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Effects of plant-soil interactions on grassland carbon dynamics in a changing world

by

Robert Kenneth Connell

B.S., University of Tennessee, Knoxville 2015

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Division of Biology
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2020

Abstract

Plants are a major conduit through which carbon moves between the atmosphere and the terrestrial biosphere. The organic inputs from plants provide energy to soil microbes which fuels microbial extracellular enzyme production. Soil microbial activity determines the proportion of plant organic inputs that remains stored in soil as organic matter or is mineralized and released back into the atmosphere as carbon dioxide. Plant-soil interactions are, therefore, a critical driver of terrestrial carbon cycling. We live in an era of human-driven change which affects every aspect of ecosystem functioning, so it is critical to understand how different global change factors modulate the plant-soil interactions that influence carbon cycling. In this dissertation I focus on the effects of four specific global change factors on plant-soil interactions in a tallgrass prairie ecosystem: (1) land-use change (i.e., fire suppression and bison removal), (2) woody encroachment, (3) plant invasion, and (4) nutrient enrichment. The overall conclusion from my dissertation research is that all four of these global change factors alter plant-soil interactions in ways that change the storage or turnover of soil carbon. First, long-term fire suppression and/or bison exclusion increases soil C content over time. This change in soil C content is associated with an increase in woody plants in the case of fire suppression or an increase in the dominance of warm-season grasses in the case of bison exclusion under a frequent fire regime. Second, potential C mineralization rates under clonal woody shrubs is higher when the microbial community is decomposing proportionally more shrub-derived organic matter, suggesting that the rate of soil C flux may be dependent on how long the soil has been occupied by woody species. Third, the invasive grass *Bromus inermis* induces legacy effects on soil microbial community composition and soil organic matter (SOM) decomposition rates. These legacy effects persist for at least six months post-invasive grass removal. Finally, phosphorus

fertilization stimulates the rate of SOM decomposition in soil undergoing woody encroachment, but nitrogen fertilization does not. Collectively, these results suggest that the effects of many global change factors on carbon cycling is dependent on spatiotemporal context and historical factors. Additionally, since each of the global change factors I studied affected carbon cycling independently, it will be important to study the combined effects of multiple global change factors acting simultaneously in order to better predict how carbon cycles through terrestrial ecosystems as the world continues to change.

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Approved by:

Major Professor
Dr. John Blair

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Chapter 1 - Introduction

Carbon is the sixth element on the periodic table, the energy currency for most biological processes, and the building block of all life. There is an estimated $40 * 10^{18}$ g of actively cycling carbon continuously being exchanged between the living and nonliving portions of the Earth (Schlesinger & Bernhardt, 2013). Because of its propensity to bond with atoms of hydrogen, oxygen, nitrogen, phosphorus, and other carbon atoms, carbon is the backbone of all organic material on the planet and is tightly associated with the transfer of energy through biological systems. Therefore, carbon is "embedded" in every element of human society (Malone et al., 2018), and understanding the carbon cycle is essential for humanity's food, energy, and climate security. On land, more carbon is stored in the soil than in the atmosphere or the living biomass combined. Due to the potential of soil as a carbon reservoir there has been substantial interest in increasing the amount of carbon stored in soil for climate change mitigation (Minasny et al., 2017). However, it is not the storage but the turnover of soil carbon that releases the nutrients bound to organic carbon that helps maintains plant productivity (Janzen, 2006). Therefore, factors that control both the flow of carbon *into* and the flow of carbon *out o*f soil deserve investigation.

Interactions between plants, microbes, and the soil environment drive soil carbon cycling. Plants can allocate a large proportion of their net primary productivity belowground, especially in grasslands (Blair et al., 2014). Through their growth, roots change the soil physical structure, increase mineral weathering, and alter the flow of water and nutrients (Cardon & Whitbeck, 2007). The area immediately surrounding plant roots, the rhizosphere, is different chemically and biologically from the bulk soil. The rhizosphere is considered a hotspot of biological activity (Kuzyakov & Blagodatskaya, 2015); it is estimated that 6 - 21% of soil carbon flux is controlled

by rhizosphere processes to a 1 m depth (Finzi et al., 2015). Plant species exude unique cocktails of chemical compounds through their roots, which select for unique microbial communities that have different nutrient cycling capabilities (Bais et al., 2006; Philippot et al., 2013). Since plant species differ in the quantity and quality of belowground inputs any factor that alters plant community composition can potentially feedback to affect soil carbon cycling.

Due to their diverse enzymatic and metabolic capabilities, soil microbes are the key players in elemental cycling. Microbes (bacteria, archaea and fungi) secrete a suite of hydrolytic and oxidative enzymes into the soil environment that liberate nutrients from soil organic matter (SOM) and hasten decomposition. As a result of this microbial activity some of this soil carbon is mineralized and released back to the atmosphere as CO_2. Microbial decomposition also releases plant-limiting nutrients that are bound within organic matter, making those nutrients available for plant uptake and increasing plant productivity. Since soil microbes are carbon limited (Soong et al., 2020), high-energy root exudates "prime" microbial activity and change the rate of SOM decomposition (Huo et al., 2017; Kuzyakov, 2002). However, whether priming results in faster or slower SOM decomposition is dependent on many other factors such as water and nutrient availability (Dijkstra et al., 2012, 2013).

The interactions between plants, soils, and microbes are susceptible to the multitude of changes that humans have made to the environment. We have altered the composition of atmospheric gases through the burning of fossil fuels (Schlesinger & Bernhardt, 2013), replaced native ecosystems with agricultural systems, interfered with natural disturbance regimes (R. C. Anderson, 2006), doubled the amount of biologically available nitrogen on the planet (Vitousek et al., 1997), accelerated the release of soluble phosphorous through mining (Smil, 2000), and facilitated the movement of species outside of their native range (Jeschke, 2014). Indeed, in

addition to studying plant-soil-microbe interactions in the context in which they evolved, it is increasingly necessary to understand how these interactions have been altered in the age of anthropogenic global change in order to fully understand contemporary carbon cycling.

The tallgrass prairie in the Central Great Plains of North America may be the most endangered ecosystem in the world with only a small fraction remaining (Samson & Knopf, 1994). Even protected areas of tallgrass prairie are subject to the influence of global change. In this dissertation, I investigated how plant-soil interactions influence soil carbon cycling in tallgrass prairie in response to four global change factors: land-use change, woody encroachment, plant invasion, and nutrient enrichment.

In Chapter 2, I addressed the long-term effects of land-use change on soil carbon and nitrogen concentration and isotopic composition. Tallgrass prairie was historically maintained by frequent disturbances including the effects of fires and large ungulate grazers, but humans have largely suppressed regular fires and extirpated native herbivore megafauna (*Bison bison*). In this chapter, I use archived soil samples and plant community data collected between 1982 and 2015 to demonstrate that soil carbon concentration increases over time in tallgrass prairie if fire is suppressed and/or bison are absent. The isotopic composition of the archived soils and plant community data indicated that increases in soil carbon content were associated with concomitant increases in woody plants or C_4 grasses, depending on the specific combinations of fire and grazing treatments.

In Chapter 3, I investigated the biogeochemical effects of woody encroachment at smaller spatial scales than normally measured. Due to fire suppression, clonal shrubs such as *Cornus drummondii* are increasing in density on the tallgrass prairie, and an individual shrub can occupy a large area on the landscape (greater than 800 m^2). In this chapter, I measured soil microbial

processes at multiple locations under individual shrub canopies of varying sizes to examine the spatial heterogeneity of elemental cycling as woody encroachment progresses. I demonstrate that soil microbial demand for carbon increases as shrubs grow larger in every location but the canopy's edge. Additionally, I found that the magnitude of the potential soil carbon mineralization rate was larger if soil microbes were breaking down proportionally more shrub-derived organic matter.

In Chapter 4, I investigated the effects of the invasive grass, *Bromus inermis*, on the rate of SOM decomposition and soil microbial community composition. *B. inermis* was planted in the tallgrass prairie as forage for cattle grazing but is currently threatening native plant diversity. In this chapter, I found that the magnitude of SOM decomposition was greater in soil conditioned by *B. inermis* than in soil conditioned by the native grass, *Pascopyrum smithii.* Interestingly, the increase in SOM decomposition persisted even in soil where *B. inermis* was removed six months prior. This indicates that the changes induced by invasive plants on the soil environment can persist long after their removal. I hypothesize that this change in carbon cycling is the result of the changes that *B. inermis* made to the soil microbial community, which persisted throughout the entire experiment.

In Chapter 5, I investigate the impact of nutrient enrichment on the rhizosphere priming effect in woody-encroached soil. The stoichiometry of available nitrogen and phosphorus is hypothesized to influence the magnitude of the rhizosphere priming effect (Dijkstra et al., 2013). Because of differences in the processes affecting plant available nitrogen and phosphorus, enhanced nitrogen limitation (i.e., phosphorus fertilization) should increase the magnitude of the priming effect while enhanced phosphorus limitation (i.e., nitrogen fertilization) should not increase it. By fertilizing mesocosms containing *C. drummondii* or *Rhus glabra* with nitrogen or

phosphorous, I tested the aforementioned hypothesis. I did not find evidence for a rhizosphere priming effect of any magnitude in any of the fertilization treatments. However, while there was no significant plant effect, phosphorus fertilization increased the overall magnitude of SOM decomposition indicating that microbial nitrogen limitation may stimulate soil carbon flux.

Chapter 2 - Three decades of divergent land use and plant community change alter soil C and N content in tallgrass prairie

Abstract

Frequent fire and grazing by megafauna are important determinants of tallgrass prairie plant community structure. However, fire suppression and removal of native grazers have altered these natural disturbance regimes and changed grassland plant communities with potential long-term consequences for soil carbon (C) and nitrogen (N) storage. I investigated multi-decade changes in soil C and N pools in response to contrasting long-term burning and grazing treatments. Fire suppression with or without grazers, and exclusion of grazers in annually-burned prairie increased soil C concentration and shifted the $\delta^{13}C$ signature of soil C over time, concomitant with changes in community composition. Cumulatively, mean soil C content was highest in grasslands that were not burned or grazed, and $\delta^{13}C$ indicated that increased soil C content was associated with an increased contribution from plants using a C_3 photosynthetic pathway (i.e., woody shrubs). Soil N content also increased when fire was suppressed, relative to frequently burned grassland, but the rate of increase was slower when grazers were present. Additionally, changes in $\delta^{15}N$ suggested that grazing increased the openness of the N cycle, presumably due to greater N losses. By coupling long-term fire and grazing treatments with plant community data and soil samples archived over three decades, I demonstrate that human-caused changes to natural disturbance regimes in a tallgrass prairie significantly alters soil C and N cycles through belowground changes associated with shifts in the plant community. Since natural disturbance regimes have been altered in grasslands across the world, these results are relevant for understanding the long-term biogeochemical consequences of these ongoing land-use changes.

Introduction

A major goal of ecosystem ecology is to identify the mechanisms regulating pools and transformations of organic matter in order to predict how global changes alter carbon (C) storage over time. Processes that influence the movement and storage of soil organic matter (SOM) are especially important because SOM is the major reservoir for C in most terrestrial ecosystems, and changes in SOM can impact the ecosystem services on which humanity relies: plant productivity, air quality, climate moderation, and water quality (Lal, 2004). Approximately 30% of the North American land surface is covered by grassland ecosystems (Pendall et al., 2018). Because grasses allocate 40 – 80% of their net primary productivity (NPP) belowground [tallgrass prairie, in particular, allocates 75% (Hui & Jackson, 2006)], grasslands have great potential to sequester large quantities of C in the SOM pool (Derner & Schuman, 2007).

Land management decisions influence many of the factors that control the inputs and processing of SOM, especially in grasslands (Conant et al., 2017; Pendall et al., 2018). For example, in mesic grasslands like tallgrass prairie, timing and frequency of prescribed fires can alter the productivity and relative dominance of perennial C_4 grasses and shift the balance of herbaceous and woody plant cover (Collins, Knapp, Briggs, Blair, & Steinauer, 1998; Collins & Smith, 2006; Heisler, Briggs, & Knapp, 2003; Spasojevic et al., 2010). The natural fire and grazing disturbance regimes that created and maintain North American tallgrass prairie (R. C. Anderson, 2006) have been altered through human activity, notably through fire suppression and replacement of native ungulate grazers with managed domestic grazers (R. C. Anderson, 2006; Collins, 1990). In addition, fire and grazing disturbance regimes that maintained grass dominance in these ecosystems in the past may not be sufficient to maintain this structure under

current conditions (Bond & Midgley, 2000; Briggs et al., 2002; Suding et al., 2004). Human alteration of these natural disturbance regimes have important implications for both plant and soil processes in grasslands (Allred et al., 2012; Fynn et al., 2003), though the net effects on SOM accumulation are not well documented.

The frequency of fires in tallgrass prairie affects pools and fluxes of both C and nitrogen (N). If tallgrass prairie remains unburned over multiple growing seasons, accumulated plant litter increases moisture and available N, but decreased light availability results in lower aboveground NPP (Knapp & Seastedt, 1986) and belowground plant biomass (Kitchen et al., 2009). Fire oxidizes aboveground biomass and accumulated detritus, which increases light availability and creates an environment favorable for new shoot growth (Knapp & Seastedt, 1986). Fire also volatilizes N in plant biomass and litter, so that combustion losses of N in frequently burned prairie, rather than denitrification or leaching, is the primary pathway of N loss in ungrazed tallgrass prairie (Blair et al., 1998). Therefore, frequent fires induce greater N limitation on plant productivity (Blair, 1997). Belowground, frequent fires stimulate root biomass production (fine and total) but decrease root N concentrations (Johnson & Matchett, 2001; Kitchen et al., 2009). Because frequent fire widens the C:N ratio of aboveground and belowground plant material, this can have cascading effects on the decomposability of roots and subsequent transfer to the SOM pool.

Before European colonization of the U.S. central Great Plains, the dominant grazers of tallgrass prairie were American bison (*Bison bison*), but they were nearly extirpated due to overhunting in the late 19th century (Flores, 2016). Although their numbers have rebounded, bison do not serve the ecological role in modern grasslands that they once did (Freese et al., 2007) and in many grasslands bison have been replaced by cattle as the dominant ungulate

herbivore. Ungulate grazing alters plant community composition, above- and below-ground plant productivity, and N cycling processes (Johnson & Matchett, 2001; Pineiro et al., 2010; Winter et al., 2015). Through urine and feces deposition, grazers can increase rates of N mineralization and nitrification (Frank & Groffman, 1998; Hobbs, 1996), and potential N losses through leaching, denitrification, and ammonia volatilization which can in turn increase the $\delta^{15}N$ signature of the N that is retained in the soil.

Prescribed fire and grazing regimes have important effects on plant communities that can feedback to alter soil processes. Fire suppression has been implicated in the recent expansion of woody vegetation into tallgrass prairie (Briggs et al., 2005; Fuhlendorf et al., 2008; Taylor et al., 2012). Increased cover of clonal C_3 woody shrubs such as *Cornus drummondii* and *Rhus glabra* is a common response to reduced fire frequency in tallgrass prairie (Ratajczak, Nippert, & Ocheltree, 2014). Fire suppression allows for woody shrub recruitment and as woody plants become established, they become more tolerant of fire disturbance and can reduce fine fuels and the intensity of fires when they do occur. Once critical thresholds of woody encroachment have been passed, returning to a frequent fire interval may not be sufficient to reverse the transition from grassland to shrubland (Ratajczak, Nippert, & Ocheltree, 2014). Due to the differences in their physiology, resource allocation, and litter quality, a shift from a graminoid to a shrub dominated plant community has important implications for belowground C and N cycling. Grazers also alter plant community composition (Hickman et al., 2004). In tallgrass prairie, plant diversity increases in response to grazing (Collins & Calabrese, 2012). In the absence of grazers, tallgrass prairie plant communities are typically dominated by a few species of C_4 grasses (e.g., *Andropogon gerardii*), particularly when frequently burned. Bison preferentially feed on the dominant grasses which changes the competitive relationships between C_4 grasses and C_3 forbs

on the landscape, decreasing the dominance and cover of C_4 grasses and increasing the abundance of C_3 forbs (Knapp et al., 1999).

The ecosystem-level impacts of woody plant encroachment is still an open question, and there have been mixed results from previous studies regarding how the increased prevalence of woody species in grasslands affects carbon storage belowground (Barger et al., 2011; Eldridge et al., 2011a; Lett et al., 2004). There are concerns that woody encroachment into grasslands will lead to overall losses in stored C belowground, particularly in mesic grasslands (Jackson et al., 2002a); however, it is difficult to assess the overall influence of woody encroachment since woody plant occupancy does not occur uniformly across the landscape, and it can have opposing impacts on different C pools. Previous approaches have used changes in aboveground NPP (Knapp et al., 2008), changes in soil C content associated with soil depth (Smith & Johnson, 2003), and satellite data (Asner et al., 2003) to assess the changes in C storage with woody plant encroachment over time. Key questions regarding ecosystem effects of different types of land management include the magnitude of those effects and the timeframe over which those effects develop. In this study, I analyzed selected properties of soil samples taken across a period of three decades from the same locations within watersheds under contrasting fire and grazing regimes to infer the impacts of fire and grazing on soil C and N dynamics. To our knowledge, this is the first study to harness multi-decadal samples and associated data to document changes in soil C and N content associated with altered fire and grazing regimes and associated plant community changes, including woody encroachment.

In addition to assessing changes in total soil C and N content over time, I also examined changes in soil C and N isotopic signatures. Stable isotopes are an excellent tool for assessing the differential impacts of land management on soil C and N dynamics because C isotopes can

reveal differences in the contributions of plant functional groups that use different photosynthetic pathways to the soil C pool as a result of community shifts, such as a shift in relative abundance of C_4 grasses and C_3 forbs or a C_4 grass to C_3 woody plant transition. Similarly, changes in soil N isotopes can be used to infer changes in N cycling processes under contrasting fire and grazing regimes, though identifying the specific processes driving altered ^{15}N isotopic signatures is difficult. By tracking changes in both total C and N content and changes in the C and N isotopic signatures of soil over several decades, this study aimed to infer the impacts of multiple drivers on soil C and N content in the tallgrass prairie.

The overarching goal of this research was to assess the impacts of divergent disturbance regimes and associated changes in plant community composition on belowground C and N dynamics in a tallgrass prairie ecosystem. I tested three hypotheses. 1) Soil C and N concentrations will change over time as a function of divergent fire and grazing treatments and associated shifts in plant community composition. Specifically, I predicted that surface soil C and N concentrations would increase with fire suppression, but the magnitude of this effect would vary depending on presence or absence of grazers. 2) Because both fire suppression and the addition of native grazers shift plant community composition from C_4 grass dominance to greater abundance of C_3 plants (i.e., forbs and woody species), I hypothesized that soil $\delta^{13}C$ would reflect changes in sources of organic inputs and would differentially correlate with soil C content under different treatments. I predicted a positive relationship between soil $\delta^{13}C$ and soil C content in frequently burned, ungrazed prairie where C_4 grasses contribute proportionally more C below ground and a negative relationship in unburned and/or grazed prairie if C_3 plants contribute proportionally more C belowground. 3) Lastly, I hypothesized that changes in total soil N would generally track changes in total C, but divergent long-term grazing and burning

regimes would alter N cycling processes and the openness of the N cycle, which would be reflected by changes in the soil $\delta^{15}N$ signature. A greater $\delta^{15}N$ value for soil N would indicate a more open N cycle with more N being lost from the system, potentially via increased denitrification, leaching, or ammonia volatilization in the presence of grazers.

Methods

Study site

To assess changes in soil C and N pools and isotopic signatures and relate these to changes in plant communities under contrasting fire and grazing regimes, I analyzed archived soil samples and used plant composition data collected as part of the Long-Term Ecological Research (LTER) program at the Konza Prairie Biological Station (KPBS). Konza Prairie is located in the Flint Hills ecoregion of northeast Kansas, USA. The 34.87-km^2 KPBS site consists primarily of unplowed tallgrass prairie on a heterogeneous landscape consisting of rolling hills that divide the area into a series of small watersheds or catchments that include distinct uplands, lowlands, and slopes. The KPBS site includes watershed-level manipulations of prescribed spring burning and grazing by native ungulates (*Bison bison*) that were established in phases between 1972 and 1992. For this study, I used data from four experimental watersheds (001D, 020B, N01B, and N20B) ranging in size from 24 to 122 ha, each with comparable management histories and initial conditions, but with subsequent divergent fire and grazing treatments imposed over time. In this paper, I refer to the treatments as follows: UG1 = ungrazed, annually burned; UG20 = ungrazed, burned every 20 years; G1 = grazed, annually burned; G20 = grazed, burned every 20 years. All prescribed burns were performed in the spring. The full history of the burning and grazing regimes for each watershed, including any unplanned wildfires, is provided in Table 2.1.

Archived soil collection

Archived soil samples were available for selected dates spanning more than three decades. All soil samples were collected along four permanent sampling transects established within the lowland region of each watershed. Each transect was 50 m long, and the four transects were dispersed to maximize coverage of the watershed area and increase spatial independence. For this study, I considered each transect an independent replicate and our analyses focused on exploring temporal dynamics of change in soil C and N pools in watersheds under divergent fire and grazing regimes and linking these changes to observed plant responses. However, because fire and grazing treatments were not replicated independently across multiple watersheds, I advise caution in extrapolating these results more broadly. Soils in the lowland regions of these watersheds are part of the Tully series, which are non-rocky, silty clay loams that are deeper than other soils at KPBS (Collins & Calabrese, 2012; Ransom et al., 1998) which is why I focused solely on lowlands for this study.

Soils were sampled in 1982, 1987, 2002, 2010, and 2015 using the same methods over time. Four 2-cm diameter soil cores were taken to a depth of 25 cm along each transect and composited into a single sample. Each sample soil was passed through a 4 mm sieve to remove large rocks and roots, and the sieved soil was hand-picked to remove small rocks, large root fragments, and any coarse organic debris. Samples were dried to constant weight at 60 °C and finely ground with a ball grinder before being archived and stored at Kansas State University (Manhattan, KS, USA). Bulk density was not measured when the samples were collected, so I present C and N results as concentrations throughout the manuscript. Samples collected during the 1990s were not archived, so I was unable to include them in this study.

Plant community data

Plant community composition was assessed annually in permanent plots located along 50-m transects adjacent to the same transects from which the soil cores were collected (Hartnett et al., 2020). Five permanent circular plant sampling plots of 10-m^2 each were evenly spaced along each transect. The identity and percent foliar cover of all plants within each plot was recorded annually in both spring and autumn. Briefly, cover of each species was visually estimated using modified Daubenmire cover class categories and the maximum spring or fall value for that species was used to create annual cover values for each species in each plot. Further details on the methods used to generate plant species cover values are provided in Collins & Calabrese (2012). For this study, Daubenmire cover class category was converted to percent cover by using the midpoint in each cover class. Plant species were assigned, grouped, and summed by photosynthetic pathway (C_3 or C_4) for each transect. To calculate proportional cover of C_3 or C_4 plants in each transect, each sum was divided by the grand total of plant cover in each transect. Plant species were also grouped by growth form [grass (C_3 and C_4), forb, woody, sedge, non-grass monocot, or other] to calculate the proportional cover of each growth form in each transect.

Isotope and nutrient analysis

In the fall of 2016, a subsample of each archived soil sample was analyzed for $\delta^{13}C$, $\delta^{15}N$, and % C and %N using a ThermoFinnigan Delta Plus Mass Spectrometer at the Stable Isotope Mass Spectrometry Lab at Kansas State University.

Data analysis

Linear mixed models were used explore directional changes over time in soil and plant response variables and to investigate correlations between the selected soil variables (e.g., % soil C and $\delta^{13}C$) using samples from all available dates (1982-2015). Comparisons of slopes were done by

calculating the 95% confidence intervals (CI) for slope estimates. If the CI did not overlap, slopes were not considered statistically different. To determine the effects of the divergent burning and grazing regimes, linear mixed models were used to analyze the independent and interactive effects of burning and grazing on % soil C, % soil N, $\delta^{13}C$, and $\delta^{15}N$. Only data from 2002, 2010, and 2015 were used for these analyses since the combined burning and grazing treatments were not fully established until 1992 (Table 2.1). Tukey's HSD was used to compare least square means. For all analyses, transect was treated as a random effect and temporal autocorrelation for repeated measures of the transects was accounted for. All models were tested to ensure that they did not violate model assumptions of normality and homoscedasticity. For all analyses of $\delta^{15}N$, this required dropping one outlier data point (UG20: Year = 2002, $\delta^{15}N$ =13.57). All analyses were conducted in R (R Core Team, 2019) with the packages *nlme*, *lsmeans*, and *multcomp* (Hothorn et al., 2008; Lenth, 2016; Pinheiro et al., 2019). All figures were created using the packages *tidyverse* (Wickham, 2017) and *cowplot* (Wilke, 2019).

Results

Trajectories of change in soil carbon

Percent soil C increased over time under every treatment except the annually burned, grazed treatment (G1; Figure 2.1). Although the 95% confidence intervals of the estimated slopes slightly overlapped, of the treatments that experienced an increase in soil C concentration, the increase was slowest in the annually burned, ungrazed treatment (UG1) at 0.008% yr^{-1} and most rapid in the infrequently burned, ungrazed treatment (UG20) at 0.027% yr^{-1} suggesting an effect of fire frequency on rates of soil C accrual (Table 2.2). The $\delta^{13}C$ value of soil C also changed over time in all treatments (Figure 2.2), though the direction and trajectory varied. In the

infrequently burned treatments, soil $\delta^{13}C$ values decreased over time regardless of grazing treatment (UG20: 0.075‰ yr^{-1}; G20: 0.046‰ yr^{-1}). In contrast, soil $\delta^{13}C$ values increased in the annually burned, ungrazed treatment (UG1) at 0.019‰ yr^{-1}. In the annually burned, grazed treatment (G1), soil $\delta^{13}C$ values also increased initially until 2002, and then decreased between 2002 and 2015.

Trajectories of change in soil nitrogen

Soil N concentration increased over time in the infrequently burned treatments regardless of presence or absence of grazers (Figure 2.3). However, the rate of N accumulation was significantly more rapid, by a factor of three, in the infrequently burned treatment without grazers (UG20: 0.003% yr^{-1}) compared to the grazed treatment (G20: 0.00094% yr^{-1}) (Table 2.2). Soil $\delta^{15}N$ values changed nonlinearly with time in the treatments G1, G20, and UG20 (Figure 2.4). In both of the grazed treatments, $\delta^{15}N$ values decreased until 2002, ten years after the bison introduction, and then increased between 2002 and 2015. Soil $\delta^{15}N$ values ultimately decreased over time in the unburned, ungrazed treatment (UG20).

Cumulative effects of burning and grazing on soil C and N

Mean soil C and N concentrations averaged over the latter part of the temporal sequence (2002-2015) were impacted by the divergent burning and grazing regimes. On average, soil C content was 10% higher in the infrequently burned treatments vs. annually-burned and 10% lower in grazed vs. ungrazed treatments (Figure 2.5). Mean soil $\delta^{13}C$ values also diverged in response to burning, grazing, and their interaction (Figure 2.5). Soil $\delta^{13}C$ was highest (-14.1‰) in the annually burned, ungrazed treatment (UG1) and lowest (-16.5‰) in the infrequently burned, ungrazed treatment (UG20). Soil N content and $\delta^{15}N$ values were also impacted by burn regime (Figure 2.5). On average, annually burned treatments had 10% lower soil N content and soil $\delta^{15}N$

values that were 24.3% higher than infrequently burned treatments. Grazing decreased average soil C/N ratios by 10.4% (from 12.7 to 11.5) but only in the annually burned treatment, which also had the lowest C/N ratio (Figure 2.5).

Relationship between soil isotopes and nutrient concentrations

Soil $\delta^{13}C$ values and soil C content were correlated across soil samples from all dates in treatments UG1, UG20, and G20, but the nature of the relationship varied with treatment (Figure 2.6). In the annually burned, ungrazed treatment (UG1), this relationship was positive (0.37% C $‰^{-1}$). However, in the infrequently burned treatments, the relationship was negative (G20: -0.29% C $‰^{-1}$; UG20: -0.33% C $‰^{-1}$). Soil N content was negatively correlated with $\delta^{15}N$ values across all treatments (Figure 2.7). The slope of the negative relationship between soil N content and $\delta^{15}N$ values was steepest in the infrequently burned, ungrazed treatment (UG20; -0.029% N $‰^{-1}$) and shallowest in the annually burned, grazed treatment (G1; -0.014% N $‰^{-1}$).

Plant community trajectories

The proportional cover of C_3 plants relative to the cover of C_4 plants increased over time in all treatment combinations except the annually burned, ungrazed treatment (UG1; Figure 2.8), where the cover of C_3 plants declined over time. The change of proportional cover of C_3 plants was most rapid under low fire frequency and the absence of grazers (UG20; 0.018yr^{-1}) and most gradual in the annually burned, ungrazed treatment (UG1; -0.0051 yr^{-1}). The rate of plant community change was significantly slower in the annually burned, ungrazed treatment compared to the other three (Table 2.2). Under an annual fire regime, C_4 grasses remained dominant when grazers were absent, but C_4 grass dominance decreased and relative cover of C_3 forbs increased when grazers were present. In infrequently burned treatments, C_4 grasses became less dominant as woody plants became more abundant (Figure 2.9).

Discussion

Hypothesis 1: Soil C and N concentrations will change over time as a function of divergent fire and grazing treatments and associated shifts in plant community composition

Soil C content increased in three out of the four fire and grazing treatment combinations over the 33-year period encompassed by our study (Figure 2.1), and the mean C content of soils was affected by manipulations of both burning and grazing. By the later portion of the study (2002-2015), mean soil C content was lowest in soils under disturbance regimes that included frequent burning or grazing (Figure 2.5). Additionally, the increase in soil C content over time was greatest in treatments in which fire was suppressed and grazers were absent (Figure 2.1). In the absence of major disturbances, it appears that the C content of tallgrass prairie soils can increase over time, which may contribute to additional C sequestration (Barger et al., 2011) although in the case of greatly reduced fire frequency, the increase in soil C may come at the expense of loss of tallgrass prairie vegetation to encroachment by woody species. For example, in another study in this region, an increase in soil organic C content in the absence of prescribed burning was associated with encroachment by the woody species, *Juniperus virginiana* (McKinley & Blair, 2008). Such replacement of tallgrass prairie vegetation by woody species is associated with both ecological (e.g., loss of native biodiversity) and economic (e.g., loss of grazable rangelands) impacts that offset the potential benefits of increased soil C storage with reduced fire frequency.

Contrasting fire treatments had a larger effect on N dynamics than did presence or absence of grazers (Figure 2.5). Cumulatively, mean soil N content was higher in the infrequently burned treatments (Figure 2.5), reflecting the gradual increase in soil N

accumulation over time in the absence of frequent fires (Figure 2.3). Burning increases N limitation in mesic grasslands through combustion and volatilization of N in aboveground litter (Blair, 1997; Fynn et al., 2003), widening of C/N ratios in organic inputs, and an increase in the N immobilization potential of soils (Dell et al., 2005). These results are consistent with frequent fire as a mechanism for maintaining chronic N limitation in grasslands. Grazers did not significantly influence total soil N content in this study. Ungulate grazers amplify the magnitude and spatial heterogeneity of N mineralization and nitrification rates (Frank & Groffman, 1998; Johnson & Matchett, 2001) through the deposition of labile N in urine and feces (Hobbs, 1996). Additionally, through the consumption of aboveground biomass and deposition of more labile forms of N, ungulate grazers often decrease the C/N ratio of plant tissue which could subsequently increase the quality of SOM. Grazing did decrease the C/N ratio of SOM in our study but only when there was annual burning, which resulted in the widest C/N ratio I observed (Figure 2.5). Based on deposition of urea in ungulate urine and enhanced rates of N transformation, one might predict that grazed grasslands could be more prone to losses via ammonia volatilization, leaching, and denitrification pathways compared to ungrazed grasslands (Frank & Evans, 1997). However, these results indicate no change in total soil N content with grazing in the annually burned treatment, and an increase in N content regardless of grazers in the infrequently burned treatments (Figure 2.3). In the grazed and burned treatment, any enhanced losses of N due to microbial transformation may have been offset by the reduced volatilization of N during fire (i.e., due to reduced aboveground biomass and litter in the presence of grazers). In the grazed and infrequently burned watershed, N increased over time, but the rate of increase was lower than in the absence of grazers (Figure 2.3), potentially reflecting greater N losses due to microbial activity in the presence of grazers when fire is

infrequent (see Hypothesis 3). Since bison at KPBS have the ability to freely move between watersheds of different burn regimes, the effect of grazers on N loss might have been weaker in the infrequently burned treatment than the annually burned treatment because bison preferentially forage in areas that have been recently burned (Raynor et al., 2017). In total, these results suggest that the effect of grazers on soil N is dependent on fire regime.

Hypothesis 2: Soil $\delta^{13}C$ values reflect changes in sources of organic inputs and will differentially correlate with soil C content under different treatments.

The overall contribution of C_3 plants to soil C increased over time in response to fire suppression and to grazing following the reintroduction of bison in the annually burned treatment, as indicated by the decreasing $\delta^{13}C$ value of the soil (Figure 2.2). Interestingly, the contribution of C_3 plants decreased and the contribution of C_4 plants to soil C increased in the annually burned treatment with no grazers present (UG1; Figure 2.2). These results reflected plant community composition changes under divergent fire and grazing treatments. In all treatments except for UG1, the proportion of C_3 plants increased over time (Figure 2.8). In the rarely burned treatments, the increase in C_3 plant cover and associated change in the $\delta^{13}C$ signature of the soil were driven, in large part, by encroachment of woody plants in the absence of regular fire disturbance (Figure 2.9). Additionally, preferential grazing of C_4 grasses allows C_3 grasses and forbs to increase in abundance, even with frequent burning, offsetting the effects of annually burning alone (Collins et al., 1998), as observed in the annually burned and grazed treatment in this study.

There are several potential reasons why the $\delta^{13}C$ signature of the soil decreased so quickly in the soils of the infrequently burned treatments. Assuming that aboveground plant cover is a reasonable proxy for proportional plant inputs to the soil, there were increases in

relative contributions from C_3 plants over time. According to the preferential substrate utilization hypothesis, soil microbes prefer recent plant inputs, rather than older C contained within SOM, as a C source (Blagodatskaya et al., 2011). If microbial products derived from the most recent plant inputs are the major precursors to stabilized SOM (Cotrufo et al., 2013), it follows that the fraction of C_3–derived inputs to SOM pools will increase over time as the plant community shifts to greater cover of C_3 forbs and woody plants, and the $\delta^{13}C$ signature of the soil C will decrease. Since grasses maintained dominance in the annually burned, ungrazed treatment, an increase in C_4 grass cover would also explain why the $\delta^{13}C$ increased in those soils over the same time periods. Alternatively, since this site was grazed by cattle for an extended period of time prior to implementation of burning and grazing treatments, this trend could reflect the turnover of C_3-derived organic matter that accumulated prior to the start of this study. In the annually burned and grazed treatment, soil $\delta^{13}C$ values also initially increased between 1982 – 2002 and then decreased after 2002 in response to the competitive release that grazers provide to C_3 forbs, reflecting the occurrence of bison on the landscape starting in 1992 (Figure 2.9). The lag between bison introduction and shifts in soil $\delta^{13}C$ signatures likely reflects the time it takes for plant communities to change following the addition of a new driver and the time required for those changes to subsequently impact soil C.

In the infrequently burned treatments, soil C content was higher when the stable isotope signature indicated a higher proportional contribution of C_3 plants (Figure 2.6). However, in the annually burned and ungrazed treatment, the opposite pattern was observed. This would indicate that soil C content is higher when land management selects for a particular type of plant growth (i.e., C_3 woody shrubs vs. C_4 grasses) that contributes proportionally more C belowground. The one exception to this was in the annually burned, grazed treatment (G1) in which the plant

community became increasingly more dominated by C_3 forbs over time with no subsequent increase in soil C content. It seems that in the case of combined annual burning and grazing by a species that feeds on and reduces abundance of the dominant C_4 grasses, grazing may offset the positive effects of burning on C_4 grass production and contribution to soil C content, while annual burning maintains relatively low cover of woody vegetation and litter inputs that would otherwise increase and contribute to increased soil C concentration, as was the case in the grazed and rarely burned treatment. Therefore, it appears that the plant community changes driven by divergent burning and grazing regimes (i.e., Figures 2.8 - 2.9) have the potential to alter belowground C concentration with potential long-term impacts on belowground C storage in tallgrass prairie.

Hypothesis 3: Long-term grazing and burning regimes will alter multiple N cycling processes and, consequently, the soil $\delta^{15}N$ signature

The soil $\delta^{15}N$ signature is often used as an indicator of the openness of the nitrogen cycle (i.e., a higher $\delta^{15}N$ value indicates more N loss through denitrification or leaching whereas a lower $\delta^{15}N$ value indicates N is being retained within the system). Using soil $\delta^{15}N$ values as a proxy for N cycle openness, the N cycle has become more closed in the infrequently burned, ungrazed treatment (UG20; Figure 2.4). In the grazed treatments, soil $\delta^{15}N$ values exhibited a curvilinear change over time, most likely due to the lag effects of reintroducing grazers. Following the reintroduction of grazers in 1992, soil $\delta^{15}N$ values began to increase indicating that their activity led to a more open N cycling. There was also a clear negative relationship between soil $\delta^{15}N$ values and soil N content (Figure 2.7) across all dates and in all treatment combinations, which could indicate that when the rate of N cycling slows, organic soil N concentration increases.

Soil $\delta^{15}N$ is also positively related to the residence time of different pools of SOM within grasslands (Liao et al., 2006a). Since soil $\delta^{15}N$ was significantly higher in annually burned treatments (Figure 2.5), this could indicate that frequent N volatilization due to fire has resulted in a higher proportion of organic N being immobilized by soil microbes (Dell et al., 2005) and/or physically protected within aggregates.

Conclusions

This research demonstrates that contrasting burning and grazing regimes have divergent effects on soil C and N pools and, through their impacts on the plant community, have shifted the relative contributions of different plant growth forms to the soil C and N pool. Changes in soil C and N content were associated with three decades of land management favoring certain plant growth forms (C_3 woody encroachment vs C_4 grass dominance) in the tallgrass prairie ecosystem. The stable isotope analysis demonstrated that more C is stored belowground when woody plants heavily encroach and contribute a larger proportion of C to that pool when fire is suppressed. In addition, this research has demonstrated the unique potential for using long-term soil collections to more fully understand the biogeochemical impacts of human-altered disturbance regimes in grasslands.

Although our results suggest that woody encroachment may increase soil C and N content with potential benefits with respect to some selected ecosystem services, woody encroachment has other negative ecological consequences that should be taken into consideration (Archer et al., 2017). The tallgrass prairie is among the most endangered ecosystems in the world (Samson & Knopf, 1994), and woody encroachment threatens many plant species (Ratajczak et al., 2012) and decreases the amount of habitat available for grassland-dependent animals (Archer

et al., 2017). Woody encroachment can also decrease the economically important ecosystem services that grasslands provide. For example, modest increases in woody plant cover decreases livestock production (Anadón et al., 2014). Finally, while these results show that soil C content can increase over time in response to divergent burning and grazing regimes, observed rates of increase were lower than 0.4% yr^{-1}, the recommended sequestration rate proposed by the UN Climate Action Program (Minasny et al., 2017).

Acknowledgements

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Table 2.1 Burning and grazing history of Konza watershed-level treatments used for this study. All prescribed burns were conducted in the spring. UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, G20 = grazed, burned every 20 years.

Treatments	Konza LTER Watershed ID	Years Burned	Notes
UG1	001D	1978 - 2015	
G1	N01B	1988 - 2015	Bison added 1992
UG20	020B	1991	Wildfire
G20	N20B	1980, 1991, 1996	Wildfires in 1991 and 1996; Bison added 1992

Table 2.2 Equations, 95% confidence intervals (CI) for slope estimates, and p-values for all linear mixed models. UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, G20 = grazed, burned every 20 years.

UG1			
Response	**Equation**	**95% CI for slope**	**p**
% C	0.0079x -12	(-0.00047, 0.016)	0.062
$\delta^{13}C_{soil}$	0.019x - 52	(0.0091, 0.029)	0.001
% N	0.00059x – 0.89	(-0.00025, 0.0014)	0.156
$\delta^{15}N$	-0.017x + 38	(-0.054, 0.020)	0.344
% C vs $\delta^{13}C$	0.37x + 9.0	(0.086, 0.65)	0.014
% N vs $\delta^{15}N$	-0.015x + 0.35	(-0.024, -0.0054)	0.004
C_3 Cover	-0.0051x + 10	(-0.0084, -0.0018)	0.003
G1			
Response	**Equation**	**95% CI for slope**	**p**
% C	0.0045x – 5.8	(-0.0036, 0.013)	0.251
$\delta^{13}C_{soil}$	$-0.48x - 1.5x^2 - 15$	x: (-1.8, 0.86) x^2: (-2.9, -0.21)	0.034
% N	0.00063x – 0.97	(-0.00023, 0.0015)	0.139
$\delta^{15}N$	$-2.2x + 3.2x^2 + 3.9$	x: (-3.9, -0.35) x^2: (1.4, 5.0)	<0.001
% C vs $\delta^{13}C$	-0.053x + 2.5	(-0.22, 0.11)	0.505
% N vs $\delta^{15}N$	-0.014x + 0.35	(-0.021, -0.0074)	<0.001
C_3 Cover	0.012x - 24	(0.0088, 0.15)	<0.001
UG20			
Response	**Equation**	**95% CI for slope**	**p**
% C	0.027x - 49	(0.014, 0.039)	<0.001
$\delta^{13}C_{soil}$	-0.075x + 130	(-0.098, -0.052)	<0.001
% N	0.0030x – 5.6	(0.0014, 0.0046)	0.001
$\delta^{15}N$	$-4.8x - 1.6x^2 + 3.1$	x: (-6.5, -3.1) x^2: (-3.3, 0.081)	<0.001
% C vs $\delta^{13}C$	-0.33x – 1.2	(-0.46, -0.20)	<0.001
% N vs $\delta^{15}N$	-0.029x + 0.40	(-0.037, -0.021)	<0.001
C_3 Cover	0.018x - 35	(0.014, 0.022)	<0.001
G20			
Response	**Equation**	**95% CI for slope**	**p**
% C	0.010x – 16	(-0.0012, 0.021)	0.077
$\delta^{13}C_{soil}$	-0.046x + 76	(-0.066, -0.026)	<0.001
% N	0.00094x – 1.6	(0.00010, 0.0018)	0.030
$\delta^{15}N$	$-3.2x + 2.3x^2 + 3.7$	x: (-4.2, -2.3) x^2: (1.3, 3.2)	<0.001
% C vs $\delta^{13}C$	-0.29x – 0.90	(-0.44, -0.14)	<0.001
% N vs $\delta^{15}N$	-0.019x + 0.37	(-0.027, -0.012)	<0.001
C_3 Cover	0.013x – 25	(0.0085, 0.017)	<0.001

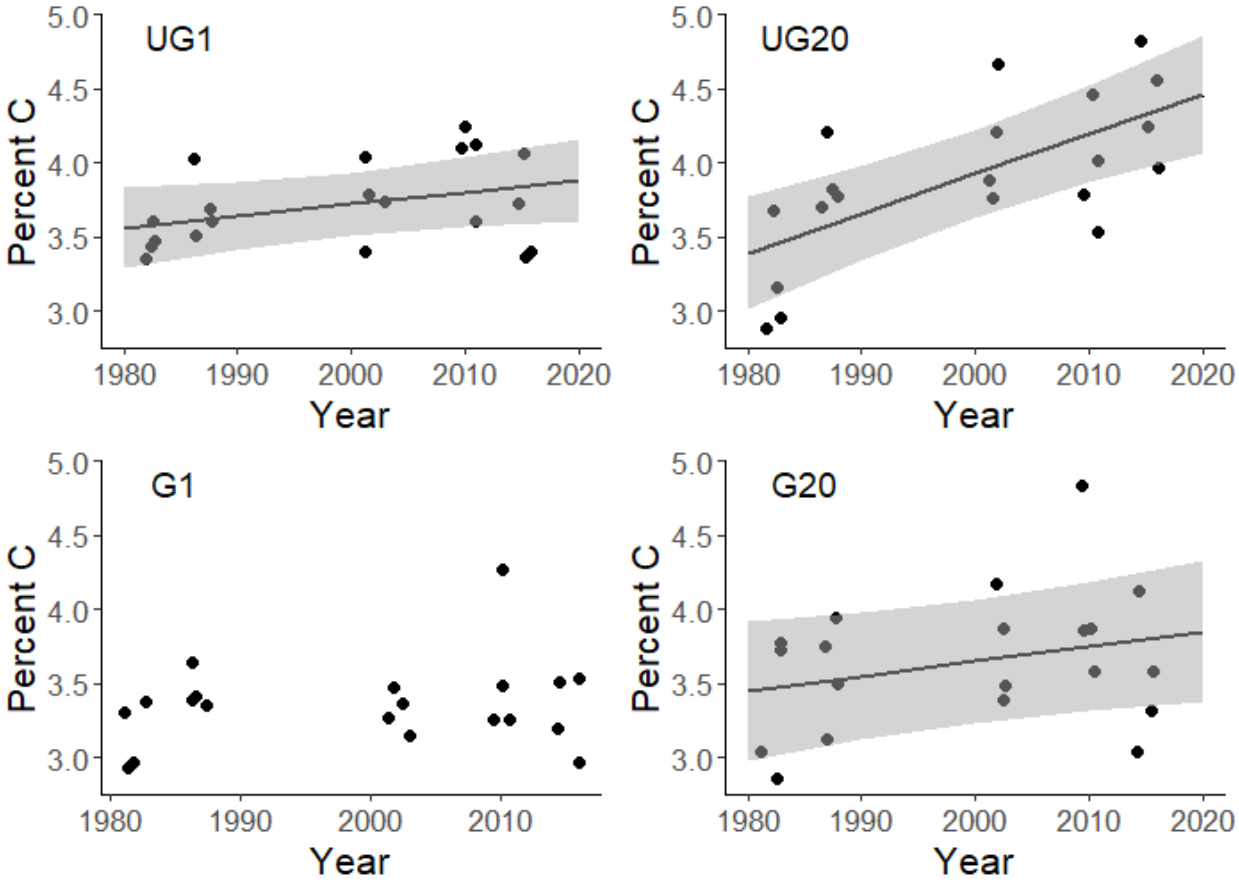

Figure 2.1 Change in soil C concentration over time in four watersheds at the Konza Prairie LTER site from 5 sample dates (1982, 1987, 2002, 2010, and 2015). Points have been jittered to minimize visual overlap. Shaded areas represent 95% confidence interval for predicted values. UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, G20 = grazed, burned every 20 years.

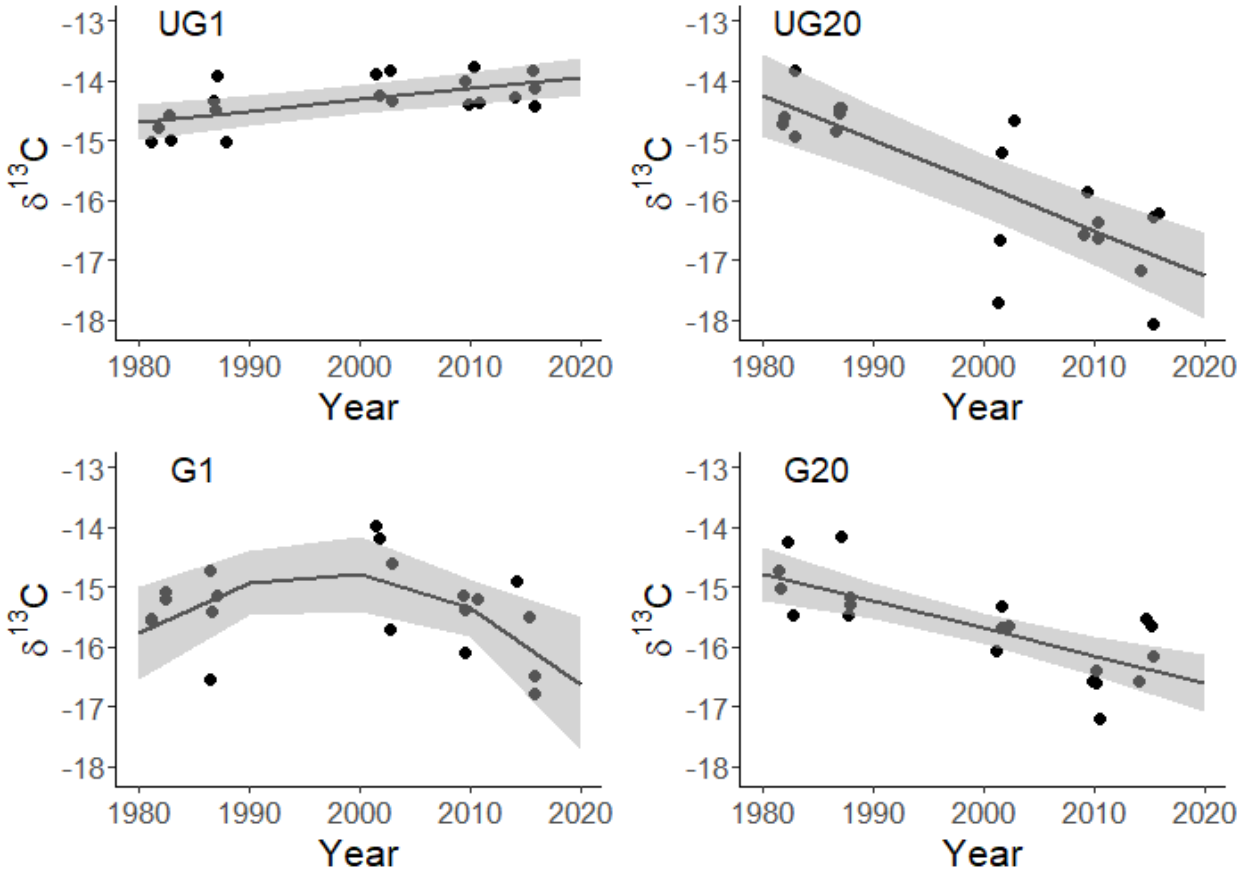

Figure 2.2 Change in the soil $\delta^{13}C$ signature over time in four watersheds at the Konza Prairie LTER site from 5 sample dates (1982, 1987, 2002, 2010, and 2015). Points have been jittered to minimize visual overlap. Shaded areas represent 95% confidence interval for predicted values. UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, G20 = grazed, burned every 20 years.

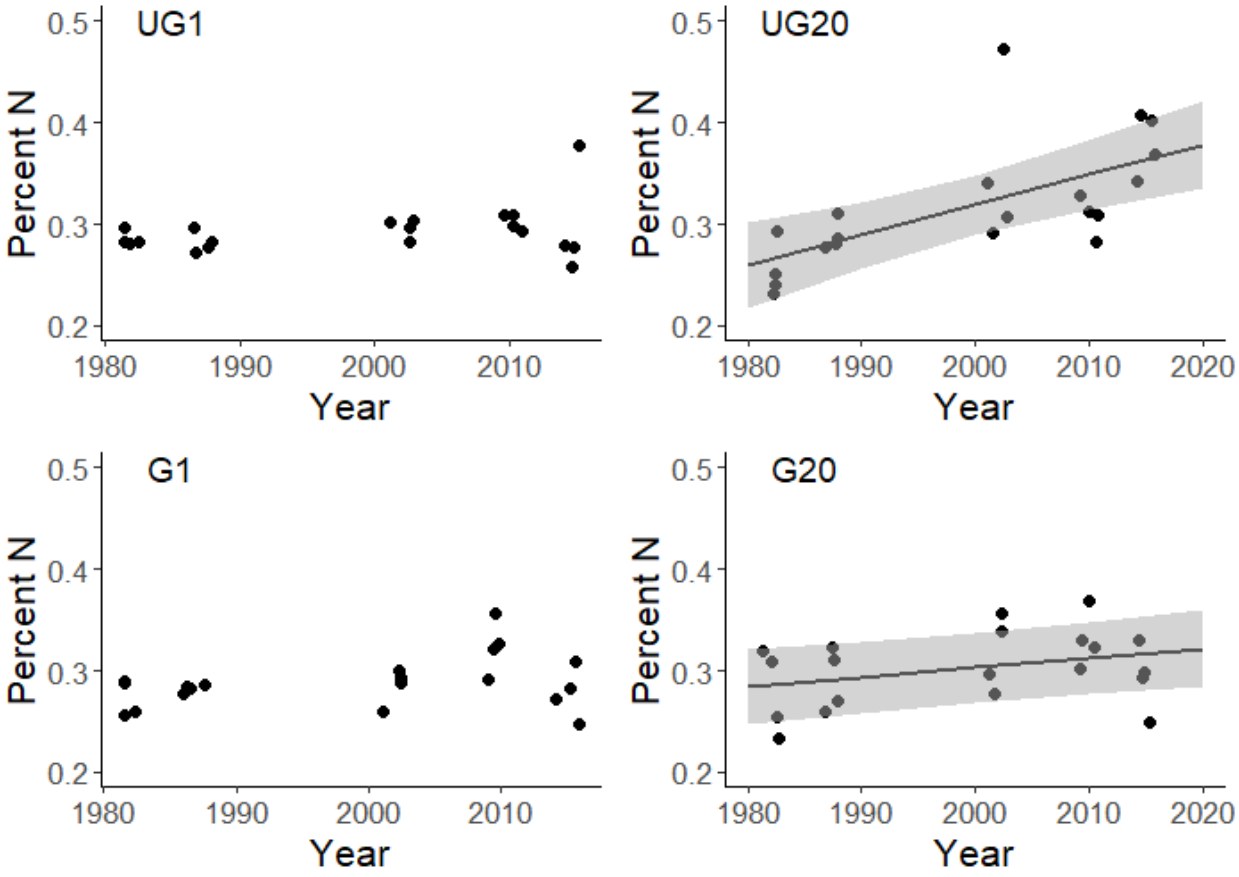

Figure 2.3 Change in soil N concentration over time in four watersheds at the Konza Prairie LTER site from 5 sample dates (1982, 1987, 2002, 2010, and 2015. Points have been jittered to minimize visual overlap. Shaded areas represent 95% confidence interval for predicted values. UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, G20 = grazed, burned every 20 years.

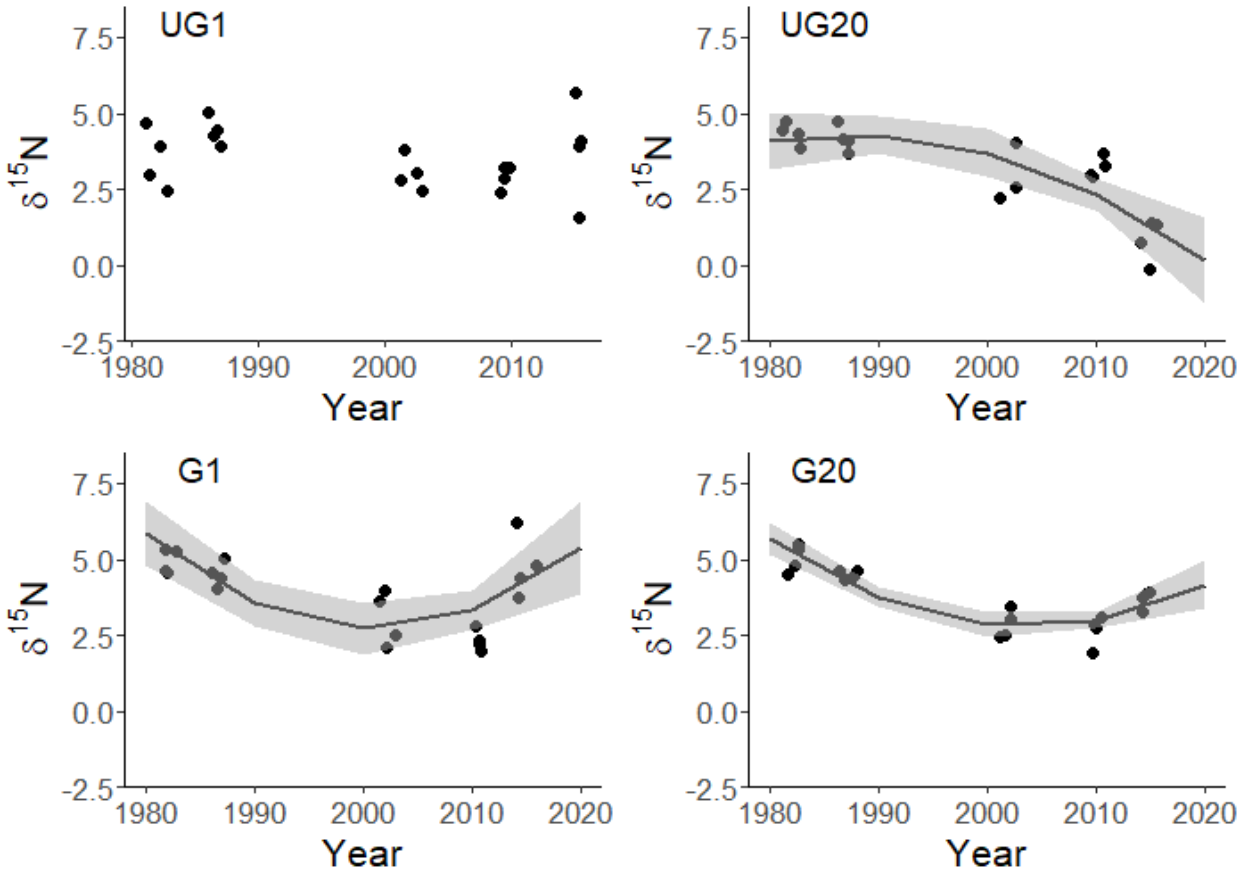

Figure 2.4 Change in soil $\delta^{15}N$ signature over time in four watersheds at the Konza Prairie LTER site from 5 sample dates (1982, 1987, 2002, 2010, and 2015. Points have been jittered to minimize visual overlap. Shaded areas represent 95% confidence interval for predicted values. UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, G20 = grazed, burned every 20 years.

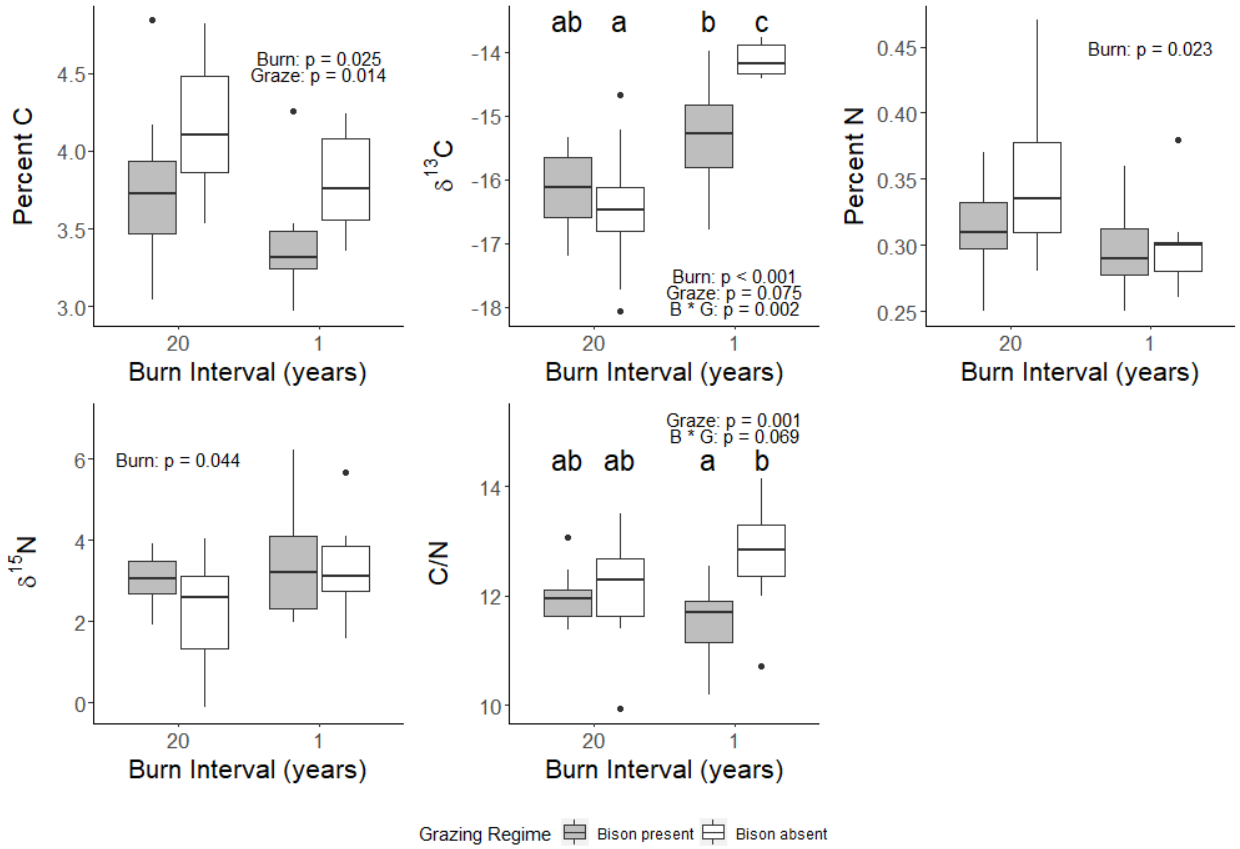

Figure 2.5 Cumulative effects of divergent burning and grazing regimes on soil %C, $\delta^{13}C$, %N, $\delta^{15}N$, and C/N. Only data from 2002 – 2015 were used in this analysis. Where $p < 0.1$ for the interaction between burn and graze regime, letters denote significant differences according to Tukey post-hoc pairwise comparisons.

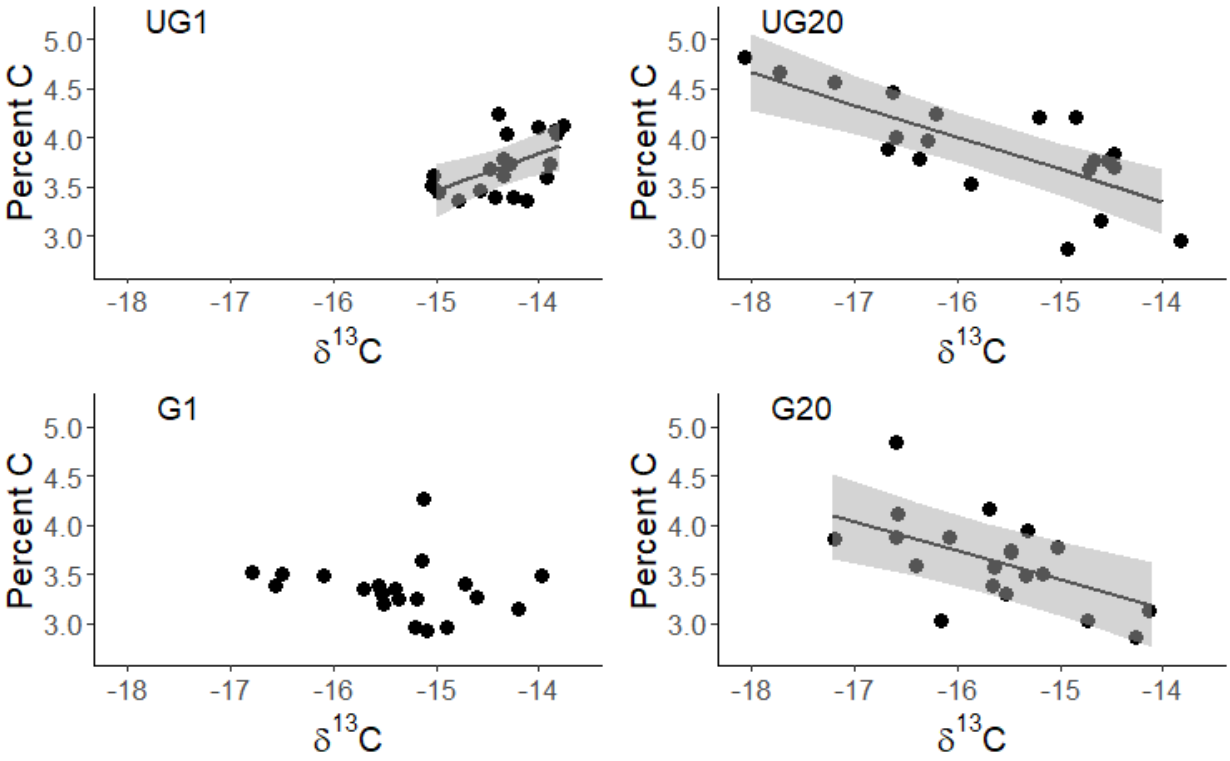

Figure 2.6 Relationship between the soil $\delta^{13}C$ and soil C content in four treatments at the Konza Prairie LTER site. Shaded areas represent 95% confidence interval for predicted values. UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, G20 = grazed, burned every 20 years.

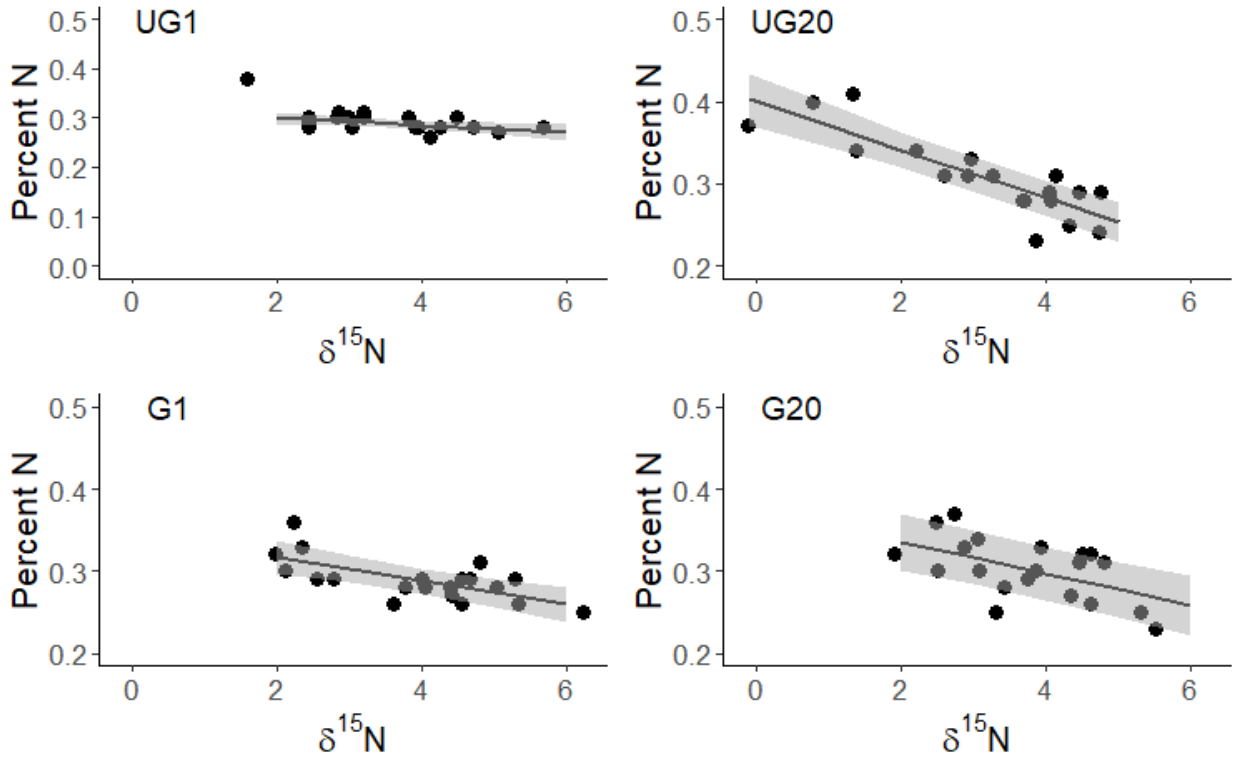

Figure 2.7 Relationship between the soil $\delta^{15}N$ signature soil N content across four watersheds at the Konza Prairie LTER site. Shaded areas represent 95% confidence interval for predicted values. UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, G20 = grazed, burned every 20 years.

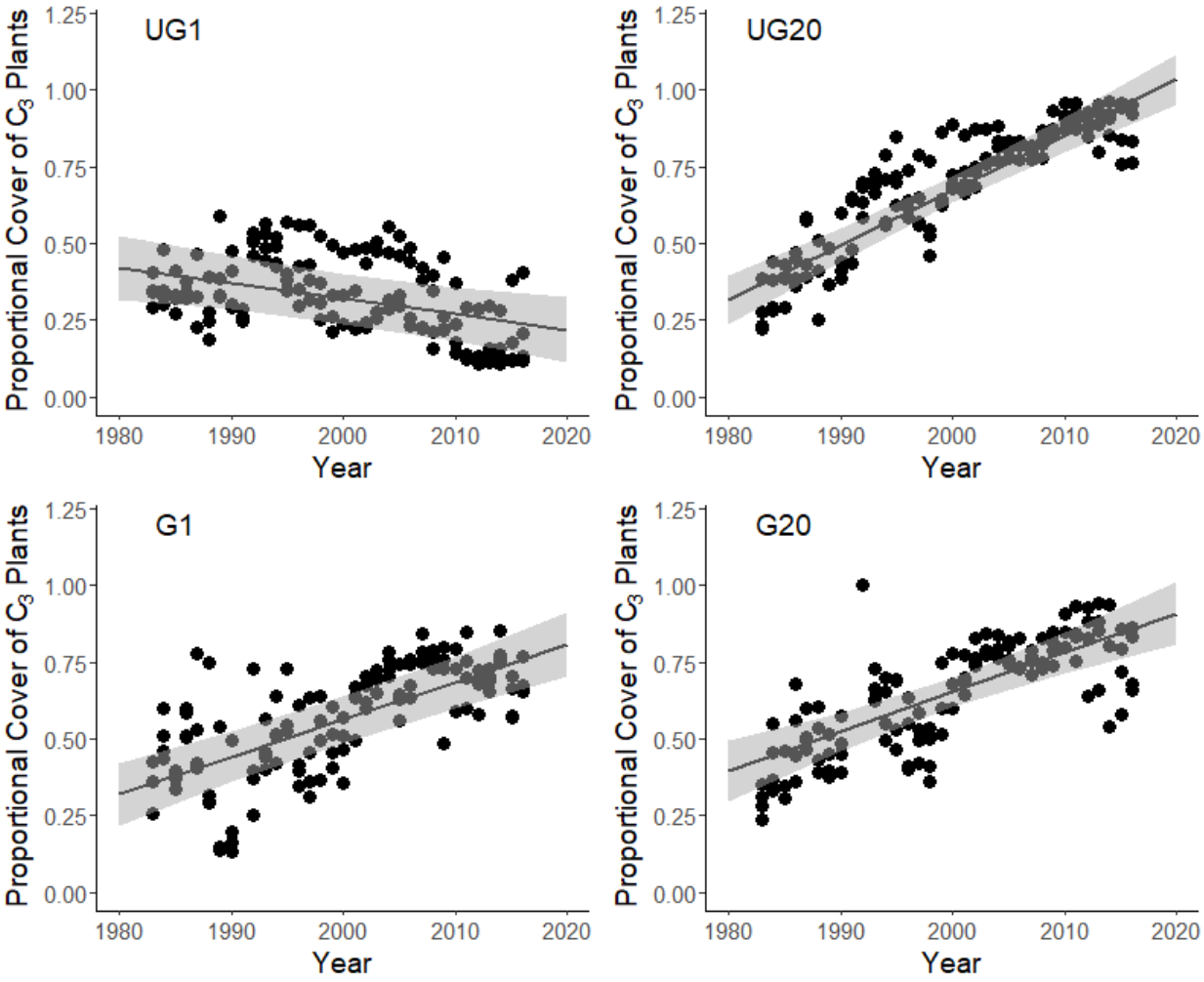

Figure 2.8 Changes in the proportional cover of C_3 plants of the plant community according to plant community composition data in four treatments at the Konza Prairie LTER site from 1982 – 2015. Shaded areas represent 95% confidence interval for predicted values. UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, G20 = grazed, burned every 20 years.

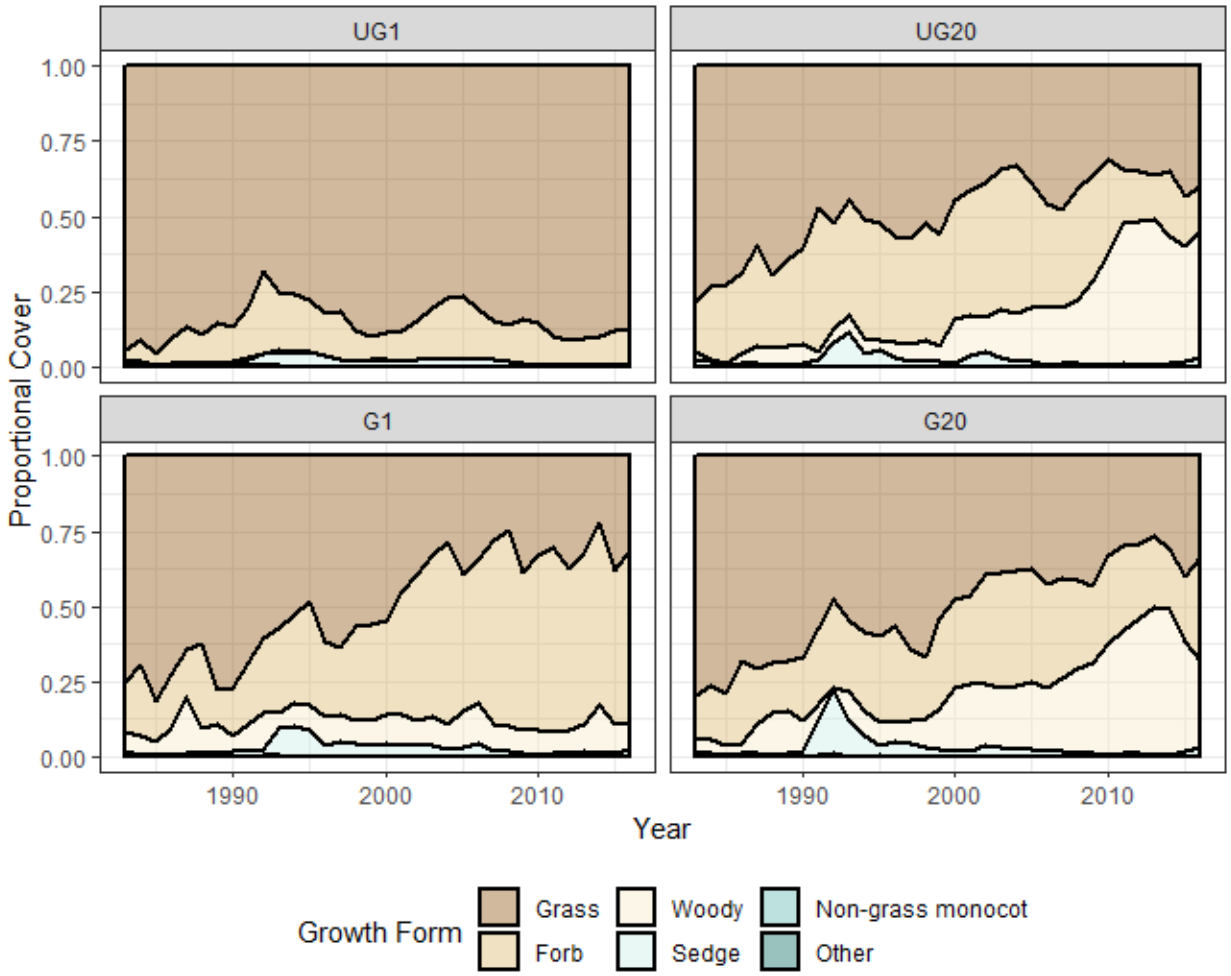

Figure 2.9 Change in proportional cover of six categories of growth form in plant communities according to data from four treatments at the Konza Prairie LTER site from 1982 – 2015. UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, G20 = grazed, burned every 20 years. Note that the Grass category represents the combined cover of C_3 and C_4 grasses.

Chapter 3 - Spatial variation in soil microbial processes as a result of woody encroachment is dependent on shrub size in tallgrass prairie

Abstract

As woody plants encroach into grassland ecosystems, we expect that altered plant inputs and plant-soil interactions will lead to changes in the microbial processes that affect carbon (C) storage and nutrient cycling. While previous studies have investigated the ecosystem-scale consequences of woody encroachment, less is known about the impact of woody plants at the scale of individual shrubs. Specifically, this research aimed to address how (1) soil chemistry, (2) microbial nutrient demand, and (3) the rate and source of potential soil C mineralization vary spatially under individual woody shrubs that have encroached into areas that previously were dominated by herbaceous tallgrass prairie species. *Cornus drummondii* is a clonal woody shrub that establishes in prairies under low fire frequency and spreads radially from a center point of establishment, creating a gradient in which soils at the shrub's periphery have been occupied by *C. drummundii* most recently while soils at the shrub's center have been occupied the longest. I collected soil samples from the center, the midpoint between the center and edge, the edge, and the shrub-grass ecotone of multiple *C. drummondii* islands across a shrub-size gradient. I found total soil C, total soil nitrogen (N), and microbial C demand increase with shrub size in every location but the edge. Additionally, microbial phosphorus (P) demand was always lowest at the shrub's edge regardless of size. Across all shrub sizes and sampling locations, potential soil C mineralization rates were higher when microbes were breaking down proportionally more shrub-derived organic matter, as determined by natural abundance ^{13}C signatures of the respired C. The

$\delta^{13}C$ values of respired CO_2 also indicated that microbes in the interior portion of the shrub respire proportionally more shrub-derived organic matter than older grass-derived organic matter. Our results suggest that the spatial heterogeneity of root function could partially explain the variation in elemental cycling across sampling locations under individual clonal shrubs. Additionally, our results suggest that the rate of soil C flux may be dependent on how long the soil has been occupied by woody species. As woody plants continue to spread through grass-dominated ecosystems around the globe, our results suggest that understanding the spatiotemporal context of woody encroachment is critical for understanding its impact on belowground microbial processes and that in our system longer occupancy by woody plants may lead to greater soil C flux.

Introduction

Woody encroachment, the increase in cover and abundance of woody plants in grasslands and savannas, threatens many grassland systems globally (Archer et al., 2017; Sala & Maestre, 2014). The change from grass-dominated to woody-dominated communities can significantly impact the cycling of carbon (C) and nutrients in these systems (Barger et al., 2011; Knapp et al., 2008a), although drawing general conclusions across grassland types is challenging. Whether woody encroachment results in shifts in the cycling of C or nutrients often depends on abiotic factors such as climate (Jackson et al., 2002b; Knapp et al., 2008a), soil texture (Li et al., 2016), or the ecosystem properties being measured [e.g., increases in soil organic C and aboveground C but no change in soil respiration (Eldridge et al., 2011b)]. Biotic factors that also can influence nutrient cycling include association with nitrogen-fixing microbes (Blaser et al., 2014),

mycorrhizal interactions (Williams et al., 2013), and interactions with grazing megafauna (Soliveres & Eldridge, 2014).

Compared to the grasses they displace, shrubs allocate a greater proportion of their biomass aboveground and generally have greater aboveground net primary productivity (ANPP) (Barger et al., 2011; Briggs et al., 2005), whereas grasses usually allocate a large proportion of their biomass belowground (Blair et al., 2014). Additionally, woody plants typically invest in larger-diameter roots than grasses and allocate roots deeper in the soil profile than grasses (Jackson et al., 1997). Woody plants also can increase the formation of soil macroaggregates, which increases the amount of stable C belowground (Liao et al., 2006b). Thus, woody encroachment likely influences multiple ecosystem functions, although local factors such as climate and identity of encroaching species may determine the specific ecosystem-level consequences.

In North American tallgrass prairie, woody encroachment can increase carbon stocks, at least in the short term. For example, encroachment by *Cornus drummondii* into tallgrass prairie increased aboveground net primary productivity and decreased soil respiration (Lett et al., 2004). Replacement of tallgrass prairie by another woody species, *Juniperus virginiana*, increased soil organic carbon (McKinley & Blair, 2008). However, this increase in carbon might only be temporary. For example, another study reported that belowground net primary productivity and soil organic carbon stocks were significantly lower in tallgrass prairie that had been encroached by *Prosopis glandulosa* and *Gleditsia triacanthos* for over sixty years (McCulley & Jackson 2012). The consequences of woody plant encroachment are expected to vary with time (Blaser et al., 2014; Creamer et al., 2011; McCulley & Jackson, 2012), so it is imperative to place conclusions regarding the ecosystem impacts of woody encroachment within a temporal context.

Clonality is a characteristic of several species contributing to the encroachment of tallgrass prairie (Ratajczak et al., 2011a). In the absence of disturbance or management, a single clonal shrub can grow to occupy a large area of the landscape. *Cornus drummondii* (hereafter, "dogwood") is a woody shrub currently increasing in density and cover in many areas of North American tallgrass prairie (Briggs et al., 2002). After dogwood establishes, it spreads radially through vegetative reproduction to create distinct "islands" that have unique aboveground properties (e.g. ANPP, LAI) compared to nearby open grassland (Ratajczak et al., 2011a). The clonal nature and radial growth pattern of dogwood makes it an ideal species to explore the effects of encroachment over time, since soils under the center of the island are assumed to have been impacted by woody plants the longest while soils near the edges of the island are more recently impacted (*sensu* Throop and Archer 2008). Soil is a complex environment in which heterogeneity exists at both coarse and fine spatial scales (Jackson & Caldwell, 1993; Lehmann et al., 2008). Woody encroachment via clonal shrubs has the potential to alter fine-scale heterogeneity in soil properties and microbial processes, though this has not been well studied.

An additional advantage to studying encroachment by dogwood, which uses a C_3 photosynthetic pathway, into areas previously dominated by grasses using a C_4 photosynthetic pathway is that I can use natural abundance of stable C isotopes to determine the source of C respired by soil microbes during organic matter decomposition at various locations from the center of the shrub islands. According to the preferential substrate utilization hypothesis, microbes prefer to break down organic matter from newer sources rather than from older, more stabilized organic matter (Blagodatskaya et al., 2011). Based on this hypothesis, the isotopic C signature of respired soil CO_2 from under encroaching shrub islands should indicate that microbes are decomposing proportionally more C_3 plant-derived organic matter. On the other

hand, some studies indicate that C_4-derived organic matter decomposes more readily in mixed C_3/C_4 soils (Wynn & Bird, 2007), such that we may expect C isotopic signatures of respired CO_2 to reflect older organic matter that was derived from the C_4 grasses which previously dominated the landscape.

By examining the properties of soils collected from multiple spatial locations within dogwood islands in a heavily encroached area of tallgrass prairie, I aimed to answer the following three questions: (1) Does soil chemistry vary predictably across spatially distinct microsites in woody encroached areas? (2) Does microsite matter for soil microbial biomass and enzymatic activity within woody encroached areas? and (3) How do patterns of potential C mineralization and sources of respired soil C change across microsites within a dogwood island? Specifically, I hypothesized that due to changes in root distribution and activity, increasing distance from the center of dogwood islands would lead to lower soil C:N. This would result in lower microbial demand for nitrogen (N) and increased demand for C and phosphorus (P) (i.e., lower N-acquiring extracellular enzymatic activity and higher C and P-acquiring extracellular enzymatic activity) in locations further from the center. Second, I hypothesized that greater nutrient availability would increase potential C mineralization rates with increasing distance from the center of dogwood islands. Because soils under the center of dogwood were expected to have been influenced by woody plant encroachment the longest, I predicted that the isotopic C signature of respired CO_2 would be most negative at the center, reflecting greater reliance on C_3-derived organic matter, and would become less negative with increasing distance from the center.

Methods

Study site

To assess spatial patterns of soil properties under shrub islands encroaching into tallgrass prairie, soils were sampled in the lowland topographic region of an infrequently burned treatment area at the Konza Prairie Biological Station (KPBS) near Manhattan, Kansas, USA. KPBS is a preserve of unplowed tallgrass prairie primarily dominated by C_4 grasses. The site is divided into large landscape units that vary in fire frequency. Areas that are infrequently burned are undergoing woody encroachment into what were C_4 grass dominated prairies (Ratajczak, Nippert, Briggs, et al., 2014). The site used in our study has a prescribed fire interval of every 20 years. Since 1978, it was burned only twice, once in 1991 and once in 2012. The primary woody species in this area are *Cornus drummondii, Juniperus virginiana,* and *Gleditsia triacanthos.*

Soil collection, preparation, and handling

Ten distinct dogwood islands were randomly selected within the previously described treatment area. The ten islands ranged in size from 85 m^2 to 830 m^2. Two 5 cm diameter x 15 cm deep soil cores were taken at four locations within each dogwood island along a linear transect: the center, the midpoint, the edge, and the ecotone with grassland. I only sampled to a 15 cm depth since the main goal of this study was to assess the response of soil microbial processes to dogwood occupation, and most soil microbial activity occurs in the topsoil. The center was defined as the intersection of perpendicular transects running the length and width of the dogwood island. The midpoint was defined as the halfway point between the center and the furthest edge. The edge was defined as the outer perimeter of the dogwood island canopy, and the ecotone was defined as the halfway point between the edge of the dogwood island of interest and the nearest neighboring dogwood island. The median distance between the edge and the

ecotone was 0.875 m. While aboveground biomass in the ecotone was dominated by grasses and forbs, dogwood roots extend several meters beyond the canopy edge (pers. obsv. R.C. O'Connor), so even soils in the ecotone could be influenced by the dogwood clone. All soil cores were kept on ice until they could be placed in short-term storage at 4 °C.

On the day after sampling, soils were passed through a 4 mm sieve. A small subsample of soil from each location was dried at 60 °C for 48 hours to determine gravimetric water content. One core from each location was used for nutrient, microbial, and enzymatic analyses. Soil from the other core was used for to assess potential C mineralization rates and isotopic signatures. Soil used for nutrient analyses was stored at 4 °C. Soil used for extracellular enzymatic activity analyses was stored at -20 °C.

Soil C, N, P, and organic matter

Total C and N content of each soil sample was determined via dry combustion using a LECO TruSpec CN combustion analyzer. Total organic matter was determined by a slightly modified loss on ignition protocol outlined in Combs and Nathan (1998). Briefly, 1 g of soil was dried at 150 °C for two hours and then combusted at 400 ºC for three hours. Total extractable P was determined by the Mehlich-3 procedure (Mehlich, 1984). The above analyses were performed at the Soil Testing Lab at Kansas State University (Manhattan, KS, USA). Fifty ml of 2N KCl was added to 12 g of field moist soil and placed on an orbital shaker table at 200 rpm for 60 minutes to extract soil NH_4^+ and NO_3^- (Bremmer & Keeney, 1966). Extracts were passed through a 0.45 polycarbonate filter and stored at -20°C until they were analyzed colorimetrically for NO_3^- and NH_4^+ in a flow analyzer at the Kansas State University Soil Testing Lab (Manhattan, KS, USA).

Microbial biomass carbon and nitrogen

Microbial biomass carbon was determined using the chloroform fumigation-extraction method (Jenkinson & Powlson, 1976). A subsample of each soil sample was placed into a chamber, fumigated by boiling chloroform under a vacuum, and kept in the fumigation chamber under a vacuum for 48 hours. A vacuum pump was used to remove all chloroform from the chamber after fumigation was completed. Fumigated and unfumigated samples were extracted by combining 15 g of field moist soil and 75 ml 0.5 M K_2SO_4 and placing on an orbital shaker table at 200 rpm for 60 minutes, then passing through a 0.45 µm polycarbonate filter. Extracts were stored at -20°C until they were analyzed for total organic C with a Shimadzu TOC-L. Microbial biomass C was defined as the difference in dissolved organic C between fumigated and unfumigated subsamples.

Total nitrogen content in microbial biomass was determined by taking a subsample of the K_2SO_4 extracts and subjecting them to a persulfate digest (Cabrera & Beare, 1993; D'Elia et al., 1977). This oxidizes all forms of nitrogen to NO_3^-. After being reduced to NO_2- by a cadmium coil, N concentrations in the extracts were determined colorimetrically using an Alpkem OI Analytical Flow Solution IV. Microbial biomass N was defined as the difference in N between fumigated and unfumigated subsamples.

Potential extracellular enzymatic activity assays

I tested the potential activity of four extracellular enzymes (Sinsabaugh et al., 1999): β-glucosidase (BG; a C-acquiring enzyme), N-acetyl-glucosaminidase (NAG; a N-acquiring enzyme), phosphatase (PHOS; a P-acquiring enzyme), and leucine-aminopeptidase (LAP; a N-acquiring enzyme). I used 200 µM solutions of 4-methylumbelliferone-b-D-glucoside, 4-methylumbelliferone-N-acetyl-b-glucosaminide, 4-methylumbelliferone-phosphate, and L-

leucine 7-amido-4-methylcoumarin as substrates, respectively. For each soil sample in each assay, I created a slurry of 1 g of soil in 100 ml of 50 mM acetate buffer (pH 5). In 96-well plates, I pipetted 200 µl of the soil slurry with 50 µl of the substrate solution. There were six analytical replicates for each sample in each assay as well as a blank, a negative control, a reference standard, three quench standards, and six soil blanks. For BG, NAG, and PHOS assays, I incubated the plates in the dark at room temperature for 2 hours. Assays for LAP activity were incubated for 16 hours. Once the incubations were complete, I added 10 µl of 0.5 N NaOH solution to raise the pH and stop the assays. Finally, I used a FilterMax F5 plate reader to collect fluorescence data.

Potential carbon mineralization

Approximately 300 g of soil from each sample was placed into an 8 cm wide x 15 cm deep mason jar (Day 0). Total CO_2 respired and the $\delta^{13}C$-CO_2 value was measured at 1, 3, 5, 7, 10, 34, and 77 days after the start of the incubations. Starting at Day 0, soils were wetted to 60% water-filled pore space. Throughout the duration of the incubation, each jar was regularly weighed and rewetted to maintain constant soil moisture. Before each measurement, the time at which the lids were sealed was recorded. Total CO_2 concentration and the $\delta^{13}C$-CO_2 value was determined by taking a gas sample of the headspace of each jar through a rubber septum. Two blanks were also measured on each day to account for background CO_2 of the room. The CO_2 concentration and isotopic composition of the gas sample was analyzed with a Picarro Cavity Ringdown Spectrometer (model G2101-I, Picarro Inc., Santa Clara, CA).

Statistical analyses

All response variables fit either normal or lognormal distributions. Generalized linear mixed models were used to analyze the effect of the categorical sampling location (i.e., center,

midpoint, edge, ecotone), shrub size, and their interaction. In each model, location and shrub size were treated as fixed effects and the identity of the dogwood island was treated as a random effect. The model was simplified for potential C mineralization rates: Rate ~ $\delta^{13}C\text{-}CO_2$ + (1|Island). This model was selected from all possible models using shrub size, location, day of incubation, and $\delta^{13}C\text{-}CO_2$ as fixed effects using AIC. All means comparisons conducted using Tukey HSD comparisons of least square means. All statistics were performed within the R statistical computing software (R Core Team, 2019) using the packages *car* [version 3.0-3; (Fox & Weisberg, 2019)], *lsmeans* [version 2.30.0 (Lenth, 2016)], *multcomp* [version 1.4.11 (Hothorn et al., 2008)], and *lme4* [version 1.1-21; (Bates et al., 2015)]. Figures and tables were generated with the packages *tidyverse* [version 1.2.1; (Wickham, 2017)], *ggeffects* [version 0.14.1 (Lüdecke, 2018)], and *cowplot* [version 1.0.0 (Wilke, 2019)].

Results

Soil chemistry and microbial biomass

There was a significant interaction between sampling location and shrub size on total soil C and N (Table 3.1), such that the direction of change in soil C and N at a specific sampling location varied as a function of shrub island size. In general, both total C and total N concentrations tended to increase with shrub island size across all sampling locations except the edge, where total C and N tended to decrease as shrub islands increased in size (Figure 3.1). There was no significant effect of sampling location or shrub size on any other soil nutrient or microbial biomass property measured (inorganic N, extractable P, C:N, organic matter content, microbial biomass C, or microbial biomass N; Table 3.1).

Potential extracellular enzymatic activity

Sampling location, shrub size, and their interaction differentially affected the potential activity of the four enzymes I assayed (Table 3.1). Potential BG activity increased logarithmically with shrub size at all sampling locations except the edge, where potential BG activity decreased as a function of shrub island size (Figure 3.2A). There was a marginally significant effect of sampling location on potential PHOS activity (Table 3.1). Across the gradient of shrub size, potential PHOS activity was significantly lower in soil collected from the edge of shrub islands compared to other sampling locations (Figure 3.2B). Finally, potential activity of the two N-acquiring enzymes I assayed (NAG and LAP) logarithmically increased as shrub size increased irrespective of sampling location (Figure 3.2C and 3.2D).

Potential C mineralization assay

According to the top model based on AIC, there was a negative logarithmic relationship between $\delta^{13}C\text{-}CO_2$ values and potential C mineralization rate across all sampling locations and shrub island sizes (Figure 3.3). The potential C mineralization rate decreased by 9% for every 1‰ increase in the $\delta^{13}C$ value of the emitted CO_2. Additionally, there was a significant effect of sampling location on respired $\delta^{13}C\text{-}CO_2$ (Figure 3.4, Table 3.1) indicating that, on average, the microbial community was not breaking down the same proportions of woody- and grass-derived organic matter across the spatial gradient within shrub islands. On average, the respired $\delta^{13}C\text{-}CO_2$ was significantly lower in soil collected from the center and midpoint than soil collected from the edge or ecotone of shrub islands (Figure 3.4). The effect of day of incubation was not included in our top model and had no statistically significant effect in any of the models in which it was included.

Discussion

By measuring soil characteristics and microbial processes in multiple locations under individual dogwood clones of various sizes, I found that woody encroachment predictably alters variation in nutrient cycling at finer scales than are often measured in studies of woody plant encroachment. Our results demonstrate that heterogeneity in soil properties and processes develops at the scale of individual clonal dogwood shrub islands, with distance from the center of the shrub being an important variable. Additionally, this within-shrub-island heterogeneity also is moderated by shrub size, which is likely a function of time since establishment (Ratajczak et al., 2011b). I hypothesize that heterogeneity in soil microbial processes under an individual dogwood island are driven by time since establishment and the competitive strategies that dogwood uses to expand outward, which results in spatially divergent root processes and subsequent effects on soil microbes and soil properties.

Clonal shrubs have both a shallow, lateral root system that is more extensive than many of their herbaceous neighbors (Casper & Jackson, 1997) as well as deep taproots (Schenk & Jackson, 2002). The roots in the oldest, more developed central parts of a dogwood shrub island grow deeper in the soil profile than grasses (Canadell et al., 1996), facilitating access to deeper water sources (Logan & Brunsell, 2015; Nippert et al., 2013; Nippert & Knapp, 2007), which alleviates competition between dogwood and the surrounding herbaceous vegetation (Ratajczak et al., 2011a). Additionally, because dogwood spreads radially, patterns in 'heterorhizy' [i.e., differences in root function within individual plants due to ontogenetic differences of individual roots within a whole root system (Hishi, 2007)] may correlate with distance from center or shrub size. Older roots often respire less (Bouma et al., 2001) and have lower N concentrations (Hishi & Takeda, 2005). Additionally, fine roots growing from older root structures turn over more

slowly (Hishi & Takeda, 2005). This study suggests that heterogeneity in the distribution or function of roots under dogwood islands may be a causal mechanism influencing the heterogeneity of soil microbial processes; however, the distribution and function of dogwood roots along a horizontal spatial gradient is currently unknown and is worth investigating.

In contrast to my initial hypothesis that total soil C:N would increase with distance from the center of dogwood islands, total soil N was lower at the edge of the canopy under larger dogwood islands (Figure 3.1B). Additionally, spatial variation in total soil C exhibited the same pattern (Figure 3.1A) such that there was no significant change in soil C:N. Previous studies of spatial gradients resulting from woody encroachment by other species in more arid grasslands have also observed that soil C and N decreases with distance from the center of a shrub (Throop & Archer, 2008) and increases with shrub size (Wheeler et al., 2007). Using shrub size as a proxy for age, then the root system in the central portion of a dogwood island has had more time to contribute greater amounts of C and N to the soil pool whereas soil at the edge of the canopy would not be occupied by dogwood roots for as long. Interestingly, as shrub size got larger, total soil C and N also increased in the ecotone (Figure 3.1), potentially indicating persistence of organic inputs from grasses that have been incorporated into SOM as well as greater inputs from the shrub as it explores for unoccupied areas to produce new ramets. Enhanced nutrient availability is often a result of the activity of range-expanding and invasive plants and is implicated as a mechanism for their success (Zhou & Staver, 2019).

Results did not support the hypothesis that N-acquiring enzyme activity would vary with distance, though it did vary with shrub island size. I did find that C- and P-acquiring enzymatic activity varied with distance. However, opposite of our prediction, potential β-glucosidase (a C-acquiring enzyme) activity was greatest in the interior of the shrub islands (i.e., the center and

midpoint) and increased as shrub islands got larger (Figure 3.1A). Two potential mechanisms could explain this pattern. First, microbial demand for C in the central locations of the shrub island might be higher because of the reduction in grass roots and their replacement with coarser, mature dogwood roots that potentially do not exude as much C as younger roots. Second, the biomass of herbaceous litter (i.e., litter originating from a non-woody source) decreases from the edge of a dogwood island to the center (Ratajczak et al., 2011a). Therefore, the center of large dogwood islands could be more C-limited, despite having higher total soil C concentrations, because of less new plant inputs from both above- and belowground. Potential P-acquiring enzymatic activity was always lowest at the edge regardless of shrub size (Figure 3.2B). Deep-rooted shrubs can translocate P from deeper in the soil profile to the surface (Zhou et al., 2018a), but soil P does not accrue as quickly as C and N in response to woody encroachment (Zhou et al., 2018b) so it follows that microbial demand for P will be higher in locations that have been occupied by shrubs for the least amount of time. However, no change in soil P occurred along the distance gradient or a decrease in P-acquiring enzymatic activity as shrubs grew larger. Finally, while potential N-acquiring enzymatic activity did not vary along the horizontal spatial gradient, it did increase as shrub islands got larger (Figure 3.2C, 3.2D) indicating that microbial demand for N increases as shrub islands expand their areal cover. Differences in quality between grass and shrub inputs or increased soil aggregation due to shrub growth could reduce the amount of microbially-available C and N under larger shrubs despite the increase in total soil C and N pools.

Potential soil C mineralization rate did not increase with distance from the center of shrub and the source of the C being mineralized was an important factor affecting potential C mineralization rates. Across all shrub island sizes and spatial locations, potential C

mineralization rates were higher if the isotopic signature of that CO_2 reflected that the microbial community was breaking down a higher proportion of C_3-derived organic matter (i.e., dogwood-derived organic matter; Figure 3.3). Since plant communities growing on the soils in this study were previously dominated by C_4 grasses, I can conclude that the microbial communities underneath dogwood islands preferentially utilize newer C_3 shrub-derived organic matter as a carbon source rather than older C_4 grass-derived organic matter. Potential C mineralization rates were higher when microbes decomposed proportionally more woody-derived organic matter for several potential reasons. First, according to the preferential substrate use hypothesis, microbial communities will break down newer organic inputs before shifting to older soil organic matter (Blagodatskaya et al., 2011). Secondly, newer carbon inputs from dogwood might initially be going to physically unprotected fractions of the soil which would increase microbial access to woody-derived organic matter (Creamer et al., 2011). Finally, C_3-derived organic matter might be intrinsically more decomposable than C_4-derived organic matter. However, this contrasts with previous research in a mixed C_3/C_4 system showing that C_4-derived organic matter decomposes more quickly (Wynn & Bird, 2007). Additionally, since the isotopic signature of respired CO_2 in the central locations of the shrub were significantly more negative (Figure 3.4), microbial communities may become more reliant on shrub-derived organic matter for carbon sources as woody encroachment progresses. A similar spatial pattern in soil $\delta^{13}C$ has been observed in a Texas shrubland (Bai et al., 2012) indicating that similar spatial heterogeneity in the isotopic composition of respired soil CO_2 may be observed in other systems beside the tallgrass prairie.

Conclusion

Using shrub size as a proxy for shrub age, these results suggest shrubs increase the amount of soil C and N in the central, and presumably older, parts of the island but not at the edge (Figure

3.5). The heterogeneity in shrub inputs results in spatial heterogeneity in microbial extracellular enzymatic activity that is dependent on shrub size. Microbial demand for N increases as dogwood shrubs continue to expand, while demand for C increases in the central locations of the dogwood island but decreases at the edge (Figure 3.5). Thus, soil properties and processes vary in space under clonal woody shrubs in the tallgrass prairie and change as islands grow larger, indicating that conclusions about the biogeochemical impacts of woody encroachment should be made within a spatiotemporally explicit context. Future studies that consider how results from our potential C mineralization assays compare with *in situ* measurements of soil CO_2 efflux along the same spatial gradient could provide insights into the role of live roots in affecting soil C dynamics across these gradients. However, based on the data here, I conclude that the rate of stored soil carbon flux to the atmosphere is dependent on how long the soil has been occupied by woody plants; longer occupancy leads to greater rates of soil C mineralization. Additionally, future studies would benefit from more refined data on clonal shrub demography, how belowground biomass of woody plants varies spatially, as well as how woody plants affect soil microbial processes deeper in the soil profile in order to more accurately predict ecosystem responses to woody encroachment.

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Table 3.1 Effect of sampling location (i.e., center, midpoint, edge, ecotone), shrub size, and their interaction on soil chemical properties, microbial biomass properties, potential extracellular enzymatic activity, and the $\delta^{13}C\text{-}CO_2$ respired during the potential C mineralization assays. P-values < 0.1 are bolded.

Total C	X^2	p	**MBC**	X^2	p
Location	2.22	0.527	Location	1.55	0.672
Size	1.16	0.283	Size	1.55	0.214
L * S	9.60	**0.022**	L * S	2.34	0.505
Total N	X^2	p	**MBN**	X^2	p
Location	2.94	0.400	Location	2.06	0.560
Size	1.38	0.241	Size	0.078	0.781
L * S	7.94	**0.047**	L * S	1.37	0.714
C:N	X^2	p	**β-glucosidase**	X^2	p
Location	2.26	0.521	Location	1.92	0.588
Size	0.115	0.735	Size	0.759	0.384
L * S	3.82	0.282	L * S	8.20	**0.042**
Inorganic N	X^2	p	**Phosphatase**	X^2	p
Location	4.78	0.189	Location	7.68	**0.053**
Size	1.63	0.202	Size	0.018	0.893
L * S	3.35	0.341	L * S	2.95	0.400
Extractable P	X^2	p	**NAG-ase**	X^2	p
Location	3.89	0.274	Location	1.21	0.750
Size	0.030	0.863	Size	3.42	**0.064**
L * S	5.87	0.118	L * S	3.17	0.366
Organic matter	X^2	p	**LAP-ase**	X^2	p
Location	1.69	0.640	Location	2.98	0.395
Size	0.563	0.453	Size	7.42	**0.006**
L * S	4.73	0.193	L * S	7.72	**0.052**
$\delta^{13}C\text{-}CO_2$	X^2	p			
Location	1.69	0.640			
Size	0.563	0.453			
L * S	4.73	0.193			

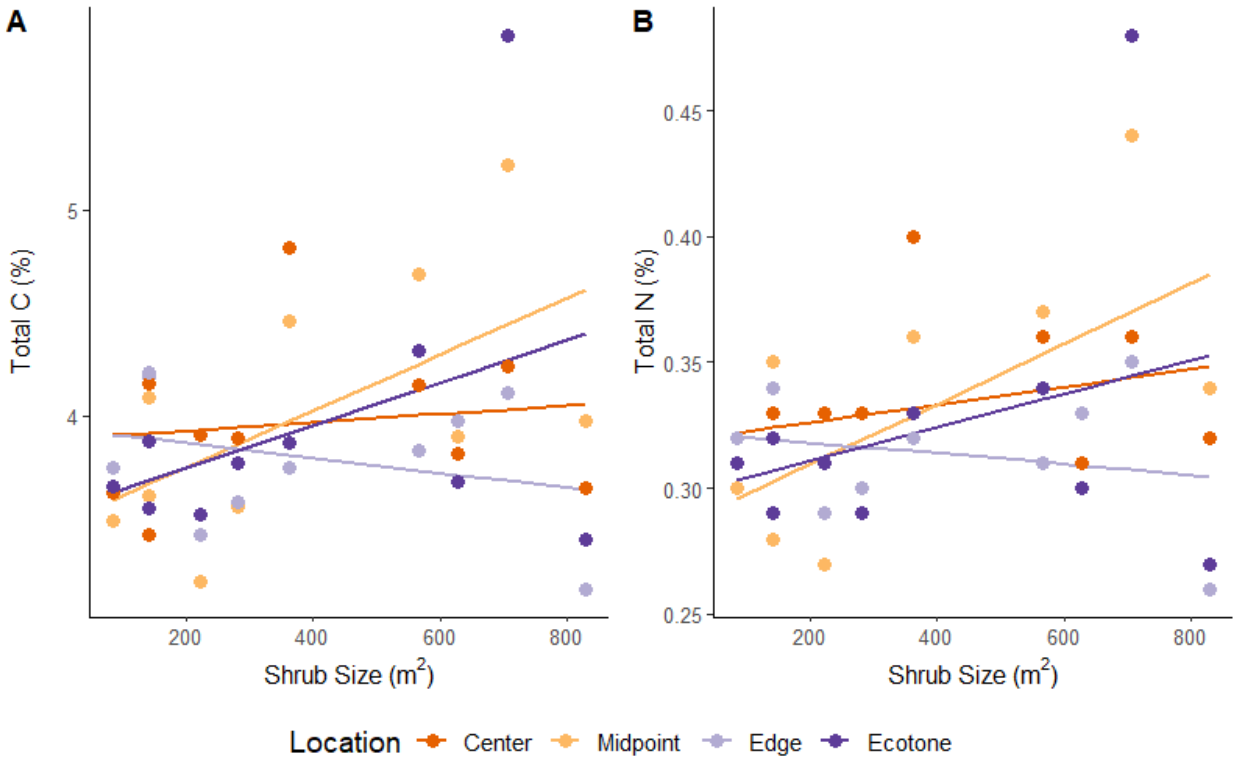

Figure 3.1 Interaction plots displaying the marginal effect of the interaction between sampling location (i.e., center, midpoint, edge, ecotone) and shrub size as lines on A) Total C and B) Total N. Marginal effects are calculated from mixed effect models using the formula: Response ~ Location * Size + (1| Island). The raw data are also displayed as points. The color of the lines and points indicate sampling location.

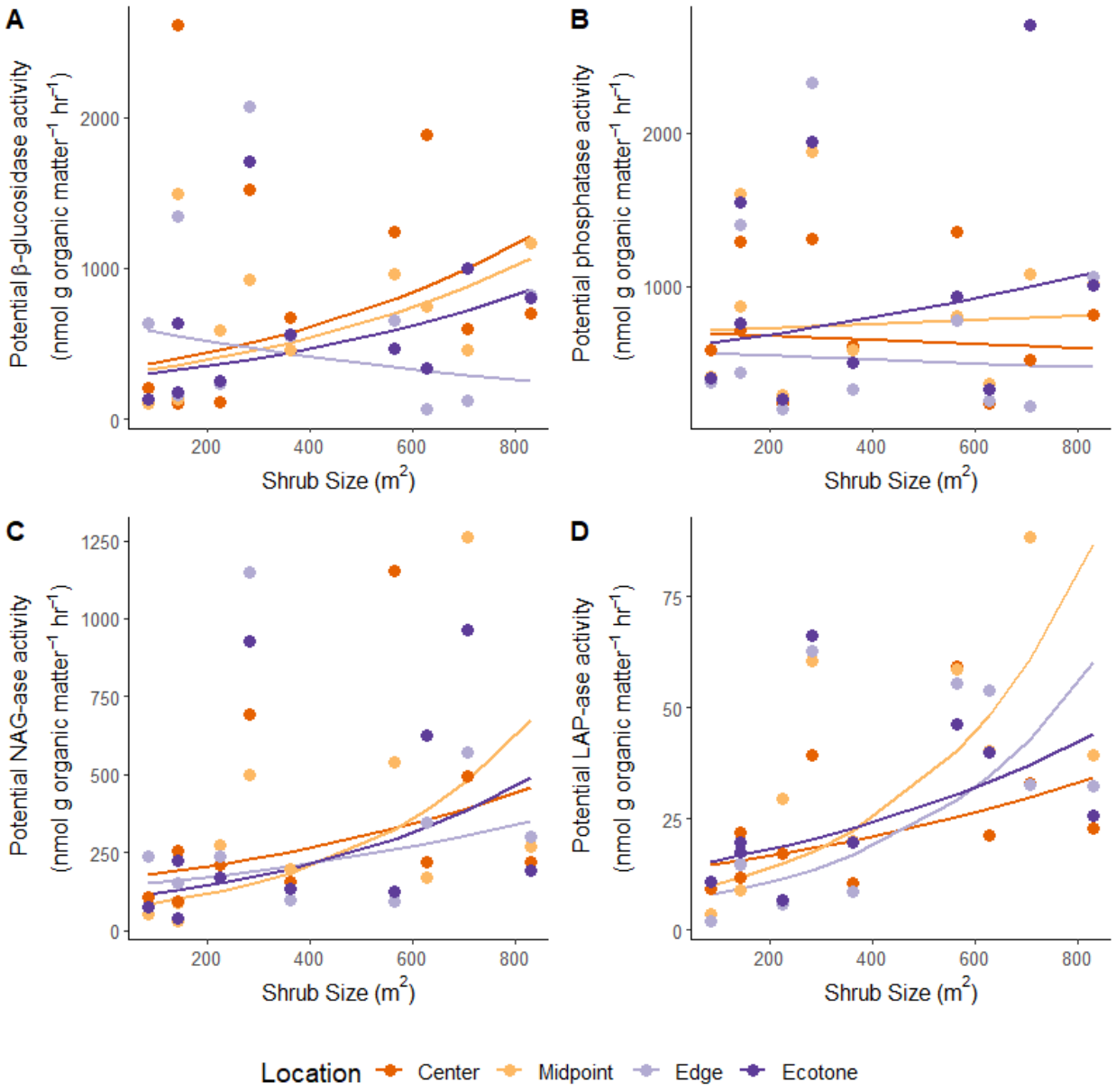

Figure 3.2 Interaction plots displaying the marginal effect of the interaction between sampling location (i.e., center, midpoint, edge, ecotone) and shrub size as lines on A) potential β-glucosidase activity, B) potential phosphatase activity, C) potential NAG-ase activity, and D) potential LAP-ase activity N. Marginal effects are calculated from mixed effect models using the formula: Response ~ Location * Size + (1| Island). The raw data are also displayed as points. The color of the lines and points indicate sampling location.

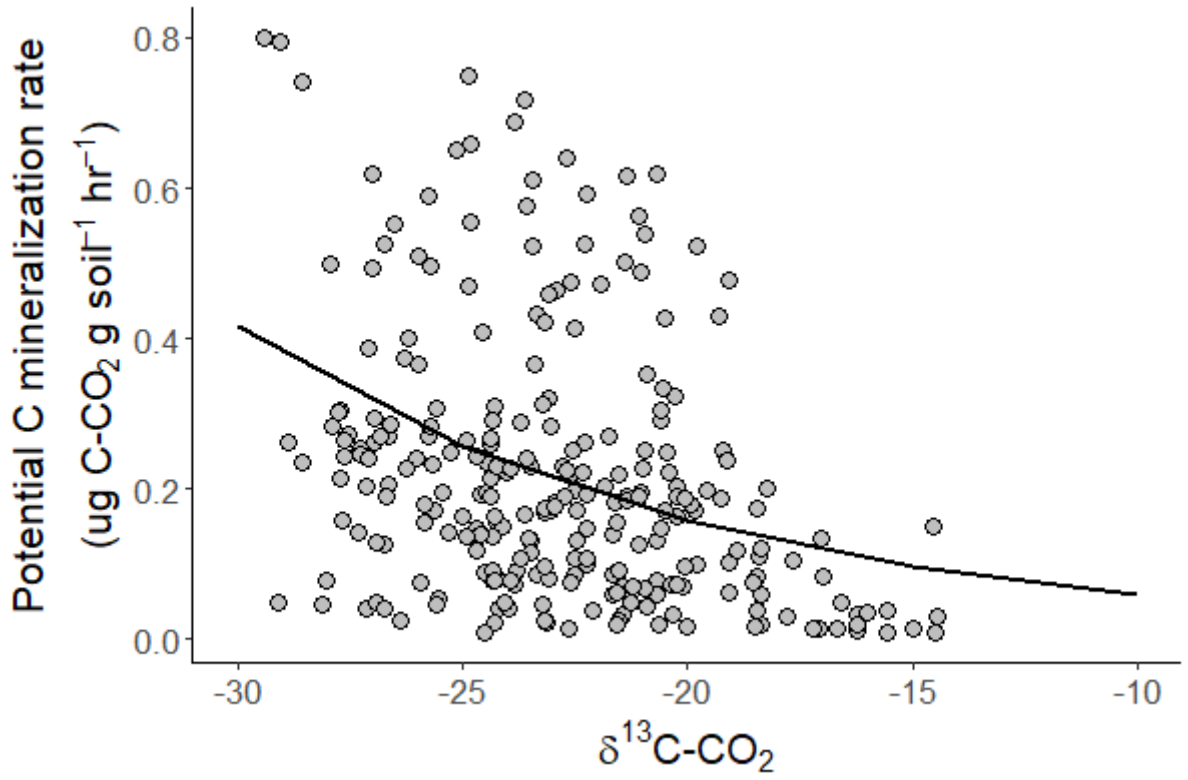

Figure 3.3 The potential C mineralization rate was negatively correlated with the $\delta^{13}C$ signature of respired CO_2 across all shrub islands and locations. The line represents the calculated marginal effect of $\delta^{13}C\text{-}CO_2$ on potential C mineralization from a mixed effects model using the formula: Response ~ $\delta^{13}C\text{-}CO_2$ + (1|Island). The raw data are also displayed as points.

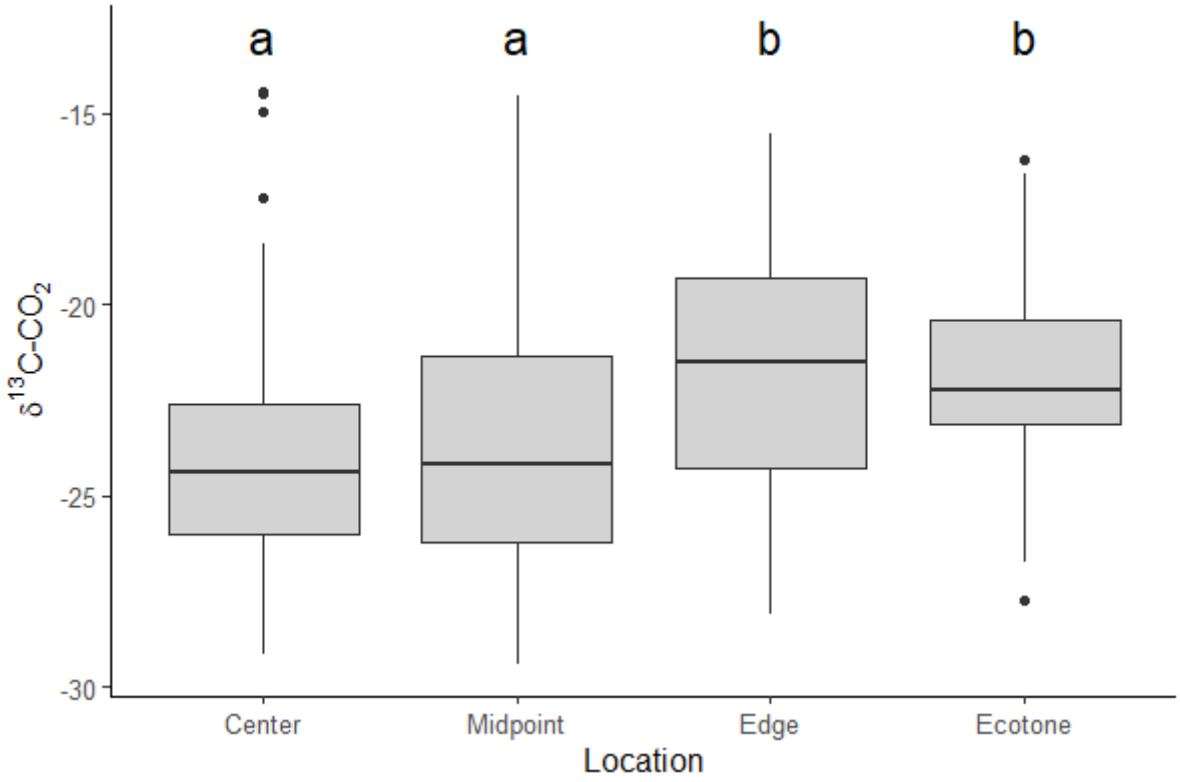

Figure 3.4 Boxplots of mean $\delta^{13}C$ of respired CO_2 from each sampling location (center, midpoint, edge, and ecotone) from the potential C mineralization incubations. The dark line represents the median and the box represents the first and third quartiles. Whiskers extend to the maximum and minimum of the data, excepting outliers (represented by points). The average $\delta^{13}C$ signature of respired CO_2 was higher in soils collected further away from the center of the dogwood shrub.

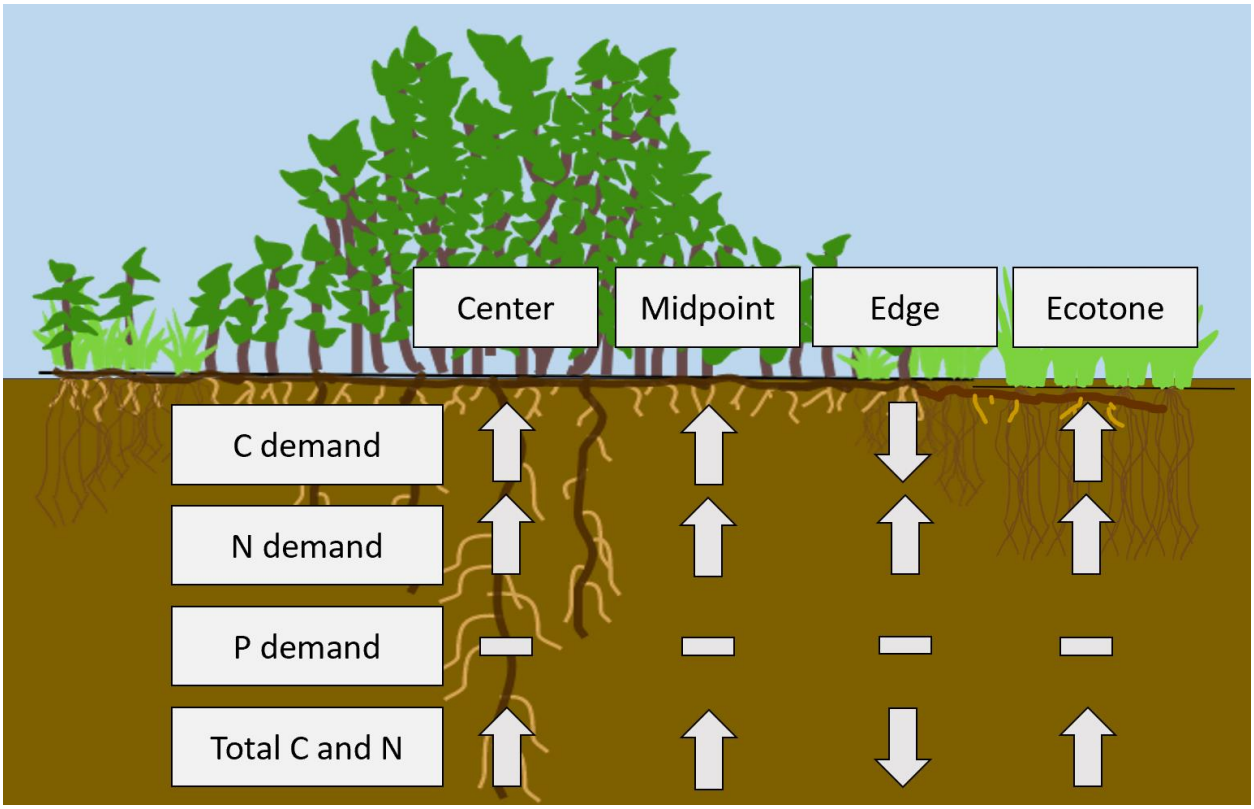

Figure 3.5 The response of soil properties and microbial processes as dogwood shrub islands grow larger. An upward arrow indicates that the property or process increases as dogwood shrubs grow larger while a downward arrow indicates the opposite. A dash indicates no change.

Chapter 4 - Plant legacies and soil microbial community dynamics control soil organic matter decomposition

Abstract

Through their litter and root inputs, plants modulate soil properties and soil microbial communities around them. In turn, these changes in soil properties and microbial community structure can impact plant performance (i.e., plant-soil feedbacks). Many studies have focused on how plant-soil feedbacks affect plant performance and successional patterns, but studies on the impact of plant-soil feedbacks on ecosystem processes are rare. The overall objective of our research was to investigate how species-specific plant-soil feedbacks affect rates of SOM decomposition. I addressed this by conducting a "home vs. away" plant-soil feedback greenhouse experiment using two C_3 grass species (*Bromus inermis* and *Pascopyrum smithii*) grown in C_4 tallgrass prairie soil. I used a closed-circuit CO_2 trapping method and isotopic analysis to differentiate between root-derived and SOM-derived CO_2 mineralization. Contrary to our predictions, plant-soil feedbacks on biomass were not connected to the effects of plant-soil interactions on SOM-derived CO_2 production, but I did detect a significant legacy effect of conditioning by *B. inermis.* During the second round, SOM-derived CO_2 production was always significantly higher in soils that were originally conditioned by *B. inermis* regardless of which plant species was being grown in those soils. Our results are likely due to the differential effects of plant species on soil chemistry and soil microbes during the original conditioning phase. This hypothesis is supported by the observation that differences in soil chemistry and bacterial community composition persisted in soils with different plant histories throughout the entire experiment. Together these results suggest that plant-soil history is important for SOM

mineralization and that changes in soil microbial community caused by shifts in plant species composition can have lasting effects on ecosystem processes.

Introduction

Plants are a critical link between aboveground and belowground processes. Through surface litter and root inputs, plants modulate the physical, chemical, and biological soil properties around them. In turn, these changes in soil properties impact plant performance (i.e., plant-soil feedbacks; Ehrenfeld *et al.* 2005; Van der Putten *et al.* 2013). Plant-soil feedbacks are considered to be positive if plant-mediated changes to the soil environment increases the growth rate or survival of that plant species (Bever, 1994). Whether plant-soil feedbacks are negative or positive may explain why some plants are rare while others are dominant (Klironomos, 2002). Multiple negative plant-soil feedbacks within a community have been posited to partially explain species coexistence, while multiple positive plant-soil feedbacks may lead to a single species dominating a community (Bever, 2003; Bonanomi et al., 2005). Plants that can accumulate beneficial mutualists are much more likely to dominate than plants that accumulate pathogens over time (Ehrenfeld et al., 2005; Jeschke, 2014). Additionally, plant-soil feedbacks can vary temporally and may switch from positive to negative (Bever et al., 1997; Diez et al., 2010). Through this accumulation of beneficial or harmful microbes, plants can create legacy effects on soil properties and soil microbial communities that persist even after the plants are no longer present, which can influence future plant success (Bartelt-Ryser et al., 2005; Hamman & Hawkes, 2013).

On average, plants allocate 17% of their total photosynthate belowground as rhizodeposits (Nguyen, 2003) and can potentially use that C investment to select microbes that

maximize their performance (Bais et al., 2006). Microbial communities use rhizodeposits and root exudates as an energy source to support growth and extracellular enzyme production (Dakora & Phillips, 2002). In return, microbes can assist plants with nutrient acquisition and protection against herbivores and pathogens (Philippot et al., 2013). Rhizodeposits vary in chemical composition and quality among plant species (Badri & Vivanco, 2009). Since plant species vary in root traits and belowground C inputs, plants have the potential to "cultivate" microbes that can best utilize the substrates released from their roots (Berg & Smalla, 2009; Grayston et al., 1998). In fact, plant species identity is the best correlate of rhizosphere microbial community composition at a local scale (Burns et al., 2015).

Rhizosphere processes also drive nutrient cycles (Singh et al., 2004). Previous research has shown that microbial diversity influences many ecosystem processes, such as N mineralization and plant N uptake (Weidner et al., 2015). However, little is known about how rhizosphere-induced variation in the functional diversity and composition of soil microbes affects SOM decomposition rates. In this study I aim to assess how changes in soil microbial communities due to plant-soil feedbacks affect SOM decomposition.

Plant-induced changes in soil microbial communities may play previously unappreciated roles in ecosystem processes (Weathers et al., 2016). Recent technical and methodological developments have allowed scientists to uncover the identities and function of microbes previously considered hidden within the "black box" of soil biodiversity (Zimmerman et al., 2014). Soil microbes are responsible for many transformations of nitrogen (N) and carbon (C). In many ways, microbes are the "gatekeepers" that mediate nutrient cycling (Docherty & Gutknecht, 2012). Therefore, it seems likely that plant-mediated changes in soil microbial communities should alter soil nutrient and C transformations. Although many studies have

focused on how plant-soil feedbacks affect plant performance and plant succession (Bever, 1994, 2003; Kardol et al., 2013), studies on the impact of plant-soil feedbacks on ecosystem processes are lacking.

In the present study, I conducted a greenhouse experiment to investigate the legacy effects of two species of C_3 grass, one native to tallgrass prairie and the other an exotic species generally considered to be invasive. In addition to characterizing potential legacy effects on plant performance, soil C fluxes, and soil communities, I tested two predictions: 1) The magnitude of SOM decomposition will be affected by plant-soil feedbacks (i.e., positive plant-soil feedbacks will increase SOM decomposition). 2) Plant-mediated changes to soil chemistry and the soil microbial community may persist after their removal which potentially may influence the magnitude of SOM decomposition.

Methods

Soil collection

All soil used in the greenhouse study was collected in 2015 from the upper 15 cm of an area of native tallgrass prairie dominated by C_4 grasses at the Konza Prairie Biological Station, Manhattan, Kansas, USA. The soil was a silty clay loam (fine, mixed mesic Pachic Argiustoll) classified as part of US Soil Survey's Dwight-Irwin complex. After collection, all soil was passed through a 6 mm mesh sieve, coarsely hand-picked to remove roots and stored air-dried in barrels until the greenhouse experiment began.

Greenhouse preparation

In June 2016, the collected soil was distributed into 180 pots (10 cm diameter x 25 cm deep) constructed from PVC pipe with airtight bottom caps. To allow better drainage and water

accumulation below the soil, a 0.5 kg nylon sandbag (~5 cm depth) was placed at the bottom of each pot. To inoculate the stored soil with a fresh microbial community, a small amount of freshly collected field soil from the field site was mixed into each pot (3% fresh soil in each pot). Commercially available seeds of the native C_3 grass *Pascopyrum smithii* and the invasive C_3 grass *Bromus inermis* (Stock Seed Farms, Murdock NE, USA) were germinated in potting soil in the greenhouse. One week after germination, individual seedlings were transplanted into each PVC pot so that half of the soils would be conditioned by *P. smithii* and half of the soils would be conditioned by *B. inermis*. After ten weeks of growth, the plants were harvested, and coarse root biomass was removed. Fine root biomass was picked from the soil and removed as much as possible. Soils that were conditioned by the same plant species were pooled, homogenized, and redistributed into new pots. Newly germinated seedlings of *P. smithii* and *B. inermis* were transplanted into pots with soil that had been conditioned by the same species for a second round of soil conditioning over another ten weeks. Ten weeks of soil conditioning has been shown to be sufficient for plants to significantly change their soil environment (Klironomos, 2002).

Greenhouse experiment

Following the second round of conditioning, the aboveground and belowground biomass of the plants was harvested. Fine root biomass was removed from the soil as much as possible. Soils that were conditioned by the same plant species were pooled, homogenized, and redistributed into new pots. For the experimental portion of this greenhouse study, the 180 pots were split into two 'Soil History' treatments based on the conditioning phase described above: 1) soil conditioned by *B. inermis* or 2) soil conditioned by *P. smithii*. Each pot was also assigned one of the three 'Current Plant' treatments: 1) one *B. inermis* seedling or 2) one *P. smithii* seedling, as well as 3) a no plant control. A subset of the pots was destructively sampled each

month over a 3-month growing period, so that after 12 weeks, 90 of the pots were sampled (Round 1: Month 1-1, Month 1-2, and Month 1-3). For the remaining 90 pots, the aboveground and belowground biomass was harvested. Fine roots were removed from the soil as much as possible. Soils belonging to the same treatment were pooled, homogenized, and redistributed in preparation for a second round of growth and sampling. A new seedling of either *P. smithii* or *B. inermis* was transplanted into each pot in the same distribution as the first round of the greenhouse experimental phase and allowed to grow for 12 weeks (Round 2: Month 2-1, Month 2-2, and Month 2-3). This was done to see if any legacy effects of the original conditioning phase on SOM decomposition or microbial properties persisted in pots that had the opposite plant growing in them during the first round of the experimental phase.

Throughout the conditioning and experimental phases of the greenhouse study, soils were kept at 35% gravimetric water content. Pots were watered with DI-H_2O via a 50cc syringe attached to a ~15 cm perforated tube inserted into the soil. The watering mechanisms were installed at the time the pots were filled. Each time pots were watered, DI-H_2O was poured into the syringe such that the perforated tube allowed water to be distributed evenly through the soil. Additionally, to prevent anoxia, the soils were aerated for ~1 hour every day via a vacuum pump connected to each pot to draw air through the soil. Pots also were rotated within the greenhouse every 2 weeks to avoid artifacts of placement in a particular location.

Carbon mineralization measurements and destructive sampling

A closed-circuit CO_2 trapping method (Cheng et al., 2003) was used after 4, 8, and 12 weeks of growth during each round of the experimental phase. Thirty pots underwent this process each trapping date (2 Soil History x 3 Current Plant x 5 replicates). Briefly, liquid silicone rubber (Silicones-Inc., High Point, NC, USA) was spread over the surface soil of each

pot to form an airtight seal separating aboveground and belowground portions of the pots and plants, which allowed sampling of CO_2 released from soil and intact plant roots. After allowing the silicone rubber to cure for 16-18 h, each pot was connected to a soda lime column and the soil atmosphere was scrubbed for 40 minutes with the closed-circuit system to ensure that I wase only trapping CO_2 produced during the measurement period. For 24 hours, all the CO_2 produced belowground in each pot was trapped by bubbling air via air stones in the trapping circuit through bottles containing 300 ml of 0.25M NaOH. After trapping was completed, the silicone rubber was removed, and the pots and plants were destructively sampled. Two subsamples of soil were collected from each pot. One subsample was stored at 4°C for subsequent nutrient and microbial biomass analysis, and the other (collected from the rhizosphere) was stored at -20°C for subsequent microbial community analysis. In pots that contained plants, aboveground and belowground biomass was collected. After rinsing the belowground biomass with DI-H_2O, all plant biomass was dried at 60°C for 48 hours and weighed.

Microbial biomass analysis

Soil microbial biomass C was determined using a fumigation-extraction method (Jenkinson & Powlson, 1976) and calculated as the difference between fumigated and unfumigated samples. For each unfumigated sample, ~15 g of moist soil was extracted with 75 ml of 0.5M K_2SO_4 on a shaker table at 200 rpm for 1 hour. Extracts were passed through a 0.4 µm polycarbonate filter and stored at -20°C. Another set of soil samples was placed into a vacuum desiccator and fumigated with chloroform under a vacuum for 48 hours. Following fumigation, the beaker of chloroform was removed, and residual chloroform was removed from soil samples by repeatedly applying a vacuum and opening the chambers. Total organic C in the

extracts was measured with a Shimadzu TOC-L dissolved carbon analyzer (Shimadzu, Kyoto, Japan).

Total inorganic nitrogen

Approximately 12 g of moist soil was extracted with 50 ml of 2N KCl on a shaker table at 200 rpm for 1 hour. Extracts were passed through a 0.4 µm polycarbonate filter and frozen for later analysis. Extractable inorganic nitrogen (NH_4^+ and NO_3^-) was determined colorimetrically at the Soil Testing Lab at Kansas State University (Manhattan, KS, USA).

Determining SOM-derived CO_2 and RPE

Total CO_2 respired from soil and plant roots was determined from the inorganic C content of the NaOH traps, measured on a Shimadzu TOC-L dissolved carbon analyzer (Shimadzu, Kyoto, Japan). To determine $\delta^{13}C$ of the respired C, trapped CO_2 was precipitated as $SrCO_3$ by adding excess 1 M $SrCl_2$ to a subsample of the NaOH traps. The precipitate was rinsed with DI-H_2O once every 24 hours for 7 days to neutralize pH, and then dried at 105°C for 24 hours. The $\delta^{13}C$ of the $SrCO_3$ was measured by mass spectrometry at the UC Davis Stable Isotope Facility. The proportion of CO_2 derived from SOM was calculated according to the following isotope mixing model equation: $\% SOM_{CO_2} = \frac{\delta_t - \delta_p}{\delta_s - \delta_p} * 100$. In this equation δ_t represents the $\delta^{13}C$ value of the trapped CO_2. The δ_p represents the $\delta^{13}C$ value of the plants. In this study, I used a value of -27.5‰ for δ_p. The δ_s represents the $\delta^{13}C$ value of the soil. I used a value of -16.24‰ for δ_s based on analysis of the collected bulk soil.

Calculating Plant-soil Feedback (PSF) and SOM-derived CO_2 Feedback

Feedback effects for plant biomass or SOM-derived CO_2 were calculated for both *B. inermis* and *P. smithii* using the following equation: $\ln(\frac{x_{home}}{x_{away}})$. In this equation x_{home} represents

the value of the response variable when a plant is grown in its "home" soil (i.e., conditioned by a conspecific) while x_{away} represents the value of the response variable when a plant is grown in the "away" soil (i.e., not conditioned by a conspecific). The *boot* package in the R statistical software was used to calculate the bias-corrected and accelerated (BCa) bootstrap 95% confidence interval (CI) for each feedback metric for each plant species using 999 permutations. The feedback effect was considered significant if the BCa bootstrap 95% CI did not cross zero. Because it takes time for feedback effects to develop, they were only calculated for the final sample date in each of the two rounds of the experiment (i.e., Month 1-3 and Month 2-3).

16S rRNA gene sequencing

Genomic DNA was extracted from soils using a MoBio PowerSoil Extraction kit according to the manufacturer's instructions. Successful genomic DNA (gDNA) extraction was confirmed using a NanoDrop spectrophotometer. The bacterial and archaeal 16S rRNA gene was targeted using universal bacterial primers (515F/806R) and amplified using PCR according to Earth Microbiome Project protocols (Caporaso et al., 2012) with a few exceptions. First, I added 2 µl of 1% Bovine Serum Albumin, 0.25 µl of $MgCl_2$ and double the amount of gDNA to each reaction well. Additionally, PCR was only run for 25 cycles instead of 35. Each sample was amplified in triplicate, and amplification was confirmed using gel electrophoresis. Each sample was normalized by DNA concentration and combined into a single amplicon library. The combined library was cleaned using a QIAquick Gel Extraction Kit according to included instructions. The library was sequenced using a 2 x 150 paired-end Illumina MiSeq run, with v2 reagents and a 10% PhiX spike, in the Integrated Genomics Facility at Kansas State University (Manhattan, KS, USA).

Processing sequencing data

Raw sequence data were initially processed with the QIIME software. Sequences were quality filtered, joined and demultiplexed, and assigned to operational taxonomic units (OTUs) at 97% sequence similarity. OTUs were aligned to the GreenGenes v. 13.8 16S rRNA gene reference database, and taxonomy was assigned using the RDP classifier. Chimeras were identified with CHIMERASLAYER and removed from further analysis. From this point forward, the data were exported for further processing using the *phyloseq* package within the R statistical software (McMurdie & Holmes, 2013). After ensuring that the DNA extraction and PCR blanks contained a low OTU richness, the *decontam* package identified and removed 168 likely contaminant sequences using a X^2 analysis. Any sample that retained fewer than 15,000 reads at this point was removed from further analysis. Next, I filtered the taxa so that the data only included OTUs from the kingdom Bacteria. Additionally, I excluded any OTUs associated with mitochondria or chloroplasts. Finally, I removed rare OTUs (<10% relative abundance). Alpha diversity, evenness, and phylum-level response to soil conditioning were estimated using these data. OTU richness and evenness were estimated by rarefying each sample to have the same number of reads as the sample with the least number of reads (14,462 reads). For our multivariate analyses, I normalized OTU relative abundances by the median number of reads across samples. The final dataset included 11,019,079 reads in 139 total samples that were affiliated with 14,770 unique OTUs.

Statistical analyses

Two-way analysis of variance (ANOVA) was used to analyze the effects of Soil History, Current Plant, and their interaction on three different aspects of plant biomass (aboveground, belowground, and total). Since I were interested in how the aforementioned variables affected

cumulative plant performance, only the data from the final month of each round (Month 1-3 and Month 2-3) were used.

For the analyses addressing SOM-derived C mineralization rates, root-derived C mineralization rates, microbial biomass C, and total inorganic N, the data were subset to include only samples from pots with plants growing in them at the time of measurement and then further subset by Round of the greenhouse experiment. Linear mixed models were used to determine the fixed effects of Soil History, Current Plant, and their interaction using the formula: Response ~ Soil History * Current Plant + (1|Month). Month was treated as a random effect in each model. SOM-derived C mineralization rates were standardized by soil C content while root-derived C mineralization rates are standardized at the pot level in order to avoid the inflation of root-specific C mineralization values when root biomass was very low.

Permutational analysis of variance (PERMANOVA) was used to assess the effects of Soil History, Current Plant, Month, and their interactions on bacterial community composition. Because there were significant Soil History * Month and Current Plant * Month interactions, each month was also analyzed separately using PERMANOVA. Canonical Analysis of Principle Coordinates (CAP) was used to visualize differences between treatments (M. J. Anderson & Willis, 2003). I used the formula ~Soil History * Current Plant * Month to constrain our CAP. This process was repeated for 100 permutations in order to calculate a mean richness and evenness for each sample. OTU evenness was calculated using the Inverse Simpson index. I used ANOVA to determine the effects of Soil History, Current Plant, Month, and all possible interactions on OTU richness and evenness. Significant phyla-level responses to conditioning by *B. inermis* was determined using the *DESeq2* package (Love et al., 2014) within the R statistical software. If a response is positive, then that particular phylum is significantly more abundant in

soils originally conditioned by *B. inermis*. Throughout the manuscript, all means are reported ± standard error of the mean.

Results

Plant biomass

At the end of Round 1, total biomass of *B. inermis* was 2.07 ± 0.347 g when grown in its "home" soil (i.e., soil that was originally conditioned by a conspecific) but only 0.766 ± 0.191 g when grown in away soil (i.e., soil that was not originally conditioned by a conspecific). Total biomass of *P. smithii* was 0.358 ± 0.045 g when grown in its home soil and 0.644 ± 0.047 g when grown in away soil (Figure 4.1). The effect of both Soil History and Current Plant on total plant biomass was significant (Table 4.1). Total plant biomass was 133% greater, on average across both species, if soil was originally conditioned by *B. inermis*. Additionally, *B. inermis* biomass was 141% greater than *P. smithii* biomass across Soil History. Aboveground biomass of *B. inermis* was 0.902 ± 0.077 g when grown in its home soil and 0.358 ± 0.064 g when grown in away soil. Aboveground biomass of *P. smithii* was 0.208 ± 0.028 g when grown in its home soil and 0.423 ± 0.029 g when grown in away soil (Figure 4.1). Similar to total biomass, the effect of Soil History and Current Plant were significant on aboveground biomass (Table 4.1). Aboveground biomass was 139% higher, on average across both species, if the soil was originally conditioned by *B. inermis*. Additionally, *B. inermis* had 88% greater aboveground biomass than *P. smithii* across Soil History. Belowground biomass of *B. inermis* was 1.17 ± 0.322 g when grown in its home soil and 0.408 ± 0.133 g when grown in away soil. Belowground biomass of *P. smithii* was 0.150 ± 0.019 g when grown in its home soil and 0.221 ± 0.019 g when grown in away soil (Figure 4.1). Only Current Plant significantly affected

belowground biomass (Table 4.1). On average, *B. inermis* belowground biomass was 176% greater than *P. smithii* belowground biomass across Soil History. Overall, the root:shoot ratio for *B. inermis* was 88% higher than *P. smithii* across Soil History in Round 1 (Figure 4.1).

At the end of Round 2, total biomass of *B. inermis* was the same whether grown in its home soil (2.15 ± 0.318 g) or grown in its away soil (2.14 ± 0.140 g). Total biomass of *P. smithii* was 0.863 ± 0.152 g when grown in its home soil and 1.19 ± 0.154 g when grown in away soil (Figure 4.1). At the end of Round 2, the only significant effect on total plant biomass was Current Plant species (Table 4.1), with *Bromus inermis* biomass being 109% higher, on average, than *P. smithii* biomass across Soil History. Aboveground biomass of *B. inermis* was 1.02 ± 0.106 g when grown in its home soil and 1.16 ± 0.099 g when grown in away soils

Aboveground biomass of *P. smithii* was 0.724 ± 0.135 g when grown in its home soil and 0.900 ± 0.159 g when grown in away soil (Figure 4.1). Again, similar to patterns in total biomass, only the effect of Current Plant was significant at the end of Round 2 (Table 4.1). On average, *B. inermis* aboveground biomass was 35% higher than *P. smithii* across Soil History. Belowground biomass of *B. inermis* was 1.13 ± 0.326 g when grown in its home soil and 0.981 ± 0.101 g when grown in away soil. Belowground biomass of *P. smithii* was 0.139 ± 0.025 g when grown in its home soil and 0.295 ± 0.055 g when grown in away soil (Figure 4.1). Consistent with patterns for the other aspects of plant biomass during Round 2, only Current Plant significantly affected belowground biomass (Table 4.1). Belowground *B. inermis* biomass was 109% greater, on average, than the belowground biomass of *P. smithii* across Soil History. Overall, the root:shoot ratio for *B. inermis* was 266% higher than *P. smithii* across Soil History in Round 2 (Figure 4.1).

Soil respiration rates

In Round 1, the average SOM-derived C mineralization rate was 220 ± 58.8 µg $C\text{-}CO_2$ g^{-1} soil C day^{-1} when *B. inermis* was grown in its home soil and 263 ± 58.6 µg $C\text{-}CO_2$ g^{-1} soil C day^{-1} when *B. inermis* was grown in away soil. The average SOM-derived C mineralization rate was 241 ± 75.6 µg $C\text{-}CO_2$ g^{-1} soil C day^{-1} when *P. smithii* was grown in its home soil and 255 ± 68.7 µg $C\text{-}CO_2$ g^{-1} soil C day^{-1} when *P. smithii* was grown in away soil (Figure 4.2). In Round 2, the SOM-derived C mineralization rate was 633 ± 50.1 µg $C\text{-}CO_2$ g^{-1} soil C day^{-1} when *B. inermis* was grown in its home soil and 547 ± 42.3 µg $C\text{-}CO_2$ g^{-1} soil C when *B. inermis* was grown in away soil. The SOM-derived C mineralization rate was 571 ± 58.6 µg $C\text{-}CO_2$ g^{-1} soil C day^{-1} when *P. smithii* was grown in its home soil and 628 ± 55.7 µg $C\text{-}CO_2$ g^{-1} soil C day^{-1} when *P. smithii* was grown in away soil (Figure 4.2). Only Soil History significantly affected SOM-derived C mineralization rates and only during Round 2 of the greenhouse experiment (Table 4.2). Soil originally conditioned by *B. inermis* mineralized 12% more SOM-derived organic C regardless of the identity of the Current Plant species.

During Round 1, the average root-derived C mineralization rate (i.e., autotrophic respiration or microbial breakdown of recent root-derived organic C) of *B. inermis* was 2.19 ± 0.782 mg $C\text{-}CO_2$ pot^{-1} day^{-1} when grown in its home soil and 1.10 ± 0.298 mg $C\text{-}CO_2$ pot^{-1} day^{-1} when grown in away soil. The average root-derived C mineralization rate of *P. smithii* was 0.433 ± 0.175 mg $C\text{-}CO_2$ pot^{-1} when was grown in its home soil and 1.32 ± 0.310 mg $C\text{-}CO_2$ pot^{-1} day^{-1} when was grown in away soil (Figure 4.2). During Round 2, the average root-derived C mineralization rate of *B. inermis* was 8.51 ± 1.63 mg $C\text{-}CO_2$ pot^{-1} day^{-1} when was grown in its home soil and 8.63 ± 1.61 mg $C\text{-}CO_2$ pot^{-1} day^{-1} when grown in away soil. The average root-

derived C mineralization rate of *P. smithii* was 3.77 ± 0.766 mg C-CO_2 pot^{-1} day^{-1} when grown in its home soil and 5.59 ± 1.02mg C-CO_2 pot^{-1} day^{-1} when grown in away soil (Figure 4.2).

During Round 1, Soil History significantly influenced root-derived C mineralization rates (Table 4.2). Across both species, root-derived C mineralization was 200% higher when grown in soil originally conditioned by *B. inermis.* During Round 2, root-derived C mineralization was significantly affected by Soil History, Current Plant, and their interaction (Table 4.2). Root-derived C mineralization was significantly lower when *P. smithii* was grown in its home soil than the other treatments (Figure 4.2).

Soil chemistry

Soils that were conditioned with *B. inermis* or *P. smithii* had distinct soil C and N concentrations that persisted throughout the study (Figure 4.3; Table 4.2). Across both rounds of the greenhouse experiment, soils that were originally conditioned by *B. inermis* had an average C concentration of 32.6 ± 0.119 mg C g^{-1} soil, about 15% more C than in soils conditioned by *P. smithii* (28.3 ± 0.133 mg C g^{-1} soil). Average total N was also 11% greater in soils originally conditioned by *B. inermis* (2.76 ± 0.011 mg N g^{-1} soil) than soils originally conditioned by *P. smithii* (2.48 ± 0.011 mg N g^{-1} soil). However, inorganic N concentrations were significantly greater in soils originally conditioned by *P. smithii* during both rounds of the greenhouse experiment (Figure 4.3; Table 4.2). In addition, extractable inorganic N concentrations were lower in soils that had *B. inermis* growing in them during Round 2. This suggests that *B. inermis* took up more N than *P. smithii* during this round of the experiment. Also, during Round 2, inorganic N was lowest when *B. inermis* was grown in its home soil, and it was highest when *P. smithii* was grown in its home soil. Concentrations of inorganic N were intermediate when either species was grown in its away soil.

Microbial biomass carbon (MBC)

During Round 1, there were significant effects of Soil History and Current Plant on MBC (Table 4.2). Across both plant species, soil that was originally conditioned by *B. inermis* contained an average 199 ± 5.65 µg MBC g^{-1} soil while soil originally conditioned by *P. smithii* contained an average 160 ± 5.56 µg MBC g^{-1} soil. Across Soil History, there was an average 172 ± 7.80 µg MBC g^{-1} soil if *B. inermis* was the Current Plant, and an average of 188 ± 5.15 µg MBC g^{-1} soil if *P. smithii* was the Current Plant (Figure 4.3). During Round 2, only Soil History significantly influenced MBC (Table 4.2). Across both plant species, soil that was originally conditioned by *B. inermis* had a higher MBC (209 ± 2.64 µg MBC g^{-1} soil) than soil originally conditioned by *P. smithii* (172 ± 6.28 µg MBC g^{-1} soil; Figure 4.3).

Plant-soil feedbacks

According to the BCa bootstrap 95% CI of plant-soil feedback, *B. inermis* experienced a significant positive plant-soil feedback in Round 1 of the experiment (Figure 4.4). However, there was no significant PSF for *B. inermis* in Round 2. In contrast, PSFs for *P. smithii* were significantly negative in both rounds of the experiment. Feedback effects on SOM-derived C mineralization rates were not consistent with PSF during either round of the experiment. There was no significant feedback on SOM-derived C mineralization at the end of Round 1 for *B. inermis* or at the end of Round 2 for *P. smithii*. However, both species experienced positive feedback on SOM-derived C mineralization rates during one of the rounds of the greenhouse experiment (Figure 4.4).

Bacterial community composition

According to our PERMANOVA, bacteria community composition was significantly influenced by Soil History, Current Plant, and Month (Table 4.3). Additionally, there were

significant interactive effects of Current Plant * Month and Soil History * Month. Because there was such a strong temporal signal in bacteria community composition, the data were also analyzed separately by month (Table 4.4). Soil History significantly influenced bacteria community composition throughout the entire experiment (Table 4.4; Figure 4.5). In contrast, the effect of Current Plant was only significant at the beginning of Round 1 (Month 1-1) and the end of the Round 2 (Month 2-2 and Month 2-3; Table 4.4).

Bacterial community richness and evenness

Throughout the entire experiment, OTU richness was significantly higher in soil that was originally conditioned by *B. inermis* (3151 ± 25 OTUs) than in soil originally conditioned by *P. smithii* (3002 ± 28 OTUs; Table 4.5, Figure 4.6). On average, soils without plants growing in them at the time of measurement had lower richness (3032 ± 32 OTUs) than soils with either *B. inermis* or *P. smithii* growing in them (3114 ± 37 OTUs and 3107 ± 31 OTUs, respectively). OTU richness also varied across time, but there was no clear temporal directionality (Figure 4.6).

Similar to patterns of richness, evenness was also significantly higher in soil that was originally conditioned by *B.* inermis (197 ± 7.75) than in soil conditioned by *P. smithii* (157 ± 7.59; Table 4.5, Figure 4.6). Throughout the greenhouse experiment, OTU evenness steadily increased over time, from 100 ± 4.84 in Month 1-1 to 255 ± 14.8 in Month 2-3 (Figure 4.6). There was no effect of Current Plant on OTU evenness, but there was a significant effect of the interaction of Current Plant and Month on evenness (Table 4.5).

Soil bacterial taxonomic response to conditioning treatment

Proteobacteria and Actinobacteria were the two most abundant phyla across all samples. The most abundant families were Chthoniobacteriaceae, Gaiellaceae, and Oxalobacteraceae. The phyla FCPU426, AD3, TM6, Nitrospirae, and TM7 were significantly less abundant in soils

originally conditioned by *B. inermis* (Figure 4.7). The phyla Acidobacteria, Bacteroidetes, Gemmatimonadetes, Cyanobacteria, FBP, Chlamydiae, WPS-2, WS4, and BHI80-139 were significantly more abundant in soils originally conditioned by *B. inermis* (Figure 4.7).

Discussion

In this study, I used a plant-soil feedback approach to compare the legacies of two plant species: *B. inermis*, a weedy species exotic to tallgrass prairie, and *P. smithii*, a native C_3 grass in the same field-collected prairie soil on plant performance, soil C fluxes, and soil communities. Overall, I found that plant species-specific legacy effects (i.e., Soil History) impacted all three, indicating that plant conditioning of soil microbial communities and nutrient pools can occur over relatively short (20 weeks) time scales.

Plant species-specific differences in root allocation within their "home" soils could suggest at explanation for the source of legacy effects in our study. In addition to having a higher biomass overall, *B. inermis* allocated proportionally more biomass belowground than *P. smithii* (Figure 4.1; Table 4.1). I suggest that because of the higher allocation belowground, *B. inermis* impacted soil chemistry by increasing soil C and converting soil inorganic N to plant organic N (Figure 4.3). In addition, greater or different belowground inputs by *B. inermis* during the conditioning period may have affected soil bacterial community structure and diversity (Figures 4.5 – 4.7) and increased microbial biomass (Figure 4.3). Together, the apparent consequences for soil C mineralization were significant but idiosyncratic: Six months after the conditioning phase, SOM-derived C mineralization was higher in soil with a history of *B. inermis*, and root-derived C mineralization was lower when *P. smithii* was grown in soil with its own history rather than a

history of *B. inermis* (Figure 4.2; Table 4.2). This suggests that the chemical and microbiological changes that plants make to soil can have long-lasting effects on ecosystem processes.

In the following sections, I discuss the role of plant species-specific legacy effects on SOM decomposition in the context of our original predictions:

Conclusion 1: PSF effects on plant biomass are not related to PSF effects on SOM-derived C mineralization rates

I originally hypothesized that plant-soil feedbacks would help explain the magnitude of SOM-derived C mineralization rates. PSFs for *P. smithii* were consistently negative over both rounds of the greenhouse experiment, while PSFs for *B. inermis* were positive after Round 1 but neutral after Round 2 (Figure 4.4). SOM-derived C mineralization feedbacks were temporally variable but were always positive when significant. At the end of Round 1, more SOM was mineralized when *P. smithii* was grown in its home soil than in away soil. The same was true for *B. inermis* at the end of Round 2 (Figure 4.4).

Contrary to our hypothesis, I did not find evidence that PSF effects on biomass helped explain the magnitude of SOM decomposition in our study since PSFs and SOM-derived C mineralization feedbacks never aligned (Figure 4.4). This is somewhat surprising because I expected that when plants perform worse in certain soils, they might not be able to allocate as much C belowground. Microbial "priming" of soil organic matter decomposition is positively correlated to root exudation rates (Bengtson et al., 2012). However, root biomass is not necessarily correlated with root exudation rates (Eisenhauer et al., 2017), so it is likely that factors other than plant biomass (such as microbial community composition) were stronger controls of SOM decomposition in our study.

Temporal variation in our feedback results could be due to changes in soil microbial community composition and diversity across the duration of the greenhouse experiment (Figures 4.5 – 4.6). If the direction and magnitude of PSFs are determined by the composition of the soil microbial community, then it makes sense that the feedbacks I measured changed as the soil microbial community changed. PSFs can vary through time because plants do not respond similarly to all members of a dynamic soil community (Hawkes et al., 2013). The results from our study suggest that this concept can be extended to feedbacks that affect ecosystem processes such as SOM decomposition and that predicting feedback effects is difficult if the soil microbial community changes over time. Overall, the lack of a link between PSFs and SOM-derived C mineralization feedbacks suggests that the mechanisms of PSF that drive population and community-level responses are likely to be different from the mechanisms that drive ecosystem-level responses. More research dedicated to placing PSF effects on ecosystem processes within a temporal context is clearly needed.

Conclusion 2: *B. inermis* and *P. smithii* leave legacy effects on soil microbial properties which affect rates of SOM decomposition

I originally predicted that each plant species would alter soil chemical and microbiological properties and that these changes may influence SOM decomposition. In line with our hypothesis, Soil History was an important factor that affected microbial community composition, diversity, and SOM-derived C mineralization in this study (Tables 4.2 – 4.5). It seems that the original conditioning phase of the greenhouse experiment established unique bacterial communities that changed over time, but with two different trajectories that never converged (Figure 4.5). Bacterial community composition turned over throughout the experiment, such that the microbial communities at the end of the conditioning phase were

completely different than the communities at the end of Month 2-3. However, despite the turnover in microbial community composition, Soil History always significantly impacted soil respiration (Figure 4.2). These results further support the idea that single manipulations of the soil community, or initial changes initiated by a particular plant species, can leave long-lasting legacies on community composition (Wubs et al., 2019).

I was able to detect a signal of *B. inermis* on microbial properties and ecosystem processes even in soils where it had been removed six months prior. At the end of the experiment, microbial biomass C and both aspects of soil respiration (SOM-derived and plant-derived) were higher in soils with a Soil History of *B. inermis* (Figures 4.2 - 4.3). The legacy effect of *B. inermis* conditioning may contribute to its ability to act as an invasive species (Gibbons et al., 2017). By increasing the rate of SOM decomposition, *B. inermis* might increase nutrient release from SOM. Increasing nutrient availability is one potential strategy that invasive plants use to succeed in novel environments (Zhou & Staver, 2019). Legacy effects are persistent after invasive plant removal (Corbin & D'Antonio, 2012; Hamman & Hawkes, 2013), and our results demonstrate that invasive plants such as *B. inermis* can leave lasting impacts on ecosystem properties.

Conclusion

First, our study suggests that the impact of plant-soil interactions on ecosystem processes depends on the temporal dynamics of the soil microbial community rather than feedback effects on plant biomass. Second, feedbacks between plants and soils that affect both performance (PSF) and ecosystem processes (SOM-derived C mineralization) will be temporally variable if there is turnover in the microbial community. Finally, through their impacts on soil microbial and chemical properties, plants can have long lasting effects on SOM decomposition that persist even

after their removal. This study suggests that a full understanding of the ecosystem processes occurring at a certain point in time requires us to understand the history of that ecosystem in addition to contemporary community dynamics.

Acknowledgements

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Table 4.1 Results from two-way ANOVA on four aspects of plant biomass responses (aboveground biomass, belowground biomass, total biomass, and root:shoot ratio) at the end of both rounds of the greenhouse experiment. Soil History was determined by the identity of the plant species that originally conditioned the soil. Current Plant was determined by the identity of the plant species growing in the pot at the time of measurement. A Kruskal-Wallis test was used instead of ANOVA for testing the effects of Soil History and Current Plant on root:shoot ratio during Round 1.

	Round 1		**Round 2**	
Aboveground biomass	F	p	F	p
Soil History	34.7	**<0.001**	0.0210	0.888
Current Plant	18.3	**<0.001**	4.91	**0.042**
S * P	0.671	0.425	1.53	0.234
Belowground biomass	F	p	F	p
Soil History	3.70	0.072	4.02	0.062
Current Plant	7.66	**0.014**	68.2	**<0.001**
S * P	0.631	0.439	3.52	0.079
Total biomass	F	p	F	p
Soil History	18.3	**<0.001**	0.687	0.419
Current Plant	19.8	**<0.001**	29.9	**<0.001**
S * P	1.29	0.272	0.629	0.439
Root:shoot ratio	X^2	p	F	p
Soil History	0.571	0.450	2.59	0.127
Current Plant	5.14	**0.023**	34.3	**<0.001**
S * P	-	-	0.798	0.385

Table 4.2 Results from linear mixed models estimating the effects of Soil History, Current Plant, and their interaction on soil respiration (SOM-derived and root-derived), soil chemistry (total C, total N, and inorganic N), and microbial biomass C at the end of both rounds of the greenhouse experiment. Soil History was determined by the identity of the plant that originally conditioned the soil (*B. inermis* or *P. smithii*). Current Plant was determined by the identity of the plant growing in the pot at the time of measurement (*B. inermis* or *P. smithii*). Month was treated as a random effect in the models.

	Round 1		Round 2	
SOM-derived respiration	X^2	p	X^2	p
Soil History	1.52	0.217	7.08	**0.008**
Current Plant	2.02	0.156	0.0569	0.811
S * P	0.204	0.652	0.0928	0.761
Root-derived respiration	X^2	p	X^2	p
Soil History	7.15	**0.008**	3.90	**0.048**
Current Plant	2.88	0.090	30.8	**<0.001**
S * P	1.31	0.253	4.25	**0.039**
Microbial biomass C	X^2	p	X^2	p
Soil History	57.0	**<0.001**	31.6	**<0.001**
Current Plant	9.87	**0.002**	1.23	0.268
S * P	0.0950	0.758	0.650	0.420
Total C	X^2	p	X^2	p
Soil History	404	**<0.001**	256	**<0.001**
Current Plant	0.0710	0.789	0.549	0.459
S * P	0.134	0.714	0.898	0.344
Total N	X^2	p	X^2	p
Soil History	213	**<0.001**	169	**<0.001**
Current Plant	0.0240	0.878	0.494	0.482
S * P	1.25	0.264	0.122	0.727
Soil inorganic N	X^2	p	X^2	p
Soil History	120	**<0.001**	89.8	**<0.001**
Current Plant	0.123	0.726	79.7	**<0.001**
S * P	0.0370	0.847	8.68	**0.003**

Table 4.3 PERMANOVA results for the effects of Soil History, Current Plant, Month, and all their interactions on soil bacterial community composition. Soil History was determined by the identity of the plant that originally conditioned the soil (*B. inermis* or *P. smithii*). Current Plant was determined by the identity of the plant growing in the pot at the time of measurement (*B. inermis, P. smithii,* or no plant).

	F	R^2	p-value
Soil History	20.2	0.091	**0.001**
Current Plant	1.68	0.0152	**0.027**
Month	11.7	0.262	**0.001**
S * P	0.989	0.00890	0.424
S * M	0.0442	0.0153	**0.019**
P * M	1.60	0.0718	**0.001**
S * P * M	1.07	0.0480	0.266
Residuals		0.468	

Table 4.4 PERMANOVA results for the effects of Soil History, Current Plant, and their interaction on soil bacteria community composition when data were separated by month. Soil History was determined by the identity of the plant that originally conditioned the soil (*B. inermis* or *P. smithii*). Current Plant was determined by the identity of the plant growing in the pot at the time of measurement (*B. inermis*, *P. smithii,* or no plant). Months 1-1, 1-2, and 1-3 comprised Round 1 of the greenhouse experiment, and months 2-1, 2-2, and 2-3 comprised Round 2 of the greenhouse experiment.

	F	R^2	p-value
Month 1-1:			
Soil History	2.83	0.105	**0.002**
Current Plant	1.45	0.107	**0.046**
S * P	1.11	0.0827	0.245
Residuals		0.705	
Month 1-2:			
Soil History	5.16	0.185	**0.001**
Current Plant	1.32	0.0947	0.077
S * P	1.07	0.0766	0.360
Residuals		0.644	
Month 1-3:			
Soil History	6.02	0.215	**0.001**
Current Plant	1.05	0.0749	0.348
S * P	0.942	0.0673	0.549
Residuals		0.643	
Month 2-1:			
Soil History	6.07	0.210	**0.001**
Current Plant	1.28	0.0884	0.146
S * P	1.15	0.0795	0.253
Residuals		0.622	
Month 2-2:			
Soil History	3.38	0.126	**0.001**
Current Plant	1.89	0.141	**0.001**
S * P	0.863	0.0642	0.772
Residuals		0.670	
Month 2-3:			
Soil History	4.23	0.177	**0.001**
Current Plant	2.14	0.178	**0.001**
S * P	1.22	0.102	0.135
Residuals		0.543	

Table 4.5 Two-way ANOVA results for the effects of Soil History, Current Plant, Month, and all their interactions on soil bacterial richness and evenness. Soil History was determined by the identity of the plant that originally conditioned the soil (*B. inermis* or *P. smithii*). Current Plant was determined by the identity of the plant growing in the pot at the time of measurement (*B. inermis, P. smithii,* or no plant).

Richness	**F**	**p-value**
Soil History	40.3	**<0.001**
Current Plant	4.78	**0.010**
Month	38.3	**0.001**
S * P	1.56	0.215
S * M	0.953	0.451
P * M	1.80	0.070
S * P * M	1.04	0.414
Evenness	**F**	**p-value**
Soil History	30.4	**<0.001**
Current Plant	1.93	0.151
Month	31.4	**<0.001**
S * P	0.006	0.994
S * M	1.79	0.121
P * M	2.06	**0.034**
S * P * M	1.36	0.208

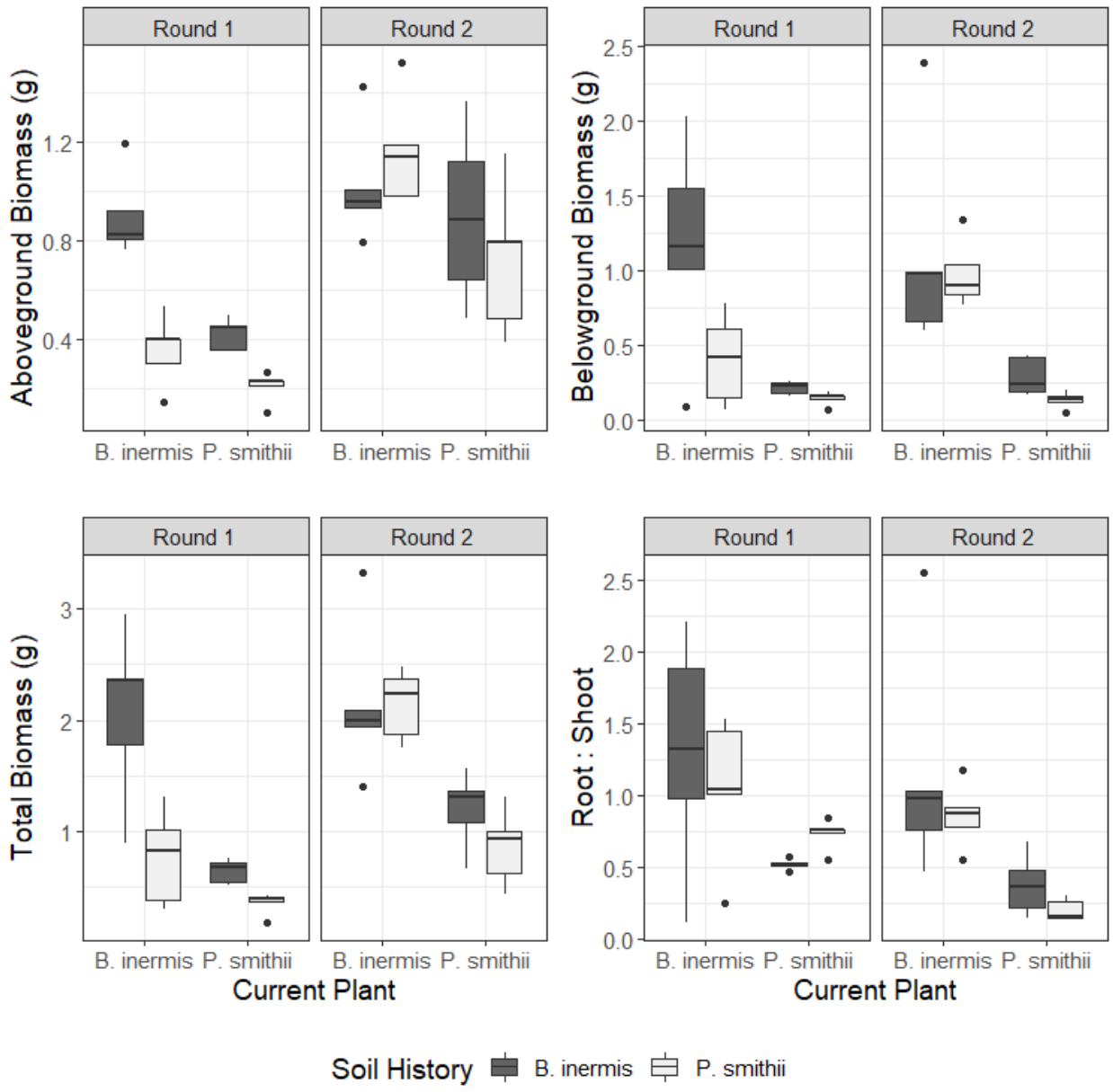

Figure 4.1 Boxplots of four aspects of plant biomass (aboveground, belowground, total, and root:shoot) measured at the end of Round 1 and Round 2 of the greenhouse experiment. The dark line represents the median and the box represents the first and third quartiles. Whiskers extend to the maximum and minimum of the data, excepting outliers (represented by points). The color of the box represents Soil History.

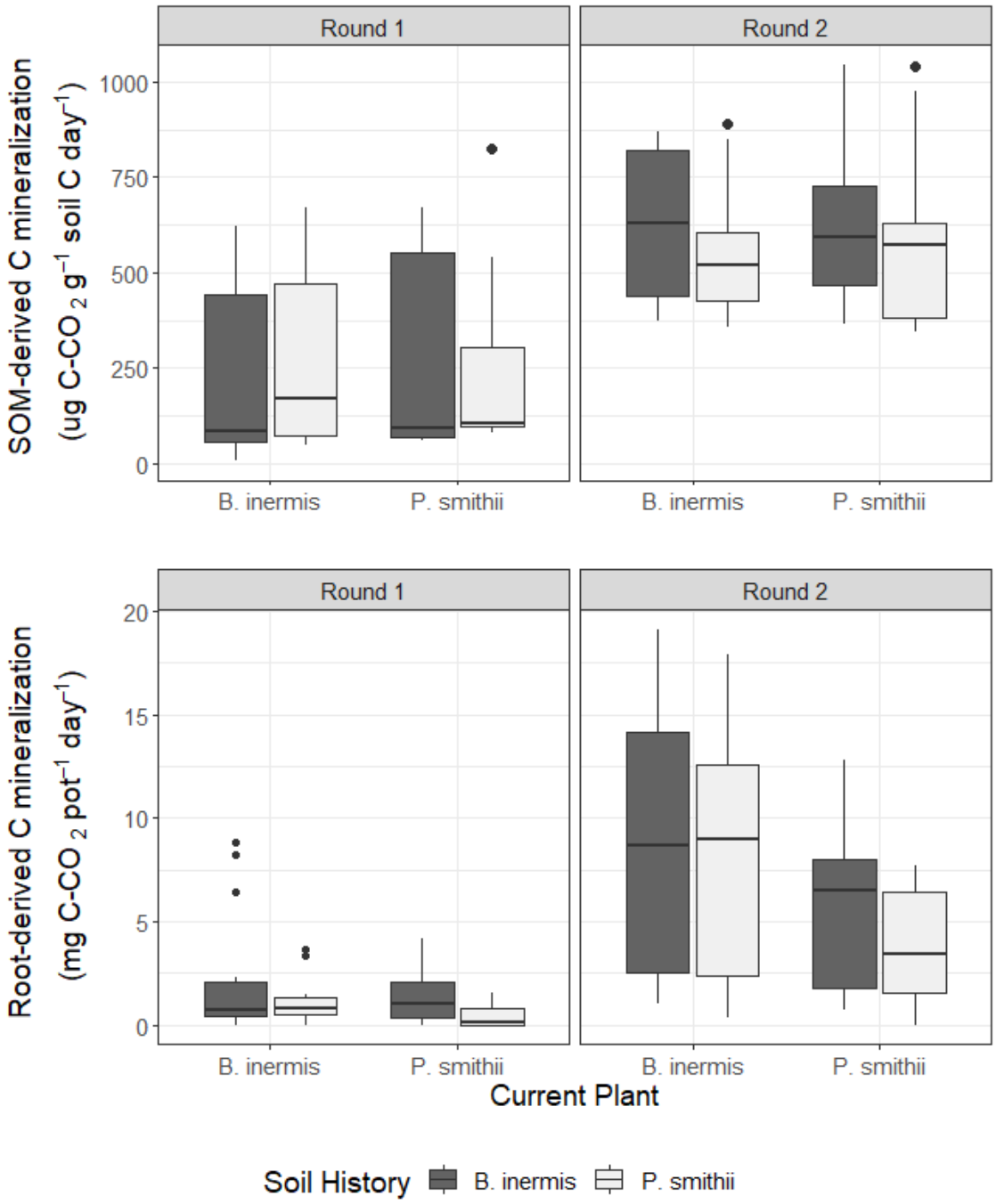

Figure 4.2 Boxplots of soil respiration (SOM-derived and root-derived) measured throughout Rounds 1 and 2 of the greenhouse experiment. Three soil respiration measurements were taken during each round of the greenhouse experiment. The dark line represents the median and the box represents the first and third quartiles. Whiskers extend to the maximum and minimum of the data, excepting outliers (represented by points). The color of the box represents Soil History.

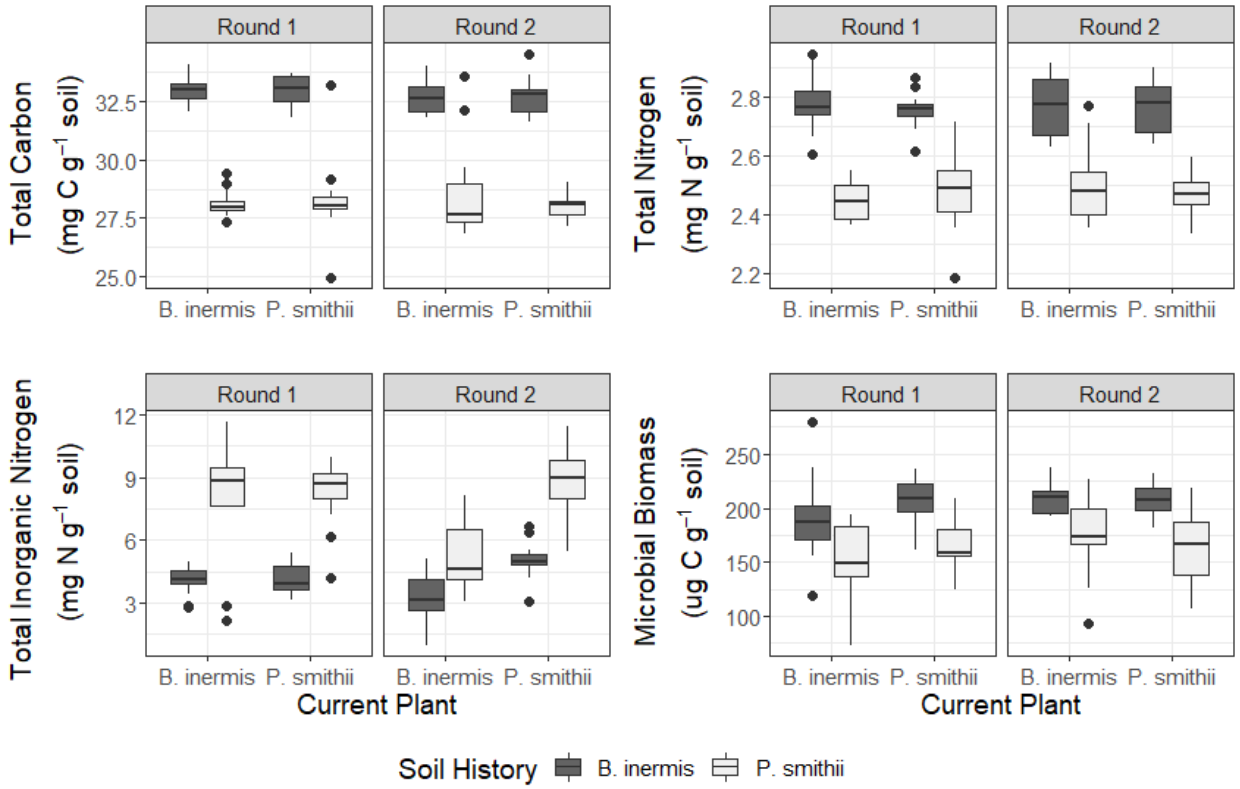

Figure 4.3 Boxplots of soil chemistry (total C, total N, and inorganic N) and microbial biomass C measured throughout Rounds 1 and 2 of the greenhouse experiment. Three measurements of each response variable were taken during each round of the greenhouse experiment. The dark line represents the median and the box represents the first and third quartiles. Whiskers extend to the maximum and minimum of the data, excepting outliers (represented by points). The color of the box represents Soil History.

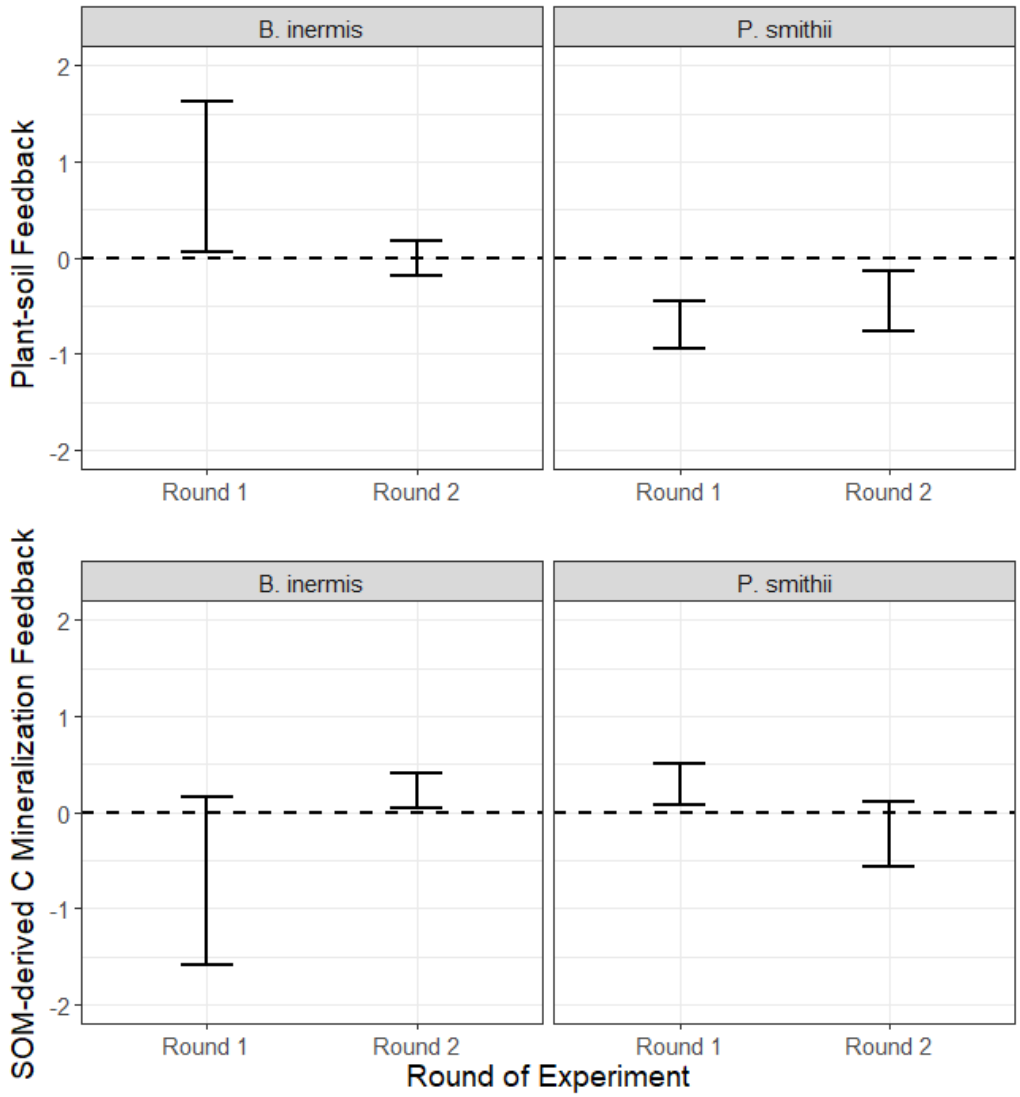

Figure 4.4 The 95% CI for plant-soil feedback and SOM-derived C mineralization feedback at the ends of Round 1 and Round 2 of the greenhouse experiment. Feedbacks are considered significant if the 95% CI does not cross zero. The response variable used to calculate PSF was total plant biomass, and the response variable used to calculate SOM-derived C mineralization feedback was the SOM-derived C mineralization rate. If the 95% CI is positive, then that particular response is higher when a plant is grown in its home soil. If it is negative, then that particular response is lower when a plant is grown in its home soil.

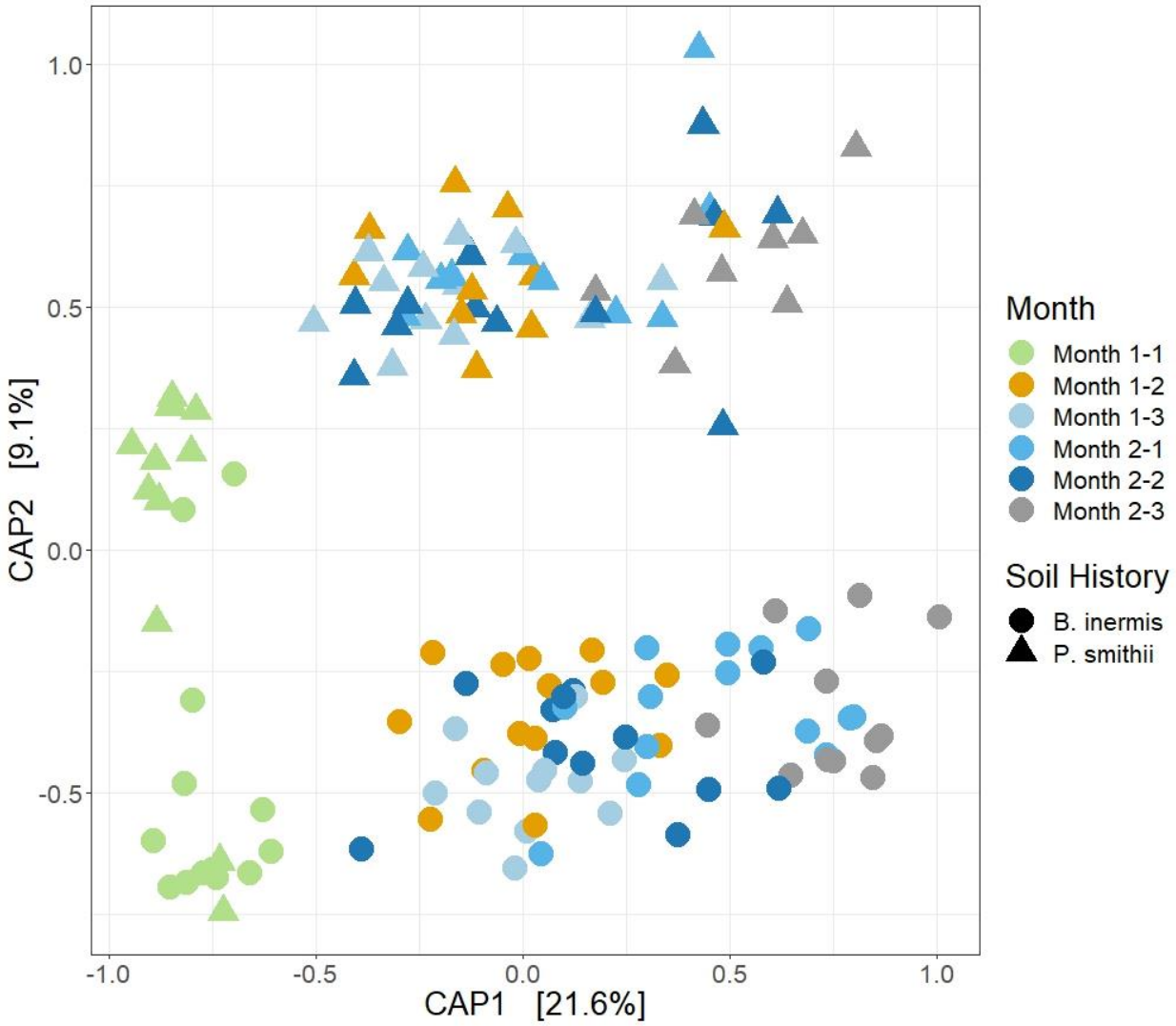

Figure 4.5 CAP ordination of soil bacteria community composition using Bray-Curtis distance. The ordination was constrained using the formula ~Soil History* Current Plant * Month. The shape of each point represents Soil History. The color of each point represents Month.

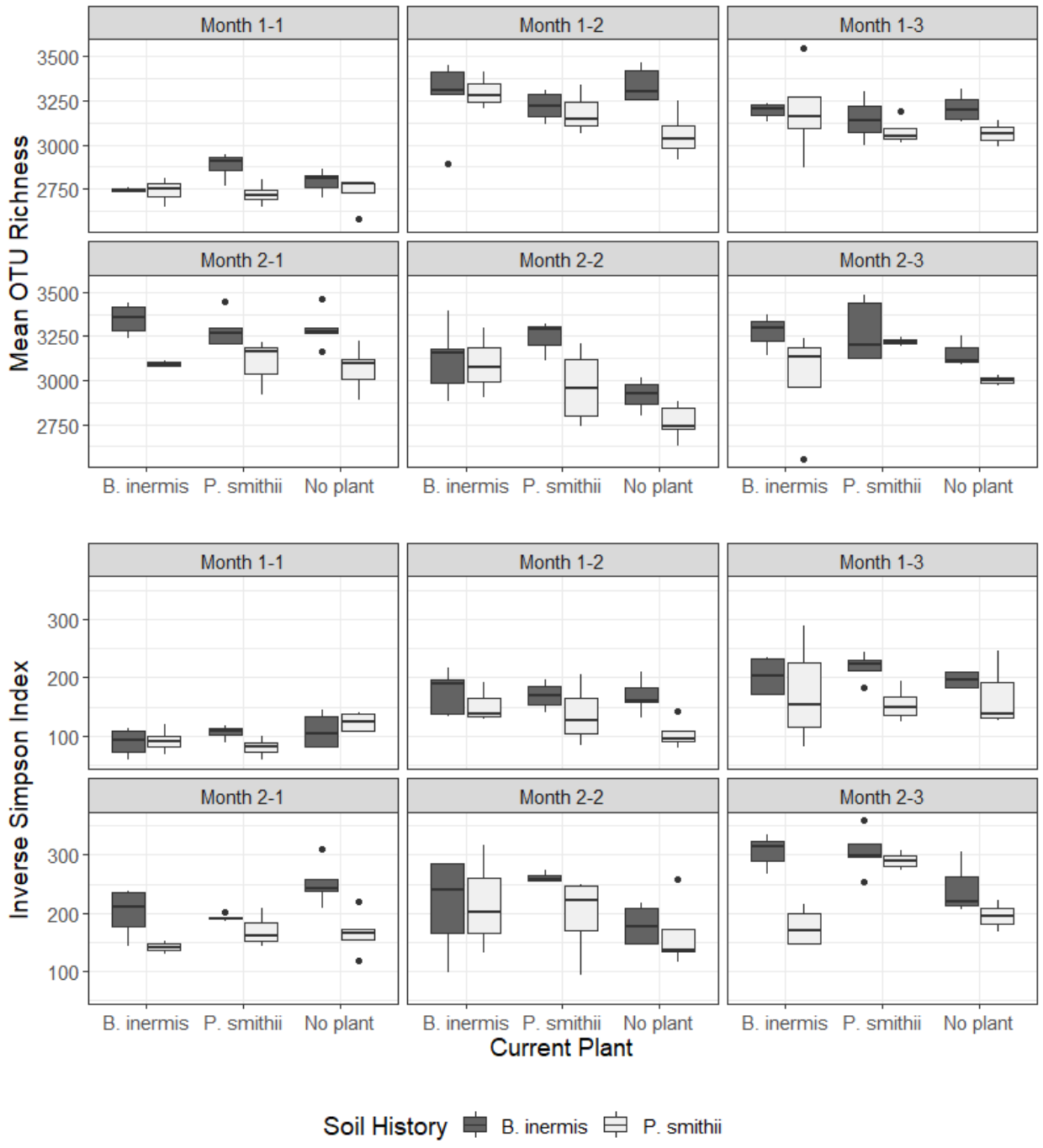

Figure 4.6 Boxplots of mean OTU richness and evenness (determined by Inverse Simpson's Index) during each month of the greenhouse experiment. The dark line represents the median and the box represents the first and third quartiles. Whiskers extend to the maximum and minimum of the data, excepting outliers (represented by points). The color of the box represents Soil History.

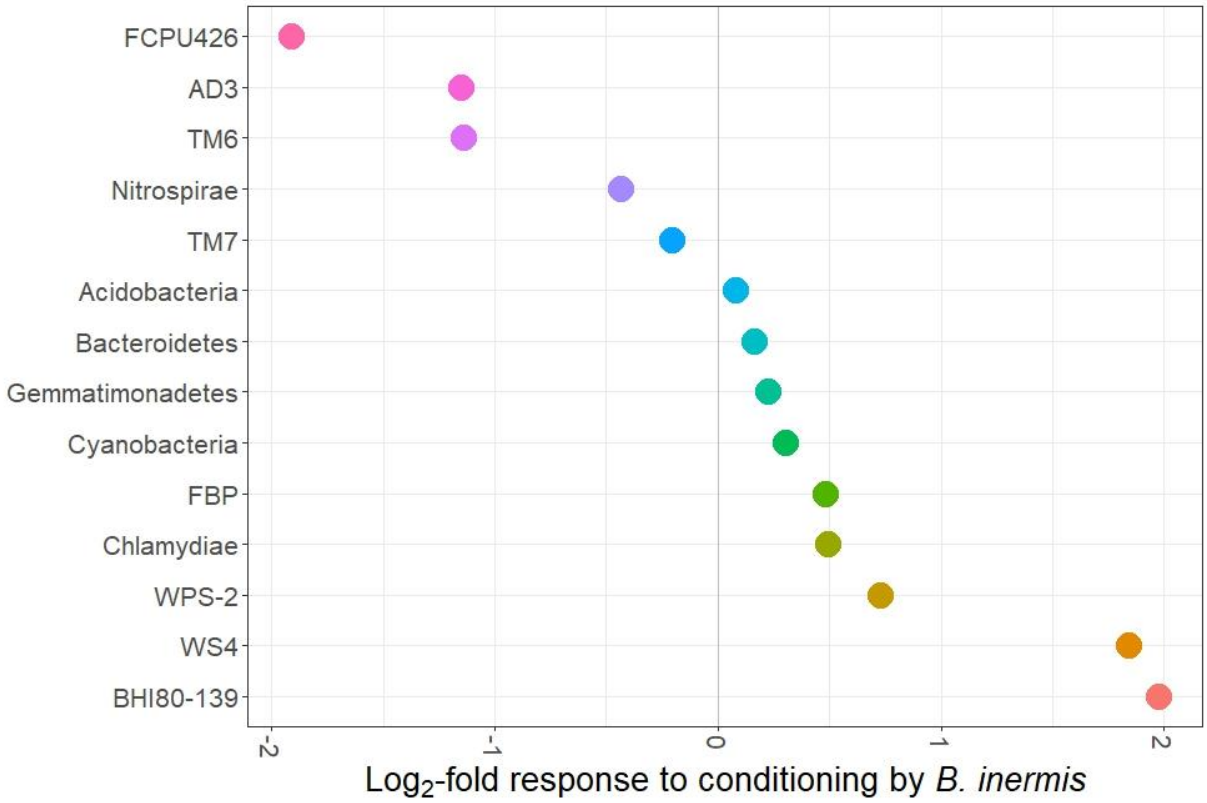

Figure 4.7 Bacteria phylum-level response to Soil History. Positive values indicate that a phylum is significantly more abundant in soils originally conditioned by *B. inermis.* Negative values indicate that a phylum is significantly more abundant in soils conditioned by *P. smithii.*

Chapter 5 - P fertilization increases the rate of SOM decomposition in woody plant-encroached prairie, but N fertilization does not

Abstract

The relative availability of soil nutrients to plants and the microbial community is hypothesized to affect the magnitude and direction of the rhizosphere priming effect (RPE). Microbial communities release nitrogen (N) contained within soil organic matter (SOM) through an oxidative process while phosphorus (P) is released through a hydrolytic process. Therefore, if microbial communities are N-limited, we would expect a greater magnitude of RPE while we would not expect a significant change in RPE if microbial communities are limited by P. In a field experiment, I tested this hypothesis by growing two woody plant species under three soil nutrient conditions: N-addition, P-addition, and a no nutrient addition control over the course of two months. Contrary to our expectations, I did not find evidence of RPE of any magnitude. However, the SOM-derived C mineralization rate varied with fertilizer treatment and was highest in the P-addition treatment and lowest in the N-addition treatment eight weeks after fertilization. Our results lend support to multiple other studies that indicate that microbial N limitation due to P fertilization can stimulate SOM decomposition.

Introduction

Soil organic matter (SOM) is the largest pool of carbon (C) in terrestrial ecosystems (Stockmann et al., 2013), and any changes in the magnitude of fluxes into or out of this pool have important implications for productivity, climate, and nutrient cycling. For this reason, much

research has been dedicated to highlighting the mechanisms that control the formation, stability, and turnover of SOM (Cotrufo et al., 2013; Lehmann & Kleber, 2015; Schmidt et al., 2011). Anthropogenic activity has altered the rates of ecosystem processes, and SOM dynamics are no exception (Crowther et al., 2016; Song et al., 2019). Nitrogen (N) and phosphorus (P) are two key nutrients that limit plant productivity (Cleveland et al., 2013; Du et al., 2020), but due to fertilizer production and elevated rates of weathering and erosion humans have greatly increased the availability of those nutrients in the terrestrial environment (Smil, 2000; Vitousek et al., 1997). Interactions between plants and microbes exert significant control of the cycling of C and other nutrients (Cardon & Whitbeck, 2007), and global change factors that increase the availability of limiting nutrients can alter plant-microbial interactions in ways that feedback to affect the turnover of SOM. However, due to stoichiometric constraints of different organisms, ecosystem processes should respond uniformly to an increase of any given nutrient. Therefore, it is important to tease apart how an important ecosystem process such as SOM decomposition responds to enrichment with different limiting nutrients.

Nitrogen is required for microbial growth and extracellular enzyme production; therefore, SOM decomposition should be constrained by N availability. However, increasing N availability may not increase the rate of SOM decomposition for two reasons. First, according to the N-mining hypothesis soil microbes can alleviate N limitation by breaking down SOM in order to obtain organically-bound N (Moorhead & Sinsabaugh, 2006). If N limitation is alleviated, then microbes will not need to invest in the energetically costly production of extracellular enzymes, and the rate of SOM decomposition will slow. Second, increased N availability may select for r-strategist microbes which do not decompose as much SOM as slower-growing K-strategist microbes which are the major producers of extracellular enzymes (Fontaine et al., 2003). On the

other hand, according to the growth rate hypothesis, microbes require substantial P in order to produce the ATP that is required for rapid growth (Elser et al., 1996). For this reason, soil microbial SOM decomposition may be limited by P, and adding additional P will stimulate SOM decomposition. In addition, enzymatic release of P that is organically bound by ester bonds does not require decomposition of SOM.

The relationship between nutrient availability and SOM decomposition is further complicated because plants also influence the rates of elemental cycling. The rhizosphere is a disproportionately important location for nutrient cycling within terrestrial ecosystems relative to its volume in soil (Finzi et al., 2015). On average, plants allocate 5-10% of C captured through photosynthesis belowground as exudates (Jones et al., 2004). The increased availability of root-derived labile C in the rhizosphere makes it a hotspot for microbial activity and growth (Kuzyakov & Blagodatskaya, 2015). Because of the complexity of biological interactions that take place within it, the factors that control the flow of nutrients from the rhizosphere warrant further investigation.

The rhizosphere priming effect (RPE) is the difference in soil organic matter (SOM) decomposition in soils with and without living roots (Kuzyakov, 2002). The RPE can vary in magnitude but is generally positive [59% stimulation of SOM decomposition, on average (Huo et al., 2017)]. Because RPE can persist over long time periods (Huo et al., 2017) it is important to determine how changes in biotic and abiotic soil factors affect the magnitude of RPE. The relative availability of soil nutrients to plants and the microbial community is hypothesized to affect the magnitude and direction of RPE (Dijkstra et al., 2013). Microbial communities release N contained within SOM through an oxidative process while P is released through a hydrolytic process. Therefore, if microbial communities are N-limited, we would expect a greater

magnitude of RPE while we would not expect a significant change in RPE if microbial communities are limited by P (Dijkstra et al., 2013).

The encroachment of woody plants into grass-dominated communities is a global phenomenon and a threat to grassland conservation. As woody plants increase in grasslands in North America, plant diversity decreases (Ratajczak et al., 2012) and nutrient cycling is altered (Barger et al., 2011). In tallgrass prairie, the woody plant species that are increasing in density use the C_3 photosynthetic pathway, while the dominant grasses use the C_4 photosynthetic pathway. Therefore, we can use natural abundance C isotopes to differentiate between root-derived and SOM-derived C mineralization. Since woody plants have a different root structure and function than the grasses they are displacing (Canadell et al., 1996; Schenk & Jackson, 2002), it is likely that woody plants may impact soil C flux via altered rhizosphere priming.

I took advantage of isotopic differences in C derived from C_3 woody plants growing in soil previously dominated by C_4 grasses to investigate the influence of two global change factors (nutrient enrichment and woody encroachment) on SOM decomposition and address the following hypotheses: (1) When P is added, there will be a greater magnitude of RPE because microbes would become more N-limited and therefore would need to decompose more SOM in order to meet their N requirements. (2) In contrast, if N is added microbes would become more P-limited. Since phosphatase activity only results in the hydrolytic removal of ester-bonded phosphate groups without a concomitant release of CO_2, I would not expect a change in the magnitude of RPE.

Methods

Soil collection

All soil used in the mesocosms was collected from the top 15 cm of a C_4 grass-dominated section of the Konza Prairie Biological Station (KPBS; Manhattan, KS, USA). KPBS is a large preserve of tallgrass prairie in the Flint Hills ecoregion of northeastern Kansas, USA. All soil was passed through a 4 mm sieve and coarsely root picked. Soil collection was completed in August 2017. After collection, the soil was stored in barrels for 9 months in a storage center at KPBS so that it could be used at the start of the experiment during the 2018 growing season.

Mesocosm design and experimental set-up

The experiment took place from June – July 2018 outdoors under a rain-out shelter at KPBS in order to allow more natural light and temperature fluctuation than would be experienced in a greenhouse, but control soil moisture content. Soil mesocosms were constructed out of 25-cm diameter PVC pipe and were capped at one end. I ensured that each cap was airtight by submerging each mesocosm in water to check for leaks. In order to assist with drainage, a nylon mesh filled with 1.5 kg of sand was placed at the bottom of each mesocosm. Each mesocosm was 40 cm deep x 25 cm diameter. Before filling the mesocosms with soil, I placed a silicone tube inside each so that one end (with a small sponge attached) was at the bottom and the other end hung out of the top. Each mesocosm was filled to the top with field-collected soil. Watering mechanisms (50cc syringe connected to ~15cm perforated silicone tubing) were placed into the soil so that each mesocosm could be watered more evenly. In order to maintain constant soil moisture, each mesocosm was weighed daily and watered back up to 60% water filled pore space through the watering mechanism with deionized water. Air was

pumped into each mesocosm using an aquarium pump for 60 minutes once every 24 hours to prevent anaerobic conditions from developing within the mesocosm.

Experimental design

Each mesocosm was assigned to a plant species treatment and a nutrient fertilization treatment. There were two plant species treatments: *Cornus drummondii* and *Rhus glabra*, hereafter "dogwood" and "sumac". These species were chosen because they are two common clonal shrubs responsible for woody encroachment of tallgrass prairie. All individuals of the same species were collected from the same clonal island at KPBS. New ramets from each clonal island were harvested and quickly transplanted into the mesocosms. There were three nutrient fertilization treatments: N-addition, P-addition, and control. Nutrients were added as a one-time pulse at a rate of 10 g m^{-2}. I used KNO_3 for the N-addition treatments and KH_2PO_4 for the P-addition treatments. Nutrients were dissolved in 300 ml deionized water and added through the watering mechanism. The control treatments received 300 ml deionized water with no nutrients. Overall, there were 72 mesocosms used in this study in the following design: (3 nutrient addition treatments * 2 plant species treatments * 10 replicates) + (3 nutrient addition treatments * 4 unplanted replicates) = 72 mesocosms. Carbon mineralization was measured in all mesocosms 4 weeks after fertilization. However, due to heat stress and browsing pressure, many plants died before the next planned C mineralization measurement at 8 weeks after fertilization, so at the 8 week measurement *C. drummondii* was the only plant species left in the following pattern: (3 control + 4 N-addition + 4 P-addition) + (3 nutrient addition treatments * 4 unplanted replicates) = 23 mesocosms.

CO_2 trapping

A closed-circuit CO_2 trapping system (Cheng et al., 2003) was used to measure belowground C mineralization over a 24-hour period at two time points: four weeks and eight weeks after fertilization. Briefly, for each mesocosm, I poured silicone over the top of the soil which cured to form an airtight barrier separating above and belowground components of the mesocosms in order to trap all belowground CO_2 production. After the silicone cured, I attached the silicone tubing from the sponge tube and the watering mechanism to a pump connected to a soda lime column for 40 minutes to flush out any CO_2 that had accumulated before I began trapping. For 24 hours, I pumped all CO_2 produced belowground through an airstone placed in a bottle of 300 ml of 0.25 M NaOH. The CO_2 was trapped in the NaOH, and then CO_2-free air was recirculated back into the mesocosms. After trapping was finished, I removed the silicone sealant from each mesocosm with a knife, careful to avoid damaging the plants in the pot. Because I was limited by trapping equipment, CO_2 trapping took place over a 3-day period during the 4 weeks after fertilization timepoint (24 mesocosms day^{-1}).

Harvesting the mesocosms

A subset of mesocosms that were not needed for C mineralization assays at the 8 weeks after fertilization time point was harvested 6 weeks after fertilization. The rest were harvested immediately after trapping at the 8 weeks after fertilization timepoint. During harvesting, a subsample of the soil was collected from each mesocosm for soil chemistry and microbial biomass measurements and stored at 4°C. All belowground plant biomass was collected and cleaned with deionized water to remove soil. The biomass was then dried for 48 hours at 60°C. All belowground biomass < 2mm in diameter was considered fine root biomass and weighed.

Analyzing NaOH traps

I determined the total dissolved inorganic C (DIC) content of each NaOH trap by analyzing a subsample for DIC using a Shimadzu TOC-L (Shimadzu, Kyoto, Japan). Additionally, I precipitated the CO_2 dissolved in the NaOH traps as $SrCO_3$ by adding excess 1 M $SrCl_2$ to a subsample from each bottle. The precipitated $SrCO_3$ was rinsed with DI-H_2O once a day. I waited 24 hours and removed the supernatant using a vacuum. The rinsing process was repeated for 7 days in order to neutralize the pH of the precipitates. The precipitates were dried for 24 hours at 105 °C and analyzed for $\delta^{13}C$ content using GC/MS at the Stable Isotope Facility at the University of California-Davis.

Isotope calculations

I used an isotopic mixing model to calculate the proportion of captured CO_2 attributable to SOM decomposition according to the following equation: $\% SOM_{CO_2} = \frac{\delta_t - \delta_p}{\delta_s - \delta_p} * 100$. In this equation δ_t represents the $\delta^{13}C$ value of the trapped CO_2. The δ_p represents the $\delta^{13}C$ value of the plants. In this study, I used a value of -27.5‰ for δ_p. The δ_s represents the $\delta^{13}C$ value of the soil. I used the average $\delta^{13}C$ value of the trapped CO_2 from the plantless plots for δ_s.

Soil chemistry

I measured total %C and total %N using a couple combustion-gas chromatography Flash EA 1112 C/N autoanalyzer. To measure soil inorganic N, I took a 12 g subsample of each soil and extracted it with 50 ml of 2N KCl (Bremmer & Keeney, 1966). After shaking the soil-extract mixtures on an orbital shaker table for 60 minutes at 200 rpm, I filtered the extract through a Whatman No. 1 filter. Total NH_4^+ and NO_3^- were measured colorimetrically at the Kansas State University Soil Testing Lab (Manhattan, KS, USA). Extractable soil P was measured using the Mehlich-3 procedure at the Kansas State University Soil Testing Lab (Mehlich, 1984).

Microbial biomass carbon

I used the chloroform fumigation-extraction protocol to assess microbial biomass C (Jenkinson & Powlson, 1976). Briefly, each soil sample was split into two 15 g subsamples. One subsample was immediately extracted with 75 ml of 0.5 M K_2SO_4 and filtered through Whatman No.1 filter paper. The other subsample was placed inside of a fumigation chamber and exposed to chloroform under a vacuum for 48 hours before extraction and filtration. I calculated microbial biomass as the difference in dissolved organic C between the fumigated and nonfumigated extracts.

Data analysis

I used ANOVA to test the effects of fertilization (control, N-added, or P-added), plant species (Dogwood, Sumac, or No plant), and their interaction on total C, total N, inorganic N, total P, microbial biomass C, and fine root biomass. For analyses of SOM-derived C mineralization, I split the data by measurement date [4 weeks after fertilization (June) or 8 weeks after fertilization (July)]. Since CO_2 trapping took 3 separate days in June, for our analyses of SOM-derived C mineralization I ran a mixed effects model using day of measurement as a random effect. I used the following equation: (Response ~ Fertilization * Species + (1|Day of measurement). For our analyses of SOM-derived C mineralization and RPE in July I ran a one-way ANOVA using the following equation: (Response ~ Fertilization). For all mean comparison analyses, I used Tukey's comparisons of least square means. I ran all analyses within the R statistical computing platform (R Core Team, 2019) using the packages *lme4* (Bates et al., 2015), *car* (Fox & Weisberg, 2019), *lsmeans* (Lenth, 2016), and *multcomp* (Hothorn et al., 2008). I used the package *tidyverse* to produce all figures used in this study (Wickham et al., 2019).

Results

SOM-derived C mineralization

Four weeks after fertilization, across all plant treatments, the average SOM-derived C mineralization rate in P-fertilized soil (786 ± 0.0456 µg C-CO_2 g C^{-1} day^{-1}) was 12% higher than the control (704 ± 0.0519 µg C-CO_2 g C^{-1} day^{-1}). The rate was 6% higher than the control, on average, in N-fertilized soil (744 ± 0.0323 µg C-CO_2 g C^{-1} day^{-1}). Eight weeks after fertilization, across all plant treatments, the average SOM-derived C mineralization rate in P-fertilized soil (841± 0.0387 µg C-CO_2 g C^{-1} day^{-1}) was 21% higher than the control (695 ± 0.0898 µg C-CO_2 g C^{-1} day^{-1}). The rate was 25% lower, on average, in N-fertilized soil (524 ± 0.0727 µg C-CO_2 g C^{-1} day^{-1}). Overall, there was a statistically significant effect of fertilization on SOM-derived C mineralization rates eight weeks after fertilization but not four weeks after fertilization (Figure 5.1; Table 5.1). There was no significant effect of plant species on SOM-derived C mineralization at any point throughout the experiment.

Soil chemistry and microbial biomass

All of the following soil chemistry and microbial biomass results were averaged over the samples collected at both timepoints. Total soil C was affected by whether a plant was present in the soil. Soil that had dogwood (32.2 ± 0.270 mg C g^{-1} soil) or sumac (31.8 ± 0.397 mg C g^{-1} soil) growing therein had 7% and 8% less soil C than soil with no plant present (34.6 ± 0.133 mg C g^{-1} soil), respectively (Figure 5.2). Total soil N was also affected by the presence of a plant. Soil that had dogwood (2.66 ± 0.0229 mg N g^{-1} soil) or sumac (2.64 ± 0.0354 mg N g^{-1} soil) growing in it had 8% and 9% less soil N than soil with no plant present (2.89 + 0.0204 mg N g^{-1} soil), respectively (Figure 5.2). Soil inorganic N was most strongly affected by plant species. Across all fertilization treatments, soil with dogwood had 48% less inorganic N (65.5 ± 9.81 µg

N g^{-1} soil) and soil with sumac had 17% less inorganic N (104 ± 20.6 µg N g^{-1} soil) than soil without a plant (126 ± 11.2 µg N g^{-1} soil; Figure 5.2). Interestingly, the effect of fertilization on total N or inorganic N was not statistically significant (Table 5.2). In contrast, the effect of P fertilization on extractable soil P was statistically significant. Extractable P was 71% higher in soil that was P-fertilized (40.5 ± 3.64 µg P g^{-1} soil) than the control (11.7 ± 0.441 µg P g^{-1} soil), on average (Figure 5.3). Additionally, the effect of plant species on extractable P was significant, but Tukey HSD pairwise comparisons did not identify any significant differences. Finally, there was no statistically significant effect of fertilization or plant species on microbial biomass C (Table 5.2). To summarize, most aspects of soil chemistry that I measured were affected by the identity of the plant species growing in the soil (i.e., total C, total N, inorganic N), while extractable P was the only response significantly affected by the fertilization treatment.

Fine root biomass

Fertilization had a strong effect on fine root biomass throughout the experiment. Six weeks after fertilization, the average fine root biomass across both plant species was 275% greater in P-fertilized soil (0.192 ± 0.0514 g) than control soil (0.0512 ± 0.0129 g; Figure 5.3). Fine root biomass was also 199% greater in soil fertilized with N (0.153 ± 0.0316 g). Fine root biomass did not significantly differ between the two plant species (Table 5.2).

Eight weeks after fertilization, dogwood individuals had 415% more fine root biomass, on average, in soil that was P-fertilized (CTRL: 0.326 ± 0.147 g, P: 1.68 ± 0.258 g) eight weeks after fertilization (Figure 5.3). Dogwood individuals had 14% less fine root biomass, on average, in soil that was N-fertilized (0.280 ± 0.147 g).

Discussion

In this experiment, I investigated the role of N and P fertilization on the rate of SOM decomposition and RPE under field conditions. I hypothesized that N fertilization would decrease the magnitude of RPE while P fertilization would not change the magnitude of RPE. In contrast to our hypotheses, I did not detect a significant difference in SOM decomposition rates between soil that had plants growing therein versus soil that did not (Table 5.1; Figure 5.1). Because of this, I conclude that I was unable to detect RPE of any magnitude in our experiment. Most studies of the RPE report that the presence of plant roots stimulates the rate of SOM decomposition (Huo et al., 2017), and this pattern is consistent across plant species (Cheng et al., 2003) and soil types (Zhu et al., 2014). While it is possible that this result could be explained by the constraints of our experimental set-up (i.e., small plant biomass, short experimental duration), there have been reports of RPE of 0% in the literature (Moinet et al., 2018). Although I was unable to detect an effect of plant roots on SOM decomposition in our study, plant-soil interactions exert a strong influence on the cycling of C and nutrients (Čapek et al., 2018), so future studies of SOM decomposition should continue to be conducted with plants present. Indeed, since average total C and N was lower in soil with plants in them (Figure 5.2), the presence of plant roots may have stimulated SOM decomposition overall, but this stimulation was not captured during either of our 24 hour measurements. This suggests that we need measurements of RPE over finer temporal scales.

Fertilization did affect the rate of SOM decomposition by the end of our experiment. Nitrogen addition suppressed SOM decomposition while P addition accelerated SOM decomposition rates (Figure 5.1). Suppressed soil respiration under N fertilization may be due to a decrease in microbial CUE (Spohn et al., 2016), a decrease in "N mining" by microbes (Craine

et al., 2007), or a shift in microbial community composition (Fontaine et al., 2003). At the global level, P addition does not stimulate heterotrophic soil respiration; however, it does stimulate total soil respiration (autotrophic + heterotrophic) in certain ecosystem types (Feng & Zhu, 2019). On average, P fertilization has no effect in grasslands on total soil respiration, but it does have a stimulatory effect in forests (Feng & Zhu, 2019). P addition might stimulate SOM decomposition by alleviating microbial P limitation. However, since soil microbes tend to be C limited (Soong et al., 2020), an alternative explanation is that inorganic P addition might stimulate SOM decomposition by desorbing organic compounds from the soil matrix (Spohn & Schleuss, 2019).

It is interesting that N fertilization did not affect total or inorganic soil N (Figure 5.2), potentially indicating that the microbial community immobilized all added N. Additionally, since soil N was lower in soil with plants, this could indicate that plant uptake facilitated additional removal of N from soil. On the other hand, extractable P remained elevated in P-fertilized soil throughout the entire experiment (Figure 5.3). Under these conditions, N would have become more limiting to plants. I hypothesize that in order to meet their N demands both plants species allocated more resources toward fine root biomass production (Figure 5.3).

In conclusion, our results suggest that P fertilization increases the rate of SOM decomposition while N fertilization does not. The cycles of C, N, and P are linked to one another, and anthropogenic changes to the N or P cycle will feedback to affect the C cycle. Overall, our results add to the growing evidence that predicting the dynamics of SOM requires scientists to understand the interactions between multiple elemental cycles.

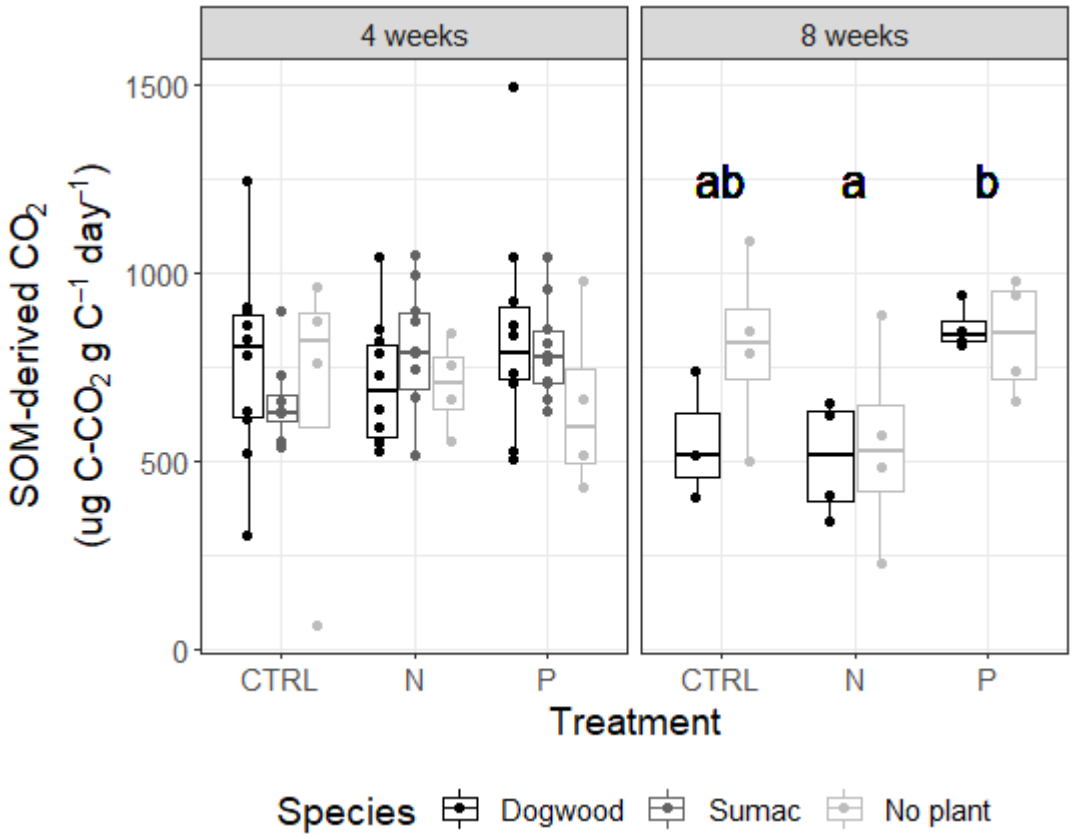

Figure 5.1 SOM-derived C mineralization rates in soil subject to different fertilization treatments four weeks and eight weeks after fertilization (CTRL = no nutrients added, N = nitrogen added, P = phosphorus added). Letters denote significant differences between means of each fertilization treatment using Tukey HSD comparisons of least square means.

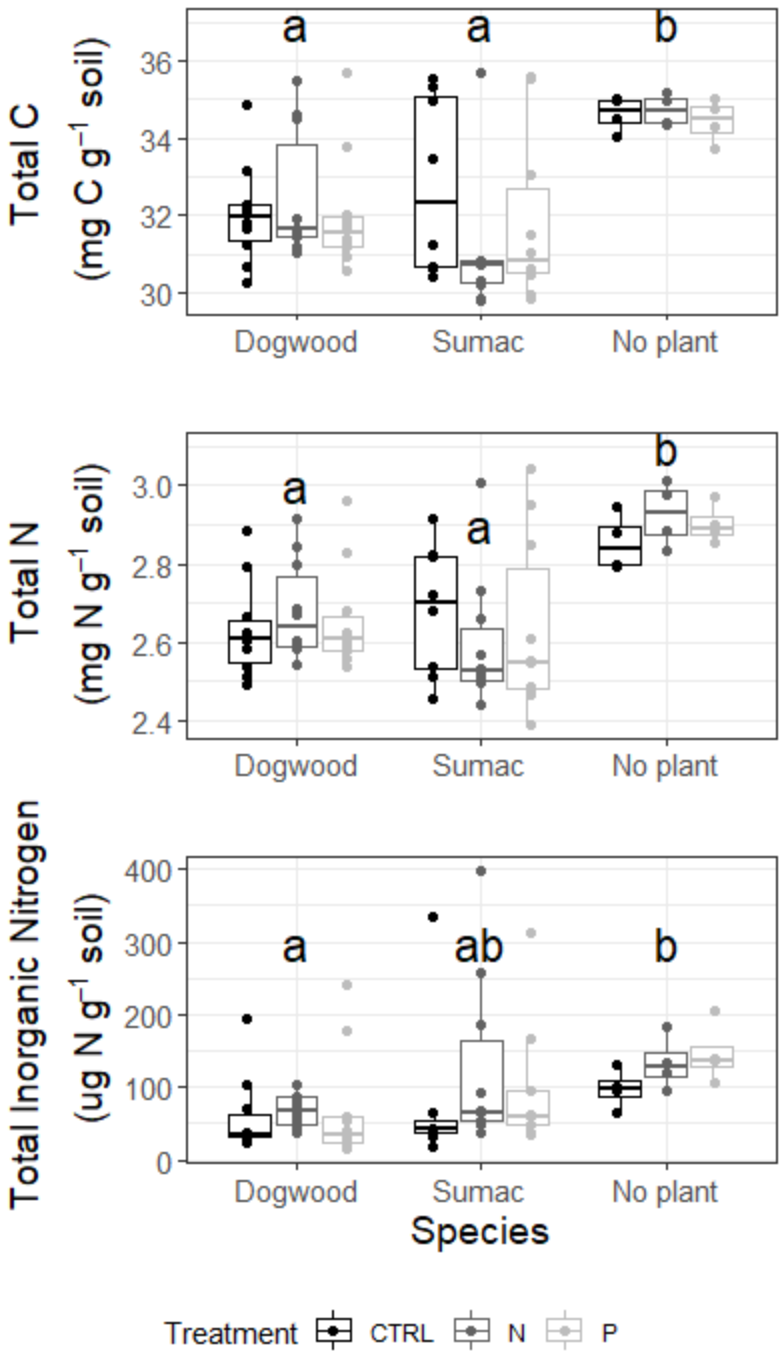

Figure 5.2 Three aspects of soil chemistry (Total C, Total N, and Total inorganic N) in soil subject to different fertilization and plant treatments (CTRL = no nutrients added, N = nitrogen added, P = phosphorus added). Letters denote significant differences between means of each plant treatment using Tukey HSD comparisons of least square means.

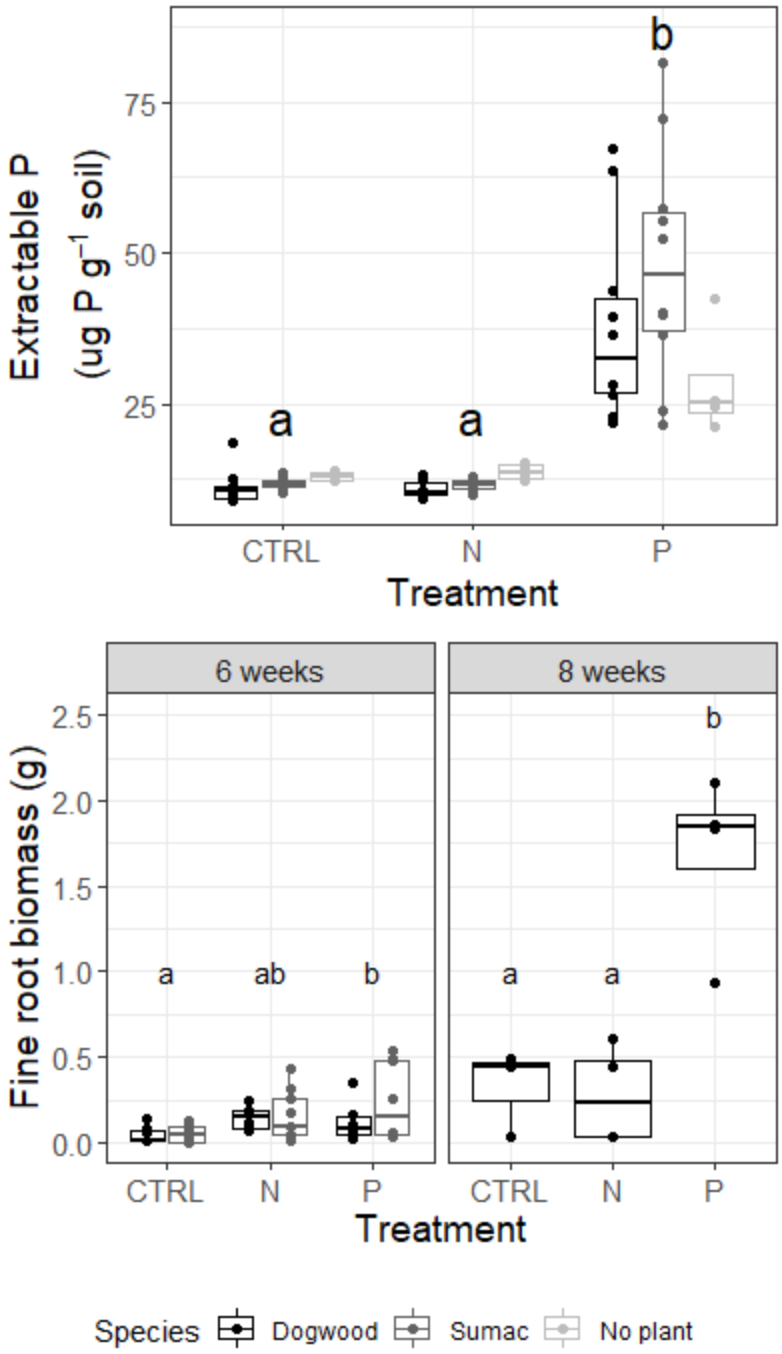

Figure 5.3 Extractable P and fine root biomass in soil subject to different fertilization and plant treatments (CTRL = no nutrients added, N = nitrogen added, P = phosphorus added). Letters denote significant differences between means of each fertilization treatment using Tukey HSD comparisons of least square means.

Table 5.1 Effects of fertilization (N-addition, P-addition, or control), species (dogwood, sumac, or no plant), and their interaction on the SOM-derived C mineralization rate. Linear mixed model results are shown for four weeks after fertilization. ANOVA results are shown for eight weeks after fertilization.

4 weeks after fertilization		
SOM-derived C mineralization rate	**X^2**	**p**
Fertilization	1.83	0.401
Species	2.97	0.226
*F*S*	3.01	0.556
8 weeks after fertilization		
SOM-derived C mineralization rate	**F**	**p**
Fertilization	5.63	**0.013**
Species	1.02	0.326
*F*S*	1.06	0.367

Table 5.2 ANOVA results for the effects of fertilization (N-addition, P-addition, or control), species (dogwood, sumac, or no plant), and their interaction soil chemical properties, microbial biomass, and fine root biomass. Only the effects of fertilization on fine root biomass eight weeks after biomass are shown since there was only data from the dogwood plant treatment.

Soil and plant properties		
Total C	**F**	**p**
Fertilization	0.130	0.879
Species	44.8	**<0.001**
*F*S*	0.972	0.429
Total N	**F**	**p**
Fertilization	0.375	0.689
Species	31.4	**<0.001**
*F*S*	0.630	0.643
Inorganic N	**F**	**p**
Fertilization	2.01	0.143
Species	6.65	**0.002**
*F*S*	0.363	0.834
Extractable P	**F**	**p**
Fertilization	28.6	**<0.001**
Species	6.17	**0.004**
*F*S*	1.77	0.146
Microbial biomass C	**F**	**p**
Fertilization	1.76	0.181
Species	0.328	0.721
*F*S*	1.71	0.161
Fine root biomass (6 weeks AF)	**F**	**p**
Fertilization	7.03	**0.003**
Species	0.391	0.535
*F*S*	0.522	0.597
Fine root biomass (8 weeks AF)	**F**	**p**
Fertilization	9.17	**0.009**

Chapter 6 - Conclusion

Humans have greatly altered the cycling of carbon and other elements on a global scale, with significant environmental consequences (Schlesinger & Bernhardt, 2013). A drastic reduction in anthropogenic carbon emissions will be necessary to avoid the most severe consequences of climate change (IPCC, 2018). In addition to cutting further emissions, using soil to sequester additional carbon from the atmosphere has been touted as an important climate mitigation strategy (Bossio et al., 2020; Minasny et al., 2017). At the same time, the benefits of soil carbon to plant productivity comes from its turnover (Janzen, 2006). Striking the balance between these two seemingly opposing goals will require enhanced understanding of plant-soil interactions since they are important drivers of the storage (Cotrufo et al., 2013) and turnover (Finzi et al., 2015; Kuzyakov, 2002) of soil carbon. Including plant-soil interactions in carbon models changes our predictions about global productivity and carbon-climate feedbacks (Shi et al., 2019), so it is crucial that we continue to explore how plant-soil interactions influence the flow of carbon in and out of soil in order to improve the accuracy of our predictions.

In addition to elevating atmospheric CO_2 concentrations, humanity has altered ecosystems through other mechanisms which have biogeochemical impacts that are not fully understood. In tallgrass prairie post European colonization, for example, humans have disrupted the natural fire and grazing disturbance regimes that are required to maintain it (R. C. Anderson, 2006). Fire suppression and the local extirpation of browsing megafauna have allowed woody plants to expand in density (O'Connor et al., 2020), threatening grassland plant and animal diversity. Additionally, bison have been replaced by cattle as the dominant grazer, and much of the tallgrass prairie is currently used as rangeland. Exotic grasses such as *Bromus inermis*,

originally introduced as forage for cattle, have become invasive (Cully et al., 2003). Large areas of the tallgrass prairie with appropriate topography and soil depth have been plowed and replaced with row-crop agriculture which require external inputs of nitrogen and phosphorus in order to maximize yields.

In this dissertation, I investigated how plant-soil interactions influence soil carbon cycling in tallgrass prairie under the context of multiple global change factors. Human manipulation of burning and grazing regimes alters plant community composition such that soil carbon content increases over time (Chapter 2). In soil under encroaching shrubs, microbes potentially mineralize more carbon if they are breaking down proportionally more shrub-derived organic matter (Chapter 3). The invasive grass, *B. inermis*, changes soil microbial community composition and stimulates soil organic matter decomposition (Chapter 4). Finally, while I did not find any evidence for a significant rhizosphere priming effect within woody-encroached soil, phosphorus fertilization within these soils did increase the rate of soil organic matter decomposition (Chapter 5).

Overall, each of the four global change factors that I investigated significantly impacted soil carbon pools or fluxes. However, the impact of each factor was dependent on spatiotemporal context and historical factors. The effects of a one-time pulse of phosphorus fertilization affected soil organic matter decomposition within a few weeks (Chapter 5). The effects of the invasive *B. inermis* on soil microbial community composition and soil organic matter decomposition persisted for months even if *B. inermis* was removed and replaced with a native grass (Chapter 4). Using shrub size as a proxy for age, *Cornus drummondii* differentially impacts soil microbial extracellular enzymatic activity as it expands over several years (Chapter 3). Finally, it took decades for appreciable effects of long-term burning and grazing regimes to develop on soil

carbon content (Chapter 2). The results of my dissertation indicate that the effects of global change on soil carbon cycling will not remain constant over time. Additionally, it is necessary to identify any factors such as land-use history or plant community history that may interact with global change to influence our predictions of carbon cycling.

This dissertation improved our understanding of global change's impact on carbon cycling in tallgrass prairie, but there is more to be done. For example, I investigated the impacts of global change factors independently of one another. However, multiple global change factors will affect ecosystems simultaneously. Since different global change factors can have opposing effects on plant-soil interactions (Tylianakis et al., 2008), multiple interacting global change factors may result in different outcomes on biogeochemical cycling than we would predict from experiments of single global change factors. Additionally, the responses of microbes and plants to global change are dynamic, and phenotypic plasticity and evolutionary processes may drive future responses to global change. For example, increases in microbial respiration due to warming are ephemeral due to shifts in microbial physiology (Allison et al., 2010), and there is evidence that legumes evolve different responses to their rhizobia partners if they come from environments that differ in nitrogen availability (Magnoli & Lau, 2020). Teasing apart whether shifts in responses to global change are a result of community turnover, phenotypic plasticity, or evolution will become increasingly important as the world continues to change.

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