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Long-term fire management history affects N-fertilization sensitivity, but not seasonality, of grassland soil microbial communities

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ABSTRACT

Nitrogen (N) availability is a driver of soil microbial diversity and function, and is affected by prescribed burning (N removal through volatilization) and fertilization (N addition). Because soil microbes control critical feedbacks to ecosystem function, it is important to understand the dynamics and responses of microbial populations under conditions of contrasting N availability. This study took place at a long-term field manipulation in which native tallgrass prairie was annually burned or not burned, and annually fertilized or not fertilized, in a factorial design, since 1986. Composite surface soil samples (0–15 cm) were collected monthly between November 2014 and December 2015 from replicate plots to evaluate event-based (post-fire, post-fertilization), seasonal, and long-term responses of soil microbial communities to management and environmental changes. Bacterial 16S rRNA gene and fungal ITS population sizes were estimated using qPCR, and bacterial community composition (BCC) was measured using Illumina MiSeq sequencing of 16S rRNA genes. We expected seasonal and event-based change in all parameters, and that total microbial population sizes and diversity would be lower in soils with higher N availability, due to greater competitive dominance of nitrophilic or copiotrophic taxa. Bacterial and fungal population sizes varied significantly by sampling month, in that bacterial populations were approximately 10× greater in summer (June–August), but did not change in response to management events or long-term treatments. In contrast, very few individual taxonomic groups displayed seasonal or event-based responses, and there was no significant whole-community turnover on weekly or monthly time-scales; instead, BCC was strongly impacted by both the long-term fire and fertilization treatments. Specifically, there were increases and decreases in putatively "copiotrophic" and "oligotrophic" prokaryotic Phyla in response to long-term N fertilization, which were significantly stronger and more predictable in soils following long-term fire suppression. These results reveal that while long-term grassland management changes BCC beyond the detected range of seasonal variability, total bacterial populations change coherently month-to-month, potentially due to significant plant inputs of labile carbon during the growing season. Furthermore, because prescribed burning reduces soil N availability, the interactive responses to fire suppression plus fertilization suggest that higher background levels of soil N availability may increase the magnitude of soil microbial sensitivity to N fertilization.

1. Introduction

As multiple global environmental factors continue to change, it is essential to understand how soil biota respond to environmental variability, particularly in threatened ecosystems such as the tallgrass prairie (Seastedt et al., 2008). Tallgrass prairie ecosystems compose < 5% of their original range due to conversions to agriculture, urbanization, and woody encroachment (Hoekstra et al., 2005). Ecologists and land managers recognize the need to manage the remaining tallgrass prairie to ensure habitat conservation for species that rely on this ecosystem, as well as for the maintenance of regionally and globally important ecosystem functions. Soil microbes mediate valuable grassland functions, including decomposition, soil fertility, and carbon storage, and understanding the environmental controls over microbial communities will help inform future land management decisions that maintain these critical ecosystem services (Torsvik and Ovreas, 2002; Van Der Heijden et al., 2008; van der Putten et al., 2013).

Microbes are often sensitive to environmental change due to their relatively short generation time, small size, and large surface area to volume ratio (Schmidt et al., 2007; Shade et al., 2013) and within the heterogeneous soil habitat, soil microbial communities are taxonomically and functionally diverse. Therefore, environmental drivers

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of changes in soil microbial dynamics have the potential to occur on multiple time scales with differential effects on specific populations with different niche preferences (Bardgett et al., 2005; Fierer et al., 2010). It is well-known that tallgrass prairie ecosystems have undergone considerable long-term changes due to anthropogenic activities such as fire suppression, which has led to woody encroachment (Ratajczak et al., 2014) and accumulations of soil organic carbon (C) and nitrogen (N) (Dooley and Treseder, 2012; Turner et al., 1997), and increases in biologically available soil N from atmospheric deposition, caused primarily by increased use of fossil fuels and N fertilizers (Farrer et al., 2013; Galloway et al., 2004). However, less is known about whether microbial turnover on shorter time scales is reflective of cumulative long-term ecosystem change. An understanding of the typical range of microbial cell and community composition variability in the short term is needed to provide context to understand the novelty of the impacts of long-term change on microbial communities (Shade et al., 2012).

In addition to long-term changes in tallgrass prairie ecosystems, these regions have distinct seasonal changes in climate and plant phenology, ranging from hot, dry plant growing seasons to colder and wetter dormant periods (Knapp et al., 1998), which could also drive microbial community or population dynamics. In winter, freeze-thaw of surface soils might impose physiological limitations on cell survival to the summer season and affect the decomposition of soil organic matter (SOM), which could have variable feedbacks on N availability during the growing season (Bardgett et al., 2005; Schimel et al., 2007). Seasonal patterns of soil microbial turnover have been described in alpine and arctic ecosystems, and can be characterized such that saprophytic fungi and slow-growing bacteria with heightened depolymerization strategies dominate in winter, whereas mycorrhizal fungi and fast-growing bacteria dominate in summer (Schimel and Mikan, 2005; Schmidt et al., 2007). In grasslands, total soil microbial biomass tends to peak late in the growing season (Bardgett et al., 1997; Garcia and Rice, 1994), but less is known about how seasonal turnover is impacted by long-term changes in fire and fertilization management.

In a managed tallgrass prairie, drivers of microbial dynamics could also occur on relatively short time-scales. For example, event-based pulses of rhizodeposited soil carbon (C) from root growth, spikes of N availability from direct fertilization events, or mobilization of nutrients following rainfall, can stimulate microbial activity in the short-term, sometimes in association with community composition change (Armstrong et al., 2016; Fauci and Dick, 1994; Kuzyakov and Blagodatskaya, 2015; Stark et al., 2004). In addition to greater root production following spring burning (Johnson and Matchett, 2001), initial responses to fire can include higher microbial activity via increased soil temperature in the weeks following combustion of surface litter (Ojima et al., 1994; Treseder et al., 2004). However, microbial responses to events that modify nutrient availability may be ephemeral, or different from, longer-term responses (Bardgett et al., 2003; Kuzyakov et al., 2000; Ramirez et al., 2010). Altogether, there is currently a lack of understanding of how short, seasonal, and long-term management and change interact to alter soil microbial communities and populations, and how these interacting time-scales may impact the tallgrass prairie ecosystem.

The objective of this study was to assess microbial community and population dynamics, at event-based, seasonal and decadal resolutions, in response to two management practices that drive N availability in tallgrass prairie: prescribed annual fire and N fertilization. Fire volatilizes organic N in plant litter, maintaining an N-limited situation in which native prairie plants with low N demand are competitively dominant (Seastedt et al., 1991; Tilman and Wedin, 1991; Yu et al., 2015); in contrast, the lack of fire allows available soil N to accumulate (Blair, 1997; Johnson and Matchett, 2001; Turner et al., 1997). We sampled a 30-year field manipulation of annual burning and fertilization once per month for one year to address the following questions: 1) What is the response of the soil microbial community to event-based (fire, resource pulse addition) and seasonal environmental variation? 2) Does long-term management of N availability impact the seasonal turnover of soil microbial communities, or modify the microbial community beyond the seasonal range of variability?

We predicted that: 1) Microbial populations would display distinct responses to fire and fertilization pulses, at both initial event-based and long-term time scales, with spring burning increasing microbial population sizes by promoting greater plant belowground production (Johnson and Matchett, 2001), and N pulses decreasing microbial population sizes through direct shifts in microbial community composition to favor a more “copiotrophic” community (Fierer et al., 2007; Ramirez et al., 2012), including a reduction in fungal populations (Treseder, 2008). Additionally, we expected to detect seasonal changes in population sizes, with higher microbial populations in the summer due to availability of labile C from plant rhizodeposition, and lower in the winter when heterotrophic microbes rely on decomposition of soil organic matter for energy and C (Schmidt et al., 2007). We also predicted that: 2) Microbial community composition would differ between winter and summer, due to a higher relative abundance of populations that grow well on complex soil organic matter in the winter turning over different populations that grow faster on labile C in summer. Also, we expected that increased N availability, through either fertilization or lack of fire, would create an environment that favors a greater proportion of fast-growing copiotrophic taxa, as opposed to slower-growing oligotrophic taxa, and as evidenced by a greater community mean rRNA operon copy number (Roller et al., 2016).

2. Materials and methods

2.1. Study site and experimental design

This study was conducted at Konza Prairie Biological Station (KPBS). Konza Prairie Biological Station is located in the Flint Hills region of Kansas (39°05′N, 96°35′W) and is characterized by warm, dry summers and wet, cool winters, with MAP of 835 mm and MAT of 26.6 °C. During the one-year sampling period, total monthly precipitation ranged from 6.2 mm in March 2015 to 147.3 mm during July 2015. Daily mean soil temperature ranged from 2.3 °C in December 2015 to 23.5 °C in July 2015, and daily mean air temperature ranged from 0.7 °C in January 2015 to 37 °C in July 2015. While mean temperatures were near or only slightly above average during the study period, the total annual precipitation of 1002.5 mm was 20% greater than average, reflecting a growing season with soil water content rarely much below field holding capacity (approximately 0.25 $g \cdot g^{-1}$ (Zeglin et al., 2013)). Although specific micro-meteorological variables were not measured in each treatment, meteorological data for KPBS were collected for a site near the experimental plots. The vegetative cover of grasslands at KPBS is dominated by perennial C_4 grasses, such as *Andropogon gerardii*, *Sorghastrun nutans*, *Panicum virgatum*, and *Schizachyrium scoparium*, while unburned plots feature more woody plants, such as *Juniperus virginiana, Cornus drummondii,* and *Rubus occidentalis* (Ratajczak et al., 2012).

The Belowground Plots Experiment (BGP) was established in May 1986 at KPBS as part of the Konza Prairie Long-Term Ecological Research (LTER) program. The experiment is located on Irwin silty clay loam (fine, mixed, mesic, Pachic Arguistolls), and arranged in a split-strip block design, where whole-plot treatments are manipulated by fire, a split-plot mowing treatment was randomly assigned to half of the whole-plot, and within each treatment split, the plots were stripped and randomly assigned a nutrient enrichment treatment (no fertilizer addition (control), N fertilizer addition (10 $g\,N \cdot m^{-2}$ as NH_4NO_3), phosphorus (P) addition, or N and P fertilizer addition). For the purposes of this study, we sampled soils from just the control and the N fertilizer addition plots under annually burned and unburned management history.

2.2. Sample collection

Soils were collected once per month from November 2014 to December 2015, excluding December 2014 and February 2015. Soils from all treatment plots were collected one week following the annual burn treatment in April 2015, and one week following the annual fertilization treatment in June 2015. Three random 2 cm diameter soil cores from the top 15 cm of mineral soil were collected in each subplot and mixed to create a composite sample. Soils were collected using aseptic techniques, placed on ice in the field and immediately carried back to the laboratory and frozen at −20 °C until further analysis. For each of these 192 samples, soil gravimetric water content (GWC) was measured as mass lost from soil after drying at 105 °C overnight. Soil organic matter (SOM) and pH were estimated for all samples collected during just one month (June 2016). SOM was measured by loss-on-ignition (LOI), and pH was measured in 1:1 slurry of deionized water.

2.3. DNA extraction and polymerase chain reaction (PCR)

Total genomic DNA (gDNA) was extracted from approximately 0.5 g of homogenized soil per sample using physical lysis, cetyltrimethylammonium Bromide (CTAB) and phenol: chloroform extraction and overnight precipitation in PEG 6000 (DeAngelis et al., 2010). From these gDNA extracts, the 16S rRNA gene was targeted for Illumina bacterial sequencing using universal bacterial primers (515F/806R) following established protocols (Caporaso et al., 2012), with two exceptions: PCR was run for 25 cycles instead of 35, and a final concentration of 0.04% Bovine Serum Albumin (BSA) was included with each reaction. Gel electrophoresis was used to confirm amplification of each reaction. Triplicate technical replicates were run for each barcoded sample, amplicon amounts normalized and combined into one library and cleaned using a QIAquick Gel Extraction Kit. These samples were sequenced with a 2 × 150 paired-end read Ilumina MiSeq run (Zeglin et al., 2016).

The number of bacterial 16S rRNA gene copies and fungal ITS copies was estimated in all gDNA extracts using a Quantitative Polymerase Chain Reaction (qPCR) on a Bio-Rad CFX CONNECT system with Bio-Rad SsoAdvanced Universal SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA). For 16S and ITS assays respectively, 1–10 ng template soil gDNA was used at 10 μL assay volume, 0.02% and 0.04% final BSA concentration, 100 nM and 500 nM final primer concentrations, using established primer sequences and thermal cycler programs (Fierer et al., 2005; Zeglin et al., 2016). Standard curves for 16S assays were prepared using *E. coli* ATCC 25922 at 5×10^{0} - 5×10^{-6} ng ul^{-1} DNA concentrations (efficiency = 85%–114%, R^2 = 0.979–0.991). ITS standard curves were prepared using *Candidia albicans* SC5314 at 5×10^{0} - 5×10^{-6} ng ul^{-1} concentrations (efficiency = 81%–95%; R^2 = 0.988-0.998). All assays included three technical replicates of gDNA per sample, as well as no-template controls and melting curves to confirm there was no amplification of non-target genes. Bacterial 16S rRNA gene and fungal ITS copy number was compared among field treatments on a g^{-1} dry soil basis, which was derived by normalizing the qPCR results by soil gDNA yield.

2.4. Bioinformatics and statistical analyses

The QIIME software package (Caporaso et al., 2010) was used to process raw Illumina sequence data. Sequences were quality filtered, joined and demultiplexed, and assigned to operational taxonomic units (OTUs, representing 97% DNA sequence similarity) that were picked using the open-reference workflow. OTU sequences were aligned to the GreenGenes v.13.8 16S rRNA gene reference database, taxonomy was assigned using the RDP classifier, and non-aligned OTUs, chimeric sequences (identified using CHIMERASLAYER) and singletons and doubletons were removed prior to further analysis. The resulting dataset included 10,719,215 reads and 59,641 OTUs, and the dataset was trimmed to include an equal number of sequences per sample. This final rarefied dataset included 172 samples with 19,000 reads per sample, for a total 3,287,000 reads and 49,968 OTUs. The same steps were used to estimate average mean weighted 16S rRNA gene copy number per sample as a proxy for bacterial life history strategies, with the exception that OTUs were picked using the closed reference workflow and OTUs were aligned to the GreenGenes v.1.3.5 reference database, then the closed reference OTU table was imported into the web-based Galaxy version of PICRUSt to derive weighted mean 16S rRNA gene copy number (Nemergut et al., 2016). Weighted copy numbers were calculated by multiplying copy number by relative abundance of each OTU, then summing these values within a sample. Sequences are archived in the NCBI SRA database under BioProject number PRJNA398249.

For all soil microbiological response variables, the individual and interactive effects of burn history, fertilizer additions and sample date were evaluated with a repeated-measures Analysis of Variance (RM-ANOVA) test using mixed-effect models with block nested within replicate as a random variable, in the “lme” package in R Studio. All data were checked for normality and log-transformed when appropriate to meet assumptions of normality before statistical analysis (16S rRNA gene and ITS copy numbers, 16S:ITS gene copy ratios, Simpson evenness, dominance, and taxon relative abundance). A Bray-Curtis dissimilarity matrix from QIIME was used to create a nonmetric multidimensional scaling (NMDS) ordination using the metaMDS function in the vegan package of R Studio, and the permuational analysis of variance (PERMANOVA) was determined by the “adonis” function of the vegan package in R Studio (Oksanen et al., 2007). The strength of correlation between NMDS axes and taxon relative abundances was evaluated using Pearson's R coefficient and associated p-values (R Commander; R Core Development Team, 2010).

Prokaryotic taxon responses to N additions were calculated by $\log_{10}$-transforming the ratio of the average relative abundance in N-amended soils to the average relative abundance in non-amended soils for both burned and unburned treatments. For this analysis, high-order taxa were selected that were either predicted to be copiotrophic or oligotrophic, as indicated from previous studies (Leff et al., 2015; Ramirez et al., 2012). For each response, paired t-tests specified responses that were significantly greater or less than zero, and one-way ANOVA indicated significant differences between burned and unburned treatments for each taxon.

3. Results

3.1. Soil characteristics and microbial abundances

Soil GWC ranged from 0.093 to 0.587 $g \cdot g^{-1}$ and showed an interactive effect of sampling date and burning treatment (Month*Burn: $F = 4.54$, $P < 0.001$): Soils in burned plots had greater GWC than unburned plots in November 2014, September 2015 and November 2015 (Fig. S1). Soil organic matter (SOM) ranged from 0.05 to 0.11 $g \cdot g^{-1}$, and pH ranged from 5.50 to 6.80, but did not vary significantly between treatments (Table 1).

Bacterial 16S rRNA gene and fungal ITS copy numbers ranged from 5.69×10^{6} to 5.86×10^{10} g^{-1} dry soil and 2.8×10^{6} to 5.50×10^{9} g^{-1} dry soil for 16S rRNA gene and ITS copy number, respectively, and the ratio of ITS copies to 16S gene copies (F:B) ranged from 0.0016 to 4.1. These proxies for bacterial and fungal abundance did not differ between the long-term management treatments, but all displayed seasonal variation (Fig. 1). Bacterial 16S rRNA gene copy number was significantly greater during the summer (June, July and August) than in other months, showing an order of magnitude increase between May and June 2015, and an order of magnitude or greater decrease between August and September 2015 (Fig. 1a). Fungal ITS copy number also decreased in the fall, being significantly lower in October 2015 than other sampling dates, but was otherwise relatively constant (Fig. 1b). Variability in F:B was primarily driven by the strong

Table 1
Soil environment and microbial diversity and abundance variables.

	Burn	Fertilizer	GWC	pH	SOM	OTUs	Diversity	Evenness	NMDS Axis 1	NMDS Axis 2	16S wm copy #	log 16S copy #	log ITS copy #	F:B
	B	**N**	M, B	–	–	M, MxB	M	BxN	N	M, BxN	–	M	M	M
BC	1y	–	0.270 (0.009)	6.44 (0.15)	0.067 (0.006)	3610 (29)	9.35 (0.03)	0.019^{b} (0.001)	0.11^{b} (0.02)	-0.06^{a} (0.01)	2.33 (0.004)	8.92 (0.06)	8.19 (0.07)	0.55 (0.12)
BN	1y	+N	0.267 (0.010)	6.35 (0.15)	0.065 (< 0.001)	3682 (28)	9.29 (0.03)	0.015^{ab} (< 0.001)	-0.11^{a} (0.02)	-0.12^{a} (0.02)	2.32 (0.005)	9.00 (0.06)	8.23 (0.07)	0.56 (0.13)
UBC	–	–	0.305 (0.039)	6.31 (0.18)	0.067 (0.005)	3589 (23)	9.31 (0.03)	0.017^{a} (< 0.001)	0.12^{b} (0.02)	0.02^{b} (0.02)	2.32 (0.006)	9.02 (0.05)	8.17 (0.05)	0.58 (0.17)
UBN	–	+N	0.288 (0.012)	6.00 (0.28)	0.086 (0.008)	3482 (34)	9.31 (0.03)	0.021^{ab} (0.001)	-0.11^{a} (0.04)	0.15^{c} (0.03)	2.33 (0.006)	8.82 (0.06)	8.11 (0.05)	0.50 (0.10)

Results of two-way repeated measures ANOVA denoted for variables significantly ($\alpha = 0.05$) different between sampling months (**M**), annual (1y) or burn suppression (−) history (**B**), nitrogen fertilized (+N) or unfertilized (−) history (**N**), and interactive effects (x). Subscript letters indicate significant treatment differences by Bonnferoni post-hoc tests. Significant monthly differences, including interactive effects, are shown in Fig. 1 and Fig. S1-3. Means (SE) of variables are shown (N = 4). Abbreviations: SOM, soil organic matter (g g^{-1}); GWC, gravimetric water content (g g^{-1}); 16S wm, 16S rRNA gene copy weighted mean; F:B, fungal ITS copy number: bacterial 16S gene copy number ratio.

seasonal patterns in bacterial 16S rRNA gene abundance, and correspondingly tended to be lower in summer, and was significantly higher in September 2015 than in other months (Fig. 1c). Bacterial 16S rRNA gene copy numbers were correlated with monthly changes in air temperature ($R = 0.65$; $p = 0.022$), but not changes in soil water or precipitation ($R = 0.03$, $P = 0.69$; $R = 0.20$, $P = 0.52$; respectively), whereas fungal ITS copy numbers were correlated with soil water content ($R = 0.27$, $p = 0.0003$), but not air temperature ($R = 0.31$, $p = 0.33$) or monthly mean precipitation ($R = 0.35$, $p = 0.26$). Despite differences in ribosomal gene abundance, there were no significant effects of management treatment or month on bacterial 16S rRNA weighted mean copy number (Table 1), thus no indication of changes in the dominant growth rate strategy integrated across the prokaryotic community.

3.2. Microbial diversity and composition

Bacterial 16S rRNA gene richness ranged from 2632 to 4863 observed OTUs per sample, evenness ranged from 0.0069 to 0.0562, and Shannon's diversity ranged from 8.35 to 10.40. While richness and diversity varied primarily by month, evenness data reflected a management treatment effect (Table 1, Fig. S2). OTU richness was significantly lowest in all treatments in March 2015, and in the unburned fertilized treatment, richness remained significantly lower through April 2015 (Fig. S2a). The temporal variation in bacterial diversity was primarily driven by low diversity in the unburned fertilized plots in April 2015 and an increase in diversity in all soils in August 2015 (Fig. S2b). Bacterial 16S rRNA gene evenness did not vary seasonally: Instead it was higher on average in the burned, unfertilized plots than the unburned, unfertilized plots (Table 1, Fig. S2c).

The best NMDS ordination model of prokaryotic community distance among all samples had 2 axes and a stress of 0.127 (Fig. 2). Soil microbial communities from fertilized plots had significantly higher NMDS Axis 1 scores than unfertilized plots, but there were no significant differences between burned and unburned treatments, while NMDS Axis 2 scores showed an interaction between burning and N additions, with bacterial communities from fertilized unburned soils grouping separately from the other treatments (Table 1). PERMANOVA results confirmed that a significant amount (11%) of variability in community composition was explained by the direct and interactive effects of long-term burning and N additions (Table 3), but there was no significant seasonal variability in community composition (Fig. S3).

NMDS Axis 1 scores were most strongly correlated with differences in the relative abundance of sequences affiliated with (sub-)Phyla Verrucomicrobia, Acidobacteria, Nitrospirae, and δ-Proteobacteria, and NMDS Axis 2 scores were best correlated with differences in α-Proteobacteria, Chloroflexi, γ-Proteobacteria, Crenarchaeota, and Planctomycetes relative sequence abundance (Table 2). Notably, genus DA101 of phylum Verrucomicrobia, class Spartobacteria, composed 20% of the dataset on average, in agreement with other findings from native tallgrass prairie soils that report high abundances of DA101 (Fierer et al., 2013). Relative abundance of sequences affiliated with "Other Verrucomicrobia" (excluding genus DA101) varied significantly with N addition, and were typically higher in the unfertilized treatments (Fig. S4e), while DA101 relative abundance was highest in burned, fertilized soils (Fig. S4a). Other major (sub-)Phyla relative abundances that varied significantly with nitrogen addition treatment included α-Proteobacteria, δ-Proteobacteria, Gemmatimonadetes, Nitrospirae, and Crenarchaeota (Fig. S4). While no significant treatment differences were observed for β-Proteobacteria, a dominant β-Proteobacterial order, Burkholderiales, was more abundant in the long-term fertilization treatment (Fig. S4l). Only δ-Proteobacteria and genus DA101 showed an interactive response to nitrogen additions and burn treatment (Fig. S4).

While there was less overall seasonal variation in prokaryotic community composition, some (sub-)Phyla relative abundances did vary significantly with sample time. Planctomycetes relative abundance generally increased from May through December 2015. δ-Proteobacteria and Nitrospirae relative abundance was lower in spring (March/April), Nitrospirae and δ-Proteobacteria were more abundant in July, δ-Proteobacteria were most abundant in October, and Gemmatimonadetes were most abundant in April (Fig. S4). While Crenarchaeota varied significantly by month, there were no post-hoc differences. While subphylum β-Proteobacteria did not vary significantly by month, the relative abundance of Order Burkholderiales did show a significant month by burn interaction in April, one week post-fire (Fig. S4l).

3.3. Microbial group-specific response to N fertilization

Response ratios to long-term N fertilization indicated significant positive responses for all putative copiotrophic (sub-)Phyla, except Actinobacteria and γ-Proteobacteria, which had negative and no response, respectively, in burned soils (Fig. 3). All copiotrophic taxa had significantly stronger responses to N additions in unburned than burned soils (*t*-test, $P < 0.05$). Of the putative oligotrophic taxa, only δ-Proteobacteria and Planctomycetes had consistently negative responses to N additions in both burned and unburned soils, and only Verrucomicrobia and δ-Proteobacteria significantly differed between burned and unburned soils, in that Verrucomicrobia had a significant positive response to N additions in burned soils (Fig. 3).

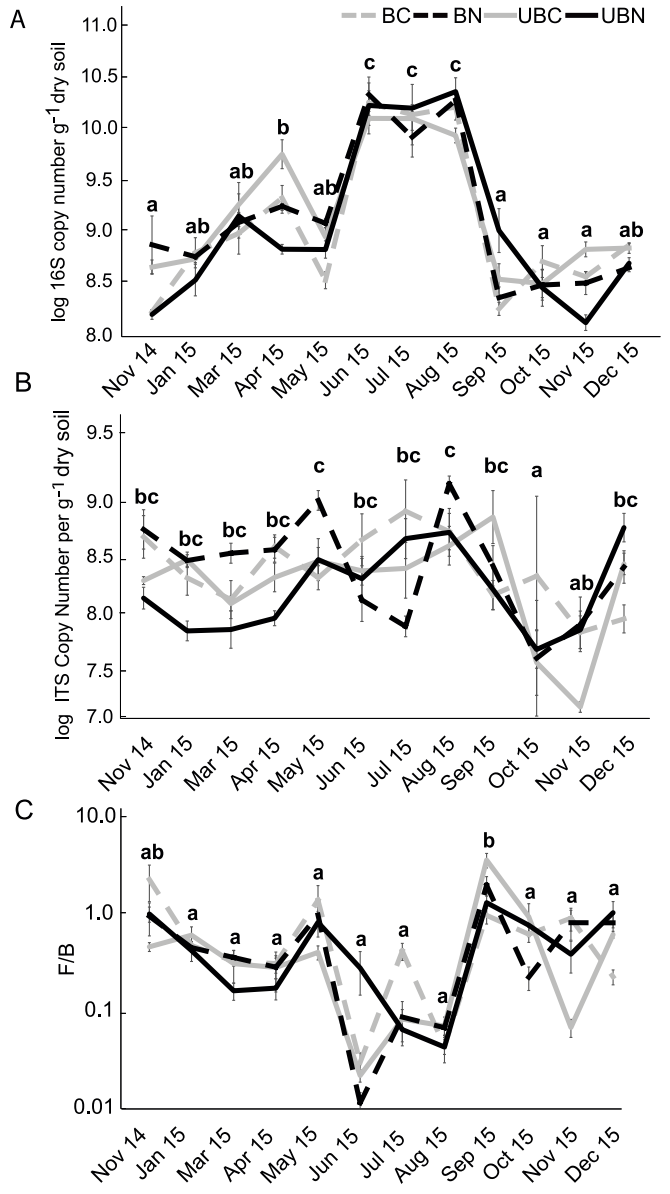

Fig. 1. Quantitative Polymerase Chain Reaction (qPCR) data for (a) log 16S rRNA gene copy numbers g^{-1} dry soil, (b) log ITS copy number g^{-1} dry soil and (c) fungal:bacterial (F/B) ratios for all soils collected between Nov 2014 and Dec 2015 (BC = Burned control, BN = Burned fertilized, UBC = Unburned control, UBN = Unburned fertilized). Repeated measures ANOVA post-hoc results (bold letters) indicate significant ($p < 0.05$) changes by month; there were no significant treatment differences. Bars indicate standard error of the mean.

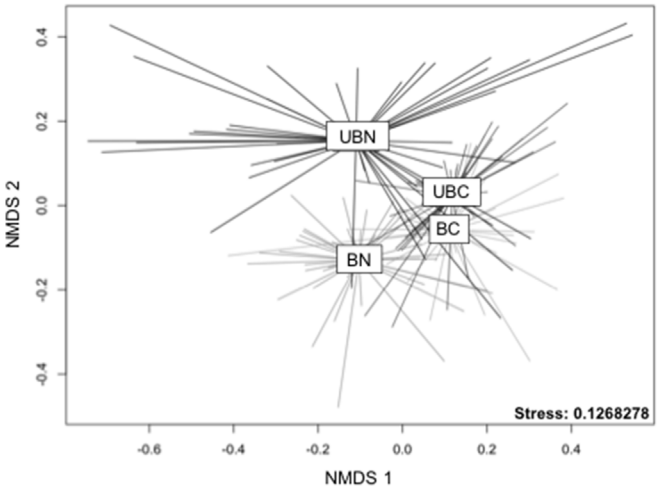

Fig. 2. Nonmetric multidimensional scaling (NMDS) ordination model axes one and two of Bray-Curtis dissimilarity in soil 16S rRNA gene OTU composition, comparing four treatments (BC = Burned control, BN = Burned fertilized, UBC = Unburned control, UBN = Unburned fertilized) and including all samples collected between Nov. 2014 and Dec. 2015. Each line endpoint represents where a sample falls in the ordination space, and the connecting centroid box notes the long-term treatment associated with the sample.

Table 2
Prokaryotic taxon correlation with NMDS axes (p-value, Pearson's R).

Taxonomic group	NMDS1	NMDS2
Other Verrucomicrobia*	*< 0.0001, 0.68*	1.0000, 0.12
δ-Proteobacteria	*< 0.0001, 0.55*	*0.0009, 0.34*
Acidobacteria	*< 0.0001, 0.48*	0.0425, 0.27
Nitrospirae	*< 0.0001, 0.45*	1.0000, 0.16
Bacteroidetes	*0.0031, 0.36*	1.0000, 0.00
α-Proteobacteria	*0.0002, -0.36*	*< 0.0001, -0.60*
DA101 (Verrucomicrobia)	*< 0.0001, -0.41*	*< 0.0001, 0.39*
Chloroflexi	0.0115, 0.29	*< 0.0001, 0.42*
Planctomycetes	1.0000, 0.16	*< 0.0001, 0.41*
Actinobacteria	0.5592–0.21	*0.0001, -0.40*
γ-Proteobacteria	1.0000, −0.11	*< 0.0001, -0.40*
Crenarchaeota	0.3616, 0.22	*< 0.0001, -0.40*

Italicized values indicate a significant correlation ($p < 0.01$) with the NMDS axis noted; groups with no significant correlations are not shown (β-Proteobacteria, Firmicutes, Gemmatimonadetes, Other Bacteria). *Other Verrucomicrobia refers to all Verrucomicrobia that are not classified as genus DA101.

Table 3
Permutational ANOVA results.

Factor	Sum of Squares	F	R^2	P
Month	1.1168	1.1164	0.06692	0.099
Nadd	*0.8138*	*8.9481*	*0.04876*	*0.001*
Burn	*0.6367*	*7.001*	*0.03815*	*0.001*
Month*Nadd	0.7996	0.7993	0.04791	0.998
Month*Burn	0.8867	0.8864	0.05313	0.916
*Nadd*Burn*	*0.3935*	*4.327*	*0.02358*	*0.001*
Month*Nadd*Burn	0.7654	0.765	0.04586	0.999
Residuals	0.09095	–	0.6757	–

Italicized values indicate a significant amount of variation explained ($p < 0.05$) by the variable(s).

4. Discussion

Soil microbial abundance and community turnover can occur on both relatively long and short time scales. In this study, we found that grassland microbial communities have the potential to change on event-based, seasonal, and historical time-scales, although the magnitude and response at each temporal scale varies depending on populations of interest or the metric used to describe changes in microbial turnover. In our study ecosystem, native tallgrass prairie, long-term changes in disturbance regime and N addition had a greater propensity to change soil bacterial community composition than seasonal variability. Seasonality had a greater impact on total bacterial abundance, and event-driven responses to fire and N addition were subtle and not necessarily predictive of the long-term soil microbial community turnover.

4.1. Soil microbial responses to discrete events at short time scales

Because microbial communities have the potential to respond

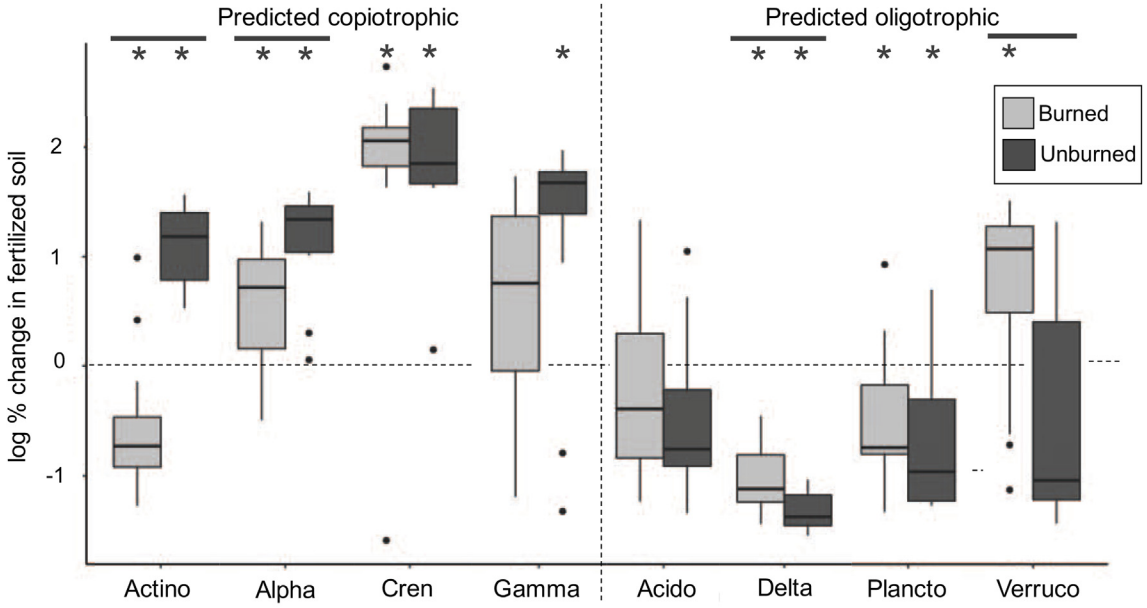

Fig. 3. Log response ratio (response to long-term N additions, averaging across all time points sampled) of the relative abundance of dominant prokaryotic taxa in burned (light grey) and unburned (dark grey) soils. Taxa are sorted into those predicted to be copiotrophic (left of vertical dashed line) and oligotrophic (right of vertical dashed line) based on previous studies (Ramirez et al., 2012; Leff et al., 2015). Boxes represent the quartile values for each taxon, and dots indicate outliers. Asterisks represent values significantly ($p < 0.05$) greater or less than zero, and a bar above a taxon response represents a significant difference between burned and unburned treatments.

quickly to fertilization pulses (Kuzyakov and Xu, 2013; Stark et al., 2004; Woods et al., 1987) and to the direct or indirect effects of burning events (Dooley and Treseder, 2012), we expected to observe pulses in microbial population sizes or changes in the relative abundance of certain taxa immediately following burning in April and fertilization in June. Surprisingly, responses to these events at both the population and community levels were minimal, except for two responsive populations that displayed transient dynamics in response to management events (Fig. S4). Order Burkholderiales relative abundance increased in only burned treatments immediately following the burn, indicating sensitivity to prescribed burning. The dominant fire-responsive Burkholderiales OTUs in our dataset had a taxonomic affiliation at the genus level with the soil-associated, cultured representative of this order *Janthinobacterium lividum* (Pantanella et al., 2007): the *Jacinthobacterium* OTU increased in burned soils between March and April from 0.099 ± 0.02% to 1.05 ± 0.2% relative abundance. We speculate that this transient response could be associated with indirect effects of fire, such as higher temperature or ultraviolet radiation on the bare soil, or changes in phosphorous and/or cation availability, rather than to the direct heat of the fire event. Only Crenarchaeota appeared to respond to the fertilization event, particularly in the unburned, N amended soils (changed from 0.015 ± 0.002% to 0.024 ± 0.004% between May and June). Crenarchaeota are generally more abundant in fertilized than unfertilized soils (Leff et al., 2015), and are a widespread and often dominant ammonia oxidizer group (Leininger et al., 2006; Prosser and Nicol, 2008; Taylor et al., 2012), suggesting that their response to the fertilizer pulse may be associated with transformations of available ammonium to nitrate. The Crenarchaeota were higher in relative abundance in long-term fertilized soils on average (Fig. 3), in addition to being sensitive to the fertilization event. In contrast, the Burkholderiales order was responsive to the fire event but not indicative of a long-term change in burning regime. This is most likely due to the nature of the environmental changes that each factor affects at different time scales: N retained in the soil post-fertilization can remain available throughout the year, but increased light following fire is a transient phenomenon, as plant growth closes the canopy in a matter of weeks.

There were many more changes in soil microbial community composition in response to long-term management regime than to episodic management events. Overall, the dampened short-term responsiveness of bacterial community and microbial population dynamics may be explained by the lack of direct heat penetration, and therefore mortality, from grassland fire, or the persistence of elevated available soil N associated with long-term fertilizer additions. Because some microorganisms are known to have generation times shorter or longer (ranging from hours to days to many weeks) than our one-week post-event sampling window, there may have been dynamics of certain bacterial taxa that were not detected. Direct fertilization may have more influence on soil nutrient status and microbial sensitivity on daily time scales, whereas fire may have more indirect impacts over multi-week time scales (post-fire soil exposure increasing soil temperature, water loss, or increased light).

4.2. Seasonal variation in soil microbial abundance and diversity

Although we did not observe evidence for pulses of growth or death in total soil bacteria or fungi following fire or fertilization events, bacterial population size dynamics were notably variable by season (Fig. 1). Our estimates of bacterial and fungal population size are in a consistent range as compared to other studies addressing growing-season microbial population sizes (Barnard et al., 2013; Fierer et al., 2009; Lauber et al., 2008). However, we know of no studies that estimated bacterial and fungal population sizes during the non-growing season using qPCR, and few that have examined F:B biomass in winter using other methods (Bardgett et al., 1997; Schadt et al., 2003). Based on the patterns in our data, we suggest that high F:B ratios may be typical of local conditions that do not favor bacterial growth, such as lack of active rhizodeposition. Among different biomes, high F:B ratios are generally found in forest soils, under conditions including higher ectomycorrhizal colonization of bulk soils and a greater presumed microbial dependence on less labile organic substrates due to lower soil C:N ratios (Fierer et al., 2009; Lauber et al., 2008; Strickland and Rousk, 2010). In this way, the high F:B ratio in our study soils during the non-growing season supports our prediction that bulk soil microbial community seasonal turnover would reflect greater reliance on soil organic

matter during this time. However, more studies investigating over-winter microbial activity and diversity are needed to better understand the dynamics of soil microbial substrate use preferences.

The mechanism for the sharp increase in prokaryotic population size in summer may be plant rhizodeposition providing labile C resources (Kuzyakov and Xu, 2013), as bacterial population size increases were correlated with changes in monthly mean air temperature, and thus roughly correlated with the timing of onset of the growing season (Garcia and Rice, 1994), and decreases were roughly correlated with the timing of plant resources shifting in major allocation to above-ground reproductive structures, followed by senescence. If each soil bacterial cell contains 100 fg C (Whitman et al., 1998) and 2.325 copies of the 16S rRNA gene (Table 1), the average summer increase in 16S rRNA gene copies of 1.46×10^{10} is roughly equivalent to 626 μg C g^{-1} dry soil, which would comprise a substantial proportion (50–100%) of the estimated total soil microbial biomass C in these soils (Ajwa et al., 1999; Zeglin et al., 2013). There is a considerable amount of uncertainty associated with this estimate, both in the amount of cellular C associated with each 16S rRNA gene based on within and among-taxon variability in cell size, morphology and intra- and extracellular C storage (Paul, 2015), and in the amount of cellular C extracted from the soil during chloroform incubation, fumigation and extraction assays (Jenkinson et al., 2004). Still, this observation suggests that bacterial growth and death associated with availability and cessation of plant rhizodeposits over the growing season can have a large impact on the soil C cycle.

An alternative explanation of the seasonal variation in microbial population sizes could be climatic variability or soil properties, because the tallgrass prairie has distinct seasonal changes in temperature and water availability (Knapp et al., 1998) and soil factors are well-known to drive microbial community processes (Regan et al., 2014; Schimel et al., 2007). Soil GWC was assessed for individual treatments; unfortunately, soil temperature was not, however local monthly air and soil temperature was used as a proxy for monthly changes in temperature. Neither soil water nor precipitation was correlated with 16S rRNA gene copy numbers: soil GWC varied relatively little, particularly over the growing season, during this wetter-than-average year (Fig. S1), in contrast to the sharper shifts in 16S rRNA gene abundance in June–August. However, ITS copy numbers were correlated with annual shifts in precipitation and GWC, possibly indicating differences in the spatial distribution of bacteria and fungi in the soil habitat. A reason that fungi may be sensitive to changes in soil water is their location on surfaces of soil aggregates, whereas smaller bacterial cells are more likely found in smaller, more consistently wet soil pore spaces (Strickland and Rousk, 2010). On the other hand, fungi are generally expected to be resistant to low soil water (Schimel et al., 2007), so indirect or other mechanisms may be at play. We were also surprised that the long-term fertilization and fire treatments did not affect estimated population sizes of soil bacteria or fungi. This result is in contrast to many studies in which increased available soil N has reduced microbial biomass (Treseder, 2008). Since QPCR population estimates are related to the amount of DNA, or number of genomes, in microbial cells, it is possible that N-driven biomass reductions detected with PLFA or chloroform fumigation methods may be due more to changes in cell size than cell number. Taken together, these results suggest that the availability of labile C resources could be a stronger driver of bacterial growth, and thus soil F:B cell ratio, than the monthly changes in air and soil temperature and soil water content or the differences in plant community composition and soil N availability between long-term land management treatments.

We also expected to observe seasonal shifts in prokaryotic community composition, particularly in relation to the striking seasonal fluctuations in bacterial population sizes. However, prokaryotic community composition was not significantly temporally variable (Fig. S3), and the only temporal pattern in diversity was an increase in bacterial richness between March and September (Fig. S2). While this change in richness could be related to increased growth of taxa that were rare before the growing season, the pattern was quite subtle, and occurred with different timing, relative to the growing-season 16S rRNA gene copy number increase, suggesting that all detected co-occurring bacterial populations increased and decreased in size nearly synchronously in June and September. While these results do not fit our original predictions regarding temporal turnover, they do corroborate patterns of temporal variability in bacterial community turnover in other managed grassland ecosystems. In cropped soils, where the growing season was marked by seeding and harvesting corn, the bacterial community showed a high degree of monthly turnover relative to a temporally stable bacterial community in adjacent successional grassland soil (Lauber et al., 2013). This suggests that in addition to rhizodeposition influences on bacterial growth, the year-round presence of plant cover could dampen variation in bacterial community turnover due to some alleviation of microbial C limitation via leaching and depolymerization of labile C from fresh litter. Also, compared to the predictable seasonal patterns in temperature and/or soil water, as well as soil microbial community composition, in alpine or Mediterranean ecosystems (Cruz-Martinez et al., 2009; Schmidt et al., 2007), our study site experiences less predictable drought/storm cycles during the summer and freeze/thaw cycles during the winter, with no stable snowpack. In this temperate grassland, bacterial communities may not show significant seasonal turnover because selective pressures from freezing and thawing are akin to pressures from drought, resulting in similar constraints on community composition through the growing season and over-winter (Schimel et al., 2007).

Another, non-exclusive, explanation for the strong seasonal variation in bacterial population size coupled with a lack of seasonal community turnover may be a shift of all populations comprising the bacterial community to a “dormant” state during the non-summer months (Lennon and Jones, 2011). We do not have data to assess this hypothesis, which might be addressed using microbial activity or rRNA analyses. We do note that our DNA-based results are unlikely to be strongly influenced by the presence of microbial extracellular DNA, which could bias measurements of microbial diversity, because previous evidence suggests that extracellular DNA is relatively insignificant in this soil type (< 1% prokaryotic DNA, < 8% fungal DNA), due to low soil sorption capacity for these molecules (Carini et al., 2016). In addition, the sharp decrease in 16S rRNA gene copy number between August and September (Fig. 1a) is evidence for degradation of DNA molecules in the soil. Most microbial taxa in these soils appear to be resistant to the challenges presented by seasonally dynamic environmental conditions, which include substrate limitation, variable temperatures, drying/re-wetting and freezing/thawing.

4.3. Decadal management treatments affect soil microbial abundance and diversity

Instead of event-based or seasonal variability, soil microbial communities differed most strongly with historical long-term management treatment (Fig. 2). Specifically, N fertilization explained the most variability in prokaryotic community composition, and there were important interacting effects of fire history and fertilization. Individual high-level taxonomic responses to long-term N manipulations supported predictions based on previous work from other studies (Leff et al., 2015; Ramirez et al., 2012), in that putatively copiotrophic taxa (Actinobacteria, Crenarchaeota, A-Proteobacteria and γ-Proteobacteria) generally had positive responses to N additions and were more abundant in unburned soils where N is more available, and putatively oligotrophic taxa (Acidobacteria, δ-proteobacteria, Planctomycetes and Verrucomicrobia) generally had negative responses to N additions and were more abundant in burned soils where N is less available (Fig. 3). Overall, our data support the copiotrophic hypothesis, which states that community shifts associated with N additions are a function of increased competitive dominance of fast-growing taxa (Ramirez et al.,

2012). Further, our results suggest that while the phylum-level affiliation of N-responsive taxa may be consistent, the magnitude of response, which is highly variable among systems (Leff et al., 2015), may be related to the baseline level of soil N availability. In annually burned soils, only half of the phyla responded significantly to long-term N-addition as predicted, and two phyla significantly responded contrary to predictions (Actinobacteria were less abundant in fertilized soils and Verrucomicrobia were more abundant in fertilized soils); while in unburned soils, all phyla responded as predicted, most (75%) in a significant manner, and the expected changes in relative abundance were generally greater in magnitude in unburned soils versus burned soils. In grasslands, the lack of fire increases the amount of organic N returned to the soil, and subsequently N availability is higher due to greater mineralization of plant litter N (Blair, 1997; Hobbie, 2015). Our data suggest that the sensitivity of soil microbial responses to N fertilization may be greater in soils with already-elevated N availability due to changes in land management.

Overall, these results highlight the need to consider the interactions between the factors controlling soil N and C availability, particularly over the decadal time scales at which the soil fertility outcomes of land management decisions accumulate, to better understand and predict microbial responses to environmental change. The temporal resolution of any study affects data interpretation, since microbial communities and populations have the propensity to respond on multiple time scales. The timing of changes in soil microbial populations and communities is critical to plant nutrition and nutrient cycling in ecosystems, because microbial turnover releases nutrients that support plant growth, and soil microbial communities are responsible for retention of nutrients during the non-growing season (Schimel and Bennett, 2004; Schmidt et al., 2007). Our data suggest that there are strong plant phenological effects on soil microbial populations, and that microbial community sensitivity to global change, such as N deposition, can be mediated by management, such as disturbance from fire. Because increased biologically available N from deposition events may have undesirable effects on plant or soil microbial diversity, decrease root and microbial biomass, or alter plant-soil feedbacks, prescribed fire might be considered a potential mechanism for mitigating these changes. This study aids in elucidating soil microbial responses to global change by adding temporal resolution to the current knowledge of microbial community turnover.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2018.03.023.

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