restoration efforts in multiple soils types as different soil types can results in different effects of treatments additions and plants on both soil and microbial characteristics that may potentially set restorations on different trajectories. Overall, similar trends were observed in these similar soils but there were still differences, suggesting that soil types that differ more in texture may result in very different results. In addition, biomass addition may work more on coarse soils with less organic matter than in soils with a finer texture and larger amounts of organic matter, but more work is needed on soil types that vary in both their texture and organic carbon levels to discern these varying effects.

Implications for Management

Our results demonstrate that the addition of plant biomass into new prairie restorations on two different soil types leads to increases in metrics related to carbon storage (SOM and POXc) when plants are present in a relatively short period of time. This suggests that when installing new prairie restorations on former agricultural land, tilling biomass into the soil before planting may incur similar benefits as observed in our study. While it is unrealistic to expect managers to till plant biomass 30 cm into the soil profile, Ma et al., (2018) found that addition of plant biomass 15 cm into the soil increases soil organic carbon, so this depth could likely be decreased.

Also, the amount of biomass used, 2.8 kg/m^2 of soil, may also be an impediment to implication on larger scales that are normal for prairie restorations. However, less biomass can potentially be used as long as enough biomass is added to offset the priming effect that is likely occurring with biomass addition, but further study is required to determine if the addition of less biomass will work in a prairie setting. While plant biomass addition is a promising strategy for increasing carbon stores in the short term, there were relatively few effects on the microbial community, especially in the fine loam. We do not know if these increases in soil carbon metrics will still be apparent longer-term or if biomass addition will lead to decreases in carbon stocks in the long term, so further study is required as these restorations age.

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APPENDIX A

Supplemental Information for Chapter 1

Supplementary Methods: We conducted *a priori* analyses to ensure that underlying variation would not influence experimental results. Prior to burning, we examined the soil edaphic factors and the soil bacterial communities in all our plots to make sure that the control (plots left unburned) and experimental plots (to be burned) were not substantially different due to underlying variation in each prairie. Overall, we did not find any differences between the control and experimental plots in either prairie before the prescribed burn occurred. Briefly, we examined the Shannon diversity Index, ASV richness, and bacterial community structure using the methods stated in the main text. We also tested whether within-treatment community variation differed between control (unburned) and (experimental) burned plots pre-fire using the betadisper function in R (Anderson, 2006). We also determined whether plots that were closer together had more similar bacterial communities than plots that are more spatially distant. Distance matrices based on plot GPS coordinates were generated using the spDists command in the sp v1.4-1 package (Pebesma and Bivand, 2005), and Mantel tests were used to determine correlations between weighted UniFrac and plot distance matrices (Legendre et al., 2012). 16S rRNA-based bacterial community structure in experimental and the control plots did not differ significantly pre-fire in both the restored and remnant prairie (permaova; $p = 0.181$ and $p = 0.06$, respectively; Fig. S2). There was no difference in variation (or distance to centroid) between control and experimental plots in the restored prairie ($p = 0.543$, Table S3). There were differences in variation (or larger distances from centroid) in bacterial communities in the experimental versus control plots in the remnant prairie ($p = 0.031$, Table S3). Neither Shannon diversity or ASV richness differed between experimental and control plots in either the restored

or remnant prairie pre-fire ($p > 0.05$; Table S4) and there were no differences in bacterial community structure based on their plot locations in either the remnant prairie or the restored prairie pre-fire (Mantel $r = 0.29$, $p = 0.086$ and Mantel $r = -0.27$, $p = 0.904$; respectively). There were also no differences in soil pH, nitrate concentration, ammonium concentrations, SWC, and SOM based on their plot locations in either the remnant prairie or the restored prairie pre-fire ($p > 0.05$, Table S4). Given these results, we concluded that there was no underlying variation which would confound the results at either field site.

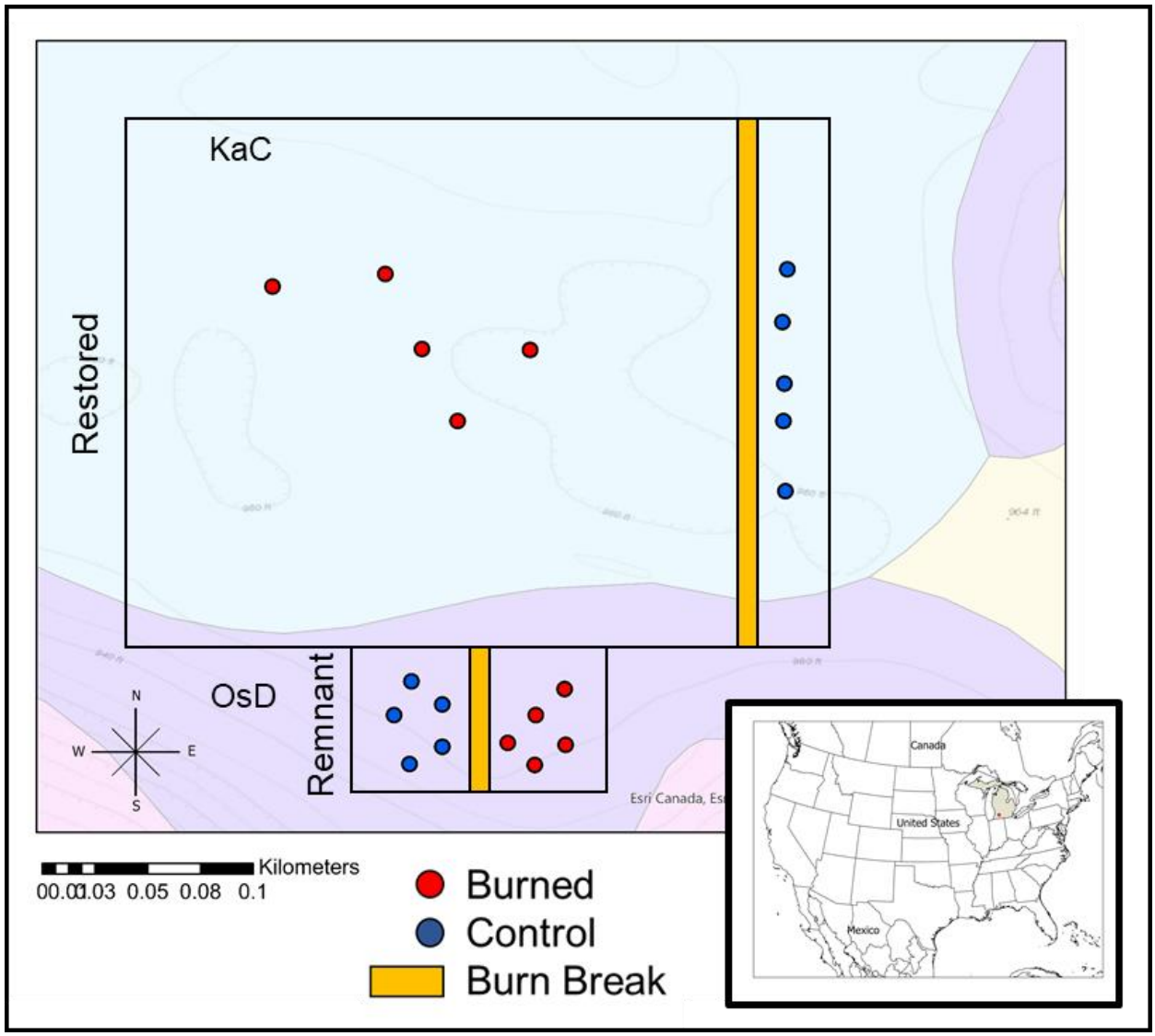

Figure S1. Experimental design of prescribed burn in both the restored and remnant prairies. Red circles denote locations of the plots in the burned portion of each prairie while blue circles represent the locations of the unburned (control) plots. The yellow rectangles indicate where the burn breaks were in each prairie. Contour lines are provided to indicate changes in elevation. The two soil types (KaC and OsD) that make up each prairie are indicated on the map.

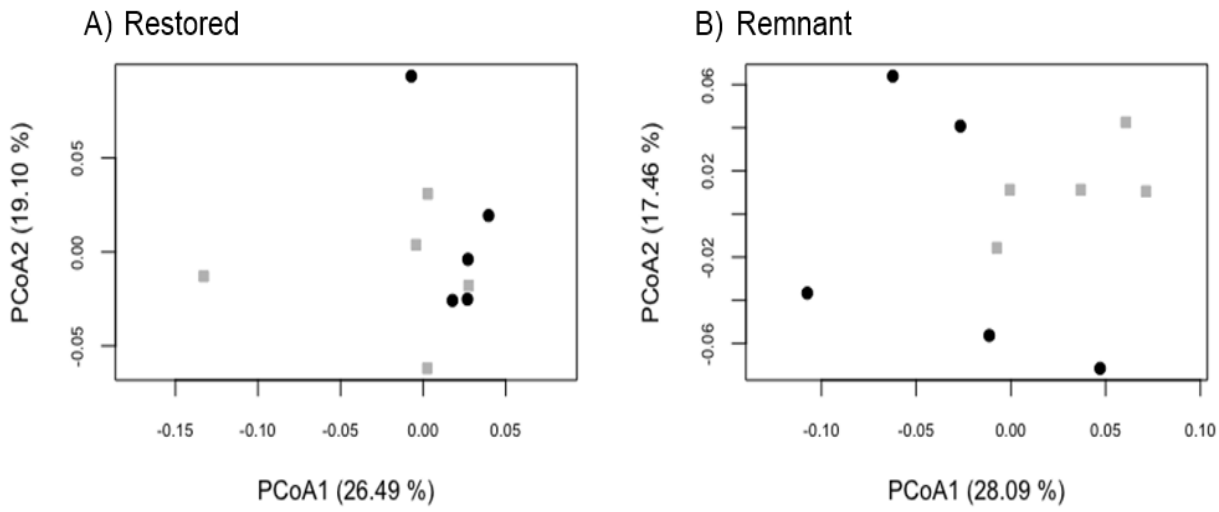

Figure S2. Principal coordinates analysis (PCoA) ordinations based on weighted UniFrac distances between pre-fire bacterial communities identified using 16S rRNA amplicon sequencing for control (black circles) and experimental (gray squares) plots in **A)** the restored prairie and **B)** the remnant prairie. Significance was determined at $\alpha = 0.05$. In both the restored and remnant prairie, the bacterial communities did not differ between the control and experimental plots pre-fire ($p = 0.181$ and $p = 0.06$, respectively).

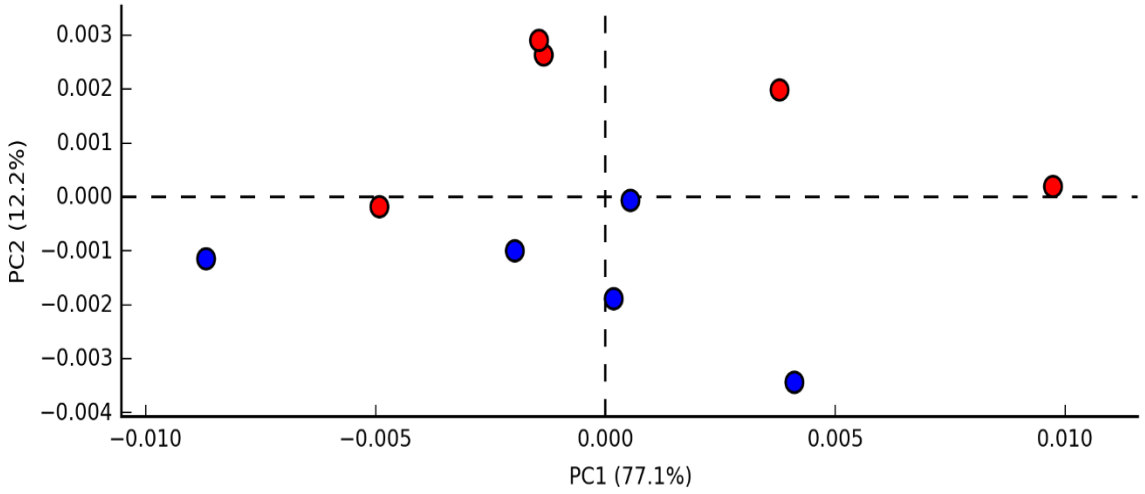

Figure S3. PCA ordination based on the STAMP analysis of the relative abundance of functional gene categories at KEGG level 3 for burned (red) vs control (blue) plots 1-day post-fire in the remnant prairie. Significance was determined at $\alpha = 0.05$. *P*-values were corrected using the Storey false discovery rate ($p < 0.05$). There were no significant differences in the relative abundance of functional gene categories between the burn treatments.

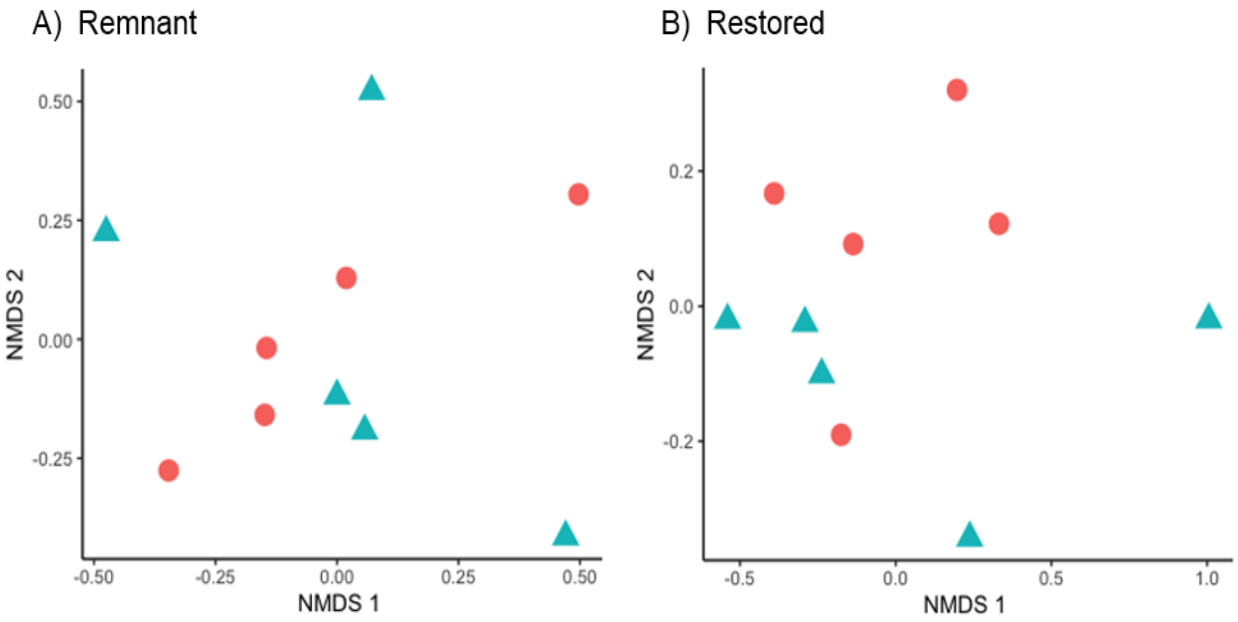

Figure S4. NMDS ordinations of plant communities 11-months post-fire in the **A)** remnant prairie and **B)** the restored prairie. Blue triangles represent the control plots while red circles represent the burned plots. Significance was determined at $\alpha = 0.05$. There were no significant differences between treatments in the remnant and restored prairies ($p = 0.434$ and $p = 0.34$, respectively).

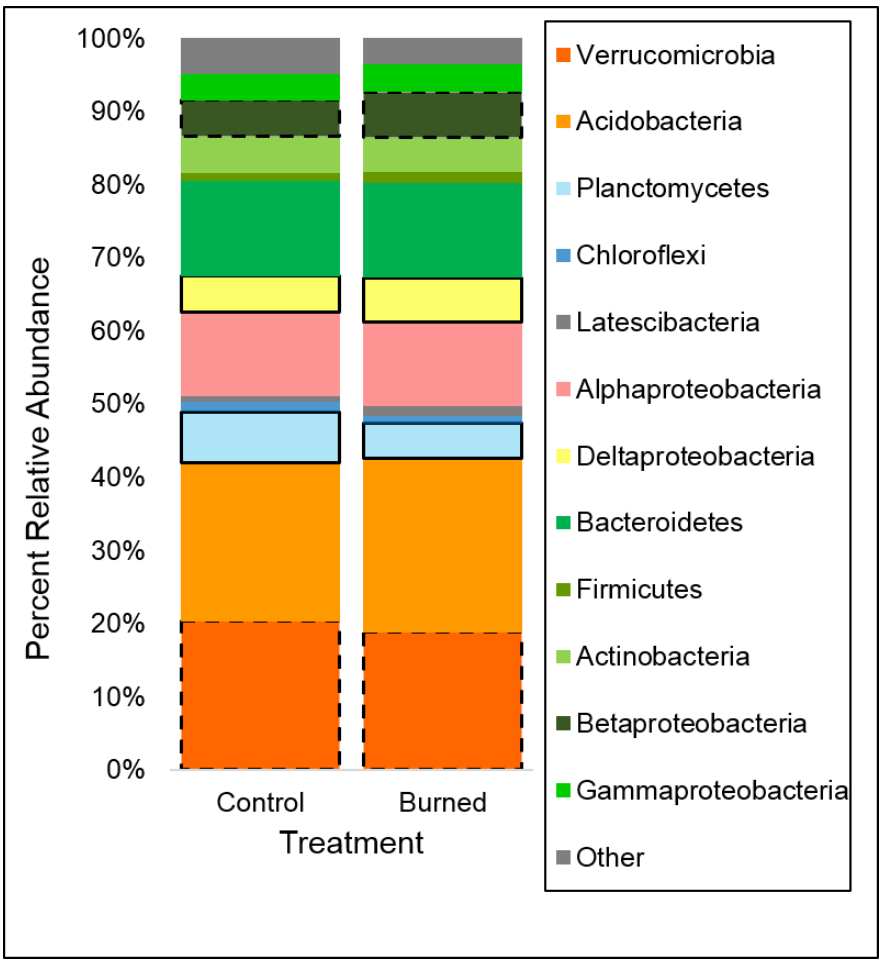

Figure S5. Percent relative abundance of bacterial phyla that comprise >1 % relative abundance in control and burned treatments in the restored prairie 11-months post-fire. Phyla that differ significantly between treatments are indicated with bold lines and phyla that were marginally different were indicated with a dashed line. Significance was determined at $\alpha = 0.05$.The relative abundance of Deltaproteobacteria was significantly higher in the burned treatment while the abundance of Planctomycetes was significantly lower as compared to the control in the restored prairie ($p = 0.046$ and $p < 0.001$, respectively). The relative abundance of Betaproteobacteria marginally increased in the burned plots of restored prairie while the relative abundance of Verrucomicrobia marginally decreased ($p = 0.086$ and $p = 0.088$, respectively).

Table S1. GPS coordinates of each plot in both the restored and remnant prairie.

Prairie Type	Treatment	Plot Number	GPS Coordinate
Restored	Control	Con_1	42.3296492N, 85.6707167W
Restored	Control	Con_2	42.3294143N, 85.6707380W
Restored	Control	Con_3	42.3291410N, 85.6707295W
Restored	Control	Con_4	42.3289745N, 85.6707337W
Restored	Control	Con_5	42.3286627N, 85.6707252W
Restored	Burned	Burn_1	42.3295724N, 85.6729973W
Restored	Burned	Burn_2	42.3296279N, 85.6724976W
Restored	Burned	Burn_3	42.3292948N, 85.6723353W
Restored	Burned	Burn_4	42.3292905N, 85.6718570W
Restored	Burned	Burn_5	42.3289745N, 85.6721773W
Remnant	Control	Con_1	42.3278171N, 85.6723823W
Remnant	Control	Con_2	42.3277146N, 85.6722456W
Remnant	Control	Con_3	42.3276676N, 85.6724592W
Remnant	Control	Con_4	42.3275267N, 85.6722456W
Remnant	Control	Con_5	42.3274498N, 85.6723908W
Remnant	Burned	Burn_1	42.3277829N, 85.6717032W
Remnant	Burned	Burn_2	42.3276676N, 85.6718313W
Remnant	Burned	Burn_3	42.3275437N, 85.6719552W
Remnant	Burned	Burn_4	42.3275352N, 85.6716989W
Remnant	Burned	Burn_5	42.3274455N, 85.6718356W

Table S2. Number of sequences per sample by time point for the restored and remnant prairie.

	Restored	Remnant
Time point	Sequences per sample	Sequences per sample
Pre-Fire	1533	1425
1-day post-fire	1533	1448
1-month post-fire	2033	2586
7-months post-fire	28204	52973
11-months post-fire	47408	40579

Table S3. Pre-fire soil bacterial community characteristics in the restored and remnant prairie. Average diversity and richness ± 95% confidence intervals are presented.

	Restored		Remnant	
Characteristics	Control	Experimental	Control	Experimental
Shannon diversity (ASVs)	6.67 ± 0.27	6.59 ± 0.083	6.2 ± 0.21	6.19 ± 0.15
ASV Richness	183.6 ± 35.88	183.4 ± 11.73	166 ± 22.95	147.2 ± 13.84
Distance To centroid	0.074	0.068	0.104	**0.131**

ASV: Amplicon Sequence Variant
Significance was assessed at $\alpha = 0.5$
Bold text indicates significance at $p < 0.05$

Table S4. Mantel test results between plot locations for five different edaphic factors and bacterial ASV composition in the remnant and restored prairie pre-fire. No significant differences were detected at $\alpha = 0.05$.

	Remnant		Restored	
Soil Characteristic	Mantel r	p value	Mantel r	p value
pH	-0.054	0.54	-0.225	0.88
SWC	0.034	0.37	0.041	0.35
SOM	-0.290	0.99	-0.061	0.58
NO_3^-	-0.057	0.58	-0.029	0.51
NH_4^+	0.081	0.27	-0.200	0.86
Bacterial Community	0.290	0.09	-0.270	0.90

SWC: Soil Water Content; SOM: Soil organic matter

Table S5. Average soil and plant characteristics over time in the restored prairie (± 95% confidence interval).

Characteristics	Control Sep. 2013	Burned Sep-13	Control Oct. 2013	Burned Oct. 2013	Control April 2014	Burned April 2014	Control Aug. 2014	Burned Aug. 2014
pH	6.1 ± 0.2	**7.3 ± 0.4**	6.4 ± 0.1	6.6 ± 0.2	6.5 ± 0.2	**7.2 ± 0.2**	6.7 ± 0.2	**7.0 ± 0.1**
Soil Temperature (°C)	16.7 ± 0.3	**14.6 ± 1.1**	7.2 ± 0.5	7.8 ± 0.8	6.8 ± 0.4	**8.1 ± 0.6**	13.7 ± 0.4	14.3 ± 0.7
SWC (%)	10.3 ± 1.1	**12.4 ± 0.7**	19.3 ± 1.3	21.4 ± 2.5	22.7 ± 1.5	20.1 ± 3.6	9.6 ± 0.7	12.6 ± 3.3
SOM (mg)	231.5 ± 23.7	ND	277.5 ± 52.4	324.4 ± 86.8	322.9 ± 68.4	402.7 ± 72.4	421.5 ± 18.1	**553.0 ± 56.9**
NO_3^- (µg • g dry soil^{-1})	99.8 ± 86.1	71.9 ± 33.0	195.9 ± 73.5	276.2 ± 85.4	23.6 ± 16.5	55.1 ± 38.1	13.0 ± 7.8	**125.0 ± 33.0**
NH_4^+ (µg • g dry soil^{-1})	144.2 ± 75.5	134.6 ± 55.2	128.5 ± 12.1	100.5 ± 22.5	156.1 ± 50.3	119.3 ± 16.6	142.1 ± 56.6	178.5 ± 81.9
Total Phosphorus (ppm)	7.6 ± 3.5	6.2 ± 1.4	5.7 ± 2.3	4.6 ± 2.6	7.7 ± 3.1	7.7 ± 3.7	4.7 ± 1.8	3.3 ± 1.3
AGB (g • m^{-2})	648.0 ± 126.4	ND	121.1 ± 56.8	**5.1 ± 6.0**	ND	ND	744.4 ± 160.9	877.7 ± 228.6
AGL (g • m^{-2})	916.0 ± 171.1	ND	1276.8 ± 249.7	**399.2 ± 154.7**	ND	ND	870.2 ± 253.8	**381.8 ± 162.3**
BGB (g)	1.7 ± 0.2	ND	3.5 ± 2.3	1.9 ± 0.9	ND	ND	5.0 ± 1.2	5.3 ± 3.3
β-gluosidase (nmol•[g ODE soil] $^{-1}$• h^{-1})	521.2 ± 163.3	**167.2 ± 93.4**	571.4 ± 378.5	819.8 ± 221.8	361.9 ± 69.2	**657.1 ± 213.7**	66.7 ± 33.4	104.1 ± 32.8
Cellobiohydrolase (nmol•[g ODE soil] $^{-1}$• h^{-1})	129.8 ± 60.5	96.3 ± 69.9	148.7 ± 39.4	197.0 ± 54.6	55.6 ± 17.7	96.9 ± 61.6	36.2 ± 21.2	57.2 ± 16.0
NAG (nmol•[g ODE soil] $^{-1}$• h^{-1})	211.1 ± 56.7	286.3 ± 188.8	345.0 ± 105.5	295.8 ± 66.2	102.9 ± 11.9	129.9 ± 67.6	50.2 ± 26.8	87.1 ± 26.2
Phosphatase (nmol•[g ODE soil] $^{-1}$• h^{-1})	3480.1 ± 921.1	**1489.8 ± 522.8**	3817.9 ± 1643.7	4235.6 ± 763.1	2474.1 ± 347.9	1990.1 ± 644.2	259.8 ± 49.3	**408.7 ± 62.8**
Shannon diversity (ASVs)	6.7 ± 0.3	**6.3 ± 0.2**	6.8 ± 0.2	6.9 ± 0.1	9.4 ± 0.2	9.3 ± 0.2	9.4 ± 0.3	9.1 ± 0.1
ASV Richness	184.0 ± 35.9	156.0 ± 25.2	205.0 ± 21.0	215.5 ± 16.8	1289.0 ± 274.4	1386.0 ± 146.8	1480.0 ± 209.5	1217.0 ± 170.9

SWC: Soil Water Content; AGB: Above Ground Biomass; AGL: Above Ground Litter; BGB: Below Ground Biomass; ODE: oven-dried equivalent; NAG: N-acetylglucosaminidase; SOM: Soil Organic Matter; ASV: Amplicon Sequence Variants

Bold Text indicates significance at $p < 0.05$ in the comparison of burned and control treatments at each time point.

Table S6. Average soil and plant characteristics over time in the remnant prairie (± 95% confidence interval).

Characteristics	Control Sept. 2013	Burned Sept. 2013	Control Oct. 2013	Burned Oct. 2013	Control April 2014	Burned April 2014	Control Aug. 2014	Burned Aug. 2014
pH	6.1 ± 0.2	**7.1 ± 0.4**	5.9 ± 0.3	**6.8 ± 0.6**	6.5 ± 0.2	**7.0 ± 0.3**	6.4 ± 0.2	6.6 ± 0.1
Soil Temperature (^{o}C)	15.1 ± 0.8	**19.2 ± 2.7**	8.0 ± 0.5	8.0 ± 0.9	8.7 ± 0.3	9.0 ± 1.0	18.4 ± 0.9	20.8 ± ND
SWC (%)	12.8 ± 1.5	10.8 ± 2.2	21.5 ± 1.7	22.2 ± 1.7	21.2 ± 1.7	21.5 ± 1.2	12.3 ± 1.0	12.2 ± 2.8
SOM (mg)	206.5 ± 17.6	ND	286.7 ± 72.5	346.4 ± 33.4	379.6 ± 26.9	367.8 ± 57.8	476.2 ± 78.2	574.0 ± 87.2
NO_3^- (µg • g dry soil^{-1})	20.3 ± 19.5	56.0 ± 42.5	105.0 ± 59.6	115.8 ± 36.5	55.3 ± 38.6	12.7 ± 7.8	25.5 ± 26.9	**134.0 ± 21.4**
NH_4^+ (µg • g dry soil^{-1})	53.9 ± 11.5	**153.8 ± 44.5**	68.4 ± 14.5	100.1 ± 92.0	86.2 ± 41.9	**183.5 ± 57.1**	124.7 ± 31.1	162.6 ± 48.1
Total Phosphorus (ppm)	4.5 ± 1.6	5.4 ± 1.7	3.8 ± 1.6	7.3 ± 7.4	4.8 ± 1.5	7.3 ± 3.8	2.7 ± 1.0	4.2 ± 2.0
AGB (g • m^{-2})	459.7 ± 186.3	ND	192.3 ± 92.1	**16.1 ± 27.5**	ND	ND	605.6 ± 106.0	688.2 ± 181.7
AGL (g • m^{-2})	414.0 ± 264.6	ND	926.8 ± 138.3	560.5 ± 311.0	ND	ND	417.6 ± 74.4	334.5 ± 174.5
BGB (g)	3.9 ± 1.4	ND	3.1 ± 0.8	3.4 ± 2.1	ND	ND	8.0 ± 2.2	7.3 ± 2.7
β-gluosidase (nmol•[g ODE soil] $^{-1}$• h^{-1})	166.1 ± 81.9	91.1 ± 82.8	359.8 ± 80.6	643.9 ± 266.7	228.8 ± 132.8	383.7 ± 203.1	62.7 ± 26.3	**114.3 ± 74.3**
Cellobiohydrolase (nmol•[g ODE soil] $^{-1}$• h^{-1})	141.9 ± 65.4	98.4 ± 45.4	77.6 ± 50.0	116.2 ± 39.2	121.6 ± 98.3	115.1 ± 69.2	71.4 ± 11.8	138.1 ± 175.2
NAG (nmol•[g ODE soil] $^{-1}$• h^{-1})	368.8 ± 225.5	297.3 ± 200.3	124.9 ± 47.9	**257.1 ± 100.4**	346.9 ± 287.9	277.4 ± 229.3	76.3 ± 30.5	165.0 ± 49.5
Phosphatase (nmol•[g ODE soil] $^{-1}$• h^{-1})	2047.5 ± 1157.4	2402.1 ± 1269.2	2746.8 ± 857.2	2909.2 ± 798.7	2697.3 ± 1269.0	3000.8 ± 1146.0	377.3 ± 140.3	399.1 ± 226.3
Shannon diversity (ASVs)	6.2 ± 0.2	**6.8 ± 0.1**	6.5 ± 0.1	6.7 ± 0.3	9.2 ± 0.4	9.0 ± 0.3	9.0 ± 0.2	9.0 ± 0.2
ASV Richness	166.4 ± 23.0	**203.2 ± 5.3**	189.6 ± 7.8	207.4 ± 39.7	1370.4 ± 243.9	1156.4 ± 244.8	1212.2 ± 166.3	1068.8 ± 152.8

SWC: Soil Water Content; AGB: Above Ground Biomass; AGL: Above Ground Litter; BGB: Below Ground Biomass; ODE: oven-dried equivalent; NAG: N-acetylglucosaminidase; SOM: Soil Organic Matter; ASV: Amplicon Sequence Variants

Bold Text indicates significance at $p < 0.05$ in the comparison of burned and control treatments at each time point.

Table S7. Distance to centroid between the control and burned plots in both the restored and remnant prairie. No distances were significant at $\alpha = 0.05$.

	Restored		Remnant	
Month	Control	Burned	Control	Burned
September	0.081	0.068	0.068	0.073
October	0.081	0.093	0.036	0.036
April	0.020	0.020	0.020	0.015
August	0.032	0.026	0.011	0.011

Table S8. Correlation coefficient (r) between functional gene categories and phyla that significantly differed in abundance between burned and control plots 1-day post-fire.

	Negative Fire-Responders		Positive Fire-Responders	
Gene Category	Betaproteobacteria	Gammaproteobacteria	Deltaproteobacteria	Verrucomicrobia
Cell Division	**0.83**	**0.85**	0.65	-0.61
DNA Replication	**0.79**	0.53	0.66	-0.49
DNA Replication Proteins	0.60	0.51	0.68	-0.41
Translation Proteins	0.42	0.37	0.54	0.04
Base Excision Repair	**-0.85**	**-0.81**	-0.50	0.47
Nucleotide Excision Repair	-0.72	-0.76	-0.37	0.53
Alanine, Aspartate, and glutamate metabolism	**-0.84**	-0.72	-0.45	0.53
Glycine, serine, and threonine metabolism	**-0.84**	**-0.85**	-0.62	0.39
Tyrosine Metabolism	-0.60	-0.66	-0.76	0.34
Carbohydrate Metabolism	**-0.82**	**-0.82**	-0.57	0.74
Galactose Metabolism	**-0.85**	-0.73	-0.51	0.38
Fructose and Mannose Metabolism	-0.73	**-0.79**	-0.57	**0.88**

Bold Text indicates significance at $p < 0.01$

Table S9. Correlation coefficient (r) between functional gene categories and families that significantly differed in abundance between burned and control plots 1-day post-fire.

	Negative Fire-Responders			Positive Fire-Responders
Gene Category	*Desulfuromonadales*	*Flavobacteriaceae*	*Micropepsaceae*	*Xiphinematobacteraceae*
Cell Division	**0.82**	**0.79**	**0.80**	**-0.90**
DNA Replication	**0.83**	**0.87**	0.65	**-0.81**
DNA Replication Proteins	**0.81**	**0.74**	0.48	**-0.78**
Translation Proteins	**0.74**	0.62	0.32	-0.54
Base Excision Repair	-0.50	-0.57	-0.74	0.69
Nucleotide Excision Repair	-0.35	-0.41	-0.71	0.59
Alanine, Aspartate, and glutamate metabolism	-0.35	-0.55	-0.71	0.62
Glycine, serine, and threonine metabolism	-0.71	-0.66	-0.69	**0.77**
Tyrosine Metabolism	-0.73	-0.42	-0.52	**0.78**
Carbohydrate Metabolism	-0.69	-0.71	**-0.86**	**0.85**
Galactose Metabolism	-0.45	-0.56	-0.72	0.62
Fructose and Mannose Metabolism	-0.54	-0.50	-0.73	**0.83**

Bold Text indicates significance at $p < 0.01$

Table S10. Relative abundances of plant species in the restored and remnant 11-months post-fire.

	Restored		Remnant	
Plant Species	Control	Burned	Control	Burned
Andropogon gerardii	56.47	56.69	25.22	21.08
Sorghastrum nutans	-	-	2.92	2.98
Quercus velutina	-	-	1.82	1.98
Helianthus divaricatus	-	-	6.20	3.39
Solidago speciosa	-	-	2.15	0.57
Sassafras albidum	-	-	8.35	8.50
Rhus copallina	0.83	-	3.08	0.91
Schizachyrium scoparium	-	-	0.70	0.29
Desmodium 1	-	-	2.48	8.93
Solidago altissima	17.47	19.66	3.43	0.34
Solidago nemoralis	-	-	0.36	-
Solidago juncea	-	-	16.10	15.38
Euthamia graminifolia	1.55	1.18	-	2.91
Lespedeza hirta	-	-	0.36	0.00
Crataegus sp.	-	-	1.01	0.82
Rosa carolina	-	-	0.36	-
Lupinus perennis	-	-	0.66	-
Desmodium 2	-	-	0.77	0.29
Galium Circaezans	-	-	0.66	0.24
Asclepias tuberosa	-	-	0.33	-
Rubus flagellaris	-	-	3.89	1.47
Lespedeza violacea	-	-	0.33	-
Euphorbia corollata	-	-	0.44	-
Desmodium rotundifolium	-	-	-	5.86
Rumex acetosella	-	-	-	0.24
Celastrus orbiculatus	-	-	-	0.29
Quercus alba	-	-	-	2.88
Baptisia alba	-	-	-	1.44
Carex radiata	-	-	-	0.29
Acer rubrum	-	-	-	0.06
Ranunculus arboretums	-	-	-	0.06
Achillea millefolium	-	-	-	0.29
Monarda fistulosa	-	-	-	0.29
Hieracium gronovii	-	-	-	0.34
Desmodium conescens	-	-	-	0.34
Juglans nigra	1.50	-	-	-
Silphium integrifolium	5.48	1.18	-	-
Coreopsis tripteris	0.71	-	3.99	6.07
Rubus allegheniensis	3.86	3.16	3.99	4.93

Erigeron Annuus	0.36	-	-	-
Oxalis fontana	0.21	0.27	-	-
Taraxacum officinale	0.13	0.13	-	-
Oenothera biennis	0.67	-	-	-
Elymus repens	1.60	12.43	0.36	-
Verbascum blattaria	0.13	-	-	-
Rumex crispus	0.36	-	-	-
Ratibida pinnata	2.38	-	-	-
Lactuca canadensis	0.36	-	-	-
Osmorhiza sp.	0.36	-	-	-
Asplenium platyneuron	0.36	-	-	-
Silene pratensis	0.36	0.09	-	-
Prunus serotina	0.36	-	1.61	0.29
Trifolium repens	0.36	0.57	-	-
Symphyotrichum lateriflorum	0.36	-	-	-
Dactylis glomerata	0.36	-	-	-
Carex blanda	0.36	-	-	-
Apocynum cannabinum	0.93	0.54	0.77	1.69
Panicum virgatum	0.36	-	-	-
Vitis riparia	0.36	-	-	-
Toxicodendron radicans	0.11	-	-	-
Carex bicknellii	0.56	-	-	-
Rosa mulitflora	0.56	-	-	-
Rudbeckia hirta	0.11	0.45	-	-
Conyza canadensis	-	0.57	-	-
Poa pratensis	-	2.36	6.98	4.33
Ambrosia artemisiifolia	-	0.09	-	-
Hypercum perforatum	-	0.09	0.66	0.24
Barbarea vulgaris	-	0.09	-	-
Asclepias syriaca	-	0.45	-	-

Table S11. Correlation between plant and soil characteristics and bacterial phyla that significantly differed in abundance between burned and control plots 11-months post-fire.

Characteristic	Betaproteobacteria	Deltaproteobacteria	Planctomycetes	Verrucomicrobia
AGL	-0.30	-0.35	**0.80**	0.17
NO_3^-	**0.70**	0.60	**-0.77**	-0.48
pH	0.55	**0.66**	-0.38	-0.25
SOM	0.25	0.25	-0.62	-0.58

Bold Text indicates significance at $p < 0.05$

Table S12. Correlation between plant and soil characteristics and bacterial families that significantly differed in abundance between burned and control plots 11-months post-fire

Characteristic	*Opitutaceae*	*Phaselicystidaceae*	*Planctomycetales*	Subgroup 5
AGL	**0.70**	0.47	0.47	-0.54
NO_3^-	**-0.75**	**0.88**	**-0.76**	**0.87**
pH	-0.63	**0.77**	-0.41	**0.66**
SOM	-0.65	**0.66**	-0.53	**0.88**

Bold Text indicates significance at $p < 0.05$

APPENDIX B

Supplemental Information for Chapter II

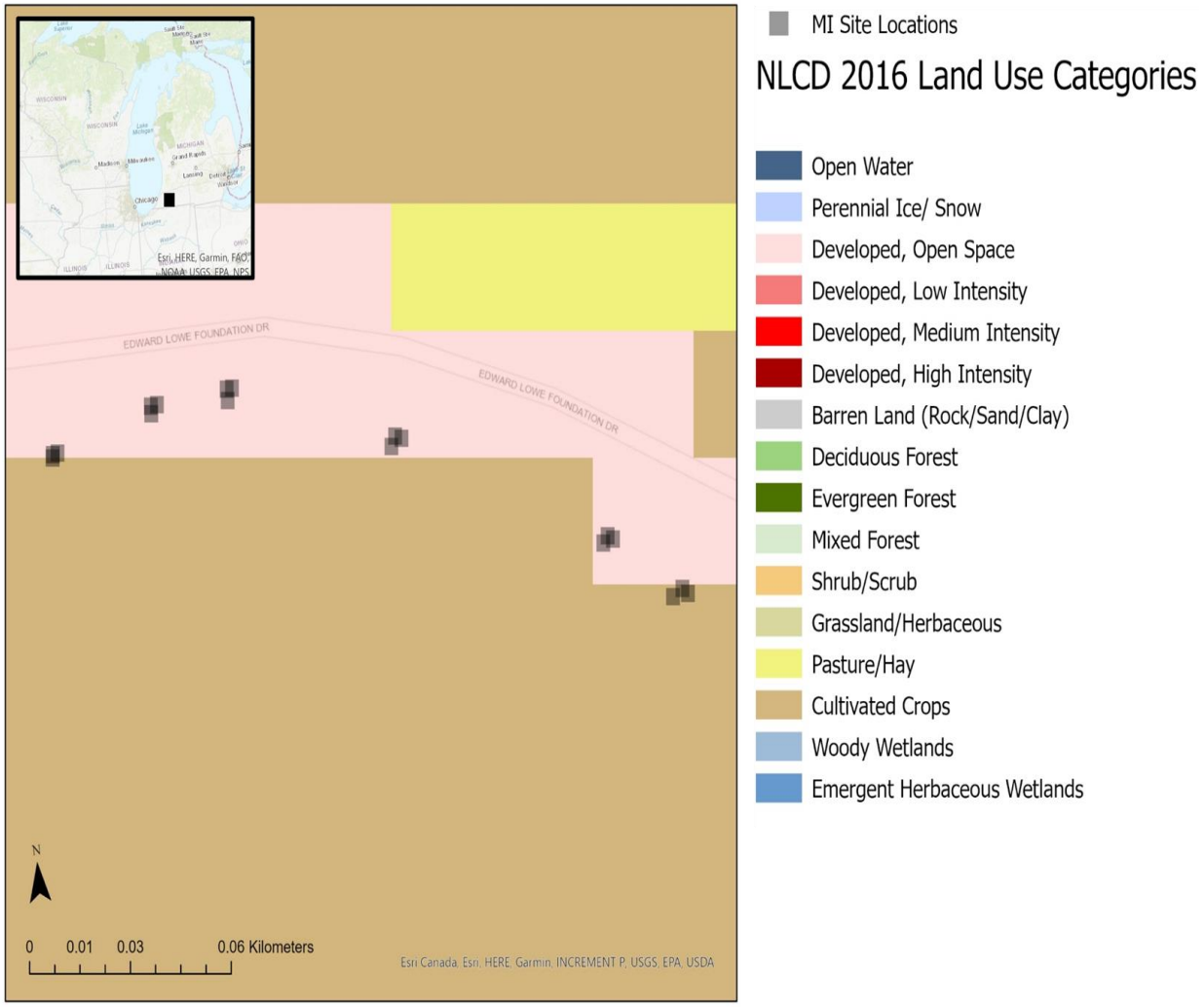

Figure S6. Map of the field plots in Michigan. USA color-coded using the National Land Cover Database 2016 Land Use Categories as defined by Yang et al. (2018).

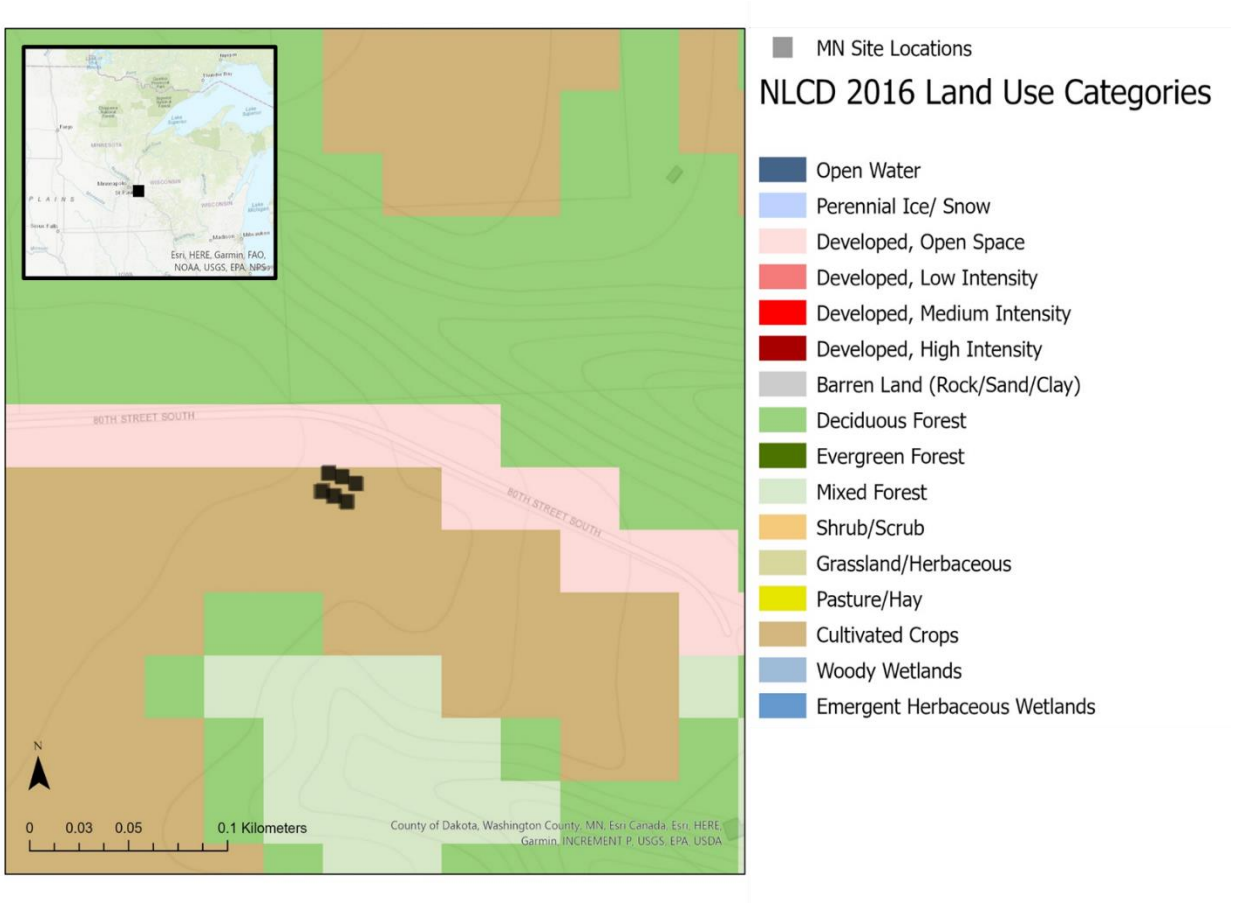

Figure S7. Map of the field plots in Minnesota. USA color-coded using the National Land Cover Database 2016 Land Use Categories as defined by Yang et al. (2018).

Minnesota Native Landscapes

8740 77th Street NE Otsego, MN 55362

MNL Upland Dry Prairie Mix
Mixed Height

Ideal for dry (xeric) or sandy sites, mixed height grasses with total height from 2-5'

	Scientific Name	Common Name	% of Mix	Seeds/ Sq Ft	PLS lbs/ac	Bloom Season
Grasses:	Andropogon gerardii	Big Bluestem	4.00	1.91	0.52	
	Bouteloua curtipendula	Side-Oats Grama	22.00	10.45	2.86	
	Bouteloua gracilis	Blue Grama	8.00	15.28	1.04	
	Elymus trachycaulus	Slender Wheat Grass	5.00	1.65	0.65	
	Koeleria macrantha	Junegrass	1.00	8.36	0.13	
	Schizachyrium scoparium	Little Bluestem	18.00	12.89	2.34	
	Sorghastrum nutans	Indian Grass	7.00	4.01	0.91	
	Sporobolus compositus	Rough Dropseed	1.50	2.15	0.20	
	Sporobolus heterolepis	Prairie Dropseed	2.00	1.53	0.26	
Sedges/Rushes:	Carex bicknellii	Bicknell's Sedge	1.50	1.22	0.20	
Forbs:	Achillea millefolium	Yarrow	0.15	1.25	0.02	Summer
	Agastache foeniculum	Fragrant Giant Hyssop	0.15	0.64	0.02	Summer
	Allium stellatum	Prairie Onion	0.50	0.26	0.07	Summer
	Amorpha canescens	Leadplant	2.00	1.53	0.26	Summer
	Asclepias syriaca	Common Milkweed	1.75	0.33	0.23	Summer
	Asclepias tuberosa	Butterfly Milkweed	1.25	0.26	0.16	Summer
	Chamaecrista fasciculata	Partridge Pea	6.00	0.77	0.78	Fall
	Coreopsis palmata	Prairie Coreopsis	0.15	0.07	0.02	Summer
	Dalea candida	White Prairie Clover	4.00	3.63	0.52	Summer
	Dalea purpureum	Purple Prairie Clover	6.50	4.66	0.85	Summer
	Echinacea angustifolia	Narrow-leaved Coneflower	0.25	0.08	0.03	Summer
	Helianthus pauciflorus	Stiff Sunflower	0.30	0.06	0.04	Fall
	Lespedeza capitata	Round-headed Bushclover	1.00	0.38	0.13	Summer
	Liatris aspera	Rough Blazing Star	0.30	0.23	0.04	Summer
	Penstemon grandiflorus	Showy Penstemon	0.50	0.33	0.07	Spring
	Potentilla arguta	Prairie Cinquefoil	0.15	1.65	0.02	Summer
	Ratibida columnifera	Long-Headed Coneflower	1.50	3.01	0.20	Summer
	Rudbeckia hirta	Black Eyed Susan	1.50	6.59	0.20	Summer
	Solidago nemoralis	Gray Goldenrod	0.25	3.58	0.03	Fall
	Solidago rigida	Stiff Goldenrod	0.50	0.98	0.07	Fall
	Symphyotrichum laevis	Smooth Blue Aster	0.15	0.39	0.02	Fall
	Symphyotrichum oolentangiensis	Sky Blue Aster	0.25	0.96	0.03	Fall
	Tradescantia bracteata	Prairie Spiderwort	0.15	0.07	0.02	Spring
	Verbena stricta	Hoary Vervain	0.75	1.00	0.10	Summer
			100.00	92.17	13.00	

Seeds/sq ft:	92.00
Grass Species:	9
Sedges/Rush Sp:	1
Forb Species:	24

Figure S8. Prairie seed mix used to seed the plants present plots.

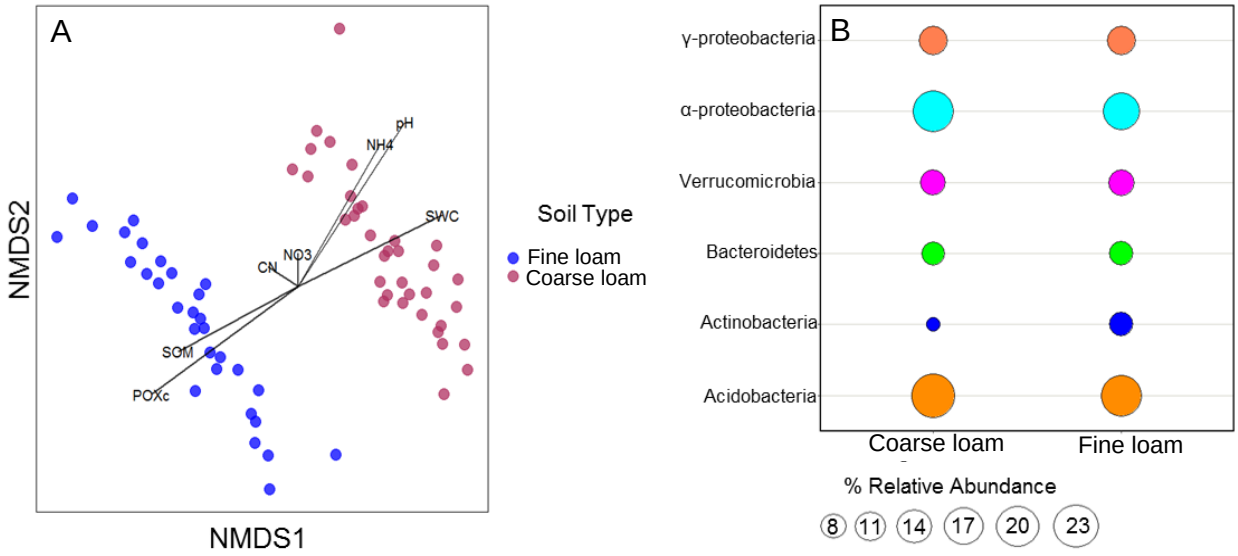

Figure S9. A) NMDS ordination and **B)** bubble plot of the bacterial communities in the MI (Fine loam) and MN (Coarse loam) sites in Summer 2020. In A) soil characteristics are represented by lines, and the length of each line is proportional to the explanatory power of each variable.

Table S12. Results of linear-mixed effect models examining the effects of carbon addition (Treatment) and plant presence (Plant Present) on soil and microbial characteristics in the fine loam soil from 2019 (± SE).

	SOM (%)	CO_2 Flux (μmol CO_2 $m^{-2}s^{-1}$)	pH	SWC (%)	NO_3^- (ug g $soil^{-1}$)	NH_4^+ (ug g $soil^{-1}$)	% C	% N	C:N	ASV Richness	ASV Diversity
p value											
Treatment	0.58	0.08	0.69	**0.033**	**0.009**	0.83	0.15	0.33	0.15	0.06	0.07
Plants Present	0.54	0.55	0.28	0.18	**0.006**	0.26	0.26	0.2	0.46	0.68	0.66
Treatment x Plants Present	0.81	0.53	0.99	0.33	**0.017**	0.052	0.54	0.63	0.93	0.67	0.64
Overall Treatment											
Control	3.32±0.14	3.26±0.34	7.46±0.17	11.8±0.86**AB**	224.9±112.4**A**	42.5±5.5	1.15±0.06	0.12±0.004	9.77±0.30	1003±49.4	6.36±0.10
Cellulose	3.59±0.22	3.95±0.53	7.34±0.26	14.4±0.78**B**	46.6±12.2**B**	41.0±4.1	1.34±0.10	0.13±0.007	10.36±0.29	872±101.7	6.24±0.16
Biomass	3.57 ± 0.19	4.46±0.24	7.27±0.19	11.4±0.72**A**	345.7±160.8**A**	47.0±8.4	1.30±0.09	0.13±0.009	10.41±0.49	1039±52.7	6.51±0.04
Plants Present											
Plants Present	3.47±0.12	3.99±0.27	7.41±0.11	12.2±0.68	104.0±52.2**A**	41.5±3.3	1.30±0.07	0.13±0.006	10.2±0.18	953±53.7	6.34±0.08
Plants Absent	3.53±0.12	3.79±0.28	7.30±0.15	12.9±0.52	308.0±88.8**B**	45.5±4.4	1.25±0.04	0.12±0.003	10.1±0.27	990±33.7	6.39±0.05

SWC: Soil Water Content; SOM: Soil Organic Matter; % C: Percent Total Carbon; % N: Percent Total Nitrogen; C:N: Carbon to nitrogen ratio; ASV: Amplicon Sequence Variant.
Bolded text indicates significance at $p < 0.05$.
Means with the same letters are not significantly different while those with different letter are significantly different ($p < 0.05$)

Table S13. Results of linear-mixed effect models examining the effects of carbon addition (Treatment) and plant presence (Plant Present) on soil characteristics in the coarse loam soil from 2019 (± SE).

	SOM (%)	CO_2 Flux (µmol CO_2 $m^{-2}s^{-1}$)	pH	SWC (%)	NO_3^- (ug g $soil^{-1}$)	NH_4^+ (ug g $soil^{-1}$)	% C	% N	C:N	ASV Richness	ASV Diversity
p value											
Treatment	**0.002**	**<0.001**	**0.004**	0.19	0.065	**0.03**	**0.011**	**0.009**	**<0.001**	0.09	0.33
Plants Present	0.75	**<0.001**	0.51	0.6	0.1	0.63	0.94	0.83	0.69	**0.043**	0.25
Treatment x Plants Present	0.42	0.75	0.64	0.57	0.97	0.86	0.42	0.34	0.5	0.29	0.09
Overall Treatment											
Control	2.38±0.89**A**	4.26±0.50**A**	6.72±0.22**A**	17.3±0.29	221.8±75.3	9.57±3.3**A**	0.74±0.04**A**	0.10±0.004**A**	7.09±0.14**A**	944±65.3	6.25±0.08
Cellulose	2.78±0.09**B**	5.58±0.58**B**	6.57±0.12**A**	17.3±0.62	65.9±20.1	13.80±2.4**AB**	0.91±0.06**B**	0.11±0.004**A**	8.31±0.37**B**	991±52.7	6.24±0.08
Biomass	2.77±0.10**B**	6.96±0.60**C**	5.89±0.20**B**	16.4±0.49	72.1±17.4	26.55±8.0**B**	0.78±0.02**AB**	0.095±0.002**B**	8.17 ± 0.21**B**	1080±59.3	6.35±0.09
Plants											
Plants Present	2.63±0.074	6.62±0.35	6.43±0.15	16.9±0.25	133.0±27.3	15.5±3.4	0.82±0.04	0.103±0.003	7.9±0.22	1043±35.8**A**	6.31±0.05
Plants Absent	2.65±0.066	4.58±0.32	6.36±0.12	17.1±0.33	107.0±34.9	17.8±3.1	0.80±0.02	0.103±0.002	7.82±0.17	967±35.6**B**	6.25±0.05

SWC: Soil Water Content; SOM: Soil Organic Matter; % C: Percent Total Carbon; % N: Percent Total Nitrogen, C:N: Carbon to nitrogen ratio; ASV: Amplicon Sequence Variant.

Bolded text indicates significance at $p < 0.05$.

Means with the same letters are not significantly different while those with different letter are significantly different ($p < 0.05$)