RESPONSES TO LONG-TERM FERTILIZATION AND BURNING: IMPACTS ON NUTRIENT DYNAMICS AND MICROBIAL COMPOSITION IN A TALLGRASS PRAIRIE

by

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Abstract

Anthropogenic activities impact ecosystems in numerous direct and indirect ways, affecting the cycling of carbon (C) and nitrogen (N) on local, regional and global scales. North America tallgrass prairie is an ecosystem profoundly altered by anthropogenic activities, with most native prairie converted to alternate land uses or heavily impacted by other environmental changes. While aboveground responses to anthropogenic drivers have received much attention, the responses of belowground biota, ecological processes, and nutrient allocation to land management and environmental change are poorly documented, especially over long timeframes. This research builds upon a long-term experiment (the Belowground Plot Experiment) initiated in 1986 at Konza Prairie Biological Station (Manhattan, KS). I utilized a subset of treatments to address the effects of annual burning vs. fire suppression and/or chronic N additions on soil C and N dynamics and microbial communities in tallgrass prairie. I measured a suite of soil variables related to C and N cycling during the 2012 growing season, including total soil C and N, microbial biomass C and N, *in situ* net N mineralization, potential N mineralization, *in situ* CO_2 efflux, and potentially mineralizable soil C. I also assessed changes in microbial community composition using microbial phospholipid fatty acids (PLFA) profiles. Annual burning significantly ($p \leq 0.05$) increased the soil C:N ratio and *in situ* CO_2 efflux, while decreasing potential ammonification and nitrification rates. Annual burning also increased total PLFA mass and relative abundance of fungi. Chronic N addition (100 kg N ha^{-1} $year^{-1}$) significantly reduced the soil C:N ratio, while increasing total soil N and potential nitrification and ammonification rates. Chronic N addition reduced potential C mineralization, microbial biomass C and N, and altered microbial community composition by increasing abundance of bacterial PLFAs and reducing fungal PLFAs. Sampling date also significantly affected many variables. These results indicate that different fire regimes and chronic N enrichment over decades affects soil C and N pools and transformations, as well as microbial biomass and composition. In total, this study highlights the importance of long-term ecological research and identifies likely changes in tallgrass prairie nutrient dynamics and soil microbial communities under increased N and frequent burning.

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Chapter 1 - Introduction

Background

In recent decades, interest in understanding and quantifying the impacts of anthropogenic activities on biogeochemical cycles at local, regional, and global scales has risen in response to the realization that ecosystem processes are affected by, and in turn feed back into, altered biogeochemical processes. For example, the amount of research on the cycling of carbon (C) in terrestrial ecosystems has increased in recent decades nearly as fast as global atmospheric CO_2 concentrations. Many of these studies have focused on understanding the dynamics of soil C and the effects of various global change drivers on soil C pools and fluxes. The turn of the century saw a rapid increase in studies to assess predictions of enhanced soil C loss from terrestrial ecosystems as a result of warming (Lu et al. 2013), the potential to enhance soil C sequestration with best management practices (Bruce et al. 1999), predictions of C saturation in loosely defined soil storage pools, and the general plea for a more mechanistic and quantitative understanding of soil C cycling across a range of ecosystem types (Falkowski et al. 2000, Schlesinger and Andrews 2000, Neff et al. 2002). It has also become apparent that the effects of altered biogeochemical cycles do not occur in isolation, and as studies of the responses of soil C dynamics to global changes rapidly increased, the field became even more complex as the effects of increasing nitrogen (N) availability were added. Some initial hypotheses about elevated N availability on soil C dynamics focused on a predicted increase in soil C storage with increasing N inputs (Falkowski et al. 2000), or potential changes in the distribution of C among different soil pools irrespective of changes in total soil C storage (Six et al. 2002). Modeling studies also have been used to test hypotheses surrounding the effects of N addition to soils on C cycling, providing potential explanations of why soil C respiration results often vary among studies under similar C or N additions, while at the same time pointing to future directions for research (Schimel and Weintraub 2003). As the foundation of today's understanding of the patterns and controls of soil C and N cycling took shape, N deposition rates and atmospheric CO_2 concentrations continued to rise ever higher.

More recently, our understanding of soil C and N cycling has continued to increase with a more comprehensive understanding of how temperature affects soil respiration (Templer and Reinmann 2011), better models of soil organic matter (SOM) turnover (Schmidt et al. 2011), and

a greater appreciation for the importance of diversity in soil ecosystems, which are arguably the most species-rich ecosystems in the world (Nielsen et al. 2011). There has been a call to expand studies of soil C and N cycling processes even more to include multi-factor experiments and analyses, though such experiments should also balance the need for complexity and statistical power, include both empirical and modeling in an iterative manner, consider temporal variation and nonlinear responses, and develop contingencies as needed (Templer and Reinmann 2011). In addition to new experiments, meta-analyses of existing studies can provide important insights. A meta-analysis of the effect of warming on ecosystem C cycling by Lu et al. (2013) analyzed 18 variables from 130 studies to conclude that under increased temperature we should expect soil C influxes (litter and plant root inputs) to increase due to increased plant production above- and below-ground. However, they also predicted that cumulative effluxes of C will likely increase by similar magnitudes, reducing sink and source potentials to near zero. Other studies have used soil from a broad range of sources to assess soil microbial respiration and N dynamics, with one study concluding that in 50 soils with a common substrate, N fertilization increased labile carbon mineralization in the short term, but would potentially reduce it by up to 40% in the long term (Craine et al. 2007). Another study at a continental scale used 84 soils to show that fates of microbial respiration can largely be predicted by only 3 variables; microbial biomass, temperature, and clay content (Colman and Schimel 2013). Clearly, we are beginning to understand broad-scale patterns and controls of soil C and N cycling processes. However, a better understanding of ecosystem-specific responses to various global change drivers and perturbations is still needed for making more practical management decisions in a changing world.

Worldwide, soils in all terrestrial ecosystems store more carbon (C) than the atmosphere and surface ocean combined and grasslands account for approximately 40% of terrestrial land cover (White et al. 2000, Baisden and Manning 2011, Schlesinger and Bernhardt 2013). Grassland soils in particular serve as a large pool for C, but grasslands are also some of the most disrupted ecosystems, and even minute imbalances in fluxes can have broad impacts on C source sink dynamics (Samson et al. 2004, Baisden and Manning 2011). In addition to storing C, grasslands have been identified as having high accumulation potential for other nutrients such as N and phosphorus (P) (Galloway et al. 2003). This is important, as anthropogenic sources of fixed N via fertilizer production and application and combustion of fossil fuels now exceeds the

rate of natural fixation (Galloway et al. 2003, Ryan 2012, Schlesinger and Bernhardt 2013). Increases in N inputs beyond biological demand (i.e., N saturation) can result in elevated losses though leaching of nitrate or dissolved organic N (DON) or enhanced gaseous fluxes via denitrification. Such enhanced export of N will undoubtedly have long-term consequences for ecosystem functioning, economic productivity, and human health. This is because humans are irrevocably linked to watersheds, which in turn are connected to, and influenced by, all sources of nutrient inputs and outputs. To this point, grasslands are of key importance serving as a primary buffer for $1/6^{th}$ of the world's major watersheds, further highlighting the need to understand how grassland ecosystems will respond to anthropogenic changes (White et al. 2000). Despite the rapid rate of change in nutrient inputs, some ecosystem processes are well buffered and responses to nutrient alterations may occur only, or be detectable only, over long timeframes, with labile C turnover in grasslands easily averaging over a decade (Neff et al. 2002). The importance and value of long-term studies of ecosystem responses to perturbations and chronic changes in resource availability is becoming more apparent, with grassland studies of key interest due to their extensive global distribution and the relatively high amount of disturbance impacting grasslands globally (Baisden and Manning 2011).

The global increase in atmospheric CO_2 and interest in global modeling has prompted many scientists to investigate C flux in a variety of ecosystems. Within one grassland system, Suyker and Verma (2001) estimated that an Oklahoma prairie fixed 804g of C/m^2/year and lost 536g of C/m^2/year to respiration and an additional 276g of C/m^2/year from annual burning. In effect, they found a zero net C flux, a conclusion that is supported by estimates of historic (pre-settlement) C balances for prairies (Schlesinger and Andrews 2000). Nevertheless, current predictions of C dynamics do not support a continued balance of C inputs and outputs, due to expectations of increasing temperature and more variable precipitation events (Peng et al. 2011). Changes in precipitation will undoubtedly change microbial and plant communities on local-to-global scales (Williams 2007, Craine et al. 2011), but predicting how these shifts will affect total respiration and C sequestration at plot, ecosystem, and global scales is not easily done. Additionally, temperature is changing both on global and regional scales. At a local management unit scale (field to watershed level), soil temperature often depends greatly on management (e.g. removal of biomass by grazing or haying or removal of accumulated detritus by burning in grasslands), and temperature generally correlates positively to soil CO_2 efflux (Schlesinger and

Andrews 2000, Bremer and Ham 2010). Taken together, the effects of changes in multiple abiotic factors on prairie C flux is still poorly understood, and soil respiration may increase as a result of a change in one factor while decreasing due to another making predictions of the net effects of future climatic changes difficult.

Aside from the effects of changing climatic conditions, C cycling can be further altered by the addition of excess N into the system, as is currently occurring in many terrestrial ecosystems as a result of increased N deposition rates. The effects of increased N inputs on C cycling vary as a result of changes in diversity, composition, and ANPP. In general, N additions alone promote greater NPP (below- and aboveground) in many terrestrial ecosystems, thus increasing total C inputs (Bruce et al. 1999). However, increased C inputs, in combination with higher levels of N availability, can also increase microbial and root respiration (Kelliher et al. 2010), potentially raising CO_2 efflux sufficiently to offset any increases in C input, leading to a near zero change in net C flux (Lu et al. 2013). Other studies have found that under the same conditions of increased N and C inputs, belowground respiration can either increase (Bol et al. 2006) or decrease (Craine et al. 2007). Although, it is generally thought that C inputs are a stronger driver of soil respiration than N at Konza Prairie Biological Station (KPBS), as microbial populations can cycle N tightly and may be more limited by C availability (Dell et al. 2005). In soils, microbial populations are often limited by available substrates and will break down available C substrates while conserving N until their C:N ratio is re-stabilized (Kelliher et al. 2010). One caveat is that with an increased level of N, overall C storage may increase, i.e. the C:N ratio remains the same with larger amounts of both elements maintained in the system (Keeler et al. 2009, Ramirez et al. 2010).

While many questions still surround soil C and N dynamics under specific conditions, one certainty is that native grassland ecosystems are rapidly declining. This is often due to land use changes such as agriculture or over grazing, but even many grasslands set aside for conservation are either experiencing or are at risk for degradation due to woody plant expansion; one of the most critical contemporary threats to the conservation of grasslands worldwide (Briggs et al. 2005). The specific causes of woody expansion into grasslands are debated, and may include factors such as overgrazing, reductions in fire frequency or intensity, elevated N deposition and increasing atmospheric CO_2 concentrations (Archer et al. 1995, Briggs et al. 2005). However, some general consequences of woody plant encroachment have been reported,

including higher ANPP and lower quality litter input (Knapp et al. 2008). Other studies report conflicting results, with ANPP nearly equivalent in grassland and shrub-encroached areas (Frank and Karn 2005). However, an increase in soil organic C and N and changes in mineralization rates are likely as woody vegetation takes over grassland areas (McKinley and Blair 2008). This suggests that it is not only aboveground diversity that is at risk, but also that soil microbial communities may be expected to change. For example, Ramirez et al. (2010) found that shifts in bacterial communities were closely linked to shifts in plant communities, but the relationship between above and belowground community structure is still not well understood. Currently, the primary drivers of soil community structure are generally thought to be edaphic factors such as nutrient availability, temperature, moisture, and pH, but the influence of aboveground communities on these drivers is often unclear due to the high interconnectedness of aboveground communities and soil properties (Fierer and Jackson 2006). Linking above- and below-ground communities together is of great interest and will allow for a more complete view of how woody encroachment in prairies could change soil communities or vice versa.

Implications of soil microbial community change extend past their relationship to the plant community structure. Neff et al. (2002) estimated that grassland soils store 3x more C than that of the aboveground plant portion. While microbial C is only a fraction of total soil C, the interactions of microbes with soil C pools will ultimately influence both microbial community structure and the net storage of soil C. Estimates of the storage of C belowground at KPBS are close to the values of Neff et al. (2002) with a mean root:shoot ratio of 3.2 and microbial populations estimated to make up 3% of the organic C pool (Blair et al. 1998, Rice et al. 1998). As microbial populations make up a significant portion of active belowground biomass it is important to understand how microbial communities change in time. PLFA analysis of restored prairies has shown that microbial community structure shifts significantly with aboveground plant changes and, ultimately, with changes in root and soil structure (Allison et al. 2005). In addition, microbial communities respond rapidly to episodic events such as precipitation, but recovery from large disturbance like tillage often takes decades. For example, 20 years of tallgrass prairie restoration has been shown to significantly shift PLFA composition from agricultural structure, but native structure was still not restored (McKinley et al. 2005). This means that even for quickly reproducing microbial communities, significant changes in community composition are a long-term process. Add this to the current lack of knowledge

regarding how microbial and plant communities are connected, and predictions of plant and soil responses to future changes in ecosystem drivers remain uncertain.

Ecosystems are in general very complex systems, and grasslands are no exception. They are in fact one of the most complex and globally extensive ecosystems. Our understanding of these complexities is just in its infancy, and with the recent (relative to ecosystem history) impact of humans on the landscape our understanding is further complicated. Human activities will undoubtedly persist in the coming decades; accordingly, a better understanding of how anthropogenic influences will change plant and microbial communities alike is of critical importance. Community change will in turn have a trickle down effect on processes such as nutrient cycling, soil CO_2 efflux and C sequestration, and ultimately to more tangible benefits that include economic value. Further complicating our predictive power, responses to anthropogenic changes may be exacerbated by positive feedbacks; meaning initial anthropogenic changes will cause community shifts which will alter nutrient dynamics resulting in further community structure changes. In all, grassland complexity supports the need for more integrated and longer-term studies, which will allow for more a more comprehensive understanding of overall grassland ecosystem dynamics and provide for more accurate forecasts of future changes.

Justification and Current Research

My research builds upon the broad foundation of prior research done on C and N dynamics globally, while focusing specifically on the long-term effects of fire and N enrichment, both independently and in combination, on tallgrass prairie soil C and N pools and fluxes. The Flint Hills region of Kansas and northern Oklahoma is home to much of the remaining tallgrass prairie in North America, a product of the rolling hills and rocky, non-arable soils of this region. The unfavorable conditions for arable agriculture led to establishment of an extensive rangeland enterprise in the region that preserved the native grasslands but led to increased management of fire (a natural disturbance) frequency to optimize grass production for target grazing densities. Fires are used today as a management tool, with ranchers commonly burning on an annual or biennial basis. In addition, regional agricultural activities and population expansion in surrounding areas has led to increased anthropogenic inputs of N. Looking to the future, an understanding of how these anthropogenic activities and inputs will influence soil C and N processes is important to ensure a stable and productive prairie ecosystem. Studies of the effects

of fire frequency in tallgrass prairie became more common starting in the mid 1900's and were eventually followed by studies of elevated N inputs. An understanding of short-term responses to fire and increased N inputs were well established by the end of the century, but longer-term effects are still poorly understood, mainly due to the difficulties surrounding the establishment of multi-decadal studies. One such long-term research project is the Belowground Plot Experiment (BGPE), established in 1986 at the Konza Prairie Biological Station to address the long-term effects of contrasting fire treatments, nutrient additions and mowing on tallgrass prairie ecosystems.

This research utilized a subset of BGPE plots that focus on fire (annual spring burning and long-term fire suppression) and N fertilization (both independently and in combination) to see how soil C and N cycling has been affected following 27 years of experimental manipulation. The ability to build on past studies conducted on this site, which assessed selected responses to the treatments during different times in the experiment's history, allows for a more complete and unique view of changes through time. Specifically, this work used intensive soil sampling in the summer of 2012 to explore how annual burning and chronic nutrient additions alter microbial composition and nutrient cycling processes in tallgrass prairie. In addressing this goal, I focused on two objectives to help elucidate the consequences of long-term contrasting fire regimes and nitrogen inputs. These objectives were to: 1) characterize soil microbial community changes after 27 years of nitrogen fertilization and/or burning, and 2) investigate how 27 years of nitrogen fertilizer and/or burning has altered soil and microbial carbon and nitrogen pools. To address these objectives I used phospholipid fatty acid analysis (PLFA) of microbial community composition and measurements of selected soil nutrient pools and transformations, including; total and mineralizable soil carbon C and N, microbial biomass C and N, *in situ* CO_2 efflux, and *in situ* net nitrogen mineralization and nitrification. Ultimately, this study will help understand the effects of anthropogenic disturbances in modern grassland ecosystems, including potential shifts in soil microbial communities as well as changes in nutrient dynamics.

Site Description

The Belowground Plot Experiment (BGPE) was established in May 1986 at Konza Prairie Biological Station (KPBS, 39°05N, 96°35W) as an integral part of the Long-Term Ecological Research (LTER) program. KPBS includes 3,487 ha of mostly native tallgrass prairie located in Kansas' Flint Hills. The dominant vegetation is comprised of C_4 grasses typical of undisturbed native tallgrass prairie in the region including: *Andropogon gerardii* (Big bluestem), *Sorghastrun nutans* (Indiangrass), *Panicum virgatum* (Switchgrass) and *Schizachyrium scoparium* (Little bluestem) (Freeman 1998). The BGPE soil is classified as an Irwin silty clay loam (fine, mixed, mesic, Pachic Argiustoll (Garcia and Rice 1994). Mean annual air temperature at KPBS is 13 °C with July ranging from 20-33 °C and January ranging between -9-3 °C (Nippert and Knapp 2007). Average precipitation is 835 mm, with ca. 75% coinciding with the growth season in late spring and early summer (Hayden 1998).

The BGPE was established near the headquarters area of KPBS to test the effects of annual burning, mowing with litter removal (simulated grazing), and nutrient additions on above- and below-ground processes. To accomplish this, plots were arranged in a split-strip random block design (Figure 1.1) with 4 main blocks, each consisting of two 25 x 50 m whole plots. Each plot was randomly assigned a burn treatment (annually burned or unburned), and split for one of two mowing treatments (mowed and raked or unmowed). Four nutrient treatments (control, +N, +P and +N+P) were applied in strips across the split plots for a total of four replicates of the 16 treatment combinations. Annual burning was done in late April to early May, and nutrient additions were applied around two weeks later. Marked changes in aboveground species composition have occurred (Figure 1.2) and in 2003 the mowing treatment was stopped due to extensive spread of the non-native grass *Bothriochloa bladhii* (Caucasian bluestem) in the mowed treatments. Aboveground biomass for all 64 plots was sampled annually each fall (October-September) from two $0.1m^2$ quadrats (not all data presented here). Accidental burns occurred in the unburned plots 17-24 (spring, 1994) and plots 1-2 (spring, 1995). For this study, I sampled the control and +N subplots in both annually burned and unburned fire treatments.

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Figures

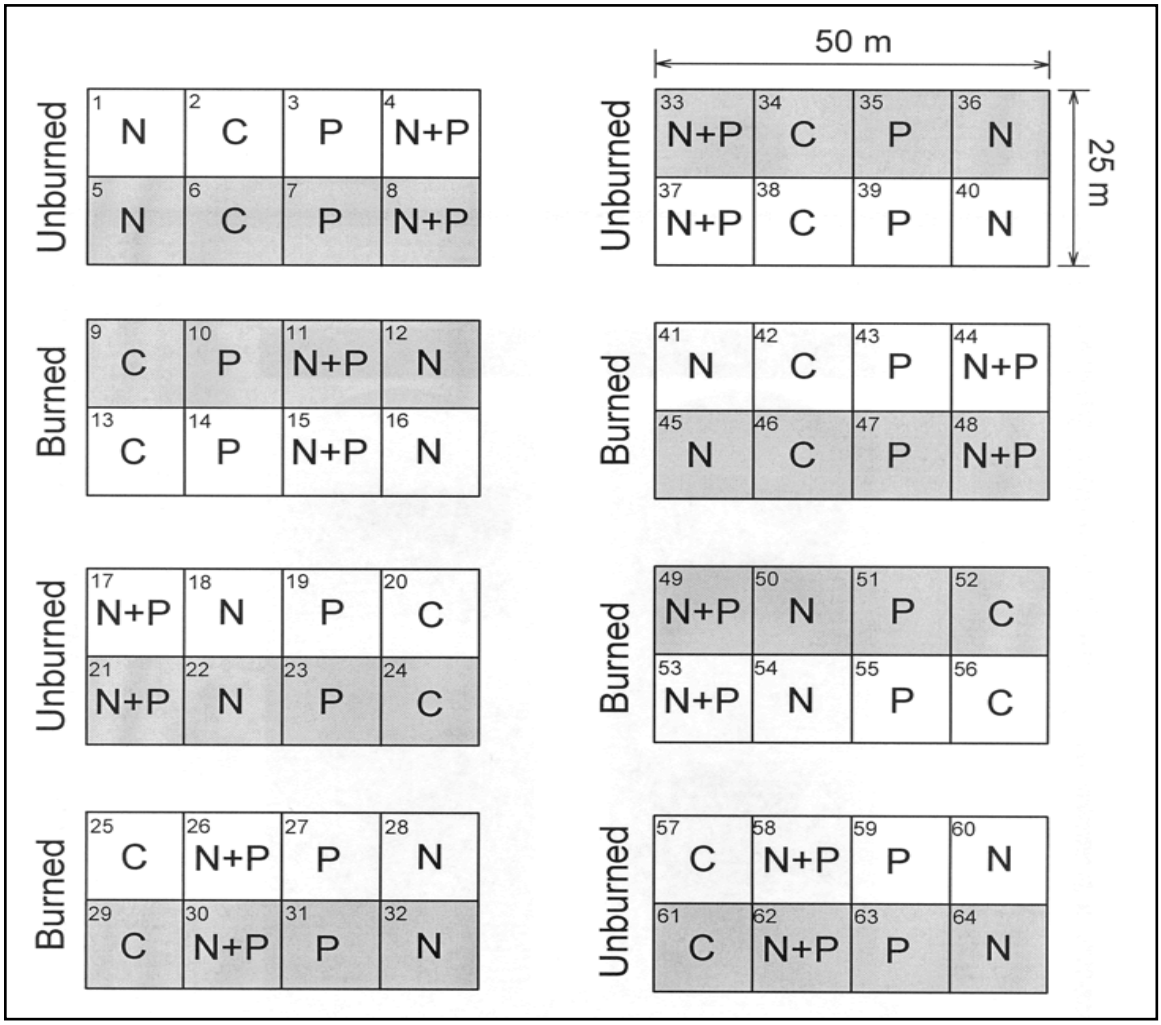

Figure 1.1 Experimental design of the BGPE where shaded plots are mowed and raked annually (stopped in 2003) in late June. Nutrients (C=control, N=10g N·m^{-2} as ammonium nitrate, P=1g P·m^{-2} as superphosphate, and N+P=10g N·m^{-2} + 1g P·m^{-2}) were applied annually by hand broadcasting in early June. Accidental burns occurred in plots 17-24 in 1994 and plots 1 and 2 in 1995 (adapted from Rice et al. 1998).

Figure 1.2 Visual comparison of the BGPE in late 1980's and in fall of 2011 taken from slightly different angles. Black arrows mark plot 1 highlighting the visible changes in aboveground vegetation through time.

Chapter 2 - Carbon and Nitrogen Dynamics

Abstract

Contrasting land-use and nutrient enrichment can alter ecosystem structure and function in numerous ways, often affecting carbon (C) and nitrogen (N) dynamics on local, regional, and global scales. In the tallgrass prairies of North America, prescribed burning is a common management practice used to control woody plant encroachment and to increase cover and productivity of dominant warm-season grasses. While the aboveground responses to contrasting fire regimes and nitrogen addition are relatively well known, there are fewer studies that have addressed responses belowground. We utilized a long-term experiment featuring contrasting prescribed fire treatments (burned annually or unburned) and nitrogen enrichment initiated in 1986 at Konza Prairie Biological Station (Manhattan, KS USA) to investigate belowground processes. Burning and nitrogen addition independently and interactively affected soil C and N dynamics, though the effects varied among specific measured response variables. Results from repeated measures ANOVAs indicated that burning increased total C:N ($p=0.0306$), *in situ* C mineralization ($p=0.0348$), total aboveground biomass ($p=0.0178$), and soil temperature and moisture ($p<0.0001$). Addition of N lowered total C:N ($p=0.0003$) and potential C mineralization ($p=0.0006$), while increasing total soil N ($p=0.0284$), inorganic N availability ($p=0.0003$), and total aboveground biomass ($p=0.0032$). An interaction effect of burning, fertilization, and date increased potential net N mineralization in unburned nitrogen fertilized plots an average of 7.75x over burned control plots ($p=0.0137$) across the summer sampling. These results indicate cumulative changes in soil processes in response to contrasting fire management and nutrient enrichment, with implications for C and N cycling and storage over longer timeframes.

Introduction

Terrestrial ecosystems globally store over twice the amount of carbon (C) as the atmosphere (Baisden and Manning 2011), with soils accounting for nearly 75% of that pool (Schlesinger and Bernhardt 2013). Warming temperatures, increasing atmospheric CO_2, and changes in total precipitation and/or patterns of rainfall events are all expected to shift the terrestrial C balance (Schlesinger and Andrews 2000, Jackson et al. 2002, Frank et al. 2002, Knapp et al. 2008, Reid et al. 2012). Ecosystems, such as the tallgrass prairie of the Flint Hills,

that acquire or maintain a mean annual precipitation (MAP) ≥ ~850 mm under changing precipitation patterns are anticipated to be a source of CO_2 via increased soil organic carbon (SOC) loss through heterotrophic respiration (Barger et al. 2011, Berthrong et al. 2012). Rising temperatures are expected to increase losses of C from ecosystems (Fang et al. 2005, Suseela et al. 2012), but regional variations in temperature and MAP must be considered together due to the expectations of strong temperature-moisture interactions on heterotrophic respiration rates (McCulley et al. 2005, Craine and Gelderman 2011). Additionally, increasing levels of reactive nitrogen (N), due to anthropogenic alteration of the N cycle (Galloway et al. 2003), are expected to greatly affect primary production as well as decomposition, further magnifying uncertainties regarding future C source-sink dynamics (Canfield et al. 2010, Guntiñas et al. 2012). In general, greater N inputs are expected to increase above- and belowground net primary productivity (A/BNPP) in most temperate terrestrial ecosystems (Baer and Blair 2008, Ziter and MacDougall 2013). Nitrogen enrichment may also increase decomposition and heterotrophic soil respiration, however this is not universal (Schimel and Weintraub 2003, Bowden et al. 2004, Kelliher et al. 2007, Peng et al. 2011, Ramirez et al. 2012). Due to the large amount of C stored in terrestrial systems, even minor changes in the balance of inputs and outputs caused by anthropogenic activities have high potential to cause large shifts the global C cycle, with possible feedbacks to future climate changes.

Grasslands are of particular interest with respect to potential changes in C and N cycling as they are broadly distributed, highly impacted, and are under recent threats of abrupt transition changes due to land-management and climate change (Frank and Karn 2005, Bestelmeyer et al. 2011, Alford et al. 2012). An understanding of grassland responses to changing climate and anthropogenic inputs is critical for effective management, and may allow for the potential to use these ecosystems in non-traditional ways such as for C storage and buffering waterways from excess nutrient inputs. A broad understanding of aboveground responses in grasslands to common management practices is already in place where, for example, diversity and species richness tend to be promoted by burning and/or grazing (Collins et al. 1998, Collins and Calabrese 2011). Loss of plant species richness is common with increased N deposition (Johnson et al. 2008), and significant losses have even been shown to result from chronic low levels of N enrichment (Clark and Tilman 2008). Precipitation frequency and amount are also known to have effects on species composition shifts, although other factors (i.e. grazing and fire) are often

appreciably more influential (Collins et al. 2012). ANPP tends to increase with greater MAP (McCulley et al. 2005, Vourlitis 2012) and increased N from deposition or following relatively infrequent burns and N accumulation in plants and soil (Seastedt et al. 1991, Ojima 1994, Blair 1997, Holub et al. 2012). The potential for C accumulation and sequestration in grasslands under increasing atmospheric CO_2 and temperatures is variable with predicted positive, negative, and zero net change in C sequestration (Suyker and Verma 2001, Fang et al. 2005). While recent research has begun to link aboveground and belowground interactions, an integrated understanding of belowground processes in grasslands and the responses to common anthropogenic activities is still not known, mainly due to a lack of belowground studies, but also because of high regional variability in soils (Price et al. 2012).

The Flint Hills of eastern Kansas and northern Oklahoma includes the largest extant tracts of tallgrass prairie in North America and has been the focus of extensive aboveground research and a growing amount of belowground studies. This provides an ideal system to expand knowledge of belowground processes especially in response to regional anthropogenic impacts. Fire, a common management tool, is known to affect belowground processes by reducing inorganic soil N availability with annual prescribed burning (Ajwa et al. 1999, Dell and Rice 2005), while increasing root biomass (Kitchen et al. 2009) and cumulative (hetero- and autotrophic) CO_2 efflux (Knapp et al. 1998). In addition to altered fire frequencies, increased N deposition as a result of anthropogenic activities is a regional factor, with a 26 year (1986-2011) average of wet N deposition at KPBS of 5.02 ± 0.187 kg-N/ha (NADP 2013). The effects of chronic low-level increases in reactive N have primarily been researched with higher levels of fertilization (>50 kg-N/ha) over shorter timeframes to simulate long-term cumulative effects of N deposition, although some studies have used fertilization gradients to address this issue (e.g. Fornara and Tilman 2012). Fertilization has been shown to increase microbial biomass (Garcia and Rice 1994) in tallgrass prairie, and a ^{15}N tracer study in these grasslands showed that soil microbes immobilize inorganic forms of N rapidly (<11% remaining after six days) before it is transferred more gradually into plant biomass (Dell and Rice 2005). Five years after application, burned prairie retained 78% of the ^{15}N tracer, while unburned prairie retained only 52%, with less than 4% detectable in inorganic forms in the soil (Dell et al. 2005). Effects of fire and nitrogen have been researched in many contexts in the tallgrass prairie, however long-term

studies in the region are less common but could play a critical role in our understanding and projections of future ecosystem responses.

This study builds on an extensive body of tallgrass prairie research, and provides a unique look into the long-term effects of annual fire and chronic N deposition on belowground processes. My main objective was to enhance our existing knowledge of potential C and N mineralization, *in situ* C and N mineralization, and total C and N storage after prolonged (27 years) annual burning and/or chronic N fertilization (100 kg-N m^{-2}), as well as the abiotic drivers that affect these soil processes. Prolonged annual burning was hypothesized to reduce soil moisture and increase soil temperature, relative to unburned prairie due increased solar radiation. Annual burning was also hypothesized to decrease *in situ* net N mineralization rates and potentially mineralizable N, as well as total soil N through greater N limitation. In contrast, I hypothesized that annual burning would increase total soil C and *in situ* and potential C mineralization as a consequence of increased above and belowground C inputs. Additionally, I hypothesized that chronic N fertilization would decrease potential and *in situ* C mineralization due to labile C loss, increase total N and total C as well as *in situ* and potential N mineralization because of reduced N limitation, while having no effect on soil moisture or temperature. Finally, an interaction of fire and N fertilization was hypothesized to have mixed effects where C and N mineralization (both potential and *in situ*) as well as total soil C and N became intermediate values of the single factors.

Methods

Soil Sampling

Sampling locations within plots were randomly assigned to locations that avoided past experimental infrastructure and were a minimum of 1.5 m away from a plot edge (Petersen and Calvin 1996, Paul et al. 1999). Within each plot (burned and unburned, control and N addition treatment plots, Figure 1.1) four PVC collars (10 cm diameter x 8 cm deep) for soil CO_2 efflux measurements were installed by driving them 5-6 cm into the ground (Figure 1.2) the day following the spring burn (April 9, 2012) and allowed to settle for a week prior to beginning measurements. For monthly estimates of *in situ* net N mineralization, a modified buried tube method was used (Raison 1987). At the start of each measurement period, a PVC nitrogen

mineralization tube measuring 15 cm long and 5 cm diameter was driven into the soil ca. 1 m from each efflux collar (different cardinal direction for each date) to a depth of 10 cm. Mineralization tubes were capped with a plastic lid to prevent rainwater infiltration and predrilled holes in tube walls above the soil surface maintained aerobic conditions. At the same time the mineralization tubes were installed, and additional soil core 5 cm in diameter was taken to a depth of 10 cm roughly halfway between the nitrogen mineralization tube and CO_2 efflux collar for initial measurements. Mineralization tubes were left to incubate in the field for 32 days for each measurement period.

Initial soil cores and post-incubation mineralization tubes with soils were immediately transferred to gallon Ziploc bags and composited by plot (4 cores per plot), and placed into a cooler over ice. Transport to the lab was a maximum of three hours after beginning sampling and samples were refrigerated upon arrival. Within 36 hours of sampling, soils were sieved to 4 mm and any remaining roots and particulate organic matter was hand-picked for 12 minutes (Boone et al. 1999). Subsamples of soil for phospholipid analyses were taken immediately after root picking and placed into a -20 °C freezer. Nitrile gloves were worn during all steps to prevent lipid contamination. Remaining soil was then placed back into refrigeration for storage prior to subsequent analyses.

Dry Matter

Dry matter content of field-collected soils was measured by weighing out two replicates of 20-30 g of field moist soil into #1 brown paper bags. Samples were dried at 60 °C to prevent ammonia volatilization for 36 hours or until constant weight was reached. Dry matter percent was then calculated using Equation 2.1 (Jarrell et al. 1999, Haney and Haney 2010).

Equation 2.1 Soil Dry Matter Percent

$$DM\% = (dry\ soil\ weight\ /\ field\ moist\ weight)\ x\ 100$$

Bulk Density

Bulk density (BD) for the top 10 cm of soil was calculated using Equation 2.2 for the June 12 sampling date, and was assumed to not change throughout the summer. Soil dry weight equivalents from all subsamples were summed and added to the oven-dried mass (60 °C for 48 hours) of remaining soil and divided by the core volume/4 due to the compositing of soil cores within plots (Elliott et al. 1999, Haney and Haney 2010).

Equation 2.2 Bulk Density

$$Bulk\ Density = \frac{dry\ weight\ (g)}{volume\ of\ core\ (cm^3)}$$

Total Carbon and Nitrogen

Dried soil samples (see *Dry Matter*) were homogenized and ground in a 8000D mixer/mill (SPEX, Metuchen, NJ) for four minutes until the consistency of talcum powder was reached. Acetone was used to clean parts between samples to prevent contamination. Then, 20-30 mg of each soil was weighed out and placed into 5x9 mm aluminum tins (Costech #41077) using acetone washed tools. Tins were folded to seal and analyzed for total C and N concentrations on a Flash EA 1112 C/N auto analyzer (Thermo Fisher Scientific, Waltham, MA). Acetanilide samples were used for calibration and a known sample was run as a check after every 16 samples.

Field Nitrogen Mineralization

Field mineralization PVC tubes were placed in the field for four sequential sampling periods throughout the summer according to methods described in the *Soil Sampling* section. Tubes were left in the field for 32 days and upon removal were treated with methods described in the *Soil Sampling* section. Replicate samples (12 g field moist) were then extracted using methods described in the *KCl Extraction* section and field net mineralization, ammonification and nitrification rates were calculated using Equations 2.3 and 2.4 where Initial [N] are concentrations of N as NO_3^- and NH_4^+ obtained from the initial soil cores and Final [N] were

concentrations obtained from the post-incubation mineralization tube soils (Robertson et al. 1999, McKinley and Blair 2008, Dell and Rice 2005).

Equation 2.3 [N] µg N·g Dry Soil

$$[N] = \frac{[(ppmN\ sample - ppm\ blank)\ x\ 50ml\ KCl\ ]}{grams\ dry\ soil}$$

Equation 2.4 Nitrogen Mineralization Rate

$$N\ mineralization/day\ = (Final\ [N] - Initial\ [N]\ )/\#\ days\ incubated$$

Lab Nitrogen Mineralization

Potential nitrogen mineralization rates were assessed concurrently with potential C mineralization rates (see *Lab Carbon Mineralization* section for description). Upon completion of the 30-day incubation and final CO_2 measurement, soil samples were extracted using methods described in the *KCL Extraction* section. Lab net N mineralization rates, ammonification rates and nitrification rates were then calculated using Equations 2.3 and 2.4, where Final [N] were concentrations of N as NO_3^- and NH_4^+ obtained at the end of the lab incubations and Initial [N] were values from the initial soil core (same as Initial [N] for Field Nitrogen Mineralization) (Robertson et al. 1999).

KCl Extraction

Initial soil samples as well as field and lab incubated soils were all extracted with 2 M KCl to determine ammonium and nitrate concentrations required to calculate nitrogen mineralization rates. Sample replicates of ca. 12 g field moist soil were measured into acid washed 125–ml Erlenmeyer flasks, and 50 ml of 2 M KCl was added to each sample (Bremmer and Keeney 1966). Flasks were sealed with Parafilm and shaken for one hour at 200 rpm on an orbital shaker. Samples were removed and allowed to settle for 45 minutes prior to filtration with a 30–ml syringe fitted with a 0.4 µm polycarbonate filter (Fisher K04CP02500). Extracts were collected in 20 ml plastic scintillation vials and stored frozen until analysis. Analysis of KCl extracts was done on an OI Analytical Flow Solution IV, following protocols described in the *Flow Solution IV* section.

Flow Solution IV

Nitrate and ammonium concentrations in extracts from nitrogen mineralization analyses and from microbial biomass nitrogen digests (persulfate digestion) were determined on an OI Analytical Flow Solution IV 3100 autoanalyzer (College Station, TX). For NH_4^+ measurements the salicylate hypochlorite procedure was used while NO_3^- was measured with the Griess-Ilosvay procedure where NO_3^- is reduced via Cd/Cu to NO_2^- for colorimetric analysis. It should be noted that only NO_3^- was measured for MBN samples (Chapter 3) as the persulfate digestion oxidizes all forms of nitrogen to NO_3^-. The standard curve for nitrogen mineralization followed Table 2.1 where nitrate Stock A was 7.218 g $KNO_3 \cdot L^{-1}$ DI (oven dried; 1,000 ppm) and Stock B (10 ppm) was 1 ml A in 100 ml DI. Ammonium stock A (1,000 ppm) was made from 4.7168 g $(NH_4)_2SO_4 \cdot L^{-1}$ DI (oven dried), Stock B (100 ppm) was made with 10 ml of A in 100 ml DI and Stock C (10 ppm) was 1 ml of B in 100 ml DI. MBN standards were established using methods in the *Microbial Biomass Nitrogen* section. The carrier solution was used for MBN analysis wash in place of the standard 2M KCl wash used during the mineralization runs. Samples with concentrations >1ppm above a high standard were diluted 1:1 or 2:1 with carrier (KCl or K_2SO_4) and reanalyzed until values within the acceptable 1ppm margin were obtained (Mulvaney 1996).

Lab Carbon Mineralization

Potential C mineralization was measured in replicate samples of ca. 12 g of field moist soil (in acid washed Erlenmeyer flasks) that was brought up to 60% water filled pore space using DI water (this was using an assumption of a bulk density of 1 $g \cdot cm^{-3}$, which was later calculated to be an average of 1.097 $g \cdot cm^{-3}$ giving an actual WFP of 64%) following Equations 2.5 and 2.6 modified from Elliott et al. (1999) and Haney and Haney (2010).

Equation 2.5 Additional Water for 60% Water-filled Pore Space

$$\%\ of\ wet\ soil\ to\ add\ as\ water\ =\ (TP\%\ x\ grams\ dry\ soil\ -\ grams\ water)/grams\ wet\ soil$$

where,

TP% is Total Porosity Percent calculated using Equation 2.6.

Equation 2.6 Total Porosity Percent

$$TP\% = (1 - (BD/PD)\ x\ WFP$$

where,

bulk density (BD) was assumed to be 1 g·cm^{-3},

particle density (PD) was assumed to be 2.65 g·cm^{-3},

and WFP was set at 0.6 (60%)

Open flasks containing soil samples were then placed into quart sized mason jars with 10 ml of DI water in the bottom to prevent drying. Lids were fit with rubber stoppers (Fisher 06-406-11B) that were sealed inside and out with clear silica caulk. This allowed for headspace sampling with a syringe on days 2, 4, 8, 12, 20, and 30 of the incubation (Robertson et al. 1999).

Measurements of CO_2 concentration were done on a Gas Chromatograph 8A (Shimadzu, Kyoto, Japan). Standard curves were done prior to the first measurement, halfway through sampling, and after sampling was complete to ensure results remained consistent. To generate the curves, 0.1, 0.2, 0.4, 0.6, and 0.8 ml of 1% CO_2 standard gas was run on the GC. Sample headspace was mixed by pumping a 10 ml syringe five times prior to drawing 0.5 ml of sample into a 1 ml syringe. The sample was then injected into the GC for analysis. After sampling, jars were vented for 30 minutes to prevent excessive CO_2 accumulation and ensure aerobic conditions. Lab air was measured with every sampling date and background CO_2 concentrations were subtracted from sample measurements to establish a "zero" baseline.

Field Carbon Mineralization and Soil Temperature

In situ measurements of soil CO_2 flux were started on June 11, 2012 and were repeated ca. every 12 days. If a precipitation event occurred around a sampling date, measurements were delayed 3 days to prevent low CO2 flux due to water-filled pore spaces or high flux values due to the flush of CO_2 following microbial turnover with wetting of dry soils. For each measurement period, I used two Li-Cor 8100 portable infrared gas analyzers (Lincoln, NE, USA) fitted with a soil temperature probes to measure fluxes at the 64 collar locations (see *Soil Sampling* section). Flux measurements lasted 45 seconds and temperature probes were inserted to a depth of 10 cm

ca. 20 cm from the outside of the PVC collar. Plot averages for temperature and flux were calculated from the four plot locations to reduce heterogeneity within plots.

Collar offset values were measured three times throughout the summer as the average of three measurements per collar (i.e. the height from soil to top of collar). These measurements were then used in Equation 2.7 (as the offset variable, other variables are recorded by the Li-Cor 8100) to correct the default collar height (2 cm) with the actual collar height and calculate more accurate flux values throughout the summer even as collars shifted (Harper et al. 2005).

Equation 2.7 CO_2 Flux Volume Correction

$$flux = \frac{((10\ x\ \left(Vtotal + \left((offset - 2.0)\ x\ area\right)\right)\ x\ IV\_pressure\ x\ (1 - (IV\_H2O/1000)))}{(8.314\ x\ area\ \ x\ (IV\_Tcham + 273.15))) * (dCdry\_dt)}$$

Continuous Soil Temperature and Moisture

Soil temperature and moisture were measured to a depth of 10 cm from April, 4th to August, 10th during the 2012 growing season in a representative subset of plots (Figure 1.1; plots 38, 41, 42) to gain an understanding of how soil temperature and moisture dynamics varied with treatment in these plots. For both variables measurements were taken every 30 seconds and a 30-minute average was recorded. Soil moisture was measured using a Campbell Scientific CS616 while temperature was measured using a Type I thermocouple using a Campbell Scientific 107C in the logger unit for a reference temperature. Battery run Campbell Scientific CR10x loggers were used and a total of four moisture and temperature readings were recorded per plot with the exception of plot 38 where only three temperature measurements were taken. The severing of a thermocouple and moisture wire in plot 42 during soil coring in mid summer lead to only three measurements for moisture and temperature in that plot. Battery issues in the woody (plot 38) logger lead to a number of gaps in data as seen in Figures 2.17 and 2.18.

Statistical Analyses

Data from four sampling intervals were analyzed in a repeated measures two-way ANOVA using a mixed effects model (PROC MIXED) with burn and fertilization treatments as fixed effects and date and plot as random effects (SAS Institute V9.2, 2008). Simple linear regressions (PROC REG) were also run to see if CO_2 efflux and temperature were correlated as well as to compare data logger temperatures and volumetric water measurements. Outliers that were more than three standard deviations from the mean were excluded from the analysis and log transformations were done when appropriate for a regression. Significance was measured at $p < 0.05$ for all tests unless otherwise noted. When an interaction between date and a main effect was significant, post hoc slices (lsmeans/slice=date), F-test contrasts, were used to identify dates where main effects were significant.

Results

Total Carbon and Nitrogen

Treatment-level mean values by date and patterns of seasonal changes in total soil C, N, and C:N ratios are shown for the summer of 2012 (Figures 2.2-2.4). There were no statistically significant differences in total C or N concentrations in the soil on a per gram dry weight basis. However, when concentrations were converted to standing stocks of soil C and N, expressed on an aerial basis to a depth of 10 cm using soil bulk density of individual plots (measured on the June 12th sampling date), significant differences emerged, despite bulk density alone not being significantly affected by the treatments. A repeated-measures ANOVA on bulk density-corrected soil C values indicated no difference in total C storage across dates or across treatments within individual sampling dates. Conversely, total soil N was affected by both fertilization ($p=0.0284$) and date ($p=0.0146$), with a tendency for higher soil N in late-summer while fertilization increased total N by an average of 8% (22.7 kg ha^{-1}) regardless of dates and burn treatments. Soil C:N ratio was significantly affected by burning ($p=0.0306$) and by nitrogen addition ($p=0.0003$) with burning tending to increase, and N fertilization decrease, the soil C:N ratio.

Inorganic Nitrogen Availability

Availability of ammonium and nitrate across summer sampling dates is shown in Figures 2.5 and 2.6, respectively. A repeated-measures mixed model ANOVA indicated a three-way interaction between date, burn, and fertilization for both ammonium ($p<0.0001$) and nitrate ($p<0.0001$) availability. A post-hoc test of slices by date for the three-way interaction indicated that ammonium availability in the burned-fertilized plots was significantly increased on the June and July sampling dates a minimum of 6.7x and 5.03x, respectively, relative to other treatments. Similarly, post-hoc slices by date for nitrate availability showed a trend for increased availability in fertilized plots on the June, July and August sampling dates (Figure 2.6). Nitrate availability for burned-fertilized plots was highest in June and decreased 2.7x throughout the summer, while unburned-fertilized plots showed increased (3.5x) nitrate availability through the summer. There was no difference in available nitrate, ammonium, or total inorganic N (sum of ammonium and nitrate, data not shown) between burned-control and unburned-control plots on any of the sampling dates.

Nitrogen Mineralization

Potential ammonification and nitrification rates are presented in Figures 2.7 and 2.8 respectively, while the net N mineralization rate was measured as the sum of ammonification and nitrification rates (Figure 2.9). A repeated-measures mixed model ANOVA indicated a three-way interaction between date, burn, and fertilization for potential ammonification ($p=0.0006$, Figure 2.7). Post-hoc test of slices for the three-way interaction effect showed that potential ammonification was different on the June 12 and July 27 incubations due to negative net ammonification (immobilization) rates that were at least 4.8x greater in the annually burned and fertilized treatment relative to all other treatments. Potential nitrification (Figure 2.8) was significantly influenced by burning ($p=0.0396$), with annual burning tending to reduce potential nitrification, and by the interaction of date x fertilization ($p=0.0358$). Post hoc analysis of the interaction term showed that nitrogen fertilization significantly increased potential nitrification in soils collected on June 12 ($p=0.0009$), July 27 ($p<0.0001$), and August 31 ($p=0.0148$). Cumulatively, net N mineralization, calculated as the change over incubation time in the sum of nitrate and ammonium concentrations, exhibited a significant three-way interaction between

burn, fertilization, and date, p=0.0137 (Figure 2.9). Again, post hoc slices were used to determine that June 12 and July 27 incubations had a burn x fertilization interaction that was significant. On June 12 (shortly after fertilization), the fertilized treatment increased net N mineralization in the unburned treatment but resulted in a negative net N mineralization rate in the burned treatment, possibly due to high initial post-fertilization values in the burned treatment soils (Figures 2.5 and 2.6). The interaction effect on the July 27 date appears to be from more "extreme" effects of fertilization on net N mineralization in the burned treatment.

In contrast to potential N mineralization incubations, estimates of net N cycling rates from *in situ* field measurements were not significantly affected by treatment for ammonification, nitrification or net N mineralization (Figures 2.10, 2.11, and 2.12, respectively). Field-based rates were much more variable both through time and in response to burn and fertilization treatments, perhaps reflecting the heterogeneity within and between plots. In general the magnitude of mineralization rates was higher for fertilized treatments with peak net N mineralization coinciding with plant growth periods in early and mid summer. Immobilization began to occur by the final sampling period initiated on August 31, which corresponded with the start of plant senescence.

Carbon Mineralization

Potential C mineralization rates (Figure 2.13) were calculated from the final 20 days of the lab incubation and a repeated-measures mixed model ANOVA revealed significant effects of both nitrogen fertilization (p=0.0006) and date (p=0.0101). Fertilization with N lowered potential C mineralization rates by an average of 62% while C mineralization rates across dates tended to increase through the spring and decline in the fall, with a maximum increase of 36% between the May 5 and June 12 intervals. While not statistically significant, there was a trend for burning to increase potential C mineralization by an average of 13.5%. A cumulative CO_2-C accumulation curve (Figure 2.14), calculated as the average μg CO_2-C for all incubations by sampling interval, highlights the general trends from individual incubation accumulation curves. A date x burn (p=0.0086) and a date x fertilization (p<0.0001) interaction was significant, with fertilization treatments separating ca. 10 days after initiation of the incubations and cumulative effects of burn treatments occurring by the final measurement. Similar to potential and field N mineralization assays, the field C mineralization measurements did not reflect the lab incubation

measurements. Field CO_2 efflux was measured on ten sample dates throughout the growth season (Figure 2.15) with the only significant result being a date x burn interaction ($p=0.0348$) with maximum differences occurring in June ($p<0.0001$ on June, 11 and $p=0.0008$ on June, 26).

Soil Temperature and Moisture

Soil temperature was measured concurrently with CO_2 efflux measurements (Figure 2.16) and exhibited significant interaction effects of date x burn ($p<0.0001$) and date x fertilizer ($p=0.031$). Fertilizer effects were less common than burn differences, and all significance occurred during the first seven dates ranging from the beginning of June to the end of August. Soil temperature was significantly correlated to the log(CO_2 efflux), with $p<0.0001$ and $R^2=0.4614$. When the same regression was run with burn treatments separated unburned plots had a $p<0.0001$ and an $R^2=0.3527$ and burned plots had a $p<0.0001$ and an $R^2=0.5597$ showing more variability in the unburned plots. Data logger results (Figure 2.17) are plotted as 24 hour averages of 30 minute intervals and were within ±3 °C of Li-Cor 8100 measurements taken the same day. To ensure temperature data were in better agreement, both temperatures were compared at the time of Li-Cor 8100 measurement (between 11am and 2pm) and agreed to within ca. ±1 °C. Based on logger data, the average soil temperature in burned plots was 3.21 °C warmer than unburned plots ($p<0.0001$, $R^2=0.9588$). Soil volumetric water content (Figure 2.18) was also continuously measured and was on average 5.97% higher in the burned treatment ($p<0.0001$, $R^2=0.8338$).

Aboveground Net Primary Productivity

Annual aboveground plant productivity, based on end-of-season sampling of biomass produced during the growing season, has been measured in the BGPE as part of the Konza LTER program since 1986. A subset of this data from the summer of 2012 is presented in Figure 2.19. End-of-season forb biomass was not affected by either burning or nitrogen fertilization. There was zero woody plant biomass in burned plots and no difference in woody biomass accumulation in unburned plots, regardless of fertilization treatment. Grass biomass was significantly affected by a burn x nitrogen interaction ($p=0.0192$) with the annual burning and fertilization treatment producing a 128% increase in ANPP over the next highest value (burned control). Total plant

productivity exhibited a slightly different trend with significant burn ($p=0.0178$) and fertilizer (0.0032) effects but no significant interaction at the $p \leq 0.05$ level ($p=0.0654$). Burning and nitrogen fertilization both increased total biomass 2.2x, due to the fact that burned control and unburned nitrogen treatments had nearly identical means.

Discussion

Total soil C and N responded differently to annual burning and chronic N fertilization. Neither total soil C nor total soil N in the upper 10 cm of soil was significantly affected by burn treatment. This was somewhat surprising, as previous studies have indicated the potential for contrasting fire regimes to alter soil C storage in the surface soil (Kitchen et al. 2009; Reed et al. 2005). While total soil C was not significantly affected by the N fertilization treatment and remained constant through the summer, total soil N was significantly increased by fertilization with an additional increase in all treatments during the late-summer sampling in August (Figures 2.2 and 2.3, plots fertilized on June 4 and 5). Previous studies of soil C and N dynamics are consistent with our non-significant findings on a per gram dry weight basis with Ajwa et al. (1999) finding no effect of burning or fertilization on total C and N throughout the summer of 1994. However, by 1998, Kitchen et al. (2009) reported that annual burning primarily affected mineral soil (top 10 cm) significantly reducing the soil %C by 11% with no significant change in total %N. However, in this study I found no differences in total soil C with burning treatment, indicating no long-term effects of annual burning on soil C in mineral soil even with increased N inputs. Additionally, Fynn et al. (2003) working in a mesic African grassland, showed that long-term chronic (50 years) burning led to significant decreases in total N, which is consistent with the general trend found in this study, although our results were not significant ($p=0.125$).

Chronic fertilization caused an increase in total N on a per unit area basis, regardless of burning treatment, while maintaining similar levels of C in all treatments. Studies in other grasslands have supported this time-dependent trend for soil C but not N, where a decade of N fertilization at similar rates did not cause changes in total C or N, but rather changed soil C pool distribution (Neff et al. 2002). A study comparable to mine conducted at Cedar Creek Ecosystem Science Reserve, a northern tallgrass prairie on sandy soils with lower overall C and N concentrations, Fornara and Tilman (2012) also found that that 17 years of annual fires did not alter total soil C or N, but that 27 years of fertilization increased both total ecosystem and soil C

and N, as a result of increased root biomass with fertilization. We know that fire alone and in combination with N increases root biomass in prairie at KPBS, however studies at KPBS generally have not detected a significant increase in total soil C with fertilization or burning (Kitchen et al. 2009, Wilson et al. 2009). This suggests that increases in root biomass alone may not drive increases in C storage across ecosystems, or that such changes may be more difficult to detect in finer textured soils with large stores of soil C and N to begin with.

Broadly, N mineralization responses to fire are affected by changes in management (i.e. prescribed fire vs. wild-fires) and are varied across ecosystems, as highlighted in the meta-analysis of Wan et al. (2001). More recent research has attempted to determine what drives differences in N mineralization from a broad range of ecosystem types or geographic locations. A continental-wide study found that predicting N mineralization potentials from a broad range of edaphic and climatic variables is not easy, with a general pattern of N mineralization decreasing as soil C:N increased (Colman and Schimel 2013). In the current study, the soil C:N ratio significantly ($p=0.0003$) decreased with N additions and significantly ($p=0.0306$) increased with burning, with expected potential and *in situ* rates roughly supporting the trend noted for a broader range of soil types (i.e. tendencies for lower rates with annual burning and higher rates with fertilization were consistent with observed changes in C:N ratios). While our results broadly matched predictions based on changes in C:N ratios, effects of burning and N fertilization treatments on net N mineralization rates varied for laboratory-based assays of potential N mineralization and field-based measurements. This likely reflected the greater heterogeneity and the use of intact soil cores in field-based measurements relative to laboratory assays using homogenized soils under more uniform incubation conditions.

Potential net N mineralization rates were significantly ($p=0.0137$) affected by a three-way interaction of date, burn, and fertilization, but there were no significant main effects of date or treatment for field incubations. Field incubation rates were variable, with N amended plots tending to have the most variability, potentially as an artifact of NO_3NH_4 application method (hand broadcasting of granular fertilizer) and high overall initial N availability in fertilized plots. Past results from the BGPE support our findings of increased inorganic N availability with fertilization and variability of available inorganic N through time. In 1994, a study conducted on the same set of subplots (i.e. burned/unburned, ±N) indicated that inorganic soil N concentrations were significantly higher in the unburned+N treatment for three of four summer sampling dates,

with the only insignificant date resulting from the fertilization and increase in burned+N treatment in June (Ajwa et al. 1999). That same year, a subsample of these treatments showed that burned plots had moderately ($p \leq 0.1$) lower ammonium availability in June (burned=0.19, unburned=0.53 g N m^{-2}) and July (burned=0.13, unburned=0.33 g N m^{-2}) but nitrate was different only on the June sampling date (burned=0.10, unburned=0.71 g N m^{-2}) (Dell and Rice 2005). These past results are generally consistent with other studies of the effects of fire in mesic grasslands that have found lower inorganic N availability and mineralization rates in frequently burned grasslands (Ojima et al. 1994, Blair 1997, Fynn et al. 2003, Seastedt et al. 1991). While our current results for potentially mineralizable N support previously reported trends of reduced N availability in burned only (unfertilized) plots, we found no significant effects of burning alone on *in situ* nitrate and ammonium availability or mineralization rates. This indicates no current differences in *in situ* N availability in non-fertilized plots as a function of annual burning and long-term fire suppression, although fire treatment did influence responsiveness to N fertilization, with burned generally showing larger increases in inorganic N availability in response to N enrichment.

Research into fertilization in restored tallgrass prairie indicates that N amendments increased extractable nitrate after 6 years continuous annual fertilization and that net N mineralization rates were significantly increased following 8 years of fertilization (Baer and Blair 2008). However research outside of grasslands has yielded contrary results with N fertilization reportedly having no effect on ammonium availability in an arctic shrub land (Sorensen et al. 2008). Woody encroachment is also a common land cover change in unburned tallgrass prairies, such as the unburned plots in the BGPE, and other studies have suggested that this may alter soil C and N pools and fluxes. For example, McKinley and Blair (2008) reported that *Juniperus virginiana* encroached areas that had potential and *in situ* rates of net N mineralization that, while not statistically different from prairie sites, tended to be higher than non-encroached grassland sites. This is consistent with results from the current study, in that unburned BGPE plots are heavily encroached with woody vegetation and similar responses were seen, with burning significantly ($p=0.0396$) lowering potential net nitrification and the added trend of N fertilization increasing potential rates significantly (date x nutrient, $p=0.0358$) for both vegetation types through time.

Soil temperature and moisture are known to be drivers of soil nutrient cycling and Guntiñas et al. (2012) used a matrix of increasing temperature and moisture to determine their collective effects on a grassland, forest and cropland soil. They found that 25 °C and 80% field capacity were ideal for maximizing N mineralization across soils. While we didn't see any increase in N mineralization in warmer, wetter plots, we did find differences in soil temperature and moisture. Representative burned and unburned plots showed a decrease in mean summer temperature (3.21 °C) and volumetric water (5.97%) to a depth of 10 cm in unburned plots compared to burned plots. Additionally, mid-day soil temperatures measured at a depth of 10 cm with the Li-Cor 8100 probe were higher in burned plots. The effect of fertilization on temperature was significant on only three dates, with a tendency to lower temperature in burned+N plots only as shown in Figure 2.16, potentially due to greater shading from an increase in canopy cover (LAI). These trends for higher temperature in burned prairie are commonly attributed to the higher solar radiation inputs received post burn due to vegetative cover loss (Ojima et al. 1994).

Soil water in burned tallgrass prairie has often been reported to be lower than comparable unburned prairie, also due to increases in solar radiation and greater surface evaporative loss (Ojima et al. 1994, Blair 1997, Seastedt et al. 1991, Knapp et al. 1998). Contrary to this expectation, we found higher soil moisture throughout the summer in burned plots. A number of key differences between the shorter- and longer-term effects of burning may have contributed to these soil moisture discrepancies. Unburned plots in other studies have typically been burned more recently (<6 years since burn). These intermittent burns tend to create a litter layer that insulates and buffers soil moisture loss, helping to maintain soil moisture at the surface. However, prolonged fire suppression in our site (>27 years) has caused vegetation cover to change to woody species. ANPP from the summer of 2012 showed significant responses with grass dominating the burned treatments (especially when fertilized), woody vegetation accumulation in unburned plots, and forbs more abundant in fertilized plots. Trends for ANPP from 2012 are comparable to the long-term results of fire and N additions to these plots, which took over six years to show treatment differences, and are comparable to trends reported both 8 (Collins et al. 1998) and 17 years (Wilson et al. 2009) after experimentation began with slight increases in total biomass through time (unpublished LTER data). Woody expansion potentially increases interception and evaporation in the canopy, reduces throughfall and may increase

evaporative losses from surface soils due to reduced vegetation density near the soil surface. These effects may have been exacerbated by the relatively dry conditions during the growing season of 2012. Despite these differences in vegetation structure, lower initial soil water content was still anticipated in the burned plots for some period immediately following the spring burn, but a number of post-burn rainfall events likely negated the effects of increased solar radiation in the burned plots.

Rates of potential C mineralization were influenced most strongly by chronic N additions, with the N additions generally reducing C mineralization rates. A potential mechanism for these low potential C mineralization rates in our fertilized soils is labile C loss through time, which is supported in other studies that have shown shifts in total C pool distributions with labile C depletion due to added N (Ziter and MacDougall 2013, Neff et al. 2002). Effectively, fertilization may in time promote the loss of easily degradable C (either chemical or spatial), lowering future potential for heterotrophic losses. In addition to fertilization reducing potential C mineralization, our lab incubations also showed that date was significant with peak efflux occurring in June and July. Frank et al. (2002) saw similar results and attributed this to peak biomass and presumably root growth and exudate production.

Field efflux measurements displayed different trends with a burn by date interaction being the only significant response. Burning has been shown to increase efflux in many prairie systems, where fire frequency tends to positively relate to CO_2 efflux (Knapp et al. 1998, Bremer and Ham 2010). This increase is often attributed to increases in soil temperature in burned sites, promoting heterotrophic respiration, and log(CO_2 efflux) has been shown to positively correlate to temperature (Knapp et al. 1998, Peng et al. 2011). Our data supported a CO_2 efflux relation to temperature with an R^2=0.4614 ($p<0.0001$) on all plots and a stronger relation to burned plots alone (R^2=0.5597, $p<0.0001$). Soil respiration has also been shown to increase across a MAP gradient (McCulley et al. 2005, Knapp et al. 1998) and soil moisture on our burned plots was significantly higher than unburned, potentially adding to the increased respiration rates. It must be noted that field CO_2 efflux is a composite measurement of total respiration (heterotrophic and autotrophic) and that increased soil CO_2 efflux in burned plots may be due to autotrophic root respiration as burned plots are known to have higher root density (Kitchen et al. 2009, Wilson et al. 2009). N fertilization has also been shown to initially promote field C mineralization, but repeated fertilizations produced no net difference in total C mineralized, which is consistent with

our limited fertilization effect in our field measurements (Peng et al. 2011). Finally, C mineralization has been shown to respond inversely in response to woody encroachment in different prairie ecosystems. Encroached tallgrass prairie has been shown to decrease potential mineralization (McKinley and Blair 2008), supporting our study, while shrub encroachment into a North Dakota mixed-grass prairie showed no change in field measurements (Frank and Karn 2005). While these may be confounding results in all likelihood abiotic drivers are changing mineralization rates, or biotic (i.e. microbial biomass or root biomass) pools are shifting causing changes in C mineralization (Fransluebbers et al. 2000).

Conclusions

This study was intended to evaluate the longer-term effects of annual fire and chronic N additions on belowground C and N cycling in a tallgrass prairie. Although past research on these plots was not always consistent with the current findings, inherent spatial and temporal variability may have caused differences or it may indicate that long-term results do not always match short-term outcomes. Significant increases in response to fire were seen in total C:N, *in situ* C mineralization, total aboveground biomass, soil temperature, and soil moisture. The addition of N fertilizer lowered soil C:N ratio and potential C mineralization, while increasing total soil N, inorganic N availability, and total aboveground biomass. Burning and/or N interacted with date to affect potential net N mineralization, soil temperature, CO_2 field efflux, and lab CO_2 accumulation, highlighting seasonal effects of burning and nitrogen on belowground processes. Abiotic factors differed between burned and unburned plots and are likely a major explanatory variable for differences between potential and *in situ* mineralization rates. Finally, our work highlights the benefits of long-term experimentation but also identifies the need for more frequent sampling and methodological foresight when developing long-term belowground studies. Yearly sampling is often more easily justified in aboveground work because common annual measurements (e.g. species composition and ANPP) are less destructive than soil sampling. However, extended field and lab incubations can be done as a part of yearly sampling regimes that minimize disturbance while ensuring beneficial data is collected. This annual resolution over decadal experiments may lead to more interpretable results, greatly improving the predictive power of ecosystem models of C and N dynamics.

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Tables and Figures

Table 2.1 NO_3^--N and NH_4^+-N standard preparation table for analysis on Flow Solution IV made with oven dried KNO_3 (A 1,000 ppm and B 10 ppm) and $(NH_4)_2SO_4$, (B 100 ppm and C 10 ppm) . Amounts are per 100 ml volumetric flask filled with 2M KCl.

Standard	ppm NO_3^--N	Stock	ppm NH_4^+-N	Stock
S0	0.00	0.00	0.00	0.00
S1	0.05	500 µl B	0.02	200 µl C
S2	0.10	1000 µl B	0.20	200 µl B
S3	0.50	5000 µl B	0.40	400 µl B
S4	1.00	100 µl A	0.60	600 µl B
S5	1.20	120 µl A	0.80	800 µl B
S6	1.60	160 µl A	1.00	1000 µl B
S7	2.00	200 µl A	1.50	1500 µl B
S8	2.40	240 µl A	2.00	2000 µl B
S9	5.00	500 µl A	4.00	4000 µl B

Table 2.2 Repeated measures mixed model F table for field and lab measurements of carbon and nitrogen. Bold values represent marginal significance (p≤0.1) and * or ** indicate significance at p≤0.05 and p≤0.01 respectively.

		Total			Field					Lab			
Effects	**DF**	**Carbon**	**Nitrogen**	**C:N**	**NO3**	**NH4**	**Net N**	**C min**	**Temp.**	**NO3**	**NH4**	**Net N**	**Cmin**
Burn	1,3	0.58	4.46	14.93*	2.69	0.40	2.09	32.11*	37.08**	12.21*	22.09*	38.24**	4.27
Nitrogen	1,6	0.22	8.25*	53.07**	0.20	0.00	0.10	3.69	2.68	55.30**	38.27**	10.3*	43.9**
Date	3,9	**3.31**	6.15*	0.30	2.05	2.41	1.23	21.21**	324.95**	7.64*	9.05**	6.74*	4.00*
B*N	1,6	0.58	0.67	0.01	0.02	0.65	0.20	**4.43**	**5.84**	1.74	18.46**	1.95	1.34
D*B	3,9	0.81	0.90	0.77	0.94	0.92	1.20	2.45*	11.64**	0.91	6.79*	5.47*	0.61
D*N	3,18	1.67	1.24	2.14	**3.01**	0.93	1.73	0.77	2.28*	3.54*	12.28**	3.30*	1.53
D*N*B	3,18	0.21	1.14	**2.7**	1.55	0.79	1.48	0.94	0.84	1.26	9.46**	4.69*	2.05

Figure 2.1 CO_2 efflux collars in (A) annually burned plot and (B) prolonged unburned showing the visible difference in early season vegetation cover.

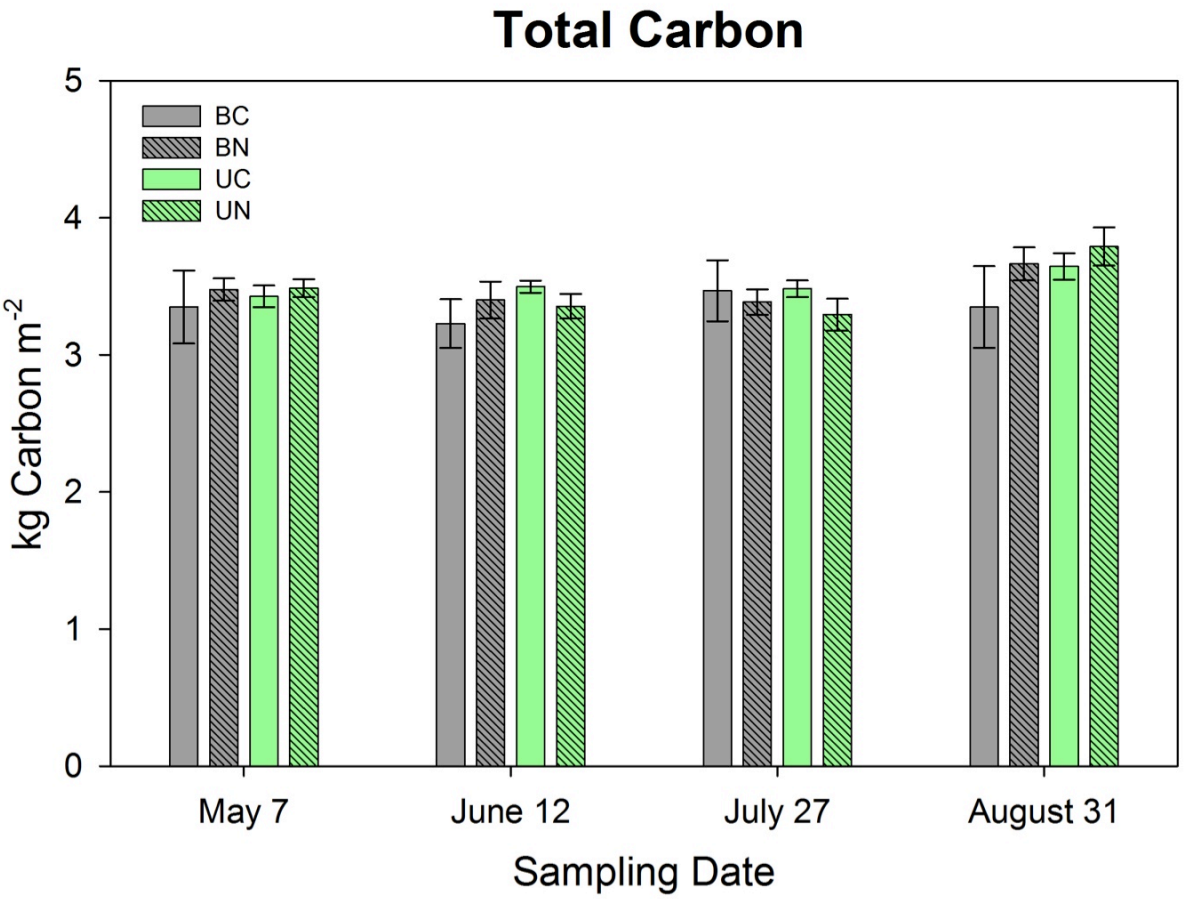

Figure 2.2 Total soil carbon (mean ± 1 SE) to a depth of 10 cm where µg C·g^{-1}·dry soil was converted to kg C·m^{-2} using bulk density measurements from the June sampling date. No effects were significant at the p≤0.05 level.

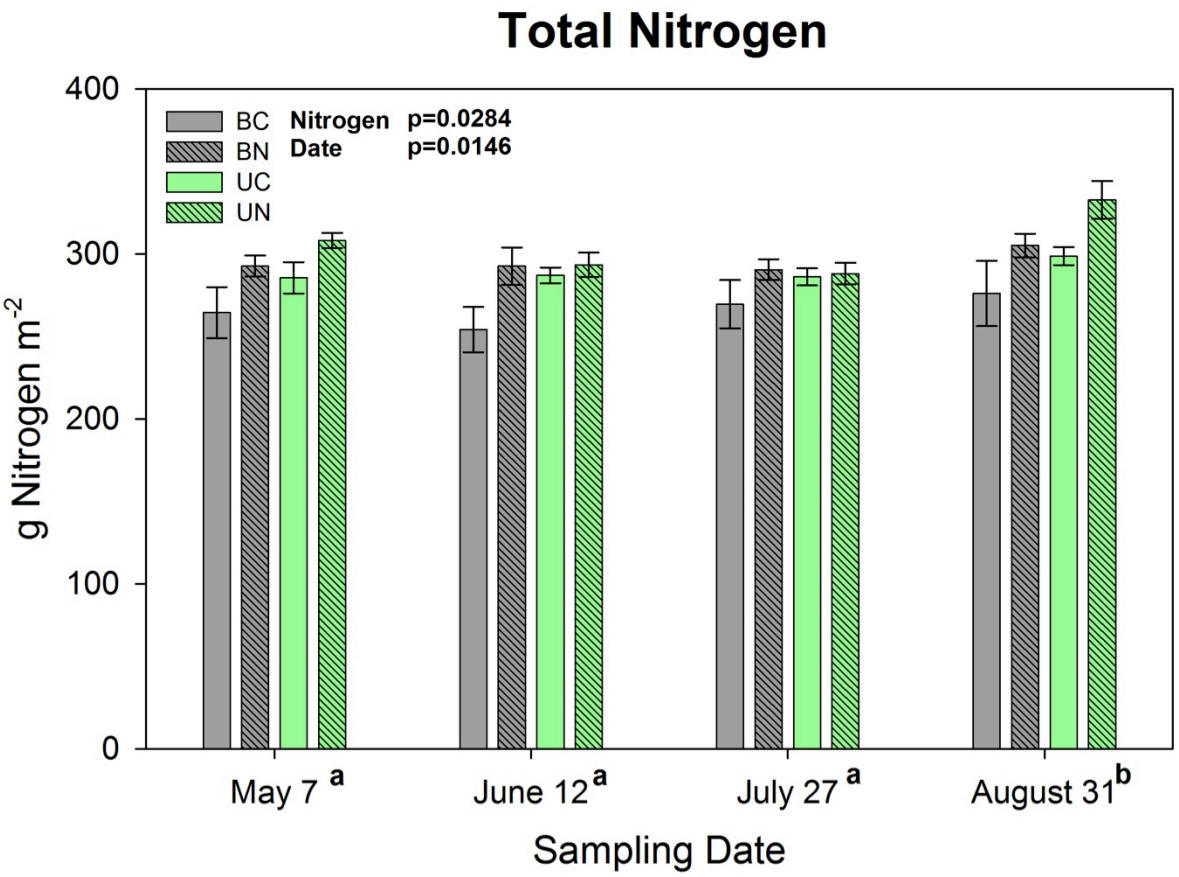

Figure 2.3 Total soil nitrogen (mean ± 1 SE) to a depth of 10 cm where μg N·g^{-1}·dry soil was converted to kg N·m^{-2} using bulk density measurements from the June sampling date. Main effects for nitrogen and date were significant at the p≤0.05 level while burn and interaction effects were not significant. Letters on sample dates indicate differences at the p≤0.05 level.

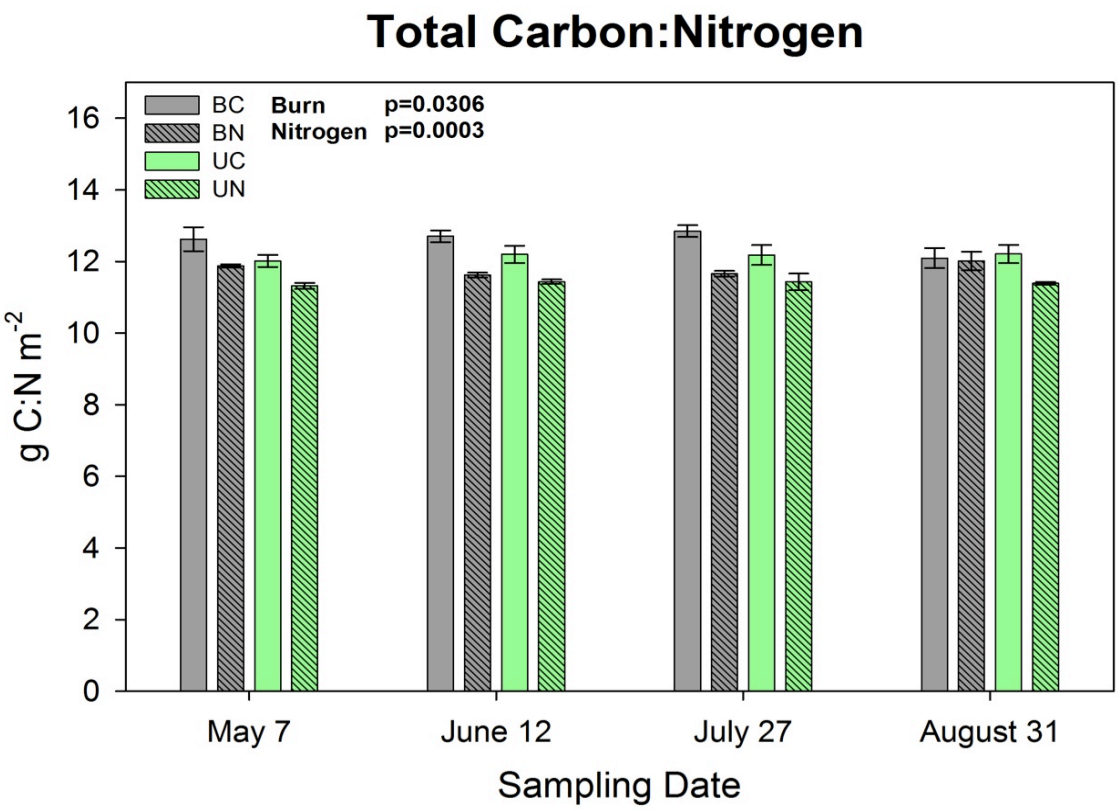

Figure 2.4 Ratio of total soil carbon to total soil nitrogen (mean ± 1 SE) to a depth of 10 cm where µg C or N·g^{-1}·dry soil were converted to kg C or N·m^{-2} using bulk density measurements from the June sampling date. Main effects for burn and nitrogen treatments were significant at the p≤0.05 level while date and interaction effects were not significant.

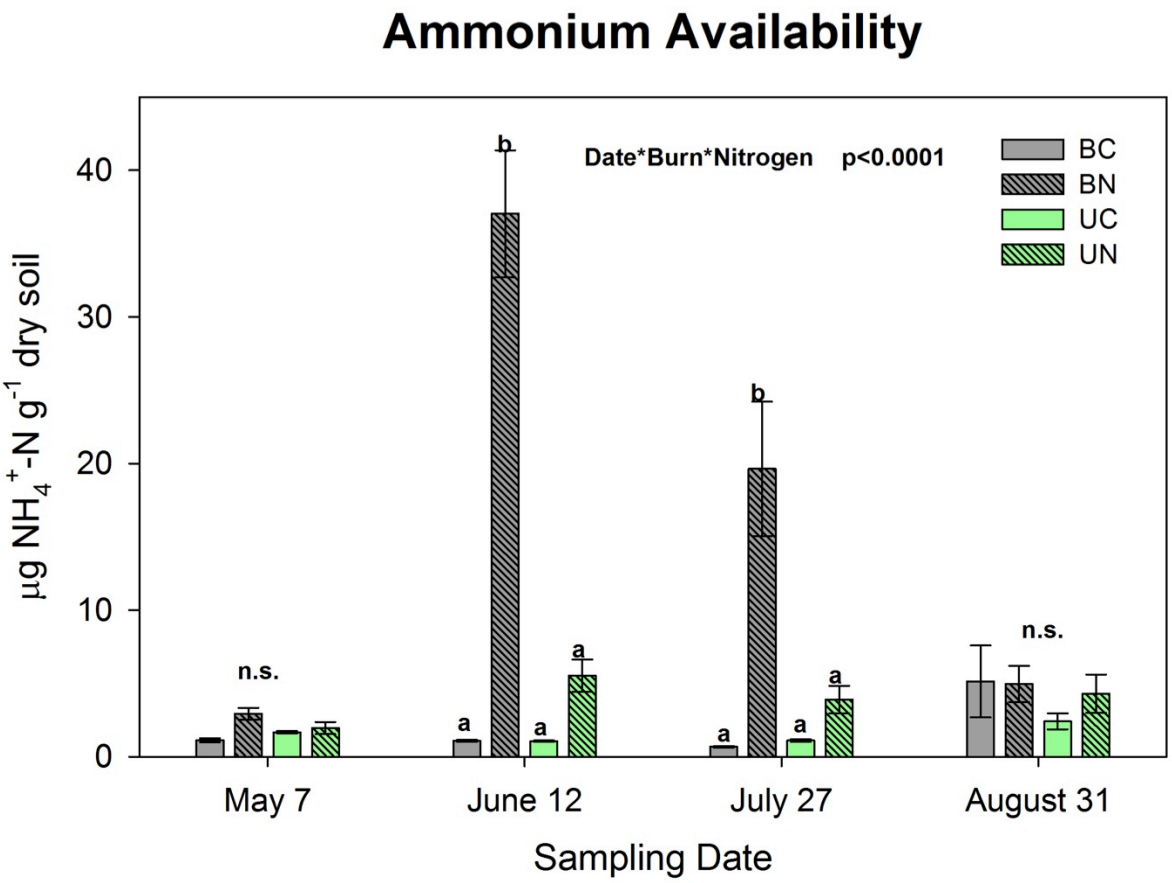

Figure 2.5 KCl-extractable soil ammonium (mean ± 1 SE) concentrations over the summer of 2012. A three-way interaction effect was significant at $p \leq 0.01$ and post hoc slices indicated within date significance as indicated with letters ($p \leq 0.05$) for the June and July sampling dates.

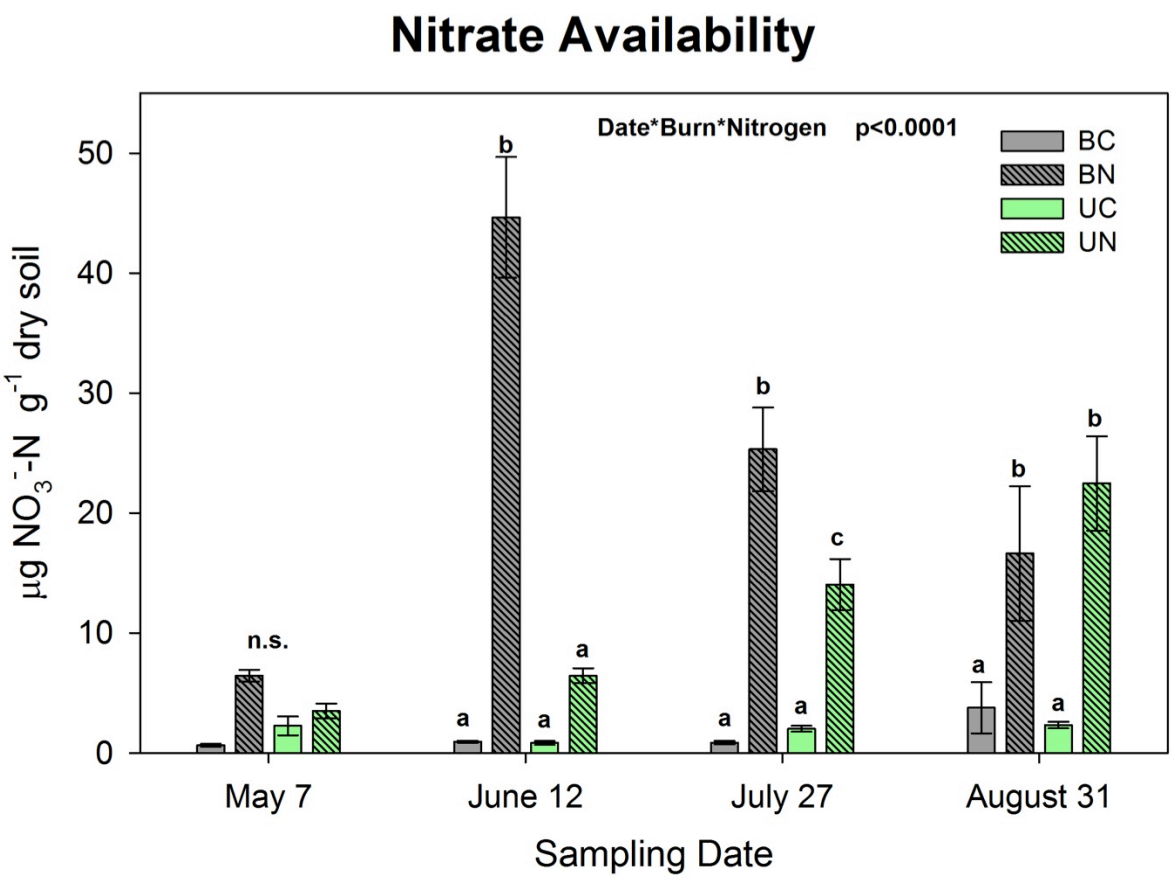

Figure 2.6 KCl-extractable soil nitrate (mean ± 1 SE) concentrations over the summer of 2012. A three-way interaction effect was significant at p≤0.01 and post hoc slices indicated within date significance as indicated with letters (p≤0.05) for the final three sampling dates.

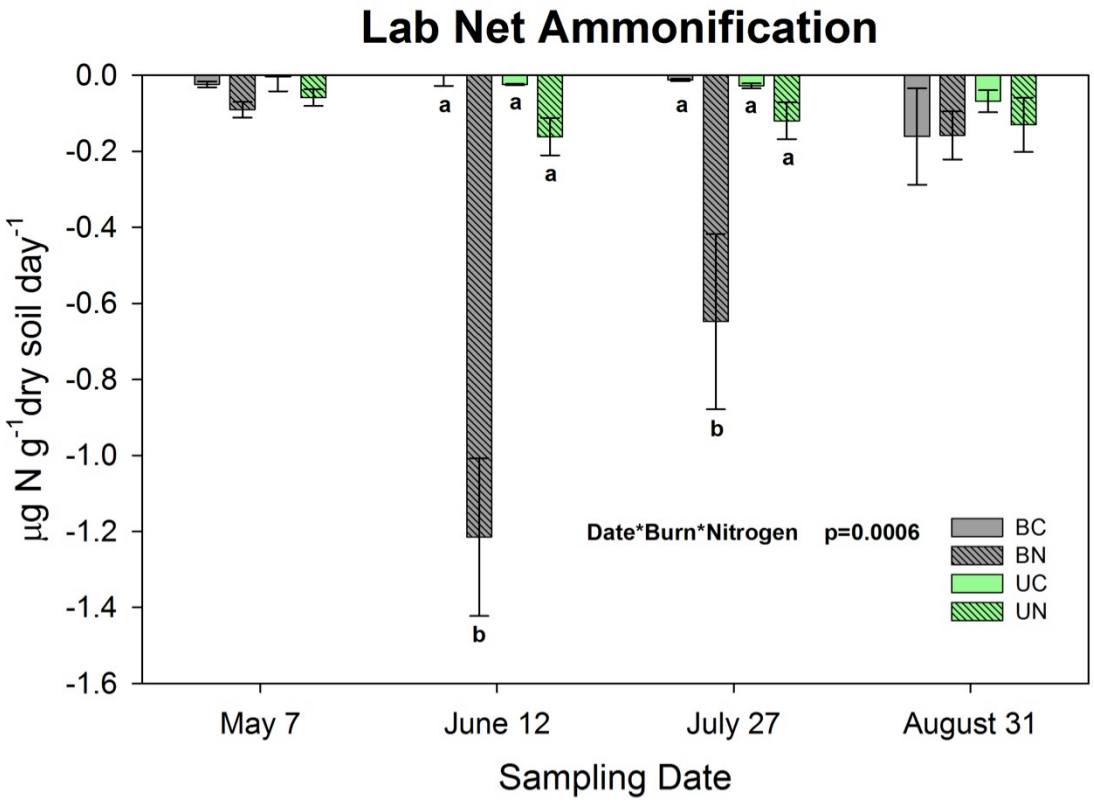

Figure 2.7 Potential net ammonification (mean ± 1 SE) for ca. 10 g dry weight equivalent soil incubated for 30 days at ca. 60% WHC and 25 °C. A three-way interaction effect was significant at p≤0.01 and post hoc slices indicated within date significance as indicated with letters (p≤0.05) for the June and July sampling dates.

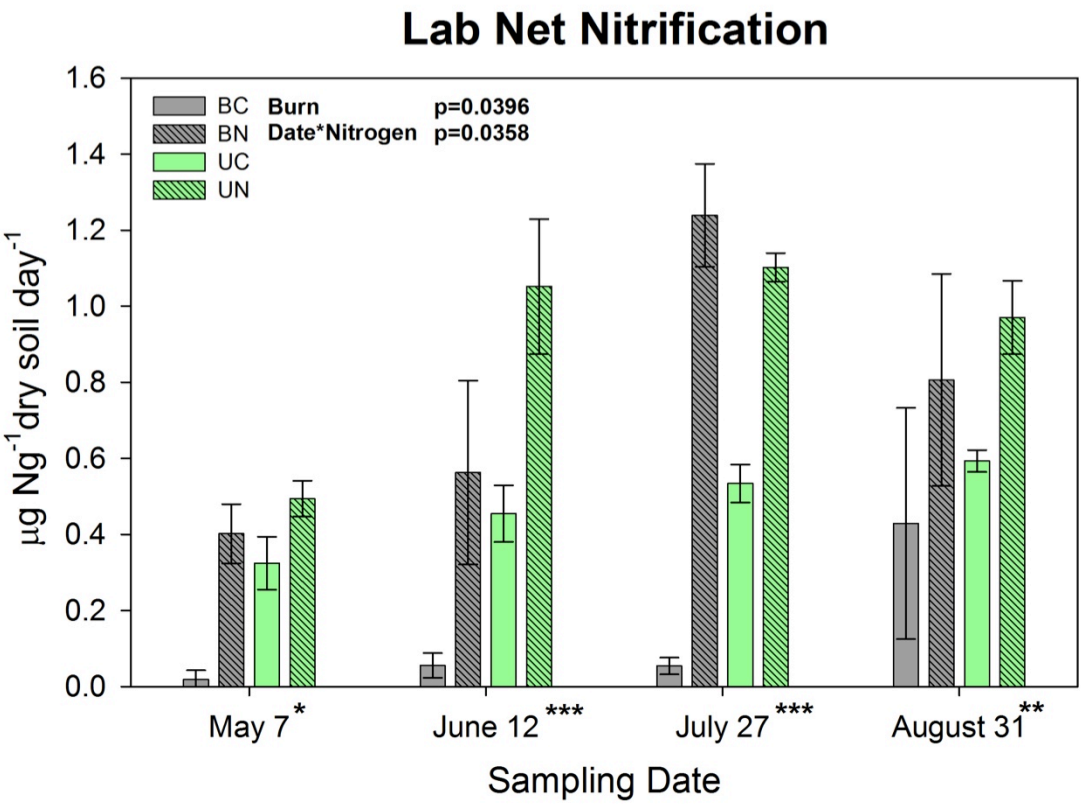

Figure 2.8 Potential net nitrification (mean ± 1 SE) for ca. 10 g dry weight equivalent soil incubated for 30 days at ca. 60% WHC and 25 °C. A main effect of burning and interaction effect between date and nitrogen were significant at p≤0.05. Post hoc slices indicated within date significance of nitrogen addition where *, ** and * indicate p values of ≤0.1, ≤0.05 and ≤0.01 respectively.**

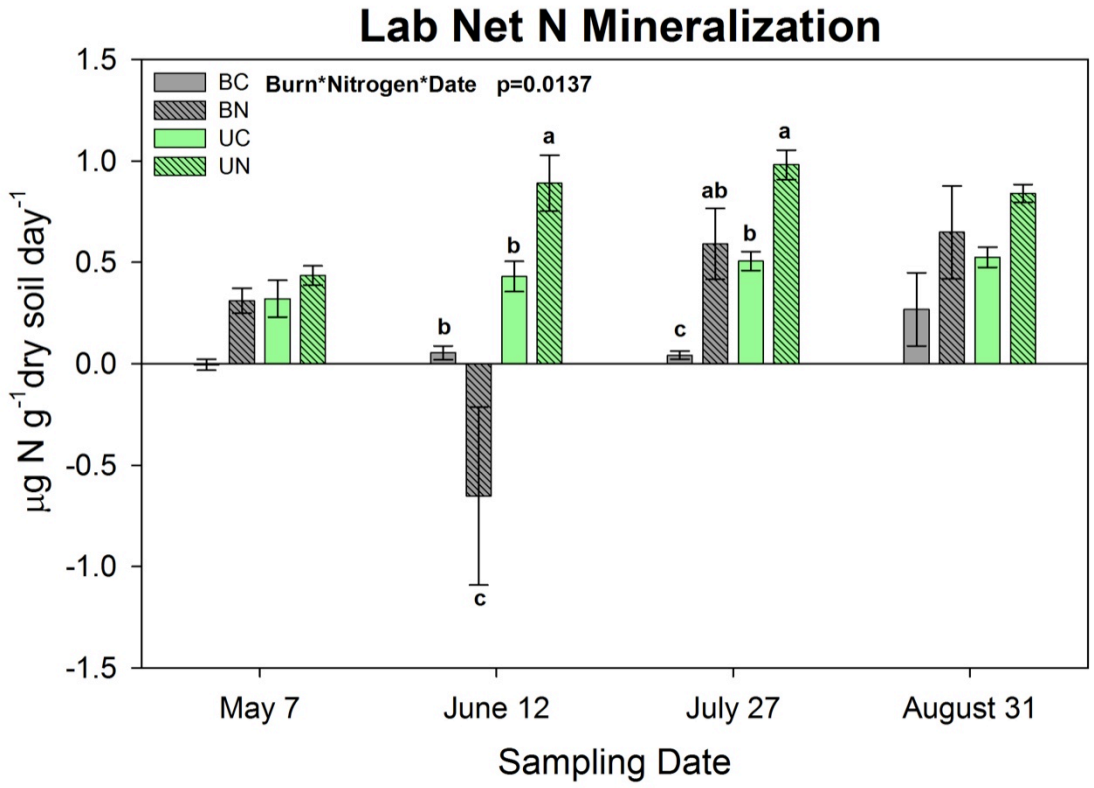

Figure 2.9 Potential net nitrogen mineralization (mean ± 1 SE) for ca. 10 g dry weight equivalent soil incubated for 30 days at ca. 60% WHC and 25 °C. A three-way interaction effect was significant at p≤0.05 and post hoc slices indicated within date significance as indicated with letters (p≤0.05) for the June and July sampling dates.

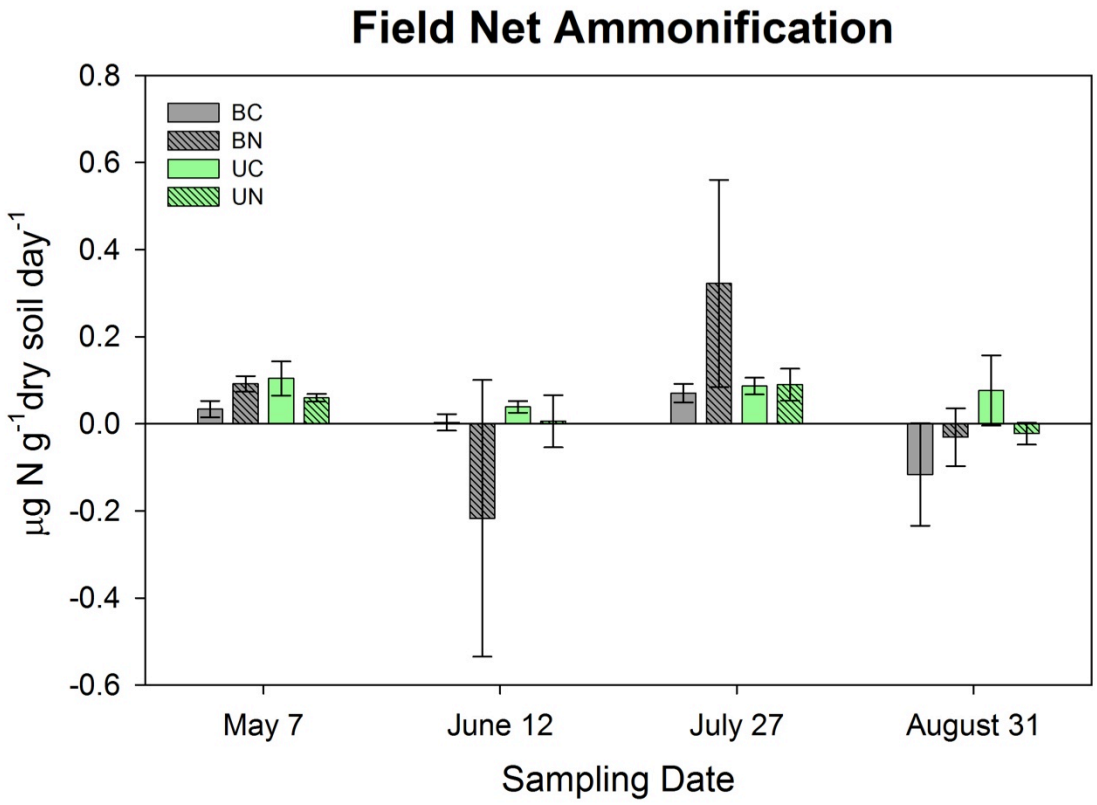

Figure 2.10 Field net ammonification (mean ± 1 SE) for 10 cm deep PVC soil cores incubated for 32 days. Repeated measures ANOVA indicated no main or interaction effects of burn, nitrogen, or date.

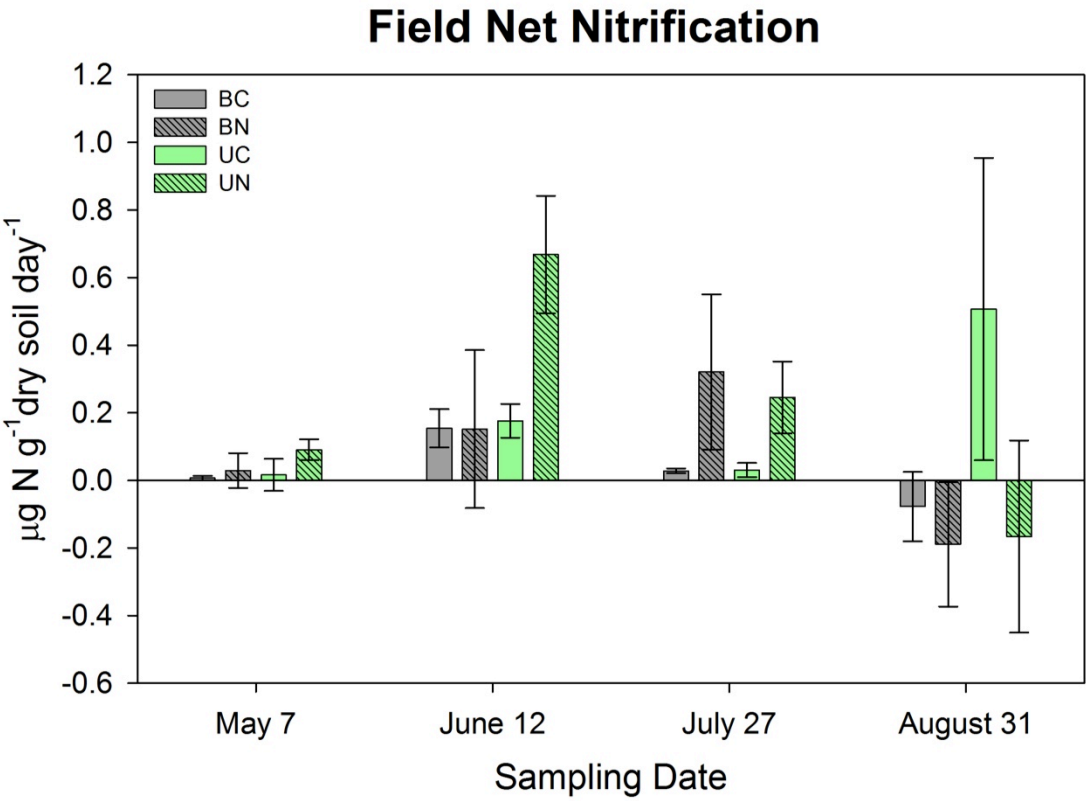

Figure 2.11 Field net nitrification (mean ± 1 SE) for 10 cm deep PVC soil cores incubated for 32 days. Repeated measures ANOVA indicated no main or interaction effects of burn, nitrogen, or date.

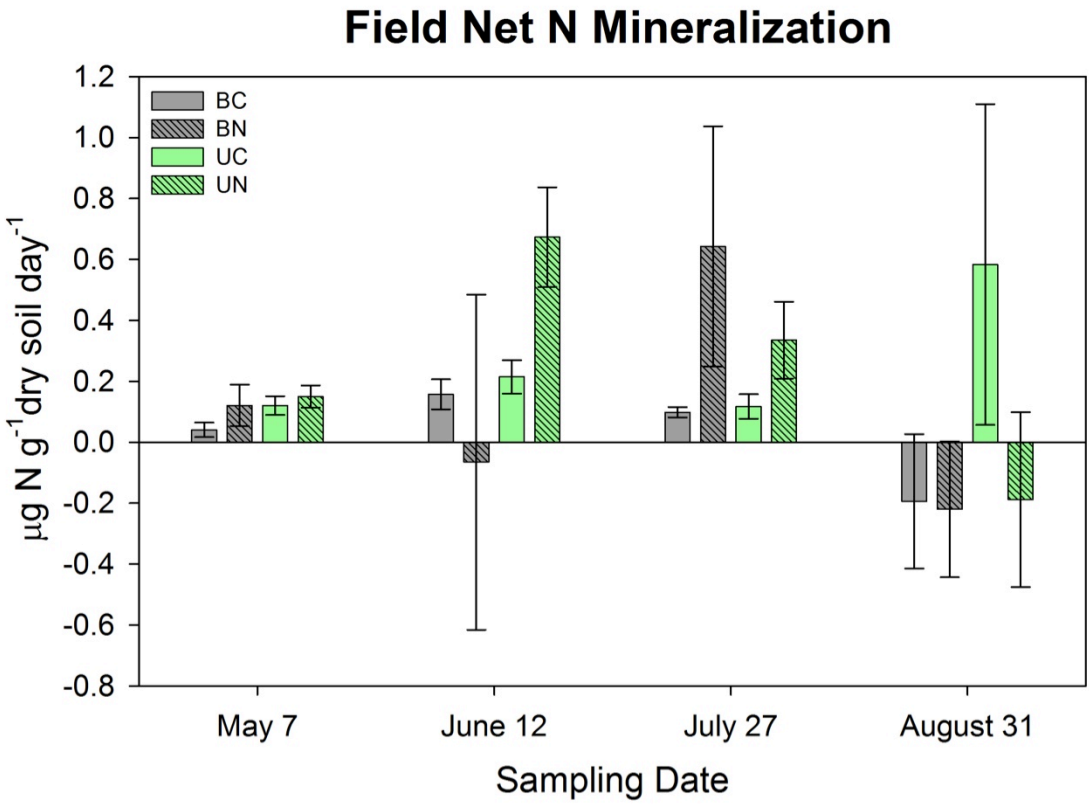

Figure 2.12 Field net mineralization (mean ± 1 SE) for 10 cm deep PVC soil cores incubated for 32 days. Repeated measures ANOVA indicated no main or interaction effects of burn, nitrogen, or date.

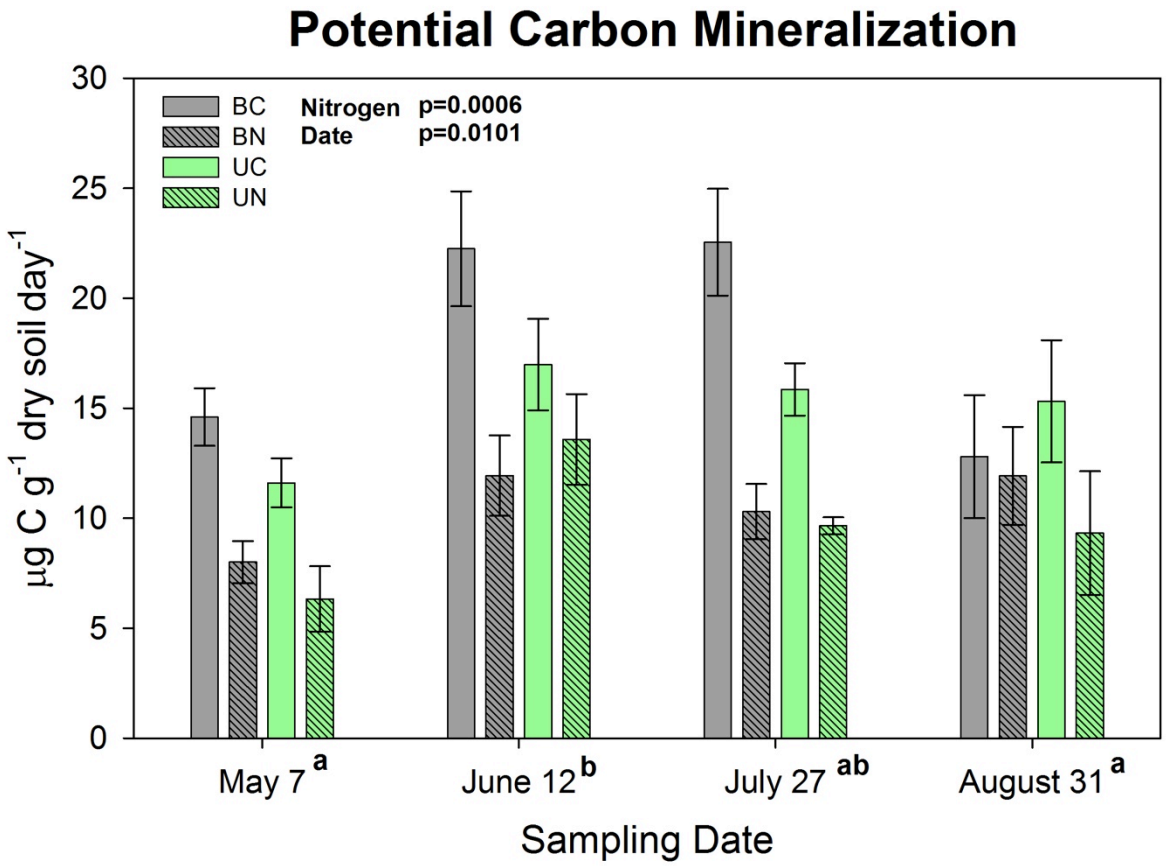

Figure 2.13 Potential carbon mineralization rate (mean ± 1 SE) for final 20 days of 30 day incubation of ca. 10 g dry weight equivalent soil at ca. 60% WHC and 25 °C. Main effects of nitrogen and date were significant at $p \leq 0.01$ and $p \leq 0.05$ respectively. Letters on sample dates indicate differences at the $p \leq 0.05$ level.

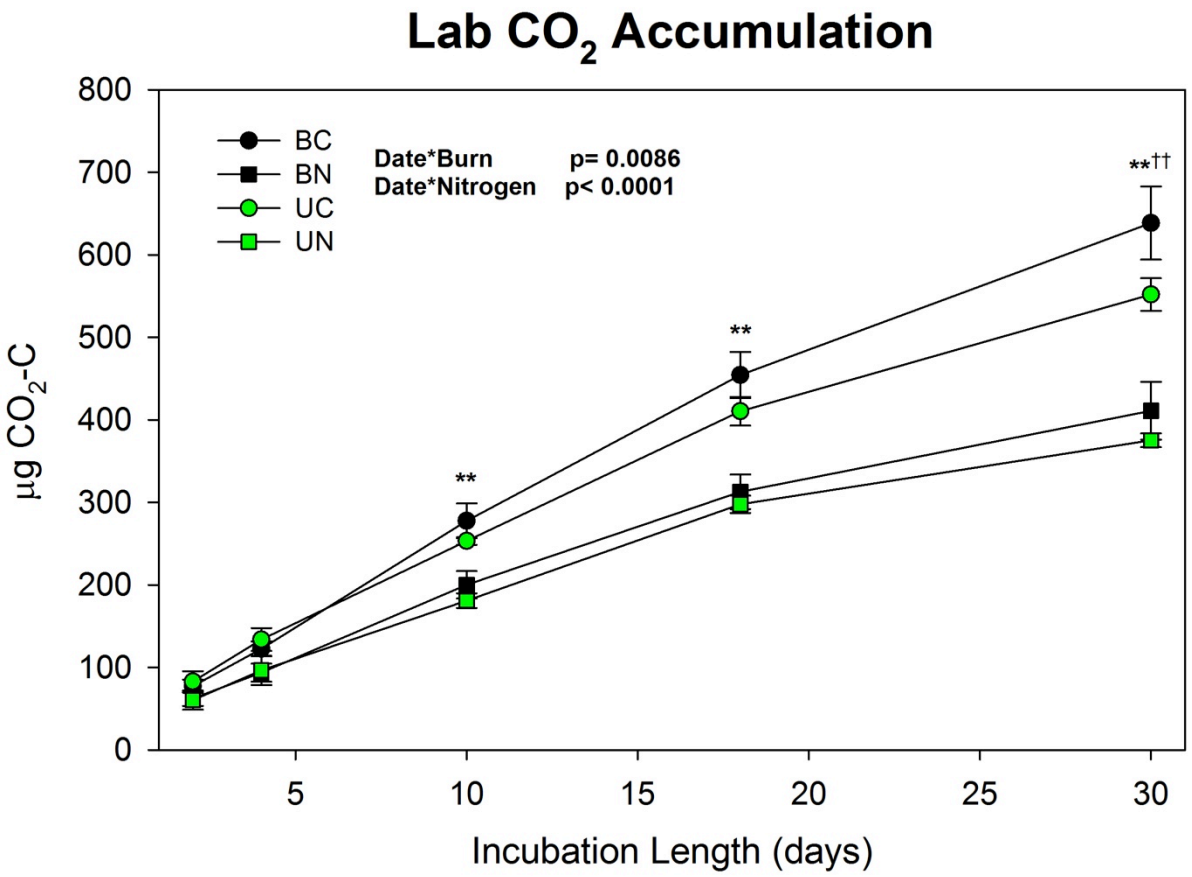

Figure 2.14 CO_2-C accumulation (mean ± 1 SE) across four incubation dates. Incubation periods where there was a date x nitrogen interaction effect are indicated by ** ($p \leq 0.01$), while †† indicates the interaction of date x burn at the same level of significance.

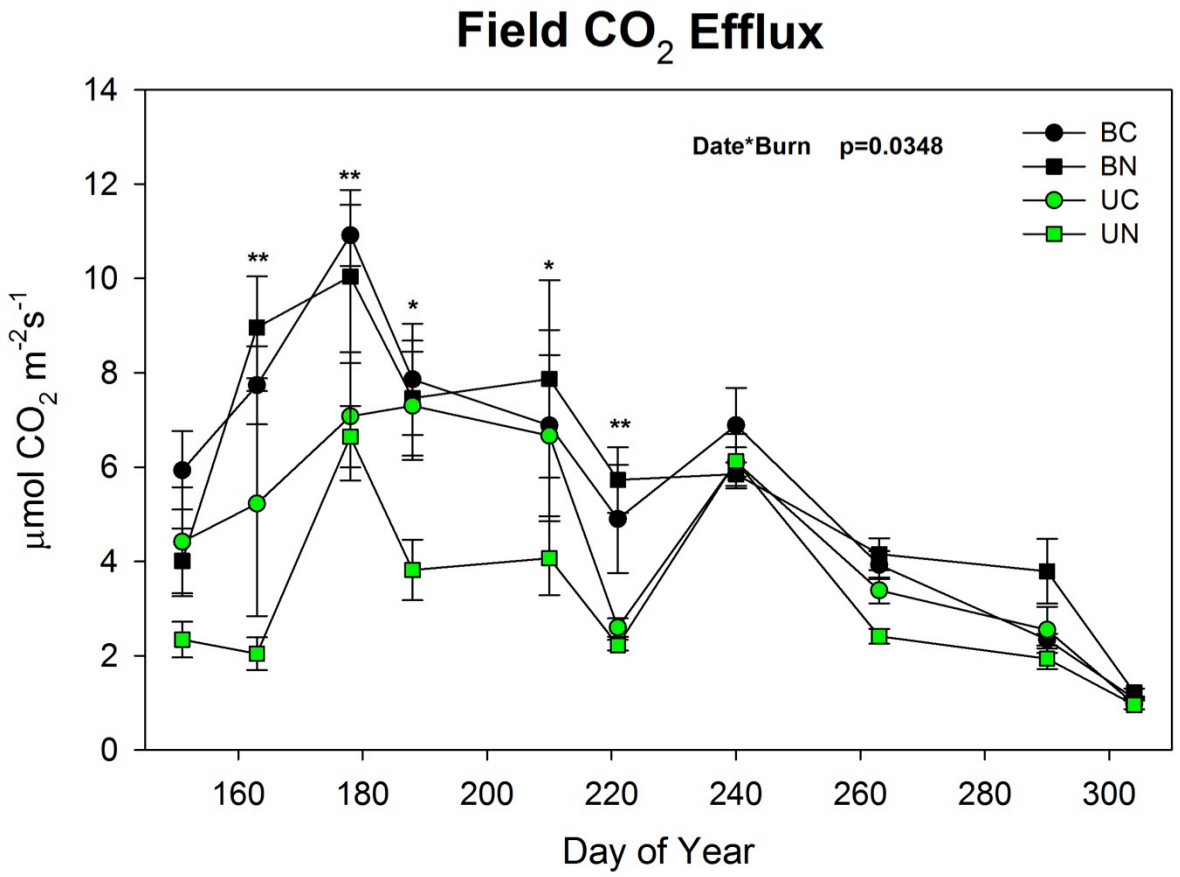

Figure 2.15 CO_2 efflux (mean ± 1 SE) for field measurements taken with a Li-Cor 8100. Post hoc slices indicated within date significance of burn where * and ** designate p values of ≤0.05 and ≤0.01 respectively.

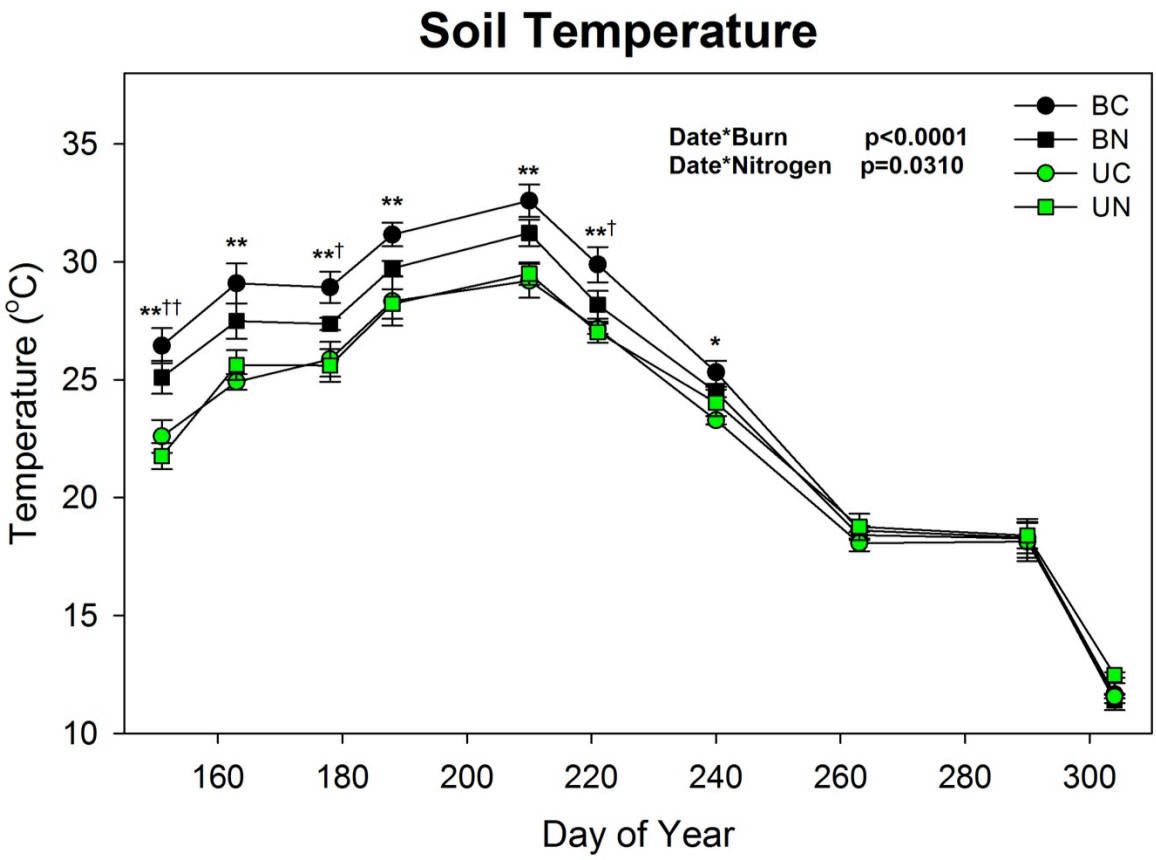

Figure 2.16 Mineral soil (10 cm) temperature (mean ± 1 SE) taken with a Li-Cor 8100 during congruent CO_2 efflux measurements. Interaction effects of date with burn and nitrogen were both significant and post hoc slices indicated within date differences of nitrogen and/or burn. Differences in nitrogen are indicated by † or †† while differences in burn are indicated by * or ** for p values ≤0.05 and ≤0.01 respectively.

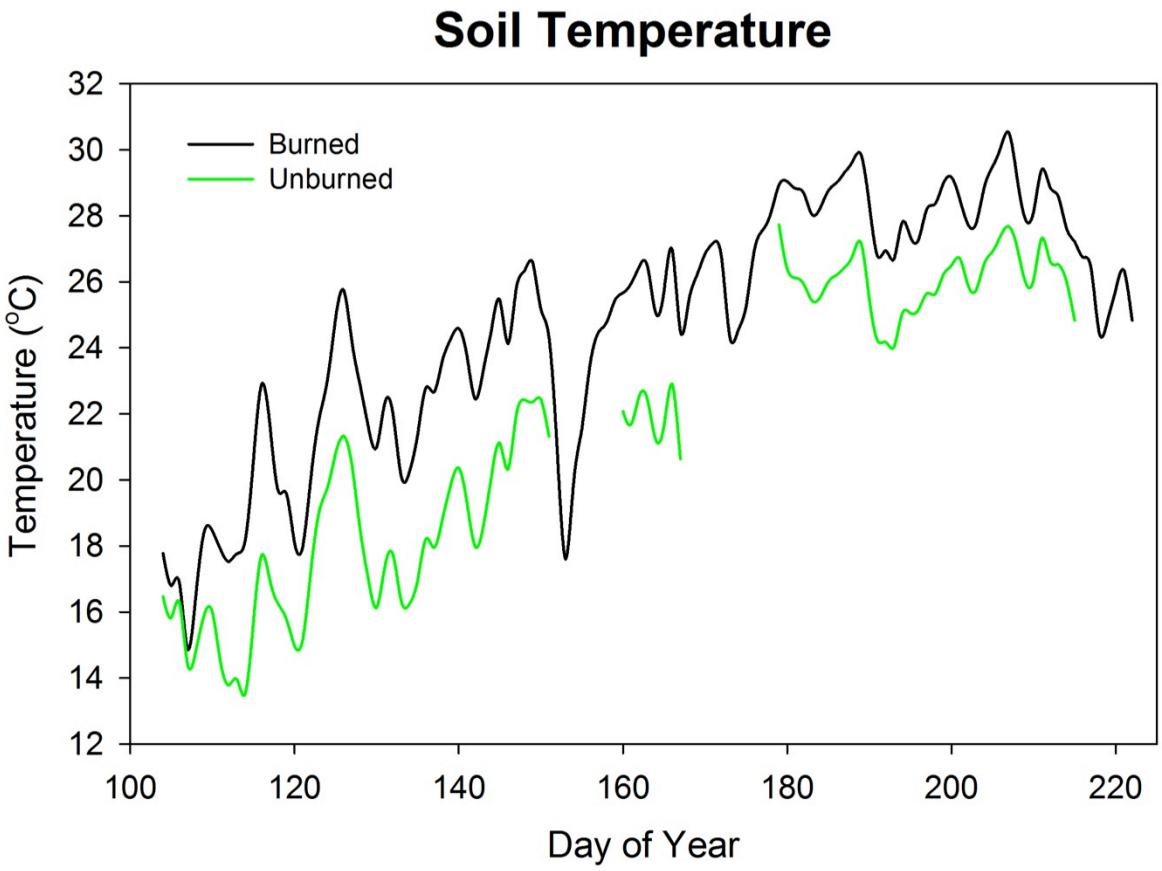

Figure 2.17 Soil temperature (mean) to a depth of 10 cm in representative burned and unburned plots. Points are 30 minute averages of 1 minute measurements recorded throughout the growing season. Gaps in the unburned record are due to battery failures and wires being chewed by rodents (burned n=8, unburned n=4).

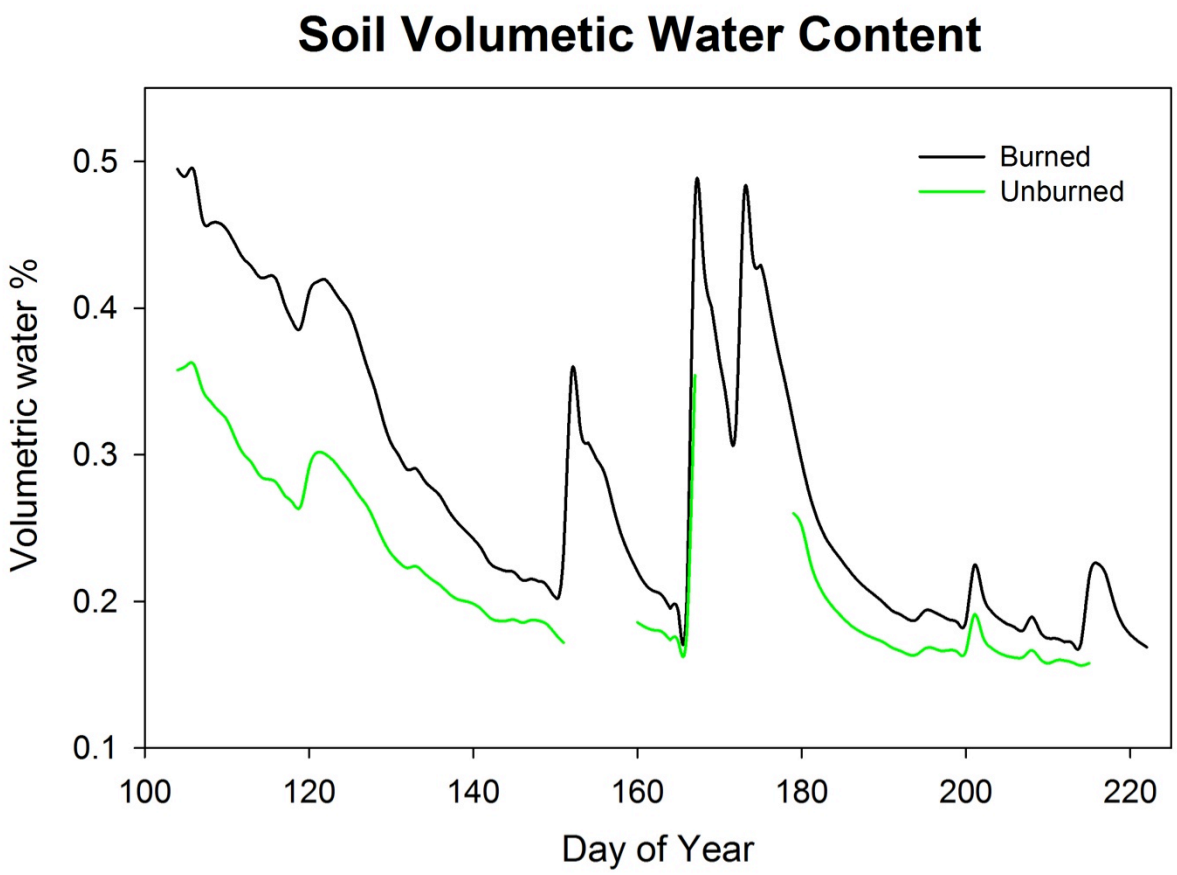

Figure 2.18 Soil volumetric water content (mean) to a depth of 10 cm in representative burned and unburned plots. Points are 30 minute averages of 1 minute measurements recorded throughout the growing season. Gaps in the unburned record are due to battery failures and wires being chewed by rodents (burned n=8, unburned n=3).

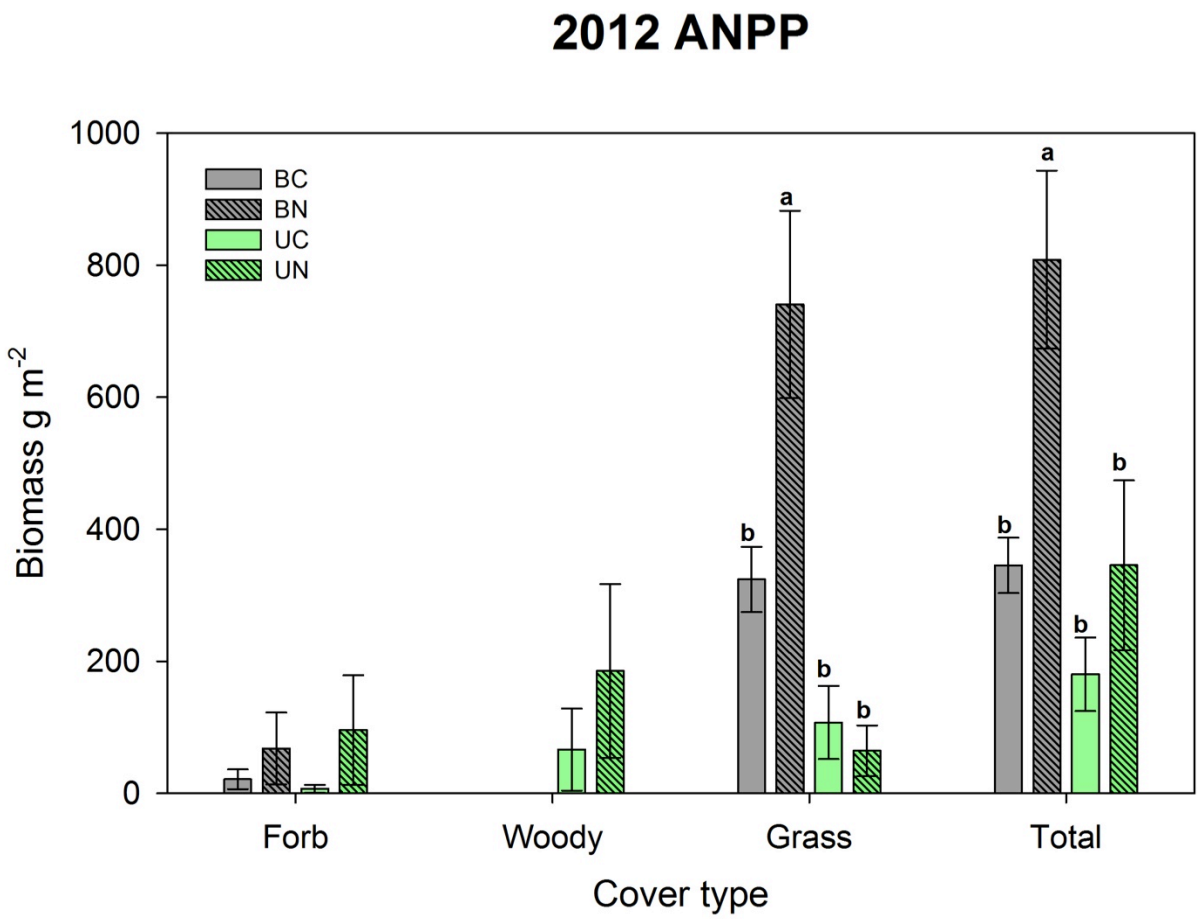

Figure 2.19 Aboveground net primary productivity (mean ± 1 SE) measured from two 0.1 m^2 quadrats in late August. Letters indicate differences between treatments within cover types at the $p \leq 0.05$ level.

Chapter 3 - Microbial Biomass and Community Composition

Abstract

Interest in soil microbial ecology has increased rapidly in recent decades, and we are now beginning to understand the drivers of soil microbial biomass and community structure from a microbial functional perspective. While broad-scale patterns of microbial biomass and community composition have been identified in response to edaphic and climatic variables, local and regional responses are known to vary, often contradicting the broad-scale tendencies. This study utilized a unique experiment, initiated in 1986 at Konza Prairie Biological Station (Manhattan, KS USA), to assess the effects of 27 years of alternate burning regimes and long-term N enrichment on microbial biomass and phospholipid fatty acid (PLFA) composition in a tallgrass prairie. Burning, fertilization, and date of sampling all affected microbial biomass and PLFA composition, although my results were often contrary to expectations based on responses in other ecosystems. Annual burning tended to increase MBC by 16% and MCN by 11%, although neither effect was statistically significant. However, both total PLFA mass and the relative abundance of fungi (expressed as mole%) were significantly increased by annual burning ($p<0.0001$ and $p=0.005$, respectively). Nitrogen fertilization significantly lowered MBC ($p=0.0116$) and MBN ($p=0.0239$) as well as PLFA fungal mass ($p<0.0001$) and common PLFAs ($p=0.0003$). Nitrogen enrichment also altered microbial community composition and increased the relative abundance of specific bacterial groups ($p<0.0001$), with actinomycetes increasing, by 13%, the most of any group. Effects of sampling date closely mirrored seasonal trends from other studies, with lower microbial biomass and increased MBC:MBN across treatments in June and July, which corresponds to peak aboveground growth. In total, this study demonstrates that decades of contrasting fire regimes and N fertilization alters microbial biomass and community structure. Future studies should use more detailed molecular approaches to determine within group changes in response to these common prairie disturbances.

Introduction

Understanding the relationships between microbial community structure and ecosystem functioning has long been a goal in soil ecology. Methodological and computational developments in the last two decades have dramatically improved our ability to explore the composition and functioning of soil microbial communities, and are beginning to shed light on the 'black box' that has traditionally represented the soil microbial compartment in ecology (Frostegård et al. 2010, Tiedje et al. 1999). On a very broad scale (e.g. continental to global patterns), we are beginning to understand some of the drivers of soil microbial community structure and function, including edaphic variables such as pH, clay content, soil moisture, and soil organic matter (SOM) content (Zhou et al. 2002, Fierer and Jackson 2006, Wallenstein et al. 2006, Manzoni et al. 2012). While these variables may be useful starting points on continental to global scales, ecosystem-specific characteristics (i.e. vegetation cover and litter inputs) as well as regional and local variability in soil characteristics can often lead to exceptions from the "rules" developed from these broad-scale studies (e.g. Ramirez et al. 2010, Dooley and Treseder 2012). Additionally, seasonal and shorter-term variation within sites leads to fine-scale temporal dynamics (Jangid et al. 2010) while predictions of climatic changes at local management scales can lead to further complex interactions between microbial populations and turnover of organic and inorganic nutrients (Sheik et al. 2011, Zeglin et al. 2013). Understanding the response of microbial populations to modern anthropogenic activities on multiple levels (e.g. microbial diversity, enzyme activity, organic/inorganic nutrient turnover) is critical for establishing a comprehensive understanding of how these activities may alter microbial community structure and function on local, regional, and global scales. In the case of mesic grassland ecosystems, two important anthropogenic alterations are N enrichment, as a result of enhanced N deposition, and changes in fire regimes, an important natural disturbance and contemporary management practice in many grasslands worldwide.

The deposition of anthropogenically-derived reactive N has increased globally (Galloway et al. 2003), and establishing an understanding of how microbially-mediated N transformations will respond to enhanced N availability is of critical importance for local to global biogeochemical budgets (Fierer et al. 2011, Börjesson et al. 2012). Current research indicates a wide range of potential microbial responses to increased N availability, with microbial biomass responses reported to be positive (Cusack et al. 2011), negative (Ramirez et al. 2012), and no

difference (Zeglin et al. 2007) in various ecosystems. Laboratory incubations with multiple soil types exposed to enhanced N level have shown a trend towards bacterial communities that more readily break down labile C (Ramirez et al. 2012), resulting in potentially decreased C mineralization in the long term (Craine et al. 2007). Some field studies have contradicted this, and indicated short term (<33 months) bacterial biomass suppression and fungal biomass stimulation with N enrichment (Docherty et al. 2012), while other studies have failed to show consistent results with regards to biomass and richness/diversity, with either varied fungal and bacterial responses to N additions (Ramirez et al. 2010, Börjesson et al. 2012), no apparent adaptations after three years of treatment (Allison et al. 2013), and even topographic interactions with increased N (Cusack et al. 2011). These diverse responses are likely due to differences in local climate, biota, and management, and will only be understood with increased research efforts, at both varied temporal and spatial scales.

Microbial responses to burning, a natural disturbance and common management tool in many mesic grasslands, are as varied as responses to increased N. Microbial biomass has been shown to increase (Jangid et al. 2010), decrease, or not change in response to fire (Ponder et al. 2009, Docherty et al. 2012). Specific groups of microbes (i.e. gram +/- and fungi) also respond in varied ways, with reports of fungi responding both positively and negatively to fire (Hamman et al. 2007, Dooley and Treseder 2012, Docherty et al. 2012). Bacteria have shown similar responses with resistance, negative, and positive changes all being reported (Docherty et al. 2012, Ponder et al. 2009, D'Ascoli et al. 2005). A meta-analysis of fire effects on soil microbial communities found that variation often results from differences in ecosystem type, with forests often showing decreases and grasslands showing increases in microbial biomass in response to fire (Dooley and Treseder 2012). Interestingly that same meta-analysis also found that fire type (prescribed/wildfire) influences the magnitude of response, with wildfires tending to decrease microbial biomass more than prescribed burns. In addition to the effects of fire per se, fire suppression in fire-dependent ecosystems can also influence microbial dynamics through changes in plant communities or the soil environment. This may be especially relevant in mesic grasslands where woody plant encroachment is often exacerbated with decreases in burn frequency (Collins and Calabrese 2011). Increased AMF associations with *Juniperus virginiana* in these invaded areas may then intensify further woody expansion while decreasing the leaf litter quality, potentially altering nutrient cycling and availability (Williams et al. 2013).

Soil microbial responses to alternate fire regimes and increasing N are very complex on their own. However, they will undoubtedly interact with changing abiotic factors; the result of both direct climatic change and shifts from altered aboveground plant communities. Precipitation patterns are predicted to change and water availability is often linked to microbial structure and function. Microbial biomass has been show to positively correlate with water content (Zeglin et al. 2013), with peak biomass often occurring around ~50% water filled pore space (WFPS) (Uhlířová et al. 2005). Shifts in microbial communities are also reported with high water availability with gram-negative (G–) bacteria often increased near saturation (Uhlířová et al. 2005), while drought and water stress are reported to increase fungi and gram-positive (G+) bacteria (Allison et al. 2013). Desiccation of soils causes osmolyte production which when released during rewetting of soils causes a flush of activity and heterotrophic respiration. This flush of mineralized C is estimated to equate to 3-6% of NPP in a grassland system for a single precipitation event (Schimel et al. 2007), and altered patterns of rainfall may significantly alter the magnitude of these effluxes (Manzoni et al. 2012). Temperature is also predicted to rise and microbial communities have potential to respond rapidly with predictions of increases in heterotrophic respiration and SOM losses (Fang et al. 2005), and changes in microbial diversity with sufficient soil moisture (Luo et al. 2001, Sheik et al. 2011). Finally, rising atmospheric CO_2 is of concern to primary production (Hu et al. 2001), but microbial responses to increasing soil C inputs (NPP) are likely to be minimal with outputs (respiration) predicted to be nearly equivalent to increased inputs (Suyker and Verma 2001, Nie et al. 2013).

Past research in microbial ecology has often concentrated on the effects of individual factors on microbial communities over shorter periods of time, with longer studies often relying on chronosequence approaches (e.g. Bach et al. 2010, Allison et al. 2005). This research is unique in that it utilized a long-term (27 years) fire and nutrient amendment experiment in a tallgrass prairie, and focused on elucidating microbial responses to both contrasting burning treatments and/or N fertilization (100 kg-N m^{-2}) over long timeframes through microbial biomass and PLFA measurements. Repeated annual burning was hypothesized to increase microbial biomass C and N, primarily due to increases in fungal abundance through stimulation of root growth and increased belowground C inputs, while having little to no effect on bacterial abundance. Additionally, chronic N fertilization was hypothesized to increase bacterial abundance while decreasing fungal abundance as well as total microbial biomass through the

loss of readily degradable C substrate through time and a reduction in carrying capacity. Date of sampling was hypothesized to affect microbial biomass as a result of seasonal dynamics, with the lowest values coinciding with high plant competition for available soil nutrients in mid-summer and peak values occurring in late summer coinciding with plant senescence and increased nutrient availability.

Methods

Soil Sampling

Soils sampling for microbial biomass and PLFA analysis occurred at the same time as initial soil collection for N and C mineralization assays (Chapter 2). Briefly, four randomly located soil collars (for C efflux measurements) were placed in each plot (burned and unburned, control and N addition treatment plots, Figure 1.1) a minimum of 1.5 m away from a plot edge (Petersen and Calvin 1996, Paul et al. 1999). On sampling dates, mineralization tubes were installed ca. 1 m from each efflux collar and initial measurement cores 5 cm in diameter were taken to a depth of 10 cm halfway between the efflux collar and mineralization tube. Cores were handled with nitrile gloves and the corer was washed with acetone between plots to prevent PLFA contamination. Soil was composited by plot (4 cores per plot) and immediately transferred to gallon Ziploc bags that were placed into a cooler over ice. Transport to the lab was a maximum of three hours after beginning sampling and samples were refrigerated upon arrival. Within 36 hours of sampling, soils were sieved to 4 mm and any remaining roots and particulate organic matter was hand-picked for 12 minutes (Boone et al. 1999). Subsamples of soil for phospholipid analyses were taken immediately after root picking and placed into a -20 °C freezer. Nitrile gloves were worn during sieving and root picking to prevent lipid contamination. Remaining soil was then placed back into refrigeration for storage prior to subsequent analyses of MBC, MBN, and mineralization assays (Chapter 2).

Microbial Biomass Carbon and Nitrogen (Direct Extraction)

For each sampling date, homogenized, sieved, and root-free soil samples were weighed (ca. 12 g field moist) into 125–ml Erlenmeyer flasks. Two replicate samples per plot were then fumigated with chloroform. Non-fumigated replicates were extracted immediately following the

fumigation to establish baseline values. Soils were extracted with K_2SO_4 and analyzed for organic carbon directly on a TOC analyzer. Organic nitrogen was measured via a persulfate digestion and analysis for nitrate on a Flow Solution IV; Griess-Ilosvay method (Paul et al. 1999).

Fumigation

Sub-samples were placed into a vacuum desiccator lined with moist paper towels containing a 150–ml beaker filled with 30-40 ml of ethanol free chloroform (amylene stabilized, Acros Organics 167735000) and 3-5 synthetic boiling chips. Two desiccators were used with one sample replicate in each desiccator to eliminate fumigation pseudoreplication. A vacuum was pulled (>25 inches Hg) on each desiccator to boil the chloroform for two minutes. Vacuum pressure was released and the process repeated a second time before sealing the desiccators under vacuum. Desiccators were covered with black trash bags to prevent chloroform degradation by light and stored for 48 hours at room temperature (24 °C). After 48 hours, samples were vented by pulling a vacuum for five minutes, desiccator lids were removed and samples aerated for five minutes. The venting process was repeated three additional times to remove any traces of chloroform prior to K_2SO_4 extraction (Jenkinson and Powlson 1976, Brookes et al. 1985, Vance et al. 1987, Brookes 2012).

Extraction

Baseline (un-fumigated) samples were extracted immediately following the fumigation procedure, while fumigated samples were extracted two days later after ventilation. In each case, 75 ml of 0.5M K_2SO_4 was added to each sample (7.5 ml solvent : 1 g soil). Flasks were then covered with Parafilm and shaken on an orbital shaker (New Brunswick Scientific G10) for one hour at 200 rpm. Samples were allowed to settle for 45 minutes before filtration using 30–ml syringes fitted with 0.4 µm polycarbonate filters (Fisher #K04CP02500). Filtered un-fumigated and fumigated K_2SO_4 extracts were collected in 20 ml plastic scintillation vials and frozen for storage at -20°C prior to analysis.

Microbial Biomass Carbon

Potassium sulfate extracts were first thawed and diluted in a 4:1 ratio of DI water to sample for analysis on a Shimadzu TOC-L fitted with a high salt kit. Instrument parameters included a best 3 of 4 rule, multiple injections, and an acceptance range of SD ±0.1 and CV max of ±2%. Values for replicate samples were averaged and microbial biomass carbon (MBC) was calculated (Equation 3.1) as the difference between post- and pre-fumigation samples (Vance et al. 1987). For ease of comparison to other studies no correction coefficient was applied, although a value of 0.45 is recommended for such conversions (see Jenkinson et al. 2004 and Joergensen et al. 2011).

Equation 3.1 Microbial Biomass Carbon

$$MBC = (Fumigated\ TOC - Unfumigated\ TOC)$$

Microbial Biomass Nitrogen

A persulfate digestion was used to quantify microbial biomass nitrogen (MBN), where all forms of nitrogen are oxidized to nitrate before analysis with a spectrophotometer. Prior to analysis, all glassware was acid washed (1M HCl) and DI rinsed three times. The following stock solutions were made using low nitrogen chemicals due to the high molar concentrations: 3.75 M NaOH (Fisher S3185-500); 0.5 M K_2SO_4 (Sigma Aldrich 31270); urea % recovery standard 3.0 ppm in 0.5 M K_2SO_4 (made from 0.536 g dried urea in 1 L of DI "Stock A" diluted to 1200 µl in 100 ml of 0.1 M K_2SO_4 "Stock B"); NO_3 standard 100 ppm in DI (made from 7.2187 g KNO_3 in 1 L of DI "Stock A" diluted to 20 ml in 200 ml of DI "Stock B"); Carrier Solution containing 200 ml 0.5 M K_2SO_4, 50 g persulfate (Sigma Aldrich 60489) 30 g boric acid, 100 ml 3.75 M NaOH, and DI to 2 L of solution; persulfate cocktail containing 100 g persulfate, 60 g boric acid, 200 ml 3.75 M NaOH, and DI to 2 L of solution. Nitrate standards were made according Table 3.1.

Replicate K_2SO_4 extracts from each sampling date and standards were used to ensure digestion went properly. Digestion standards and sample solutions were made as follows. Standards: 8 ml standard, 8 ml persulfate cocktail; urea % recovery: 8 ml "Stock B" (3 ppm), 8 ml persulfate cocktail; Spike: 4 ml urea "Stock B", 2 ml random sample, 2 ml DI, 8 ml persulfate

cocktail; Blank (S0): 1.6 ml 0.5 M K_2SO_4, 6.4 ml DI, 8 ml persulfate cocktail; Samples: 4 ml 0.5 M K_2SO_4 soil extract, 4 ml DI, 8 ml Persulfate cocktail. All solutions were made in 25–ml digestion tubes and capped with PTFE lined caps immediately after persulfate cocktail addition minimizing gaseous loss of N. Tubes were then vortexed for 15 seconds to mix and autoclaved at 120 °C for 40 minutes (D'Elia et al. 1977, Solorzano and Sharp 1980, Cabrera and Beare 1993, Allison and Vitousek 2005). Carrier solution (2 L) was also autoclaved with samples in 500 ml bottles. Autoclaved samples were refrigerated until analysis on an Alpkem OI Analytical Flow Solution IV (see *Flow Solution IV* section in Ch. 2). For each run carrier solution was used in the wash stream, establishing the baseline. Replicate standards were used for the standard curve, and a % recovery was calculated from spike samples using Equation 3.2.

Equation 3.2 Persulfate Digestion Percent Recovery

$$\%\ Recovery = \ 100 * \frac{Spike\ ppm\ average}{(average\ sample\ ppm\ +\ ppm\ of\ urea\ standard)}$$

Four % recovery samples were used per run and the average was applied to sample values (ex. for an average 75% recovery, a sample value of 2.3 ppm would be divided by 0.75 to get an actual value of 3.07 ppm). Microbial biomass N was then calculated as the difference in fumigated and unfumigated samples (Equation 3.3) and converted to μg $N \cdot g^{-1}$dry soil using Equation 2.3 where extraction volume was 75 ml instead of 50 ml. No correction factor was applied to any microbial biomass data but a value of 0.45 would be recommended (see Jenkinson et al. 2004 and Joergensen et al. 2011).

Equation 3.3 Microbial Biomass Nitrogen

$$MBN\ =\ Fumigated\ NO3-\ (ppm)\ -\ Unfumigated\ NO3-\ (ppm)$$

Phospho/Neutral-lipid Fatty Acid Analysis

Sample Preparation

Methods followed those of White and Rice (2009) as modified from Bligh and Dryer (1959) (Frostegård and Bååth 1996, White and Ringelberg 1998). Nomenclature for fatty acid description follows Bossio and Scow (1998) and Piotrowska-Seget and Mrozik (2003) while community grouping (Gram +/-, AMF, fungi) followed lipid markers used in Allison et al. (2005), McKinley et al. (2005), White and Rice (2009), Williams (2007), and summarized in Docherty et al. (2012). Sieved and root-free soil samples (see *Soil Sampling* section) were placed into 25–ml diluvials, capped and frozen at -20 °C. Within one week of sampling, frozen samples were freeze-dried at -50 °C under vacuum (0.09 Torr) using a Labcono Freezone 6. Freeze-dried samples were then ground in a SPEX 8000D mixer/mill for four minutes until a homogenized mixture the consistency of talc powder was reached. Acetone was used to clean parts between samples to prevent contamination and samples were returned to their respective diluvial for storage.

Glassware Preparation

Prior to analysis all glassware was heated in a muffle furnace to a temperature of 450 °C for four hours to burn off any lipids. All large glassware was cleaned and acid washed prior to heating, while disposable Pasteur pipettes were only heated in a muffle furnace.

Extraction

Lipid extraction of all four sample dates were done simultaneously in January 2013 due to a low yield of lipids in an earlier November run. For each sample 5 g (±0.1 g) of freeze-dried ground soil was weighed out into 50–ml glass centrifuge tube. Each tube then received 4 ml of phosphate buffer (8.7 $g \cdot L^{-1}$ potassium phosphate dibasic titrated with 3M HCl to a pH of 7.4, micro-filtered and stored with 50 ml of chloroform), 10 ml methanol, and 5 ml chloroform swirling between additions. Extraction mixtures were vortexed for 30 seconds and vented for three hours with brief vortexing every hour. Samples were then centrifuged for ten minutes at 1,500 rpm, the supernant decanted into a second 50–ml glass vial. Chloroform and nanopure water (5 ml each) was added and mixed with a brief vortexing before being capped and stored at

room temperature in a dark cabinet overnight (ca. 18 hours). The bottom organic phase was then transferred to a 10–ml test tube using a Pasteur pipette and samples were then evaporated under nitrogen (OA-SYS-N-EVAP116, NEVAP) at 50 °C before being moved directly to chromatography.

Silicic Acid Chromatography

Silicic columns (1,000 mg J.T. Baker 7086-07) were first conditioned with 5 ml of methanol followed by 5 ml of acetone and 5 ml of chloroform. Vacuum pressure of 3 inches Hg was used that resulted in a ca. 2-minute residence time for all 5 ml additions in the column. Dried lipid samples were suspended in 250 μl of chloroform and samples were transferred using a Pasteur pipette to the silicic columns. This process was then repeated two more times to ensure full lipid transfer. Columns were eluted with 5 ml of chloroform into a clean 10–ml test tube to obtain the neutral lipid fraction. Tubes were then removed, placed under nitrogen and evaporated at 50 °C. The middle lipid fraction (glycolipids) was removed into a waste tube using 5 ml of acetone, and the final phospholipid fraction was eluted into a clean test tube using 5 ml of methanol. Phospholipid samples were also placed under nitrogen and evaporated at 50 °C. Dried neutral and phospholipids were then capped and stored in a -20°C freezer.

Methylation

Frozen PLFA/NLFA samples were brought to room temperature and dissolved with 0.5 ml of chloroform and 0.5 ml of methanol. 1 ml of 0.2M methanoic KOH was added and swirled to mix. Tubes were capped and placed in the NEVAP water bath at 60°C for 45 minutes until a white precipitate formed. Hexane (2 ml) and 200 μl of 1N acetic acid was then added to each sample and swirled to mix before 2 ml of nanopure water was added to induce a phase break. Solutions were vortexed to mix for 30 seconds before being centrifuged for five minutes at 1,000 rpm. The top phase was then transferred to a clean 10–ml test tube and the process (starting with 2 ml hexane addition) was repeated a second time. The NEVAP was then set to 37 °C and samples were evaporated under nitrogen, capped, and stored at -20°C.

GC/MS Analysis

Prior to GCMS analysis samples were suspended in a 20 μg C19:0 internal hexane standard made by dissolving 20 mg of methylnonadecanoate in 1 L of hexane. Standard curve solutions contained 0, 2, 20, and 200 μl C19:0, also suspended in hexane. For analysis thawed methylated samples were dissolved in 1 ml of the 20 $\mu g \cdot L^{-1}$ C19:0 hexane solution and pipetted into 2 ml target I-D vials and capped with screw top polyethylene tops. Standard curve solutions, samples, and BAME standards were then loaded onto a Thermo Scientific trace gas GC/MS ultra. Samples took ca. 45 minutes each to run and 30 peaks were identified from comparison to the BAME standard.

Statistical Analyses

Microbial biomass and PLFA data (mass and mole%) from four sampling dates were analyzed in a repeated measures two-way ANOVA using a mixed effects model (PROC MIXED) with burn and fertilization treatments as fixed effects and date and plot as random effects (SAS Institute V9.2, 2008). Phospholipids were grouped by microbial type (gram +/– bacteria, fungi, common) and date was found to be significant for the common classification for both mass and mole%, while date was significant for fungi on a mole% basis. Due to the overall infrequency of date significance, PLFA mass and mole% from the four summer sampling dates were averaged and a two-way ANOVA was used to identify effects of burn and/or nitrogen. Simple linear regressions (PROC REG) were also run to see if microbial biomass estimates from the fumigation direct extraction and fatty acid biomass measurements correlated, as well as biomass measurements with C respiration (Ch. 2). Outliers that were more than three standard deviations from the mean were excluded from the analysis. Significance was measured at $p < 0.05$ for all tests unless otherwise noted. Repeated measures ANOVAs revealed no interaction effects so post hoc slices were not necessary. However, post hoc Tukey's HSD tests were used with the two-way ANOVAs for total PLFA mass and mole% to identify differences within microbial groupings (PROC GLM, lsmeans/adjust=Tukey).

Results

Fumigation Direct Extraction

Changes in MBC and MBN are summarized in Table 3.2 and patterns of treatment –level mean values are presented in Figures 3.1-3.3. Surprisingly, 27 years of contrasting fire treatments did not significantly affect microbial biomass C and N, potentially due to high variability within treatments and plots. There was a statistically significant effect of long-term N enrichment, however, with N additions lowering both MBC ($p=0.0116$) and MBN ($p=0.0239$). These changes in biomass C and N were proportional and, therefore, there was no net effect of fertilization on the ratio of MBC to MBN (MBC:N) ($p=0.5338$). For all three measures of microbial biomass, sample date was significant, with a general trend for both MBC and MBN to decrease in mid-summer while the MBC:N increased slightly during the same time interval, consistent with decreasing soil N availability at that time. MBC was significantly higher in August with an increase of ≥ 31% over all other sampling dates. There was a significant initial decrease (≥ 25%, $p\leq0.0035$) in MBN from May to June and July and a recovery to peak MBN in August, increasing mean values ≥ 75% over June and July ($p<0.0001$) and 31% above May ($p=0.0009$). Average values of MNC:N were higher in mid summer with a minimum increase of 32% in June and July over both May and August ($p\leq0.0001$). There were no interaction effects of date, burn and/or fertilization for any of the fumigation-based microbial biomass measurements.

Phospholipid Fatty Acids

Phospholipids were analyzed individually, and patterns of individual change closely followed microbial group changes (i.e. gram +/– bacteria, fungi, common). Due to the agreement between individual lipid and group responses, I present only grouped data here. Microbial groups were first analyzed on a mass (μg lipid·g^{-1} dry soil) and molar% (μg lipid group·g^{-1} dry soil / total μg lipids·g^{-1} dry soil) basis, using a repeated measures ANOVA (Table 3.3). Effects of sample date were only significant for common PLFA mass and molar% ($p=0.0003$, $p=0.0001$, respectively) and for fungi only on a molar% basis ($p=0.0195$). Effects of date from repeated measures ANOVA on fungi (mole%) are presented in Figure 3.7. Total fungal abundance

decreased 9% from May to August ($p=0.0114$), with both August and July having significantly lower biomass than May and June. Common PLFA mole% was different only in June, with a 14% increase in mole% over all other dates. Common PLFAs by mass changed more predictably by date, as shown in Figure 3.5, with May and June having a minimum of 19% less PLFA biomass than July or August ($p\leq0.005$). Due to a lack of date effects on other PLFA groups, subsequent analysis was simplified by averaging over the entire summer, and a two-way ANOVA was used to determine effects of burning and/or fertilization (Table 3.4). These analyses provided marginally more significant effects than the repeated measures ANOVAs, but no additional main effects became significant ($p\leq0.05$, Tables 3.3 and 3.4).

The average mass of PLFAs is shown in Figure 3.4 and significant effects are summarized in Table 3.4. PLFA mass increased with annual burning for all groups except gram-negative bacteria, with increases in specific groups as follows; total 14% ($p<0.0001$), common 11% ($p=0.0213$), gram-positive 10% ($p<0.0001$), fungi 26% ($p<0.0001$), actinomycetes 20% ($p<0.0001$), and AMF 16% ($p=0.0023$). Chronic fertilization with N had the opposite effect, decreasing all groups except gram-negative bacteria and actinomycetes as follows; total 17% ($p<0.0001$), common 26% ($p=0.0003$), gram-positive 10% ($p<0.0001$), fungi 29% ($p<0.0001$), and AMF 46% ($p<0.0001$). An interaction effect between fertilization and burning was seen in the gram-positive bacteria, with unburned-nitrogen plots having at least 16% less biomass than all other treatments ($p=0.0062$). PLFAs measured on a mole% basis (relative abundance) were less affected by burning, with only fungi significantly responding with an increase in molar% of 11% ($p=0.0005$). Nitrogen was a stronger driver of molar% PLFAs, with varied effects on bacteria vs. fungi, where bacterial groups responded positively and fungal groups responded negatively. Gram-positive bacteria increased with N fertilization by 8% ($p<0.0001$) while actinomycetes increased 13% over ($p=0.0009$). Additionally actinomycetes exhibited an interaction effect of burning and fertilization where unburned-control plots were at least 21% lower than all other treatments ($p=0.0023$). In contrast, fungal groups were suppressed with fertilization with overall fungi decreasing 16% and AMF 18% ($p<0.0001$ for both).

Correlations between total PLFA as well as MBC and MBN were done to see how well these two methods of measuring biomass correlated, due to slight differences in main effects on total and MBC/N measurements (i.e. no significant burn effects in MBC/N measurements and an increased level of significance for total PLFA mass in response to both fertilization and burning).

MBC and total PLFA mass were positively correlated with an R^2=0.5613 and a p<0.0001. Total PLFA mass and MBN were also significantly positively correlated (p<0.0001), although the R^2 was slightly lower at 0.3332. Additionally, microbial biomass measurements were regressed against potential C mineralization rates (Ch. 2), and both MBC (R^2=0.2899, p<0.0001) and total PLFA mass (R^2=0.3633, p<0.0001) was found to positively correlate to potential C soil respiration rate. Finally, I also found that MBC:N was significantly, but weakly, negatively correlated to potential net N mineralization rate, with an R^2=0.12 and p=0.0054.

Discussion

This research revealed that 27 years of chronic N fertilization of tallgrass prairie significantly decreased MBC and MBN as well as total PLFA mass. Earlier studies from the BGPE, following the initial 8 years of treatment (Ajwa et al. 1999), did not find an effect of the fertilizer treatment on MBC or MBN on soils sampled to a depth of 5 cm. In that same year, Garcia and Rice (1994) detected no change in MBC but an increase in MBN due to fertilization in soils sampled to a depth of 30 cm. Another N enrichment experiment at KPBS also indicated no change in MBC with added nitrogen in the short-term (Zeglin et al. 2007). Differences in short- and long-term responses reported to the N additions are likely due to the length of this experiment and the cumulative effects of chronic N enrichment. For example, short-term N enrichment experiments often report increases in microbial biomass across a range of ecosystems from tropical forests (Cusack et al. 2011) to arctic tundra (Sorensen et al. 2008). A potential mechanism for the observed decreases in microbial biomass in the current study comes from a yearlong laboratory incubation of 28 soils with added N. In that study, Ramirez et al. (2012) showed that microbial biomass was decreased by 35% with fertilization and that the community shifted to bacterial groups, both responses being consistent with results I obtained. This shift to bacterial likely means faster microbial turnover due to shits towards r-selected species (Kelliher et al. 2007). Another potential explanation for decreased microbial biomass with fertilization may come from plant associations with AMF. Other studies at KPBS have found that in high N environments *Panicum virgatum* wins out over *Andropogon gerardii* (both dominant C_4 grasses) and that root biomass is not increased with N fertilization (Johnson et al. 2008, Baer and Blair 2008). At the same time, *P. virgatum* has lower colonization by AMF and may be driving the loss of fungi in these high N environments due to its ability to reduce fungal associations when

they are unnecessary (Johnson et al. 2008). Although this shift to *P. virgatum* under high N is only seen in burned and fertilized plots (Collins et al. 1998), so there are likely additional causes in unburned fertilized plots.

In addition to increased N enrichment altering microbial biomass, fertilization is also known to have varied effects on diversity and richness (Ramirez et al. 2010). Although the decreased fungal abundance we measured is not universal, as highlighted in a 53-year study in Sweden that showed little change in fungi even with a variety of N and C amendments (Börjesson et al. 2012). Additionally, some shorter studies have shown 6 years of N addition stimulated fungi and suppressed bacteria in a California grassland (Docherty et al. 2012), while 7 years of N addition in a mixed-grass prairie (Cedar Creek, MN, USA) resulted in a decrease in MBC attributed to an increase in fungi, which raised the MBC:N (Keeler et al. 2009). These responses contradict our results which are more in line with the N mining theory, which predicts declines in microbial respiration rates and increasing dominance of bacterial communities with N fertilization (Fierer et al. 2007, Ramirez et al. 2012). This reasoning is further supported by our increase in G+ bacteria mass and mole% and decrease in fungi mole% and mass with N fertilization. This makes sense as G+ bacteria are known to prefer more recalcitrant plant litter (Freitag et al. 2005, Kramer and Gleixner 2008, Liu and Greaver 2010) that tends to have a wider C:N ratio, meaning bacteria are more N limited. These results are also likely due to the difference higher baseline C:N ratio of fungi compared to bacteria, where bacteria abundance is promoted under non-limiting N (fertilization or fire suppression) while fungal abundance is suppressed under the same treatments. It is also expected that under contrasting treatments (i.e. frequent burns and limited N inputs) and N limitation fungi would become more abundant while bacteria less. This conclusion and our results are supported by other studies (e.g. Bradley et al. 2006) but the variability of microbial responses to increased N across and within ecosystems further highlights the need for more fine scale work.

Fumigation-direct extraction and PLFA results both indicated that repeated annual burning increased microbial biomass in tallgrass prairie, although MBC and MBN were not significantly higher. PLFA results further indicated that these increases were primarily due to increased abundance (mole%) of saprophytic fungi in burned plots. Lack of significance in MBC and MBN responses to burn treatments are consistent with previous studies in the BGPE with soils sampled to depths of 5 cm (Ajwa et al. 1999), 15 cm (Dell et al. 2005), and 30 cm (Garcia

and Rice 1994). Lack of response in microbial biomass, and even reductions, in response to burning has been reported in other grasslands (Fynn et al. 2003) as well as in other ecosystems (Dooley and Treseder 2012). Other studies in tallgrass prairie at KPBS, however, most often report increases in microbial biomass with burning (Ojima et al. 1994, Jangid et al. 2010). This increase in microbial biomass, especially in fungi, may be related to the 48% increase in root biomass seen at KPBS in response to annual fire (Kitchen et al. 2009), a common trend seen across grasslands (Fynn et al. 2003, Dell et al. 2005, Docherty et al. 2012). In fact, grasslands are unique in that they were the only ecosystem that showed a positive response in microbial biomass to burning in a meta-analysis conducted by Dooley and Treseder (2012) who proposed that microbial communities in these ecosystems are likely adapted to fire and tolerant due to a long and frequent fire history (Allen and Palmer 2011). This is in contrast to other ecosystems that frequently see bacterial stimulation and fungal reduction in response to fire, likely due to the reduced frequency of this disturbance in ecosystems such as forests (Ponder et al. 2009, Dooley and Treseder 2012).

A significant burn by fertilization effect was detected for actinomycetes PLFA mole%, and was seen in the unburned control treatment, which had a 19% decrease from other treatment combinations. Similarly the G+ bacteria PLFA mass decreased by 16% in the unburned-fertilized plots, potentially as a product of G+ preference for more recalcitrant forms of C likely to occur in these plots (Kramer and Gleixner 2008). Historically, burning in combination with fertilization has been shown to increase MBN, root biomass, macroaggregates (>2000 μm), and to decreased extrametrical AM hyphae (also in unburned control plots) (Ajwa et al. 1999, Wilson et al. 2009). Date was also found to be a driver of MBC, MBN, common PLFAs by mass, as well as common and fungi by relative abundance. Microbial biomass measurements tended to decline in June and July, a pattern noted by Garcia and Rice (1994) over a three-year period and attributed to competition with peak plant biomass. Recovery was detected by August and PLFAs at KPBS have been shown to be highest in winter (Jangid et al. 2010).

Abiotic drivers are also commonly reported as influencing microbial community composition and biomass. Chapter 2 highlighted the abiotic differences among burning treatments with burned plots having significantly higher average summer temperatures (3.21 °C) and significantly higher soil moisture (5.97%) over unburned plots. These differences may be overriding main and interaction effects of burning and nitrogen, and have the potential to affect

SOM pools through increased levels of decomposition at higher temperatures and moisture (Fang et al. 2005, Craine and Gelderman 2011). Drought has also been shown to increase fungi by 14% and decrease bacteria by 86% (Allison et al. 2013) in other systems, however it has been shown that increased water availability at KPBS increases fungal abundance, supporting our results of increased fungal abundance in burned plots (Williams 2007). Finally, microbial biomass and soil respiration often are positively correlated (Colman and Schimel 2013) and our results supported this with MBC and total PLFA positively related to potential C mineralization. Interestingly, we also found that MBC:N had a weak negative relationship to potential net N mineralization rates measured through the summer, but not *in situ* net N mineralization rates. The reduction in potential net N mineralization rates is consistent with an increased MBC:N ratio, which may indicate greater potential limitation of N. While the lack of correlation with field measurements may reflect the greater variability of these measurements leading to a lack in correlation.

Conclusions

Understanding soil microbial biomass dynamics and shifts in community structure is critical for developing a comprehensive understanding of microbial controls over biogeochemical cycles. Aboveground biomass and diversity is rarely reflected in the soil pools (Ramirez et al. 2010, Price et al. 2012) and shifts in belowground community composition will regulate processes over long timeframes (Schimel et al. 2007). This study demonstrated shifts in both microbial biomass as well as community structure, which are likely impacting nutrient dynamics and cycling. Increased levels of N were shown to significantly lower MBC and MBN as well as total PLFA mass. Relative abundance of bacteria increased with fertilization and fungal abundance decreased, contrary to studies in some other ecosystems, but supported by other research in tallgrass prairie at this site. Burning increased MBC and MBN but not significantly, while total PLFA mass was increased as well as the mole% of fungi, indicating a shift towards more fungi in burned plots. Changes in biomass across dates were highly significant and were generally consistent with past results from the same study, with microbial biomass decreasing in mid-summer (June and July) and increasing with plant senescence. MBC and total PLFA mass were also shown to be positively correlated to each other and to potential C mineralization. We also report for the first time to our knowledge that MBC:N is negatively correlated to potential net nitrogen mineralization rates. While this correlation was weak it may

indicate that microbes are potentially N limited mid season, which supports the decrease in biomass known to occur. PLFAs are a good tool to obtain broad changes in microbial community structure, however more precise molecular approaches could be used to help clarify individual genera shifts as well as shifts within broad community groupings. This study in unique in that current results following long-term fire and N addition treatments differed from some historical results following shorter-term treatments in the same experiment, highlighting the importance of long-term studies, even in systems that seemingly change rapidly. Microbial populations may turnover rapidly in laboratory conditions, but shifts in diversity and community composition in response to disturbances may take decades to establish.

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Tables and Figures

Table 3.1 NO_3^- Preparation of persulfate digest standards. Amounts are per 100 ml volumetric flask filled with DI water. Standards were autoclaved with persulfate digestion samples to eliminate potential bias in methods.

Standard	N ppm	KNO_3 Stock B	0.05M K_2SO_4
S0	0.0	0.00µl	20ml
S1	0.20	200µl	20ml
S2	0.60	600µl	20ml
S3	1.00	1000µl	20ml
S4	1.50	1500µl	20ml
S5	3.00	3000µl	20ml
S6	4.00	4000µl	20ml
S7	5.00	5000µl	20ml

Table 3.2 Repeated measures mixed model ANOVA F-table for measurements of microbial biomass. Bold values represent marginal significance (p≤0.1) and * or ** indicate significance at p≤0.05 and p≤0.01 respectively. Microbial biomass carbon and nitrogen were measured on replicate samples using fumigation direct extraction.

		Microbial Biomass		
Effects	**DF**	**Carbon**	**Nitrogen**	**C:N**
Burn	1,3	3.36	1.29	1.39
Nitrogen	1,6	12.85*	9.03*	0.44
Date	3,9	12.99**	37.74**	42.36**
B*N	1,6	0.33	1.35	2.85
D*B	3,9	0.94	1.64	2.45
D*N	3,18	2.07	0.02	2.14
D*N*B	3,18	1.49	0.60	0.33

Table 3.3 Repeated measures mixed model ANOVA F-table for measurements phospholipid composition. Bold values represent marginal significance (p≤0.1) and * or ** indicate significance at p≤0.05 and p≤0.01 respectively. PLFAs were grouped into broad functional groups and were analyzed on a mole% (relative abundance basis i.e. μg lipid group·g^{-1} dry soil / total μg lipids·g^{-1} dry soil). "Actino" are actinomycetes and "AMF" are arbuscular mycorrhizal fungi.

Phospholipid Fatty Acid (mole%)								
Effects	**DF**	**Total**	**Common**	**Gram +**	**Gram -**	**Fungi**	**AMF**	**Actino**
Burn	1,3	NA	2.83	**1.87**	0.23	12.03*	0.49	0.37
Nitrogen	1,6	NA	0.36	54.41**	0.03	40.24**	26.01**	7.03*
Date	3,9	NA	23.06**	0.86	2.26	5.56*	5.64*	0.08
B*N	1,6	NA	0.40	0.24	0.00	0.16	1.98	**5.81**
D*B	3,9	NA	1.33	0.04	1.09	1.45	0.34	0.30
D*N	3,18	NA	0.31	3.21*	1.23	0.90	0.71	1.95
D*N*B	3,18	NA	**2.54**	2.17	1.32	1.95	0.89	0.18

Phospholipid Fatty Acid (mass)								
Effects	**DF**	**Total**	**Common**	**Gram +**	**Gram -**	**Fungi**	**AMF**	**Actino**
Burn	1,3	11.70*	4.47	**6.96**	1.69	11.39*	4.17	**8.46**
Nitrogen	1,6	24.05**	12.94*	7.73*	2.91	26.78**	24.63**	**3.8**
Date	3,9	1.18	18.94**	1.31	1.3	0.41	0.42	1.23
B*N	1,6	1.52	0.79	2.54	0.01	0.07	1.22	2.68
D*B	3,9	0.49	1.98	1.06	0.97	0.48	0.67	0.5
D*N	3,18	1.40	0.86	0.41	1.66	1.19	0.69	0.45
D*N*B	3,18	1.48	0.92	0.9	1.58	2.31	1.79	1.03

Table 3.4 Mixed model ANOVA F-table for average PLFA mass (μg lipid·g^{-1} dry soil) and average PLFA mole% (relative abundance, group mass / total mass), calculated from four summer samplings. Bold values represent marginal significance (p≤0.1) and * or ** indicate significance at p≤0.05 and p≤0.01 respectively from a two-way ANOVA. "Actino" are actinomycetes and "AMF" are arbuscular mycorrhizal fungi.

PLFA molar %							
Effects	**Total**	**Common**	**Gram +**	**Gram -**	**Fungi**	**AMF**	**Actino**
Burn	**N/A**	0.65	**3.84**	0.21	13.37**	0.90	1.75
Nitrogen	**N/A**	0.13	35.62**	0.03	38.82**	38.32**	12.15**
B x N	**N/A**	0.14	0.25	0.00	0.22	**3.28**	10.15**
PLFA mass							
Effects	**Total**	**Common**	**Gram +**	**Gram -**	**Fungi**	**AMF**	**Actino**
Burn	21.07**	5.97*	22.31**	1.85	30.74**	11.23**	26.40**
Nitrogen	39.87**	14.69**	22.66**	2.39	67.05**	64.51**	2.56
B x N	2.48	0.81	8.05**	0.01	0.14	**3.48**	**2.89**

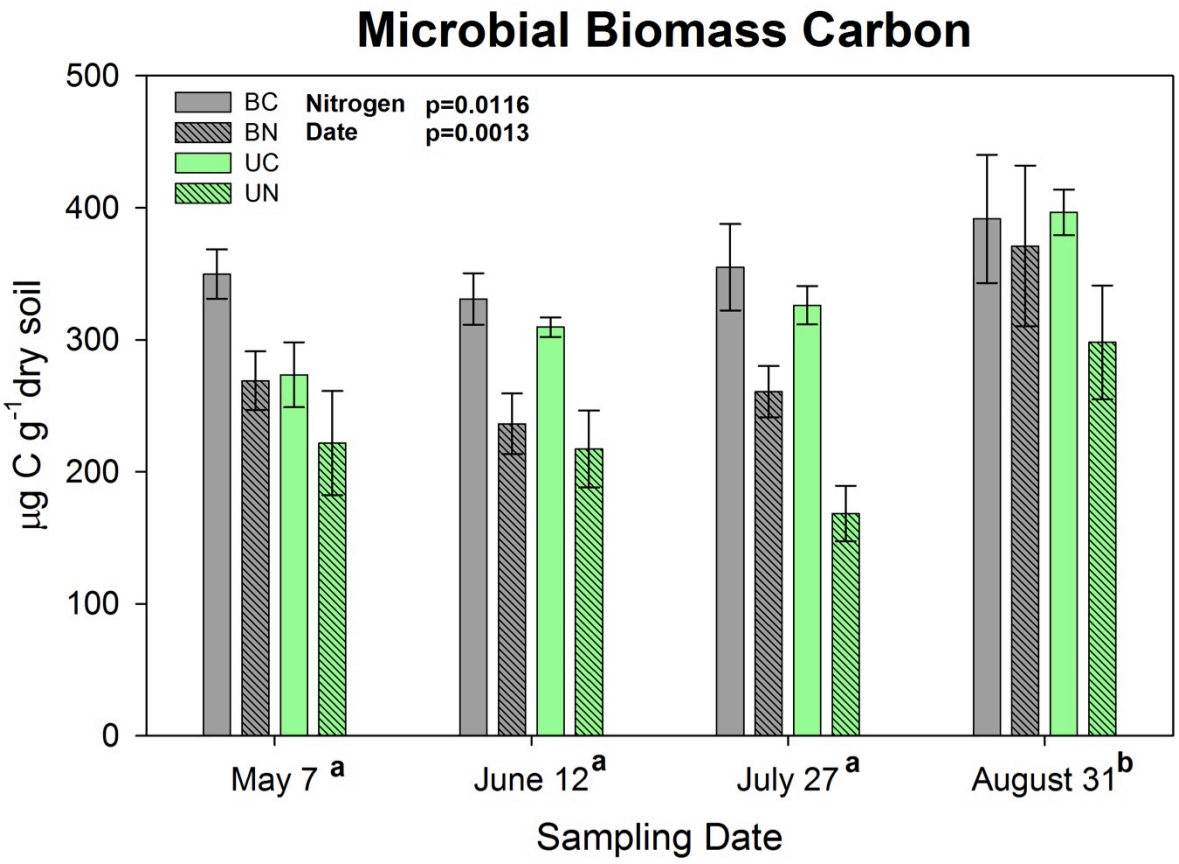

Figure 3.1 Microbial biomass carbon (mean ± 1 SE) measured on replicate samples by fumigation direct extraction. Main effects of nitrogen and date were significant at the p≤0.05 and ≤0.01 levels, respectively. Letters on sample dates indicate differences at the p≤0.05 level.

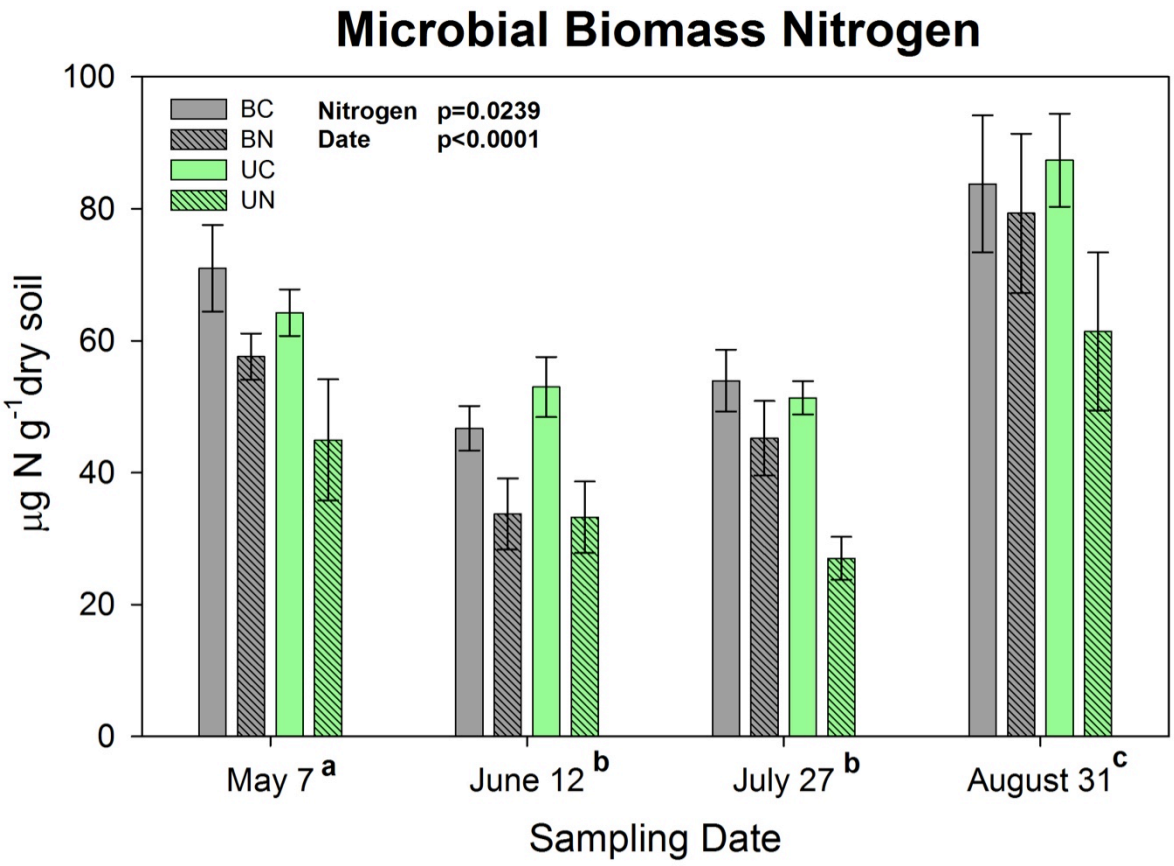

Figure 3.2 Microbial biomass nitrogen (mean ± 1 SE) measured on replicate samples by fumigation direct extraction. Main effects of nitrogen and date were significant at the $p \leq 0.05$ and ≤ 0.01 levels, respectively. Letters on sample dates indicate differences at the $p \leq 0.05$ level.

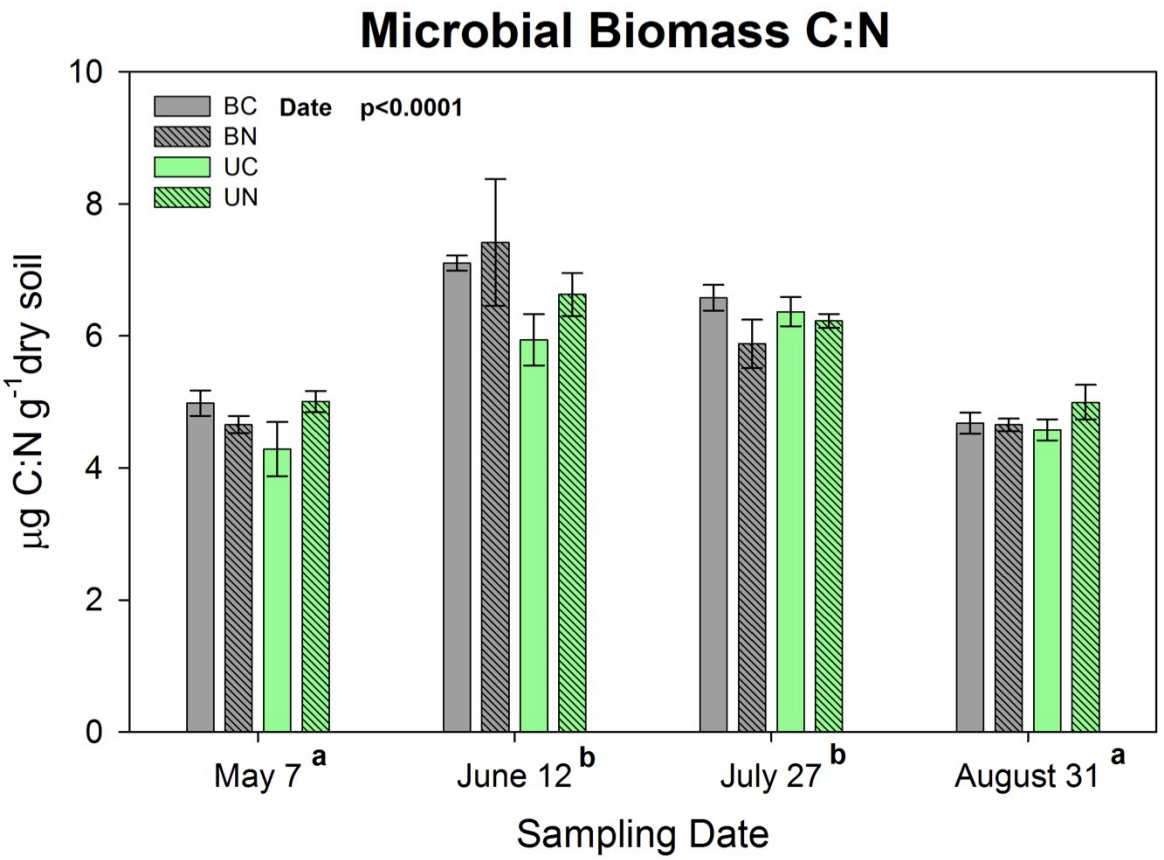

Figure 3.3 Microbial biomass carbon to nitrogen ratio (mean ± 1 SE) measured on replicate samples by fumigation direct extraction. A main effect of date was significant at the ≤0.01 level and differences between sample dates at the p≤0.05 level are indicated by letters.

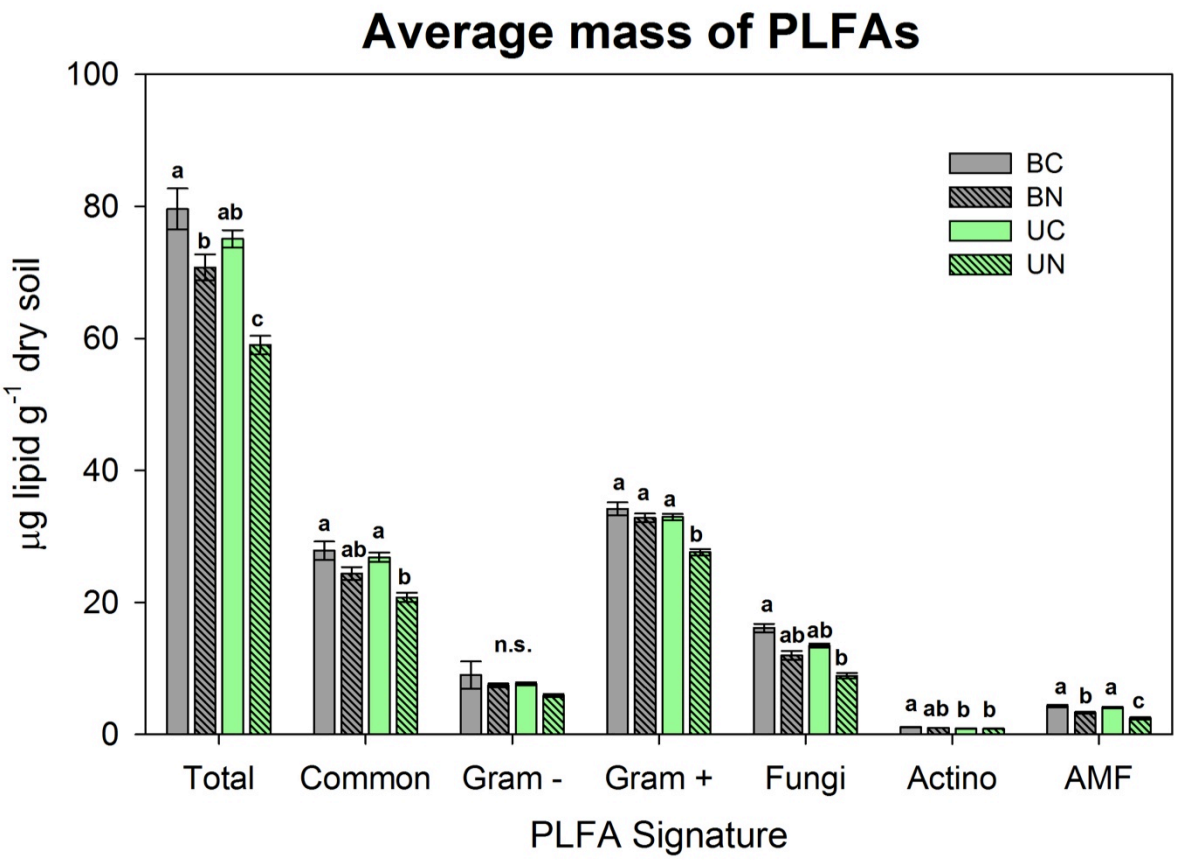

Figure 3.4 PLFA biomass (mean ±1 SE) from four sampling dates throughout the summer of 2012. Letters indicate significant ($p \leq 0.05$) differences from a two way ANOVA (Tukey's HSD).

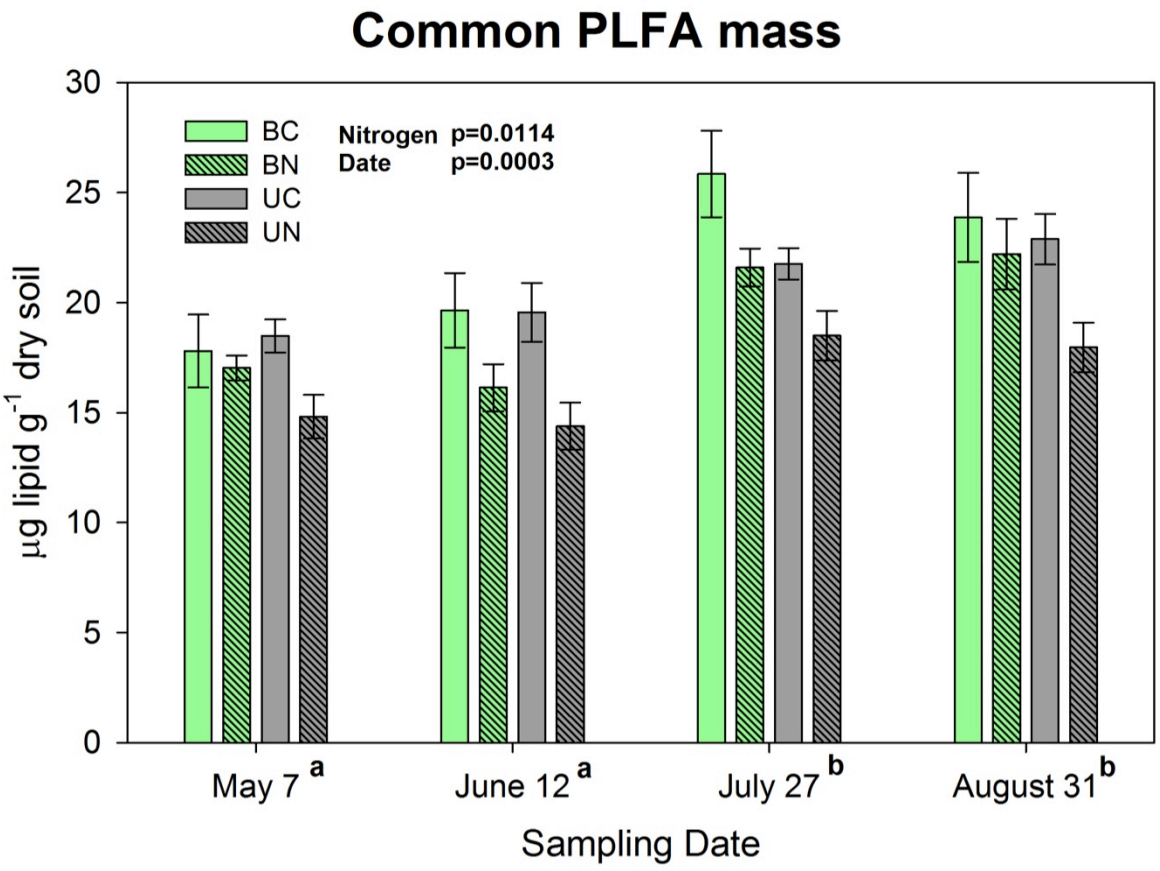

Figure 3.5 PLFA mass (mean ±1 SE) for common microbial lipids by sampling date. A repeated measures ANOVA indicated significant ($p \leq 0.05$) main effects of nitrogen and date. Letters on individual dates designate sampling dates with significantly ($p \leq 0.05$) different means.

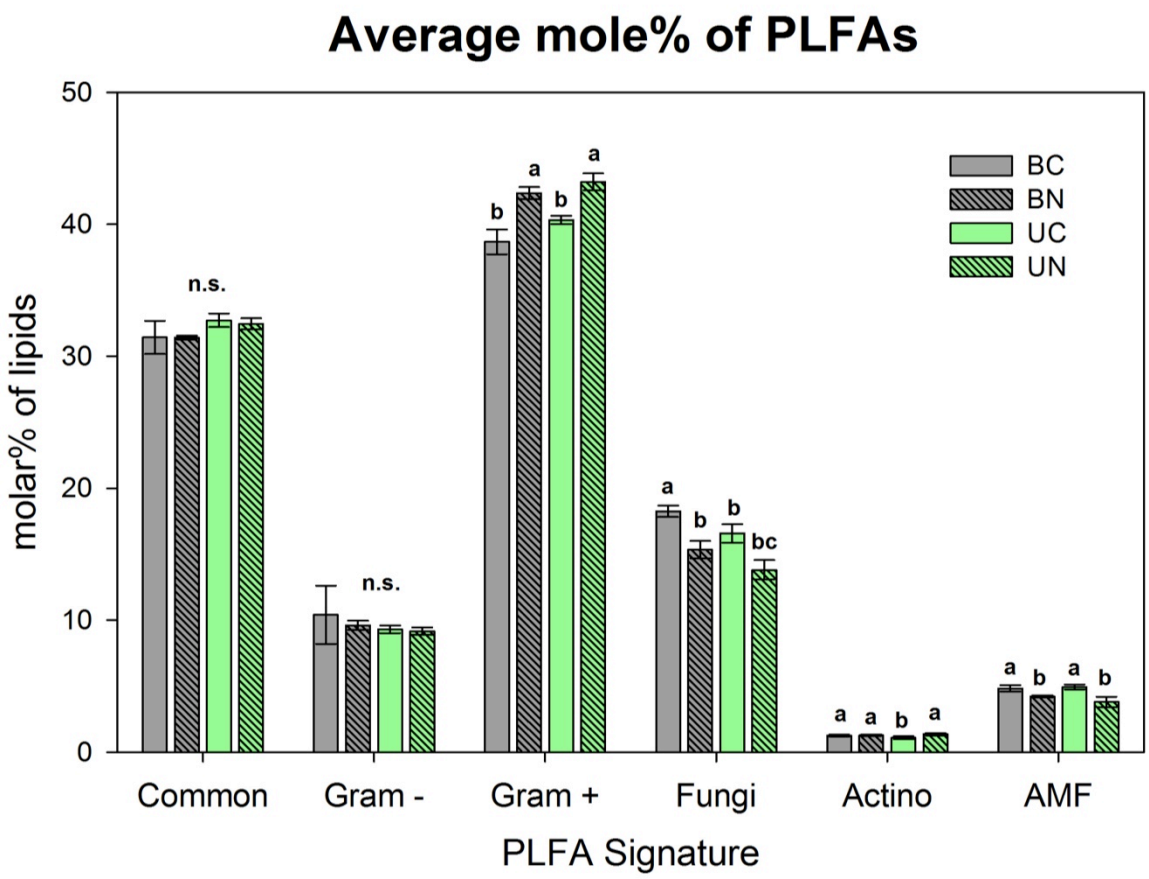

Figure 3.6 PLFA mole% (mean ±1 SE) calculated as μg lipid group g^{-1} dry soil / μg total lipids g^{-1} dry soil, from four sampling dates throughout the summer of 2012. Letters indicate significant ($p \leq 0.05$) differences from a two way ANOVA (Tukey's HSD).

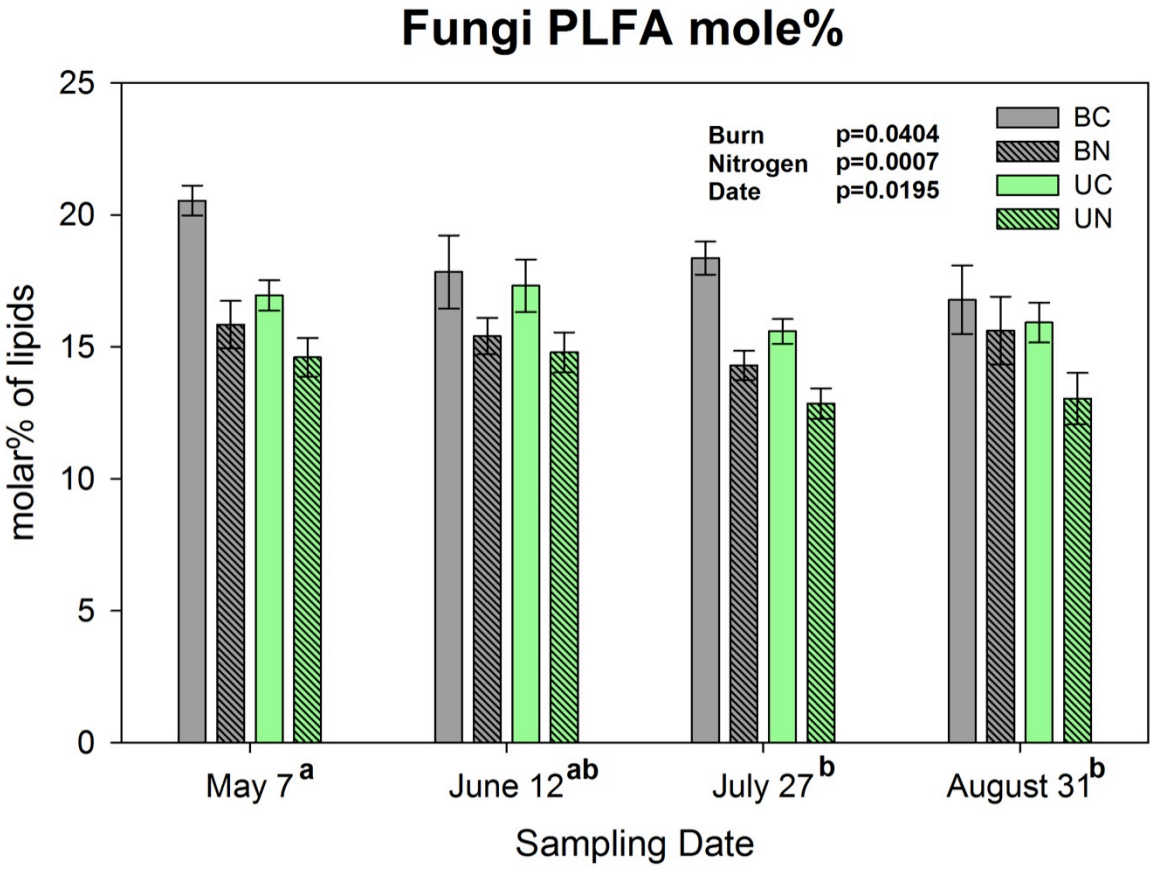

Figure 3.7 Fungal molar% (mean ±1 SE) for each sampling date, calculated as µg fungal lipids g^{-1} dry soil / µg total lipids g^{-1} dry soil. A repeated measures ANOVA indicated significant ($p \leq 0.05$) main effects of burn, nitrogen, and date. Letters on individual dates designate sampling dates with significantly ($p \leq 0.05$) different means.

Chapter 4 - Conclusions

Anthropogenic activities have transformed biogeochemical cycles on a global scale and soils are one of the largest active pools that are both impacted by and that mediate these transformations (Galloway et al. 2003). Establishing an understanding of how future environmental changes and management practices will alter cycling of nutrients as well as soil microbial communities is instrumental in accurately predicting and reacting to potential changes. Grasslands are one of the most broadly distributed and highly impacted ecosystems, making them ideal for studying the effects of anthropogenic activities on biogeochemical cycles and soil microbial communities. The intent of this thesis was to build upon past research conducted at Konza Prairie Biological Station and specifically to investigate the effects of chronic N fertilization and/or annual burning on soil C and N storage, potential and *in situ* C and N cycling, soil microbial biomass, and microbial community composition.

Historically fire was a common natural disturbance in mesic grasslands, including the tallgrass prairie of the Central Plains, but contemporary land-use practices often result in altered fire regimes in the region (Allen and Palmer, 2011). Fire is known to alter soil microclimate and current climatic models predict more variable precipitation patterns and increased temperatures throughout the Central Plains (Christensen et al. 2007). Because variations in broad- and fine-scale precipitation and temperature are known to regulate soil biogeochemical cycling (e.g. nitrification and denitrification) as well as microbial communities (Colman and Schimel 2013), I measured continuous soil moisture and temperature to a depth of 10 cm through the summer of 2012 in a subset of representative experimental plots. These results indicated a significant effect of burn treatment only, where burned plots had higher soil moisture and temperature throughout the summer. This was expected for soil temperature, but contradicted past results for soil moisture, which generally has been reported to be higher under the accumulation of surface litter and standing dead plant material that accrues in tallgrass prairie in the absence of fire (Gilliam et al. 1987). Differences between my results and other studies may be due to early season precipitation that likely counteracted the high solar radiation inputs often attributed to higher temperature and lower moisture in burned grasslands (Ojima 1994). Additionally, aboveground net primary productivity (ANPP) has been measured annually in these plots and results from 2012 are similar to long-term trends (Collins et al. 1998, Wilson et al. 2009), with burning

suppressing woody expansion and nitrogen increasing overall biomass, especially in burned plots. The relatively large increases in woody plant cover with long-term fire suppression, and associated increases in leaf area index (Briggs et al. 2005) may have contributed to increased transpirational losses of soil water, relative to annually burned grassland or unburned grassland with a major woody plant component. While soil temperature, moisture, and plant communities are not the sole drivers of chemical cycling and microbial communities, differences in these ecosystem characteristics likely contributed to the treatment-level differences seen in this experiment.

In Chapter 2, I investigated how chronic nitrogen fertilization and/or contrasting fire regimes (annual spring burning vs. long-term fire suppression) affected soil C and N pools and potential and *in situ* transformations of N and C in a tallgrass prairie. Burning caused significant changes in soil C:N ratios, *in situ* C mineralization, and potential nitrification and ammonification rates. These results highlight the potential for different prescribed fire regimes to alter soil N cycling and availability, and add to the body of literature on the effects of fire in mesic grasslands. These results indicate that frequent fires reduce potential soil N availability and increase N limitation; findings commonly reported by other studies in annually burned grasslands (e.g. Blair 1997, Fynn et al. 2003, Buis et al. 2009). The ability of tallgrass prairie to maintain relatively high productivity in spite of enhanced N limitation with annual burning may be due, in part, to mechanisms that help conserve N in tallgrass prairie, including translocation of nutrients by plants during senescence and tight cycling of soil N in microbial populations (Dell et al. 2005) as well as shifts in plant community composition towards species with higher nitrogen use efficiency (Baer and Blair 2008). Field-based measurements of soil N transformations in response to burning were highly variable, and likely the product of within and between plot heterogeneity that is common in soils. In contrast, field-based C mineralization (soil CO_2 efflux) was enhanced significantly by burning, likely due to a combination of factors including higher soil moisture and temperature, trends for higher microbial biomass (Ponder et al. 2009, Jangid et al. 2010), as well as the higher root biomass previously reported in response to the annually burned treatment (Kitchen et al. 2009, Wilson et al. 2009).

In addition to impacts of burning, anthropogenic changes in the N cycle have resulted in increasing N deposition (Sutton and Bleeker, 2013). While these grasslands are generally thought to be N-limited, increasing N inputs have uncertain consequences for soil communities and

processes. In this study, chronic N additions increased total N stored in the upper 10 cm of soil, and primarily affected potential mineralization rates with net nitrification, net ammonification, net N mineralization all enhanced with chronic N additions. These results are all generally consistent with other work on enhanced soil N availability in tallgrass prairie over long timeframes (Fornara and Tilman 2012). As with burning, field-based N mineralization rates were not significantly affected by fertilization. This was somewhat surprising, and is likely the result of highly variable field measurements of inorganic N, especially following fertilizer application. Field-based soil CO_2 efflux was not affected by N fertilization, while chronic N additions did significantly increase total soil N while reducing total soil C:N and potentially mineralizable soil C, as indexed by laboratory incubation assays. Reduced potential C mineralization with fertilization has indicated labile C loss in laboratory studies (Craine et al. 2007. Ramirez et al. 2012) which may affect C storage potential in our system, but confirmation of labile C loss in field studies is less common (Neff et al. 2002) and more research is needed to confirm this shift in C pools at KPBS.

Chapter 3 focused on microbial biomass measurements and microbial community compositional shifts in response to the same treatments as Chapter 2 (i.e. contrasting fire regimes and/or chronic N fertilization). Burning had a tendency to increase MBC and MBN although not significantly, while total PLFA mass was significantly increased by annual burning relative to long-term fire suppression, a response reported in other grasslands (Dooley and Treseder 2012) as well as at KPBS (Jangid et al. 2010). The increase in microbial biomass due to burning was accompanied by a shift in community composition, with fungi becoming more abundant. This is supported by Wilson et al.'s (2009) work on the same plots that found higher root colonization with annual burning. Fertilization with N was a much stronger driver, decreasing all microbial biomass measurements, and shifting the microbial community composition by reducing fungal abundance and increasing bacterial abundance. Previous measurements of microbial biomass C and N by Ajwa et al. (1999) showed only an interaction effect of burn x nitrogen addition for MBN eight years into the BGPE (1994), where burned unfertilized plots had significantly lower values that the other treatment combinations. While my study didn't find any interaction effects of the burning and fertilization treatments on microbial biomass, the significant effects of burning and fertilization that resulted after 27 years of continuous fire and fertilization treatments indicate the necessity of long-term studies to detect responses to chronic changes in key drivers

of ecological processes. Due to a lack of prior sampling for microbial community composition in the BGPE, I cannot say how long of a timeframe was required to detect the fungal/bacterial shifts I observed. However, other work in restoration chronosequences at KPBS has indicated that microbial PLFA compositional changes occur over decades (McKinley et al. 2005, Jangid et al. 2010).

Interest in soil ecology is at an all time high, and modern methods have provided tools that allow for previously unimagined capabilities, such as the rapid and repeatable analysis of soil microbial diversity (e.g. Fierer et al. 2011). The methods used here focused on functional changes in soil C and N pools and fluxes coupled with more coarse-scale measures of microbial community composition, and identified key changes in both nutrient turnover and microbial community changes. Future work in the BGPE should expand upon the PLFA work done in this experiment and use more fine-scale analytical methods, so that taxonomic differences in microbial communities (i.e. richness and diversity) may be determined. Additionally, there is a general need for more long-term soil ecological studies as our reliance and conclusions from chronosequences (e.g., space for time substitutions) can only lead us so far (Schmidt et al. 2011). Ideally these studies would incorporate frequent sampling regimes so that change of microbial communities, nutrient pools, and basic soil characteristics can be more confidently interpreted. Anthropogenic influences will likely increase in the coming decades and knowledge of how these impacts alter ecosystems on local, regional, and global scales over long timeframes will be critical for future predictions of ecosystem and climate change and well and conservation and remediation efforts.

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