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ENVIRONMENTAL HETEROGENEITY EFFECTS ON DIVERSITY AND NITROUS OXIDE EMISSIONS FROM SOIL IN RESTORED PRAIRIE

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by

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A Dissertation
Submitted in Partial Fulfillment of the Requirements for the
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Ecological theory predicts that high environmental heterogeneity causes high biodiversity. Theory further predicts that higher biodiversity results in greater ecosystem functioning. These theoretical predictions were evaluated in three studies using grassland restorations from agriculture.

The 'environmental heterogeneity hypothesis' has been proposed as a mechanism that enables species coexistence through resource partitioning. In accordance with this hypothesis, plant diversity is predicted to increase with variability in resources. There have been many observational studies reporting positive correlations, but only a few experiments show weak support for this hypothesis and others show no support. The objective of this research was to characterize how resource availability and heterogeneity change as plant communities develop using a chronosequence of restored prairies located at Konza Prairie (northeast Kansas, USA). More specifically, I quantified means and coefficients of variation in soil nitrate and light availability (proportion of photosynthetically active radiation [PAR] reaching soil surface) in prairies established on former agricultural lands for different times (ages) and their relationship to plant diversity and community structure using semivariograms to determine distance of spatially independent plots. Nitrate availability decreased exponentially with restoration age, but there was no directional change in nitrate heterogeneity across the chronosequence. Light availability also decreased exponentially across the chronosequence, but PAR heterogeneity

increased with restoration age. Heterogeneity in resources did not affect plant community structure, but heterogeneity in nitrate and light were positively related to plant Shannon's Diversity (H′). The positive relationship of diversity and richness to nitrate heterogeneity was weakened when considering nitrate availability interaction with nitrate heterogeneity. No significant heterogeneity effects were found for Pielou's evenness, suggesting diversity responses to heterogeneity were mostly driven by changes in richness. Overall, these results suggest that environmental heterogeneity corresponds with plant diversity as predicted by the 'environmental heterogeneity hypothesis', but high resource availability can weaken this relationship.

Plant species identity, soil depth, soil nutrient availability, and their interactions have the potential to structure soil microbial communities. If distinct communities were present within combinations of different levels of these ecosystem properties, this community specificity would indicate heterogeneity promotes soil microbial diversity at the scale of plants. I used a 20 year restored prairie located at Konza Prairie with soil depth (shallow and deep) and nutrient manipulation (reduced N availability, ambient N availability, and elevated N availability) and used three plant treatments (*Andropogon gerardii*, *Salvia azurea*, and bare soil) to evaluate the relative effects of these treatments and their interactions on the soil microbial community as measured by phospholipid fatty acid (PLFA) profiles. Permutational multivariate analysis of variance of PLFA biomass was conducted as was mixed model analysis of Shannon diversity index (H′), richness (S), and Pielou's evenness (J). Treatments had no effect on microbial community structure. The main effect of plant treatment influenced PLFA H′. This differential diversity response by plant treatment was due to differences between bare soil and the two plant-influenced soils, where plant-influenced soils had greater proportional arbuscular mycorrhizal

fungi and Gram-negative bacteria. This result indicates that increasing plant cover promotes microbial diversity. It remains unclear if aboveground plant cover or belowground plant cover would play a larger role. While I did not detect distinct microbial communities in treatment combinations, amplicon analysis may be more sensitive and indicate if environmental heterogeneity is likely to promote soil microbial diversity.

Plant diversity has been shown to increase several ecosystem functions including primary productivity, nutrient retention, and carbon sequestration. I tested if plant diversity could mitigate nitrous oxide emissions. I used an initial survey to determine study design from quadrat and semivariogram analyses and to determine cutoffs for high- and low-plant diversity. I sampled high- and low-diversity plant communities from five 10 to 12 y restorations located at Nachusa grasslands (Franklin Grove, IL, USA). I demonstrated that the diversity treatments were associated with high- and low-levels of species richness, species evenness, and functional group richness. I found the nitrous oxide emissions from high-diversity plant communities were approximately half the emissions from low-diversity plant communities. Differences in emissions did not coincide with differences in water availability, nitrogen availability, carbon availability, or microbial activity. Soils composited at the plot scale exhibited more N_2O emission hotspots from denitrification in the low plant diversity treatment. Greater denitrification in low-diversity plant communities could indicate more hotspots, i.e. small areas of high nitrous oxide production. Hotspots occur when an abundance of all necessary components of denitrification are present (e.g. available nitrate, available organic C, anaerobic microsites, ammonia oxidizing organisms, and nitrite oxidizing organisms). This result suggests that plant diversity is affecting the physiology or the community structure of soil denitrifiers. This work

suggests that nitrous oxide emissions can be managed by creating high-diversity plant communities.

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CHAPTER 1

INTRODUCTION

The tallgrass prairie in North America is an intrinsically heterogeneous ecosystem both in terms of vegetation and soil. Variation in topography, fire regime, and grazing, especially by *Bison bison*, maintain heterogeneity on the landscape (Collins and Steinauer 1998, Collins et al. 1998, 2002, Knapp et al. 1998, Joern 2005). Less than 10% of the tallgrass prairie remains intact largely because of conversion to row-crop agriculture (White et al. 2000, Samson et al. 2004). Conversion to agriculture could result in a more homogenous landscape than native (never-cultivated) prairie (Haas et al. 1957). The effect of subsequent restoration from agriculture on heterogeneity is unknown as heterogeneity effects have been examined by experimental manipulation. Uncultivated tallgrass prairie is characterized by a dominance of warm-season (C_4 photosynthetic pathway) grasses and a diverse suite of forbs (Abrams and Hulbert 1987). It has been argued that tallgrass prairie restorations should incorporate environmental heterogeneity (e.g. microtopography and soil roughness) in order to maintain diversity (Larkin et al. 2006), as heterogeneity can limit of the development of dominance in communities (Pacala and Tilman 1994, Hanski 1995) and create several different microsites (Ewing 2002). Restoring degraded systems, however, to reflect contemporary composition and function typical of less degraded plant communities can be difficult (Dobson et al. 1997), and this may be in part due to the loss of environmental heterogeneity. Conversion of prairie to a tilled row-crop agriculture homogenizes soil nitrogen, phosphorus, cation exchange capacity, pH, and soil water holding capacity (Haas et al. 1957, Pan-González et al. 2000), which may pose a challenge to reconstructing diverse prairie communities with corresponding greater ecosystem functioning compared to most monocultures,

as theory predicts (Tilman et al. 2014). However, some highly functional monocultures can outperform polycultures (Cardinale et al. 2011).

The 'environmental heterogeneity hypothesis' was developed from the observed effects of plant structure on animal communities, especially birds and insects (MacArthur 1965, 1966, Pianka 1966, Murdoch et al. 1972). This concept was first applied to plant communities on a landscape scale using tropical and temperate forest data (Ricklefs 1977) in studies that identified tree richness as a response variable along an openness gradient that was associated with changes in light, humidity, temperature, and soil nutrients. This hypothesis has also been referred to as the heterogeneity-diversity relationship (HDR), especially when using a gradient of heterogeneity. Positive correlations between plant diversity and environmental heterogeneity have been widely reported (reviewed by Huston 1979, Bakker et al. 2003, reviewed by Larkin et al. 2006), but most experimental tests of this relationship commonly report negative, unimodal, or non-significant results (Lundholm 2009, Tamme et al. 2010, Gazol et al. 2013, Laanisto et al. 2013, Bar-Massada 2014). Some of this variation may be attributed to spatial scale or different measures of heterogeneity (Lundholm 2009, Tamme et al. 2010, Stein et al. 2014, Stein and Kreft 2014), but simulated communities suggest environmental severity, i.e. low resource availability, may also affect HDR (Yang et al. 2015). High and low environmental severity caused a sigmoidal pattern in diversity while moderate severity caused a unimodal pattern (Yang et al. 2015). While most studies of environmental heterogeneity have been conducted on plants and animals, the concept has also been evoked to explain patterns in microbial communities (Zak et al. 2003). Environmental heterogeneity may interact with plant communities to influence microbial communities as light and nutrient availability and topography correlate with plant diversity (reviewed by Huston 1979, Bakker et al. 2003). Environmental heterogeneity and plant

community attributes regulating microbial communities relates to the hotspot theory of biogeochemistry (Hill et al. 2000), where several physical, chemical, and biological conditions must be right to create small areas of high biogeochemical activity.

Changes in environmental heterogeneity may occur with restoration. One example of this is decreasing heterogeneity in quantity of soil organic matter with increasing restoration age (Lane and BassiriRad 2005). Changes in light availability and light heterogeneity may occur because plant communities change dynamically during restoration (Camill et al. 2004, Manning and Baer 2018). Soil nitrate decreases exponentially following conversion of agricultural fields to grassland (Baer et al. 2002, 2003, Rosenzweig et al. 2016), and high variability in extractable N has been demonstrated in agricultural and newly restored soils compared to older restorations (Rosenzweig et al. 2016). This suggests that heterogeneity in N availability also changes dynamically as communities change over time. Further, homogenous and high concentrations of nitrate (Baer et al. 2003) correspond with homogenous and low levels of light (McCain et al. 2010). Low light conditions are also associated with low plant diversity in restorations likely due to dominance of a few plant species early in the restoration process. Less is known about how heterogeneity of plants and resources change over time during ecological restoration.

Few studies that have investigated the effects of environmental heterogeneity on biotic communities have considered spatial scale. One spatially explicit study found that decreasing resin bag nitrate corresponded with presence of dominant plants under grazing. Additionally, this study found that rare ions were more spatially heterogeneous than common ions, such as nitrate (Gibson 1986). Other spatially explicit studies considered heterogeneity of environmental variables and their correlation with plant diversity. One study found grazing increased heterogeneity in inorganic N and light availability, but only light availability was related to plant

diversity (Bakker et al.2003). Another study considering spatial expanse of rhizomatous and non-rhizomatous plants found that environmental heterogeneity was positively related to plant heterogeneity (Eilts et al. 2011).

Heterogeneity implications for ecosystem functioning

If environmental heterogeneity promotes plant diversity (Ricklefs 1977), this supports a deterministic niche-based mechanism for species coexistence (Grime 1979, Huston 1979, Tilman 1993, Caldwell and Pearcy 1994, Stein et al. 2014). Conceptual support for the 'environmental heterogeneity hypothesis' comes from studies suggesting that more variability in resources allows more species to coexist in an area through resource partitioning among species with non-overlapping (or partially overlapping) niches (niche differentiation concept; Tilman 1982, Kohn and Walsh 1994, Chesson 2000, Amarasekare 2003, Hortol 2009, Smith and Lundholm 2012, Price et al. 2014, Weisberg et al. 2014, Yang et al. 2015). Higher plant diversity has been shown to correspond with environmental heterogeneity (nutrient availability or light availability) in observational studies (reviewed by Huston 1979, Bakker et al. 2003) and theoretical modeling (Golubbski et al. 2008), but the majority of experimental tests have shown no strong effects (Lundholm 2009). Although several experimental studies in grassland have garnered weak or no support for the 'environmental heterogeneity hypothesis' influencing plant diversity (Baer et al. 2004, Baer et al. 2005, Reynolds et al. 2007, Baer et al. 2016), this discrepancy may be due to inappropriate scales of measurement for systems that contain large genets of clonal grasses (Eilts et al. 2011). It is also possible that examined factors were not limiting plant growth (Gibson 1988a). Another potential explanation for weak experimental support for the 'environmental heterogeneity hypothesis' is that vegetation patterns can drive environmental variables (Greg-

Smith 1979, Gibson 1988a and b), for example shading and nutrient uptake patterns can differ with plant species thereby creating hotspots.

If heterogeneity begets diversity, it may also increase ecosystem functioning, according to biodiversity-ecosystem functioning (BEF) theory (Naeem and Wright 2003, Tilman et al. 2014). Increased ecosystem functioning in response to higher biodiversity is presumed to result from niche complementarity, the concept of coexisting organisms using different forms of a resource (Tilman et al. 2012). Aboveground net primary productivity and secondary productivity have commonly been used as a proxy for ecosystem functioning to test BEF relationships (Tilman and Downing 1994, Naeem et al. 1994), but productivity may be an inappropriate measure of ecosystem functioning for perennial grasslands (Huston 1997, Grace et al 2007). A meta-analysis that examined nutrient uptake found evidence for nutrient use complementarity as increased species richness had a positive influence on nutrient use (decreased concentration in soil or water), though polycultures did not utilize more nitrogen than the most-efficient monocultures (Cardinale et al. 2011). Increased nutrient use plasticity of dominant species is an alternative explanation to niche-partitioning as a driver of complementarity effects (Ashton et al. 2010). Limited diversity may limit ecosystem functioning (uptake of inorganic N); causing more inorganic N to be available in the soil, as such some aspects of N cycling can be used as indices of ecosystem functioning.

Nitrogen cycling is predominately a microbialy-mediated ecosystem function. Ammonium can be biologically oxidized in a process called nitrification (Evans 2007, van Groenigin et al. 2015). This process only occurs in aerobic conditions. Nitrification has two steps, ammonia oxidation (performed by archaea and bacteria) and nitrite oxidation (performed by bacteria). While this process is generally viewed as autotrophic it also has been attributed to

some heterotrophic bacteria and fungi in a process that re-oxidizes NADPH (Stein 2011). Only two species of bacteria in the genus *Nitrospira* are known to be complete nitrifiers (van Kessel et al. 2017). Some of the nitrite produced by nitrification can be reduced to nitrous oxide (N_2O) biotically or abiotically in anaerobic microsites or as conditions become more anaerobic, such as after a precipitation event (Evans 2007, van Groenigen et al. 2015). Nitrate can be reduced to nitrogen gasses by some bacteria and fungi in anaerobic conditions, a process known as denitrification. Complete denitrifiers reduce nitrate to nitrite then nitric oxide then N_2O then dinitrogen gas. Many denitrifiers lack the nitrous oxide reductase enzyme and release N_2O (Evans 2007, van Groenigen et al. 2015). Many heterotrophic nitrifiers are also capable of aerobic denitrification (Stein 2011).

Greater nitrogen availability in soils may lead to increased nitrous oxide production in soils, and low diversity plant communities have been shown to have higher nitrate availability than high diversity plant communities (Tilman et al 2001, Ashton et al. 2010, Klopf et al. 2017). Nitrous oxide is a potent greenhouse gas that has nearly 300 times the heat trapping ability as CO_2 (US EPA 2014). Nitrous oxide is produced from ecosystems in the greatest molar quantities from denitrification (reduction of nitrate under anaerobic conditions), but can also be produced by nitrification during aerobic conditions (Evans 2007). Nitrification and denitrification occur together at the plot scale due to anaerobic microsites within soil aggregates (Stolk et al. 2011). Although produced in uncultivated ecosystems, cultivation has increased emissions of nitrous oxide (Skiba et al. 1993, Zhang et al. 2014). Restoring prairie from cultivated agricultural conditions has been demonstrated to decrease N_2O emissions. Restoring prairie with high plant diversity may further mitigate production of this greenhouse gas, which could be considered an ecosystem function potentially influenced by diversity.

Objectives

The overall objective of my dissertation research was to characterize how soil heterogeneity changes in response to time since restoration and composition of plant communities, and test how plant heterogeneity (diversity) in restored communities influence soil ecosystem services. I focused largely on heterogeneity related to soil N pools and fluxes. Soil N is ecologically important in tallgrass prairies because it is often a limiting, or co-limiting with P, nutrient to plants (Avolio et al. 2014), its availability influences microbial communities (Coolon et al. 2013), and it results in the production of the potent greenhouse gas nitrous oxide (N_2O; Evans 2007).

My second objective was to determine if heterogeneity in soils or plant species has greater influence on microbial community structure in restored prairie. This objective was examined using plots with experimental manipulations of soil depth and nutrient availability. I hypothesized soil resources (depth and nutrient availability) and plant species would have distinct effects on the soil microbial community.

The final objective of my dissertation research was to test whether plant diversity in restored prairie influences soil ecosystem functioning, specifically the production of nitrous oxide. I quantified the potential flux and relative contribution of different microbial metabolic pathways of nitrous oxide (N_2O) production in high- and low-diversity plant stands using a ^{15}N natural abundance (where relatively low values indicate more nitrification and relatively high values represent denitrification).

Hypotheses and predictions

In chapter 2, I hypothesized that soil nitrate heterogeneity would increase initially with restoration age as plants begin to establish, and then would level off. This hypothesis is

consistent with trends in variability of soil nitrate in a restoration chronosequence (Rosenzweig et al. 2016). I also hypothesized that available light heterogeneity would decrease as clonal grasses increase in dominance (Sluis 2002, Carter and Blair 2012, Baer et al. 2016). I also hypothesized heterogeneity of both soil nitrate and availability of light would be positively correlated with plant diversity and richness as predicted by the 'environmental heterogeneity hypothesis' (Ricklefs 1977).

In chapter 3, I hypothesized that plant species interacting with nutrient availability would have the greatest influence (the other treatment was soil depth) on microbial community composition. Nutrient availability can also have indirect effects through plants (Bardgett et al. 2003) on microbial community structure. Effects of plants on microbial communities are also well documented (Sasse et al. 2018, Hassani et al. 2018). I predicted that (1) altered nutrient availability would lead to shifts in microbial communities (Coolon et al. 2013, Koorem et al. 2014), (2) the identity of nearby plants would influence microbial composition (Zak et al. 2003), and (3) soil depth would affect microbial biomass by limiting root growth particularly of dominant grasses in experimentally shallow soil (Gibson and Hulbert 1987, Collins and Calabrese 2012), thereby limiting organic inputs to soil. I predicted lower microbial diversity under high nitrogen availability (Coolon et al. 2013, Yang et al. 2015).

In chapter 4, I hypothesized that diverse plant patches would have less N_2O efflux from soil as compared to low-diversity patches (Tilman et al 2001, Ashton et al. 2010, Klopf et al. 2017). I also hypothesized that initial extractable NH_4^+-N and NO_3^--N would be lower in high plant diversity patches as compared to low-diversity patches.

In summary, this research investigated the idea that environmental heterogeneity of resources (e.g. soil nitrate, light availability, and soil depth) can influence diversity of plant and

microbial communities (Fig. 1.1, Chapters 2 and 3). The high-diversity communities expected with heterogeneous environments are expected to influence ecosystem functioning. Diversity of biological communities effects on an ecosystem service, mitigation of N_2O efflux, were investigated at a landscape scale (Fig. 1.1, Chapter 4).

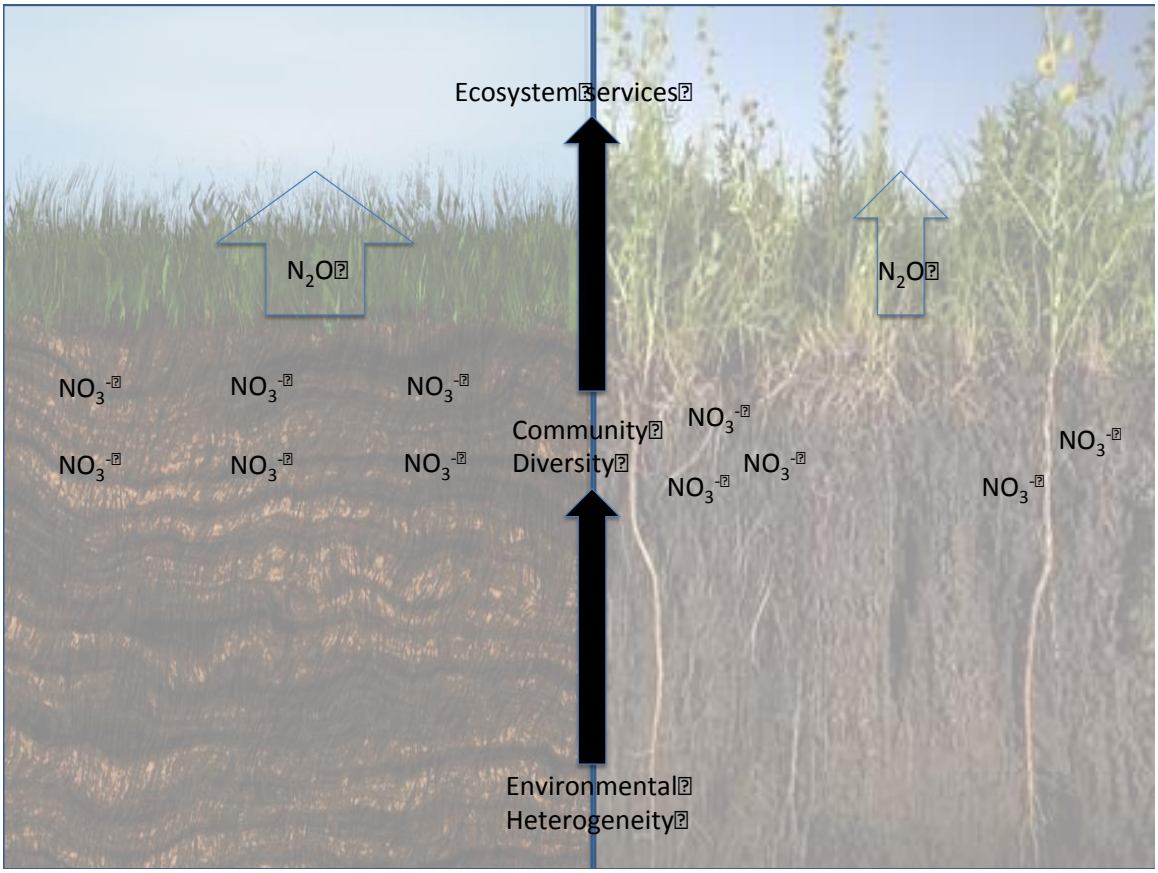

Figure 1.1. This conceptual diagram displays my expectation that environmental heterogeneity will influence diversity, which will influence ecosystem functioning. An environmentally homogenous, low-diversity, low ecosystem functioning system is represented on the left. An environmentally heterogeneous, high-diversity, high ecosystem functioning system is represented on the right. This conceptual model applies to native prairies as well as restorations, but does not account for sampling effects (e.g. highly productive species).

CHAPTER 2

DIVERSITY PATTERNS FROM SEQUENTIALLY RESTORED GRASSLANDS SUPPORT THE 'ENVIRONMENTAL HETEROGENEITY HYPOTHESIS' AT PLOT SCALE

(Pending acceptance decision in *Oikos*)

Introduction

The 'environmental heterogeneity hypothesis' (EHH) states that variation in resources promotes species coexistence, and is supported by studies demonstrating that structural heterogeneity of plants increases animal diversity (McArthur 1965, 1966, Pianka 1966, Murdoch et al. 1972). Support for this hypothesis was developed from published studies that incorporated an openness gradient of forest gaps in tropical and temperate climates. Openness gradients were known to correlate with changes in light availability and soil nutrients (Ricklefs 1977). Mechanisms underlying the EHH might include differential response of species in competition throughout space (spatial storage; Chesson 2000), and coexistence outcomes determined by minimum resource requirements and resource use rates (resource ratio hypothesis; Tilman 1982).

The high biodiversity of tallgrass prairie has been attributed to patchy grazing, burning regimes, and variability in climate that promote landscape and community heterogeneity (Collins and Steinauer 1998, Collins et al. 1998, 2002, Knapp et al. 1998, Joern 2005). Restored prairies often contain less diverse plant communities than never-cultivated prairie (Sluis 2002, Carter and Blair 2012), and a lack of heterogeneity in formerly cultivated fields has been hypothesized to contribute to this general phenomenon (Baer et al. 2003, 2004, Baer et al. 2005, Baer et al 2015). This study aims to examine the development of soil heterogeneity in response to restoration and its relationship to plant diversity.

Restored prairies often begin in homogeneous conditions because cultivation homogenizes nutrients (Haas et al. 1957, Pan-González et al. 2000). However, little research has been done on the development of heterogeneity in restored prairies. There may be a decrease in the heterogeneity of soil nitrate with restoration age as there is high variability in extractable N in agricultural and newly restored soils compared to older restorations (Rosenzweig et al. 2016). Heterogeneity in light may also decrease with restoration as C_4 grasses increase in abundance (Sluis 2002, Carter and Blair 2012, Baer et al. 2015) and limit light (McCain et al. 2010).

It is also unclear if heterogeneity in soil nutrient availability or light heterogeneity related to plant structure relate to changes in the plant community. If both soil- and plant-induced heterogeneity in resources (available nutrients and light) influence plant diversity, which has the larger influence? Because light availability is influenced by the plant community composition but can in-turn affect plant community composition, this can be viewed as a feedback. One study found that light heterogeneity was related to the plant community, but soil nitrogen heterogeneity was not (Bakker et al. 2003). By contrast, other studies found that soil nutrient heterogeneity was related to plant diversity and did not consider light heterogeneity (Golubbski et al. 2008, Eilts et al. 2011). One of these studies suggested that environmental heterogeneity interacted with plant-plant competition to influence plant community structure (Golubbski et al. 2008).

Although the 'environmental heterogeneity hypothesis' was developed from forest data, most tests of this hypothesis have been conducted in grasslands. Support for the hypothesis has come from observational studies (reviewed by Huston 1979, Bakker et al. 2003) and theoretical modeling (Golubbski et al. 2008), but there has not been strong support from experimental manipulations (Eilts et al. 2011, Baer et al. 2016). Heterogeneity in nutrient availability (Baer et al. 2003, Golubbski et al. 2008, Eilts et al. 2011, Baer et al. 2015) and light availability (Bakker

et al. 2003) has been previously shown to relate to plant diversity. However, this hypothesis is not supported by some experimental manipulations (Reynolds et al. 2007). Inappropriate scales of environmental manipulations (e.g. not considering the large expanse of genets) have been attributed to this disagreement in results (Eilts et al. 2011).

I hypothesized that soil nitrate heterogeneity will be greatest early in restoration age as plants begin to establish then decrease with age. This is consistent with trends in variability in a restoration chronosequence (Rosenzweig et al. 2016). I also hypothesized that available light heterogeneity will decrease as clonal grasses increase in dominance (Sluis 2002, Carter and Blair 2012, Baer et al. 2016). I also hypothesized heterogeneity of both soil nitrate and availability of light will be correlated with plant diversity and richness as predicted by the 'environmental heterogeneity hypothesis' (Ricklefs 1977). Many studies have shown that nitrate (Baer et al. 2003, Golubbski et al. 2008, Eilts et al. 2011, Baer et al. 2015) and light availability (Bakker et al. 2003) correlate with plant diversity. I also predicted that heterogeneity in light availability would be more strongly correlated with the plant community than heterogeneity in soil nitrate because the study that analyzed both factors found that only heterogeneity in light was correlated with plant richness, where changes in light heterogeneity were driven by grazing treatments (Bakker et al. 2003).

Methods

Site Description and Field Sampling

This research was conducted at the Konza Biological Station and Long-Term Ecological Research site (KNZ), located 9 km south of Manhattan, KS. The 30-year average annual precipitation is 835 mm/yr (75% received during the April-September growing season). The research area is located on a lowland Mollisol soil that had been in continuous cultivation for >

50 years prior to restoration. The soil is a Reading Silt loam 0-1% slope (mesic Typic Agruidoll), formed from alluvial and colluvial deposits.

Seven fields located on KNZ were used (cultivated agricultural field, 1-year restored, 3-year restored, 5-year restored, and 7-years restored). Four independent plots were established in each field. Plots were 6 m x 8 m and contained 12 subplots (2 m x 2 m). Each subplot contained two resin bags and two ¼ m^2 species composition quadrats (Fig. 1). The plot shapes were based on the design of Baer et al. (2003). Plots within a field were delineated so that sampling points in different plots would be at least 10 m apart so there was no spatial dependence, based on a pilot analysis. Subplots within a plot were not considered spatially independent. Subplots within a plot were used to calculate mean (availability of a resource) and coefficient of variation of a resource (heterogeneity of a resource). Native prairie was not included in this analysis because the soil has no spatial autocorrelation due to high heterogeneity (Bakker 2003).

All fields were in continuous cultivation for > 50 years prior to restoration. Recently the agricultural practice has been a corn-soybean-wheat rotation with winter wheat cover. Before a restoration site is seeded the field is disced to increase seed contact, then tillage is stopped. The restoration chronosequence was then seeded with a mix of 60% grasses and 40% forbs (Manning and Baer 2018). Six grass species (*Andropogon gerardii*, *Sorghastrum nutans*, *Schizachyrium scoparium*, *Bouteloua curtipendula*, *Panicum virgatum*, and *Elymus canadensis*) and 14 forb species (*Amorpha canescens*, *Baptisia autralis*, *Dalea purpurea*, *Dalea candida*, *Dalea multiflora*, *Desmanthus illinoiensis*, *Echinacea angustifolia*, *Helianthus pauciflorus*, *Lespedeza capitata*, *Liatris pycnostachya*, *Oenothera macrocarpa*, *Rosa arkansana*, *Silphium integrifolium*, *Oligoneuron rigidum*) were included in the mix.

Anion exchange resin bags were used to determine relative nitrite+nitrate-N availability (Binkley and Matson 1983) in a spatially explicit design over one growing season. Two anion exchange resin bags were buried in 0.5 m x 0.5 m quadrats NW and SE from the center of each subplot. Resin bags were prepared by placing 5 g of a strongly basic anion exchange resins (Dowex 1X8, 50-100 μm mesh) in a double layer of nylon hosiery attached to zip-ties (Baer et al. 2003). These resin bags were buried to 10 cm in late May and collected in early September.

Vascular plant species were recorded in two 0.5 m x 0.5 m quadrats NE and SW from the center of the 12 subplots in late May 2016 and September 2016 in each restored field (Fig. 2.1). Presence/absence data for each subplot were converted to frequency data at the plot level. Photosynthetically active radiation (PAR) was measured in the same quadrats where species composition was taken in early September (cultivated field not recorded). Photosynthetically active radiation was measured above and below the plant canopy using a Decagon AccuPAR LP-80 ceptometer (Decagon Devices Pullman, WA). Proportion of light available at the soil surface will calculated as PAR below canopy divided by PAR above canopy.

Spatial Reference Site

Data from restored prairie plots located at Konza Prairie (Baer et al. 2003) were used to construct an experimental variogram (i.e. semivariogram) for soil nitrite+nitrate-N to examine the spatial variability in plots with no applied treatment using resin-bag data from 2014, when this prairie restoration was 16 years old (Appendices A and B). This dataset was used because it was the youngest restoration with recently collected nitrate data available. The 2014 dataset was used for this site because it had few missing values. This restoration was sown with all the same species as restorations in the main study plus a few additional species. Rare species were sown after establishment of the restoration (Baer et al. 2015). Coordinates (x and y distance in meters

from most southern and western corners of plots) were assigned with package sp (Pebesma et al. 2005, Bivand et al. 2013) in R (R core team 2016). Nitrate and coordinate data were transformed to z scores to remove anisotropy (i.e. directionality) and normalize the attribute data (Isaaks and Srivastava 1989). The range was then estimated with a variogram model using an unweighted (ordinary least squares) fit method to preserve heterogeneity in the variance estimates. A spherical model was fitted in the R package gstat (Pebesma 2004).

Lab Analyses

Resin bags were rinsed with deionized water to remove excess soil then extracted with 2M KCl in 0.1M HCl. The extracts were filtered with 0.4 μm HTTP Isopore Membrane Filters (Merk Millapore Ltd.) and analyzed on an OI Analytical Flow Solution IV (OI Analytical Corp., College Station, TX, USA) for nitrite+nitrate-N. Nitrate was reduced to nitrite with cadmium metal. The nitrite reacted with sulfanilimide and then coupled with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting colored solution (azo dye) was colorimetrically detected at 540 nm (absorbance). The average of blanks (one for each batch of resin bags analyzed) was subtracted from samples to account for ambient nitrate/nitrite.

Statistical Analyses

Means and coefficients of variation were calculated for each plot for soil nitrate and proportion of PAR available at the soil surface. Coefficient of variation (CV) was used to measure heterogeneity in light and nitrogen. I used CV as a measure of heterogeneity because it is less likely than standard deviation to be related to the mean. Relationships between mean soil nitrate (nitrate availability), mean proportion of PAR available (light availability), CV of soil nitrate (nitrate heterogeneity), and CV of proportion of PAR available (light heterogeneity) with restoration age was explored using linear and non-linear models (e.g., linear regression, low-

order polynomial, 3-parameter Gaussian, exponential rise-to-max, and exponential decay) in R (R Foundation for Statistical Computing). All significant linear and polynomial models and all converging non-linear models were compared with Akaike Information Criterion to determine the most parsimonious model. Non-linear models were tested for significance by comparison to a null model, where the most significant parameter has been dropped.

A PCoA ordination was created in the R package vegan (Oksanen et al. 2016) to display plant species composition of each plot, relating mean and CV of soil nitrate and proportion of PAR fitted as vectors if significant. Differences in plant composition based on restoration age were tested using analysis of similarity (ANOSIM) using Bray-Curtis dissimilarities and 1000 permutations. Age groups were displayed on the ordination with 95% standard error of the weighted average of scores with the principal axis of the ellipse defined by weighted correlation using the ordiellipse function. Indicator species analysis was performed with the package indicspecies (De Caceres and Legendre 2009) using the index defined by Dufrêne and Legendre (1997) to identify the species with high fidelity and constancy in each restoration age group. All species with significant indicator values (IndVal) for a single site were reported. Variance in community composition was compared among restoration age groups (PERMDISP) using the betadisperser function. Environmental variables correlation with plant community structure were tested with permutational multivariate analysis of variance (PERMANOVA) analysis based on Bray-Curtis dissimilarities with 1000 permutations and restoration age as a block using the adonis function in the R package vegan (Oksanen et al. 2016).

Exponent of Shannon's diversity ($e^{H'}$), Pielou's evenness (J), and richness (S) were analyzed as response variables in linear mixed effects models (Type 3, Satterthwaite's approximation of degrees of freedom, maximum likelihood estimations) using the lme4 package

(Bates et al. 2015), where *P* values were calculated with the lmerTest package (Kuznetsova et al. 2017). Transformations were applied to meet the normality and homoscedasticity assumptions of linear mixed models; these transformations also reduced collinearity. Models had a variance inflation factor < 7 as calculated with the usdm package (Naimi et al. 2014), indicating little collinearity. Likelihood ratio (chi squared statistic) tests were performed using the drop1 function to remove non-significant interactions and increase parsimony. Pseudo-R^2 values for linear mixed models and adjusted coefficients of determination were calculated for fixed terms using the MuMIn package (Bartoń 2018). Significance was assigned using $\alpha = 0.05$. Data for this chapter are included in appendices C and D.

Results

The spatial reference site had a z-transformed rage of 3.3 with a back-transformed range of 10.0 m (distance of spatial independence). Mean soil nitrate and mean proportion of PAR available at the soil surface decreased with restoration age according to an exponential decay model (Fig. 2.2 A and C). However, there were only pairwise differences in mean nitrate among restoration age groups (cultivated and 1 year restored prairies had higher values than the older prairie restoration sites). There was no directional change in CV of available nitrogen with restoration age (Fig. 2.2 B). There were no pairwise differences in CV of available nitrogen among restoration age groups. However, CV of proportion of PAR available at the soil surface increased with soil restoration age according to an exponential decay function with a negative b parameter (Fig. 2.2 D). Despite this slow increase, there were no pairwise differences in CV of proportion of PAR available among restoration age groups.

Sixty-one species were encountered among 20 plots (Appendix C); *Andropogon gerardii* was the most dominant across all restored prairies. Five species had high fidelity and specify to

1-yr restored prairie: *Digitaria sanguinalis* (IndVal = 1.00, *P* = 0.005), *Eragrostis cilianensis* (IndVal = 1.00, *P* = 0.005), *Chamaesyce maculata* (IndVal = 0.937, *P* = 0.020), *Amaranthus rudis* (IndVal = 0.87, *P* = 0.020), and *Bouteloua curtipendula* (IndVal = 0.79, *P* = 0.045). Three species had high fidelity and specify to 3-yr restored prairie: *Verbena stricta* (IndVal = 1.00, *P* = 0.005), *Bromus tectorum* (IndVal = 0.89, *P* = 0.010), and *Hordeum pussilum* (IndVal = 0.87, *P* = 0.050). Two species had high fidelity and specify to 5-yr restored prairie: *Helianthus annuus* (IndVal = 0.89, *P* = 0.010) and *Solidago canadensis* (IndVal = 0.85, *P* = 0.020). Three species had high fidelity and specify to 7-yr restored prairie: *Amorpha canescens* (IndVal = 0.87, *P* = 0.020), *Schizachyrium scoparium* (IndVal = 0.82, *P* = 0.005), and *Sorghastrum nutans* (IndVal = 0.78, *P* = 0.015).

Plant community dissimilarity was displayed with a two-dimensional principal coordinates analysis (PCoA). Species scores of indicator species were labeled. Vectors representing significant correlation of site scores with environmental variables are also displayed on the ordination (Fig. 2.3). Differences in plant composition with restoration age were detected by ANOSIM (R = 0.78, *P* = 0.001); dispersion (PERMDISP: *P* = 0.038), differed among restoration ages, with the most dispersion in the youngest prairie and the least dispersion in the oldest prairie. Centroid location (PERMANOVA: *P* = 0.001) also differed among restoration ages. However, there were no significant correlations between species composition centroid location and environmental variables when blocked by restoration age (PERMANOVA; N heterogeneity: *P* = 0.865; PAR heterogeneity: *P* = 0.673; N heterogeneity*PAR heterogeneity: *P* = 0.836; N heterogeneity*N availability: *P* = 0.336; PAR heterogeneity*PAR availability: *P* = 0.995).

Resource availability and heterogeneity predictors in linear mixed models of $e^{H'}$ (marginal pseudo-R^2 = 0.36, conditional pseudo-R^2 = 0.77) and S (marginal pseudo-R^2 = 0.46, conditional pseudo-R^2 = 0.82) explained a great deal of variance, but fixed effects did not explain much variance in the model of J (marginal pseudo-R^2 < 0.01, conditional pseudo-R^2 = .98). Nitrogen heterogeneity and PAR heterogeneity main effects were detected for $e^{H'}$ and S. Significant interactions of N heterogeneity*N availability and PAR heterogeneity*PAR availability were detected for H′; only N heterogeneity*N availability was detected for S (Table 2.1). All significant interactions had a smaller linear coefficient (i.e. a shallower slope; Table 2.1). No significant effects were detected in the mixed model of J.

Discussion

The 'environmental heterogeneity hypothesis' is an explanation for species coexistence through variability in resources that can be partitioned (Ricklefs 1977). For plants in tallgrass prairie, two key resources for growth are N and light. This hypothesis has been supported by multiple observational studies (reviewed by Huston 1979, Bakker et al. 2003, Golubbski et al. 2008, Eilts et al. 2011), but many experimental tests have found weak (Baer et al. 2016) or no support (reviewed by Lundholm 2009). This study used the same scale as the Baer et al. (2016) experiment and found stronger support for EHH. This weak effect might suggest that it is difficult to experimentally replicate environmental heterogeneity found in reference ecosystems. Alternatively, this discrepancy between observational support and lack of experimental evidence could suggest that plants induce rather than respond to heterogeneity, e.g., soil ions (Greig-Smith 1979, Gibson 1986, 1988a, b).

As hypothesized, light availability, light heterogeneity, and soil nitrate availability changed with restoration age. The exponential decrease in growing-season soil nitrate

availability with increasing restoration age was consistent with a previous study that measured extractable soil nitrate (Rosenzweig et al. 2016). Inconsistent with our predictions, heterogeneity in light increased with restoration age, though very slowly. Also in contrast to our predictions soil nitrate heterogeneity did not show a directional change with increasing restoration age. This lack of increase in heterogeneity suggests if increasing soil nutrient heterogeneity is a restoration goal, it should be implemented at the onset of restoration. Without manipulation to promote environmental heterogeneity, it might be difficult to maintain high diversity plant communities that coincide with heterogeneous environments in grassland restorations.

Dynamically changing light and nitrate availability with restoration age resulted in prairie communities grouped by age in the ordination space. Plant composition was not related to environmental heterogeneity, suggesting all separation was due to changes that occur with restoration age. Restored prairie plots within the youngest restoration site were most variable in terms of plant composition because of variability in the soil seed bank, as many of the sown perennial species had not yet established. There was also greater dispersion in young restoration sites compared to older restoration sites, supporting strong community convergence with restoration age (Baer et al. 2016), which might lead to reduced ecosystem functioning because community convergence is associated with trait convergence (Grman et al. 2018). I suggest that coexistence and diversity research be extended to evaluation of functional traits, because environmental variability and trait availability influence ecosystem functioning (Hodapp et al. 2016).

Plants associated with N fixers might be an important N source when little N from agricultural fertilizer remains. Putative N fixing plants can have greater N availability in surrounding soils, which can be utilized by neighboring plants (Temperton et al. 2007). *Amorpha*

canescens, a legume, was an indicator species of 7-y restored prairie, suggesting the importance of putative N fixing plants. It has also been suggested that legumes could be better competitors than non-leguminous forbs when competing with a dominant grass that establishes early in restorations, *Andropogon gerardii* (Scott and Baer 2018).

Diversity and richness were positively related to N heterogeneity and PAR heterogeneity. When interactions of nitrate availability and nitrate heterogeneity were significant, the strength of the positive effect of resource heterogeneity on plant diversity or richness was reduced. This highlights the importance of creating heterogeneity early in the restoration process, when resources when soil nitrate levels are high. Perhaps this result should not be surprising because several studies have shown that high levels of a limiting resource decreases diversity through competitive exclusion (Al-Mufti et al. 1977, Grime 2001, Fridley 2002, Rajaniemi 2002). Because N becomes progressively limited during grassland restoration (Baer et al. 2003, Baer and Blair 2008, Rosenzweig et al. 2016), the positive effect of N heterogeneity on plant diversity and richness might be stronger in older restorations. Similar to native (never-cultivated) and grazed prairie, light heterogeneity had stronger effects on plant richness than N heterogeneity (Bakker et al. 2003), when we considered N availability modulating N heterogeneity effects. No effects on evenness were observed, suggesting that effects on diversity were mostly driven by richness. Overall, these results provide support for heterogeneity in light and inorganic N as strong drivers of plant diversity and richness as communities develop.

Our results suggest that resource ratio theory and spatial storage both contribute to maintenance of plant diversity. Differing resource ratios among communities can act as a simple form of spatial storage to promote regional diversity (Pacala and Tilman 1994). Spatial storage was developed from spatially explicit studies of plant populations (Bolker and Pacala 1998,

Bolker 2003) and has been proposed as the mechanism preserving diversity after an adaptive radiation (i.e. rapid speciation; Tan et al. 2017). However, few studies have examined resource availability and heterogeneity simultaneously as it relates to species diversity. Our results demonstrate that resource availability influences diversity responses to environmental heterogeneity, suggesting that resource ratio theory and spatial storage are not mutually exclusive even within the same site.

Table 2.1. Summary statistics (t values, pseudo-R^2 values, P values, and linear coefficients [β]) from linear mixed model analyses. Response variables include e^H = Shannon's diversity, S = species richness, and J = Pielou's evenness. Predictor variables included N heterogeneity = coefficient of variation of nitrite+nitrate–N, PAR heterogeneity = coefficient of variation of proportion of photosynthetically active radiation reaching soil, N het. * N avail. = interaction of N heterogeneity with nitrogen availability (N avail. = mean [availability] nitrate+nitrite-N).

	$e^{H'}$	S	J
N heterogeneity	$t_{15.9} = 2.41$ $R^2 = 0.27$ $P = 0.028$ $\beta = 6.59$	$t_{16.0} = 3.15$ $R^2 = 0.38$ $P = 0.006$ $\beta = 10.65$	$t_{12.3} = -0.05$ $R^2 < 0.01$ $P = 0.962$ $\beta = -0.01$
PAR heterogeneity	$t_{4.8} = 6.56$ $R^2 = 0.42$ $P = 0.001$ $\beta = 14.49$	$t_{4.9} = 7.80$ $R^2 = 0.53$ $P < 0.001$ $\beta = 22.21$	$t_{12.8} = 0.28$ $R^2 < 0.01$ $P = 0.783$ $\beta = 0.04$
N het. * N avail.	$t_{15.9} = 2.37$ $R^2 = 0.26$ $P = 0.031$ $\beta = 2.52$	$t_{15.8} = 4.13$ $R^2 = 0.36$ $P = 0.008$ $\beta = 3.95$	$t_{12.2} = 0.14$ $R^2 < 0.01$ $P = 0.894$ $\beta = 0.01$

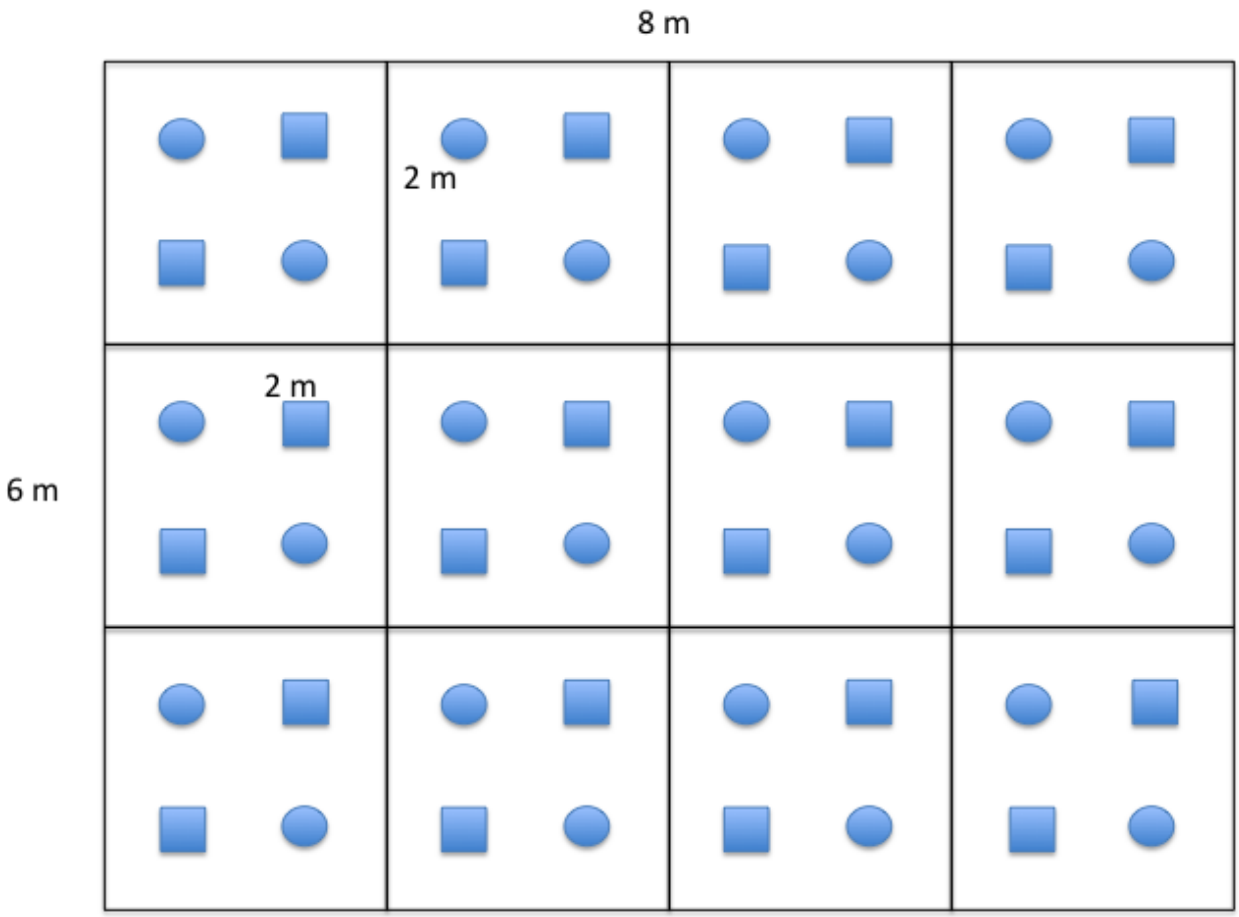

Figure 2.1. Diagram of a plot, where dots represent the location of anion exchange resin bags. Species composition quadrats are located in the other two corners of each subplot, represented by squares.

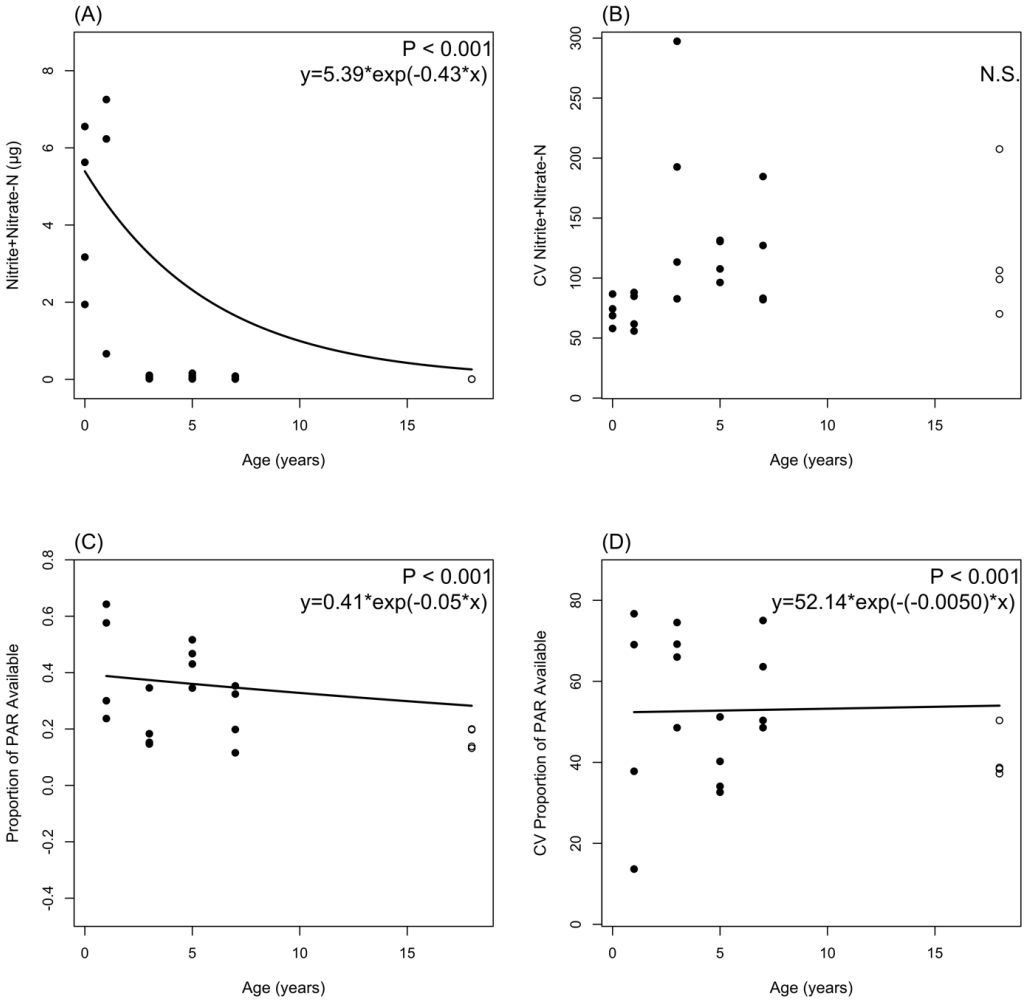

Figure 2.2. Regressions of environmental variables with restoration age. Each point represents a measurement from each plot. The lines represent a non-linear regression based on fitted parameter estimates (displayed as constants in equations on panels). Panel A represents mean soil nitrate. Panel B represents CV of soil nitrate. Panel C represents mean proportion of photosynthetically active radiation available at soil surface. Panel D represents CV of proportion of photosynthetically active radiation available at soil surface. Open circles represent an 18-y restored prairie, where spatial reference data were collected, that was not used in regression analyses.

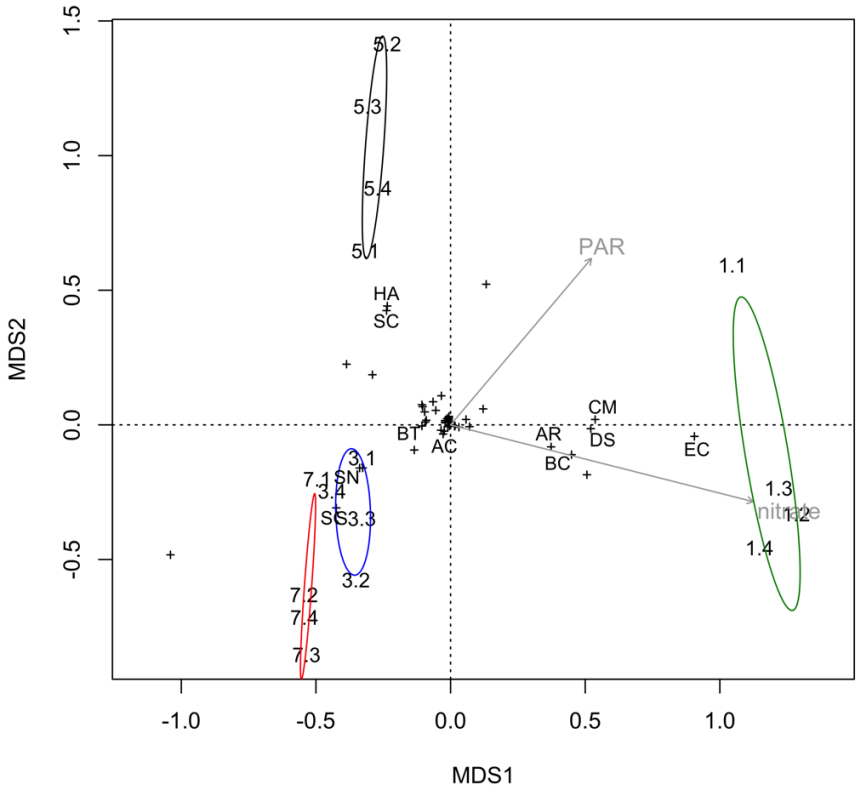

Figure 2.3. Principal coordinates analysis ordination of plots. Significant vectors are displayed, where nitrate = nitrate availability and PAR = light availability. Site points are displayed as age followed by plot number within that age. Species scores are represented + symbols. Significant indicator species are labeled according to the following abbreviations: DS = *Digitaria sanguinalis*, EC = *Eragrostis cilianensis*, CM = *Chamaesyce maculata*, AR = *Amaranthus rudis*, BC = *Bouteloua curtipendula*, BT = Bromus tectorum, HA = *Helianthus annuus*, SC = *Solidago canadensis*, AC = *Amorpha canescens*, SS = *Schizachyrium scoparium*, SN = *Sorghastrum nutans*. Two other significant indicator species, *Verbina stricta* and *Hordeum pusillum*, are located in the large group of points near the origin. Ellipses represent 95% standard error from age group centroid.

CHAPTER 3

MANIPULATED SOIL RESOURCES AND PLANT SPECIES EFFECTS ON THE SOIL MICROBIAL COMMUNITY

Introduction

Soil nutrients influence plant diversity (Bradshaw 2004, Hejcman 2014, Dias et al. 2014, Mauchamp et al. 2016), but plant species can also differentially influence soil microbial communities and nutrient availability (Hawkes et al. 2005, Hausmann and Hawkes 2009, 2010, Kilvin and Hawkes 2011). Plant soil feedbacks occur when a plant changes the soil community, which in turn affects the plant growth rate (Bever 1994, Bever 2003). Identity of neighboring plants has been shown to influence AMF in the rhizosphere of the focal plant (Morris et al. 2013), indicating plant effects on the microbial community. Plants can play a role in structuring of soil microbial communities and both directly via root turnover, litter inputs, and root exudates and indirectly through nutrient uptake, as evidenced from distinct microbial communities associated with different plant species (reviewed by Sasse et al. 2018 and Hassani et al. 2018) and genotypes within a plant species (Shakaya et al. 2013).

Soil microbial communities vary with soil profile depth. The surface microbial community may be affected by soil depth if there is more resource availability (e.g. organic C, available N and water) relative to deeper soils (Schimel et al. 1991, Turner et al. 1997). Microbial biomass and many diversity metrics decrease with depth (Blume et al. 2002, Fierer et al. 2003, Jumpponen et al. 2010) and Gram-positive bacteria and actinomycetes become proportionally more abundant with depth in grasslands (Fierer et al. 2003). Most microbial biomass is concentrated near the soil surface because of greater detritus inputs (Lenz and

Eisenbeis 1998, Fritze et al. 2000, Snajdr et al. 2008). However, possible effects of depth of soil profile on the surface microbial community have not been well studied. Soil profile depth might influence the soil microbial community by releasing forbs from competition with dominant C_4 grasses in shallow soils (van Auken et al. 1994), thus altering plant inputs to soil.

Nutrient availability could influence the microbial community through altered plant-microbe interactions (Bardgett et al. 2003), as well as microbe-microbe interactions (Yan et al. 2017). Studies in grassland and agricultural systems have revealed consistent microbial taxonomic composition responses to nitrogen addition, but microbial diversity responses are less consistent (Ramirez et al. 2010). Some grassland studies found a decrease in soil microbial diversity with nitrogen addition (Coolon et al. 2013, Yang et al. 2015). In a review of global grasslands, nutrient (nitrogen and phosphorus) addition caused consistent shifts in fungal (*Ascomyta* species increased, *Glomeromycota* species decreased), archaeal (*Crenarcheota* species increased, *Euryarchaeota* and *Parvarchaeota* species decreased) and bacterial communities (*Actinobacteria*, *Alphaproteobacteria*, and *Gammaproteobacteria* species increased, *Acidobacteria*, *Planctomycetes*, and *Deltaproteobacteria* species decreased; Leff et al. 2015). Nitrogen addition also decreased fungal and archaeal diversity, but not bacterial diversity. Changes in soil bacterial and fungal composition were related to change in plant composition (Leff et al. 2015).

Many soil microbial studies have used PLFA biomarkers as a proxy for diversity because of lower cost amplicon sequencing techniques and the ability to accurately estimate biomass of major taxonomic groups. Shifts in the Gram-negative bacteria with increased nitrogen availability have been demonstrated in tropical forests using PLFA biomarkers (Cusak et al. 2011, Liu et al. 2014), and the direction of this shift may be conditional on elevation (Liu et al.

2014). Agricultural land use (different plants) was shown to influence the AMF community using morphology of spores (Urcelay et al. 2009). An experimental removal of plant functional groups indicated that plant composition had a larger impact on AMF communities than physical and chemical soil properties in shrubland systems (Pereira et al. 2014). In P-limited tallgrass prairie such as Konza Prairie, mutualistic phenotypes of AMF are expected because AMF are more N limited than interacting plants and plants are more P limited than interacting AMF (Johnson et al 2014). Fungal hyphae (Eom et al. 1999, Wilson et al. 2009) and spore abundance (Eom et al. 1999) increased in response to N addition, but fungal species evenness decreased (Eom et al. 1999) due to N-limitations. Fungal abundance and diversity, using PLFA biomarkers, increased with plant richness in old fields that were historically tallgrass prairie (Zak et al. 2003). Positive responses of PLFA richness to restoration age have also been demonstrated (Allison et al. 2005, Bach et al. 2010, Baer et al. 2010, 2015).

This study was undertaken at a site where experimental manipulation of soil heterogeneity has been maintained for 20 years to measure plant diversity responses in restored tallgrass prairie (Baer et al. 2016). The impact of plant species and manipulating soil heterogeneity on the soil microbial community has not been investigated. Because it is unclear which potential driver has the strongest influence on soil microbial communities, the overall objective of this research was to reveal whether soil (nutrient availability and depth) or plant identity has a stronger influence on soil microbial community structure. I predicted that all main effects would have an influence microbial diversity. I predicted the strongest effect on PLFA microbial diversity would be the interaction of nutrient availability and plant species.

Methods

Site Description

The research area was located on lowland Mollisol soil that had been in continuous cultivation for > 50 years prior to restoration. The site was located 9 km south of Manhattan, KS on the Konza Biological Station and Long-term Ecological Research site. The 30-year average annual precipitation is 835 mm/yr (75% received during April-September growing season). The soil was a Reading Silt loam 0-1% slope (mesic Typic Agruidoll), formed from alluvial and colluvial deposits.

Plots containing manipulated soil heterogeneity were established in June 1998 at Konza Prairie Biological Station (Baer et al. 2003). These plots were initially sown with a mix of 42 native species at rates selected to achieve a log-normal distribution of species representative of native prairies (Baer et al. 2003) and received a second seed addition of 15 species (25 live seeds m^{-2}) that had never occurred in the plots in March 2005 (Baer et al. 2015). Starting in 2013, an additional 17 never-present subordinate species were sown at a rate of 20 seeds m^{-2} $year^{-1}$ for each species.

The design of the experiment was a strip-strip split block, with nutrient heterogeneity assigned to three strips (low, ambient, or high N availability) and depth heterogeneity assigned to four alternating strips (deep and shallow soils) perpendicular to the nutrient treatments. High N availability was created with annual ammonium nitrate fertilization (5 g N m^{-2}). Reduced N availability was originally created by incorporating sawdust in 1997 (5.5 kg dry sawdust m^{-2} with 49% C and C:N ratio = 122), and starting in 2005 sucrose was added three times during each growing season (84.2 g sucrose–C m^{-2}). These treatments were demonstrated to

significantly influence soil N availability (Baer and Blair 2008). Shallow depth treatments were created by burying limestone slabs approximately 10 cm deep.

I sampled soil below 3 plant treatments (*Andropogon gerardii* [most common C_4 grass], *Salvia azurea* [most common forb], and bare ground) within the one subplot of the soil nutrient (3 levels: labile C addition, inorganic N addition, and ambient) x soil depth (2 levels: deep and shallow [limestone buried at 10 cm]) treatment combinations (n = 72; Fig. 3.1). Soil was sampled in spring 2018 at the main stem of the two plant species (plant-influenced soils) and in bare areas between plants. These plant species were chosen because they occurred in every plot.

PLFA Profiles

Phospholipid fatty acid biomarkers were used to examine the bacteria and fungi soil community in terms of functional groups (Gram positive bacteria, Gram negative bacteria, non-specific bacteria, actinomycetes, saprophytic fungi, and arbuscular mycorrhizal fungi). Analysis followed the methods of Blye and Dyer (1959) as modified by Bosio et al. (1998) and DeGrood et al. (2005) and used by Scott et al. (2017). Extractions were made from ~2 g of freeze-dried soils. Briefly, PLFAs were extracted from the soil using a 1:2:0.8 chloroform:methanol:phosphate buffer with centrifugation (2500 rpm, 10 min). Additional chloroform and phosphate buffer was added (12 ml each) and phases were allowed to separate. The chloroform phase was removed and evaporated under nitrogen gas (N_2). From this phase, neutral-, glycol-, and phospholipid fatty acids were separated on a solid phase column (0.50 g Si, Supelco, Inc. Bellefonte,PA). Newly polarized lipids were methylated using a methanol:toluene (1:1) solution (37°C, 15 min), H_2O (2 ml), and acetic acid (0.3 ml 1.0 M). Fatty acid methyl esters were removed with hexane, dried (under N_2 at room temperature), dissolved in hexane containing an internal fatty acid standard (19:0). PLFA extracts were analyzed with a Shimadzu

GC-2010 gas chromatograph with flame ionization detector (FID; Shimadzu Corp., Kyoto, Japan) and Omegawax 320 column: 30m x 0.25mm ID, 0.25um film (polyethylene glycol phase) (Supelco, Belfonte, PA, USA). An injection of 1.0 µl was injected at a 100:1 split. The oven was set at 150° C ramping to 182.5° C at 0.5° C /min with the injector at 280° C and the detector at 260° C. Helium was used as the carrier (1.09 ml/min).

Fatty acids were identified with bacterial acid methyl ester (BAME) mix (Sigma Aldrich, St. Louis, MO, USA), 11-hexadecenoic acid (Matreya LLC, Pleasant Gap, PA, USA), and actinomycetes (16:0 Me) standard: methyl 10-methylhexadecanoate (Matreya LLC, Pleasant Gap, PA, USA). Fatty acids were quantified with an internal standard Methyl undecanoate (C11:0) (Sigma Aldrich) and recovery determined by surrogate standard (100 µl of 1 mg/ml solution of C 19:0 phosphatidlycholine; Bird *et al.* 2011, Norris *et al.* 2013) 19:0 phosphatidycholine (Avanti Polar Lipids, Alabaster, AL, USA). Recovery corrected concentrations are reported (standard recovery and uncorrected PLFA biomass values reported in Supplemental Table 1). The PLFA biomarkers were assigned as in Scott et al. (2017), according to Olsson et al. (1995), Mckinley et al. (2005), Bach et al. (2010) and Williams et al. (2012). Actinomycetes, despite being phylogenetically bacteria, were displayed with fungi, as in Scott et al. (2017), because of similar filamentous morphology and similar effects on soil structure.

Statistical Analyses

Patterns in PLFA biomarker concentration dissimilarity were analyzed with permutational multivariate analysis of variance (PERMANOVA). Biomarkers concentrations were also used to create a non-metric multidimensional scaling (NMDS) ordination. Mixed model analyses were performed to test for main effects and interactions of soil nutrient, depth and plant effects on Shannon diversity index (H), richness (S), and Pielou's evenness (J) of PLFA

biomarker concentrations. Mixed model analyses were also performed with each PLFA group (non-specific bacteria, Gram-positive bacteria, Gram-negative bacteria, saprophytic fungi, arbuscular mycorrhizal fungi, and actinomycetes). In these models, diversity metrics or PLFA biomass was the response with plant species, nutrient availability, soil depth, and all possible interactions as fixed predictors. The split block design was accounted for with random effects of the fixed effect of soil depth within vertical strips, the fixed effect of nutrient availability within horizontal strips, and blocking by whole plot and subplot within whole plot. Models were fit using the lme4 package (Bates et al. 2015) and significance was determined using *P* values calculated with Kenward-Roger approximated degrees of freedom and restricted maximum likelihood using the lmerTest package (Kuznetsova et al. 2016). To meet the assumption of normal distribution, log transformations were applied to saprophytic fungi and actinomycetes biomasses. Protected Fisher's least significant differences (LSD) were conducted using the lsmeans package (Lenth 2016) to make comparisons between levels of main effects. Plots were created to display least-squares means and associated standard errors. Fungi:bacteria PLFA biomass ratio was calculated, where actinomycetes were included as fungi. Data for this chapter are included in Appendices H and I.

Results

There was no difference in pairwise community dissimilarity of PLFA biomarkers among any treatments. This was indicated by little separation in ordination space between sampling units (Fig. 3.2) and non-significant PERMANOVA results ($P > 0.05$; Appendix E). Most biomarkers occupied a similar ordination space, with the exceptions of two Gram-negative bacteria biomarkers (cy 19:0 and 2-OH 16:0) and one non-specific bacteria biomarker (17:0), which were more variable in their presences (Fig. 3.2).

Shannon diversity index differed with the main effect of plant identity. There was greater H′ in plant-influenced soils as compared to bare soil. This was not due to richness (S; $F_{3, 36}$ = 2.28, P = 0.116) or Pielou's evenness (J; $F_{3, 36}$ = 1.97, P = 0.154) alone and was associated with greater proportional arbuscular mycorrhizal fungi and Gram-negative bacteria in rhizosphere soils. There were no significant effects of soil depth, nutrient availability, or any interaction of main effects on diversity metrics (P > 0.05; Appendix F).

Total PLFA biomass (Fig 3.4) and biomass of each major taxonomic group were all affected by nutrient availability (non-specific bacteria: $F_{2, 5.9}$ = 10.38, P = 0.012; Gram-positive bacteria: $F_{2, 5.9}$ = 11.99, P = 0.008; Gram-negative bacteria: $F_{2, 5.9}$ = 14.93, P = 0.005; saprophytic fungi: $F_{2, 5.9}$ = 13.30, P = 0.007; arbuscular mycorrhizal fungi: $F_{2, 5.9}$ = 6.22, P = 0.035; actinomycetes: $F_{2, 5.9}$ = 5.94, P = 0.038, Fig. 3.5). The fungi:bacteria ratio for ambient N was 0.38. Low nutrient availability treatment subplots had higher PLFA biomass than ambient or high nutrient availability for all PLFA groups. There were no cases of significant main effects of depth or plant species on PLFA biomass (P > 0.05). Interactions among plant species, soil depth, or nutrient availability also had no effect on PLFA biomass (P > 0.05; Appendix G).

Discussion

Diversity of PLFA biomarkers responded to plant presence/absence, while total biomass and biomass of major taxonomic groups responded to nutrient availability. This difference was in contrast to my prediction that all tested variables (soil depth, nutrient availability, and plant treatments) would influence biomass, with plant species having the strongest effect. Actinomycetes and AMF PLFA groups had less response to nutrient availability than saprophytic fungi and bacterial PLFA groups. The positive sucrose addition effect on AMF is surprising because AMF receive plant photosynthate. This positive AMF response to sucrose addition

might suggest microbial groups, such as saprotrophic fungi, are promoting decomposition of organic matter. The positive AMF response might also be due to high carbon supply to the host plant triggering uptake and transport of N by AMF (Fellbaum et al. 2012). Diversity and PLFA biomasses were unaffected by soil depth.

Recovery of PLFA biomass generally occurs on a decadal time scale with grassland restoration from cultivation (Bach et al. 2010, Baer et al. 2010, Scott et al. 2017), likely due to cessation of tillage (Gupta and Germida 1988). A restoration chronosequence study with the same PLFA methods and conducted in the same area with a cultivated field and never-cultivated prairie was used to provide context to microbial community development (Scott et al. 2017). Total PLFA biomass levels in this study (ambient N estimate = 405.16 nmol g^{-1}) were midway between values obtained from a nearby-cultivated field (~ 200 nmol g^{-1}) and a never-cultivated prairie (~ 600 nmol g^{-1}). Ratios of fungi:bacteria PLFA biomass (ambient N estimate = 0.38 nmol g^{-1}) were greater than the reference cultivated field (~ 0.25) and never-cultivated prairie (~ 0.30). The PLFA biomass of this 20 y old restoration study is consistent with the prediction of a 20 y restoration based on modeling from a chronosequence in the same area; the PLFA fungi:bacteria biomass is also similar (~0.4; Scott et al. 2017).

These results are similar to the results of Ramirez et al. (2015) demonstrating microbial biomass and community composition, but not diversity, responded to soil nutrient manipulation. In contrast to studies of native prairie under chronic nitrogen enrichment using shallow amplicon sequencing (Coolon et al. 2013) and PLFA profiles (Yang et al. 2015), these results showed no decrease in diversity with chronic nitrogen addition in restored prairie. The N addition from the Coolon et al. (2015) describes N enrichment for the same amount of time (20 y) as this study. Some, but not all, diversity metrics described in Coolon et al. (2015) decreased with N addition.

For example H and its components were not different between N addition and control, but there was greater Simpson's dominance and lower alpha log series diversity in the N addition treatments. This is likely because Shannon diversity is less influenced by the dominant species. The Yang et al. (2015) study described a shorter duration of N enrichment (9 y). It is possible that increased plant diversity in the N enrichment following two decades of treatment (Baer et al. *in review*) has overridden the expected reduction of microbial diversity, because of positive correlation between plant and microbial diversity (Zak et al. 2003) and compositional change (Leff et al. 2015). This increase in plant diversity after chronic N enrichment is attributed to increased stochasticity in community assembly (Wilcox et al. 2017, Baer et al. *in review*).

This study only considered individual plant effects on microbial diversity and only examined two plant species, but richness of plants in a small area could potentially have an effect on microbial functional diversity and abundance of functional groups (Zak et al. 2003). It seems likely that PLFA microbial groups were positively responding to the labile carbon source used to create low nitrogen availability, since there was no difference between ambient and high availability (fertilized) nitrogen treatments. This result is consistent with increased microbial biomass in response to labile C additions in herbaceous ecosystems (Jonasson et al. 1996, Michelsen et al. 1999, Baer et al. 2003, Baer and Blair 2008).

Bare soil had lower Shannon diversity of microbial PLFA groups than plant-influenced soils due to lower relative abundance of Gram-negative bacteria and arbuscular mycorrhizal fungi. This result suggests that maximizing vegetation cover would maximize PLFA microbial group diversity. The plant effect likely corresponds with differential PLFA group response to greater organic matter inputs from plants by root turnover and litter (Kuzyakov and Blagodatskaya 2015). This response to plant inputs was in contrast to the sucrose addition, which

was utilized by all PLFA groups. Although there were no distinct soil microbial communities associated with plant species or soil manipulations using PLFAs, amplicon sequencing methods might be more sensitive. If plant and soil effects contribute to distinct soil microbial communities, then heterogeneous soils and diverse plant communities would be expected to promote soil microbial diversity at the scale of individual plants.

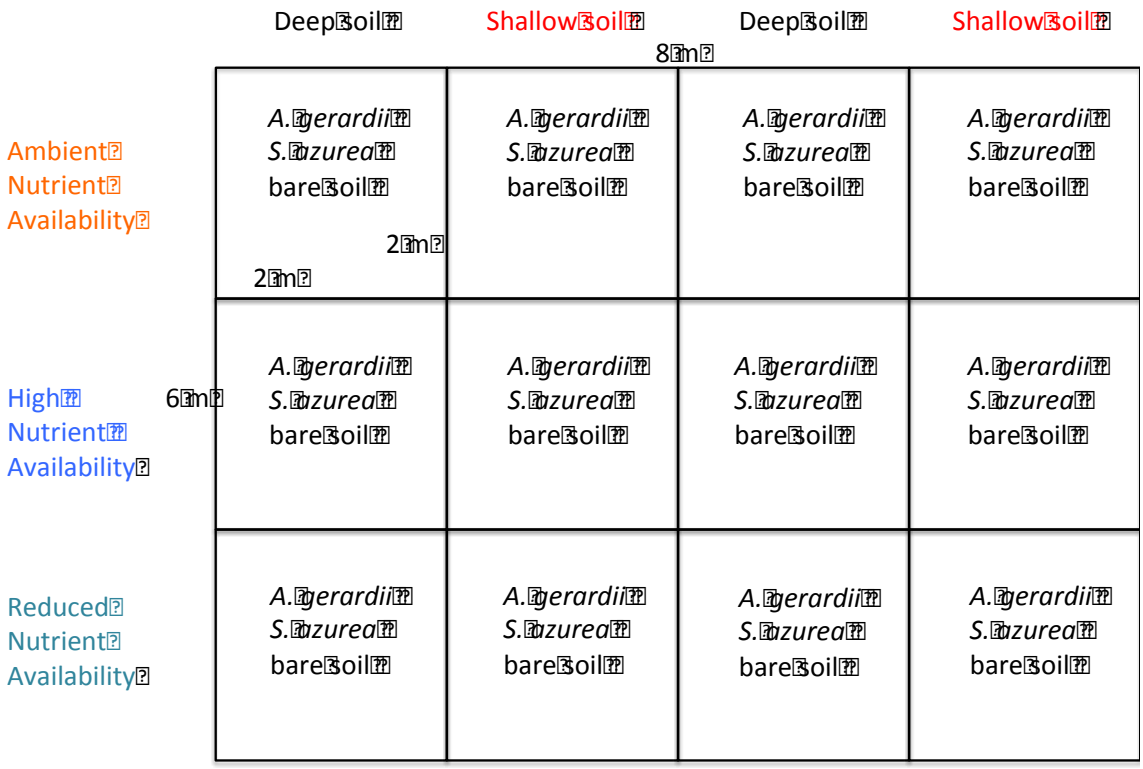

Figure 3.1. Layout of a plot with a strip-strip split plot design.

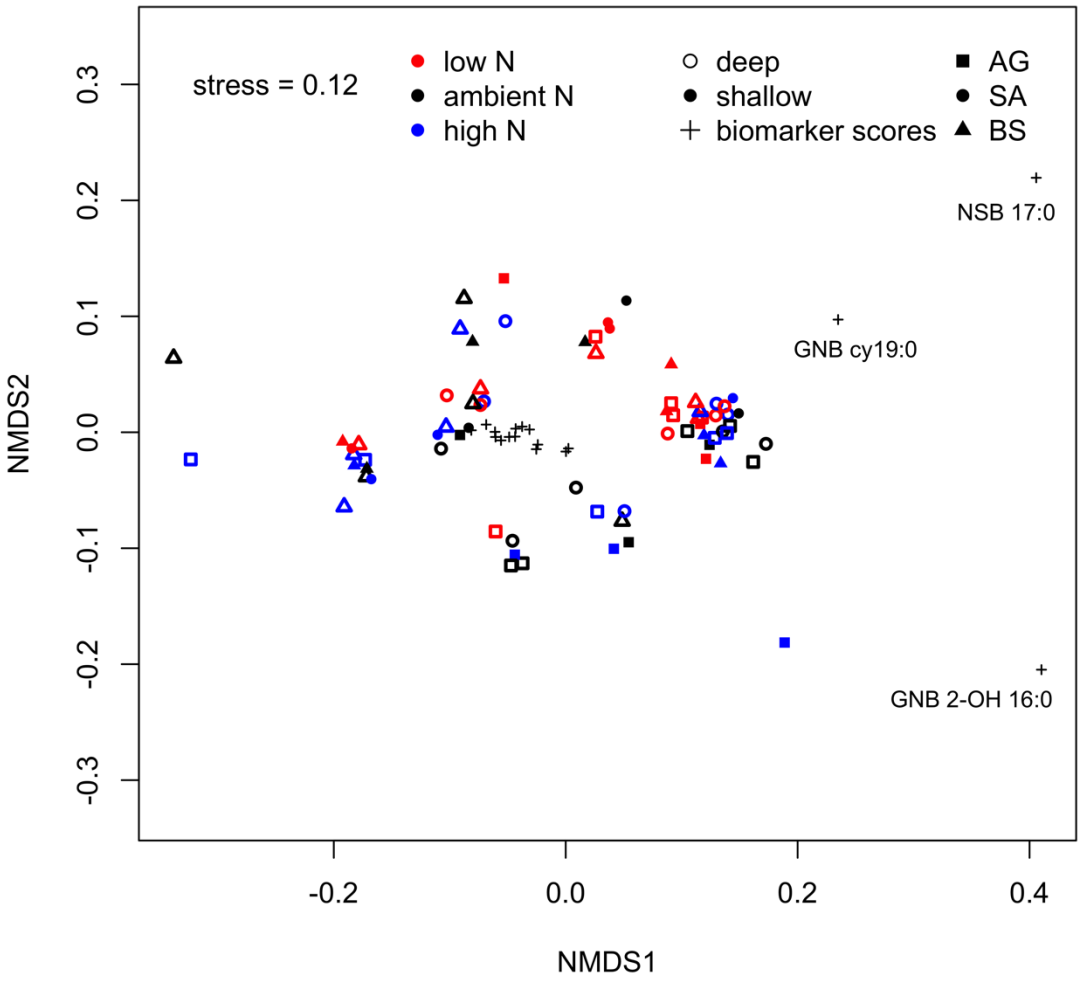

Figure 3.2. Non-metric multidimensional scaling (NMDS) ordination of whole community (all PLFA biomarkers). Plus symbols represent biomarker scores, while other symbols represent smapling unit scores. Openess of symbols represents soil depth, color represents soil N availability, and shape represents species treatment (where AG = *Andropogon gerardii*, SA = *Salvia azurea*, BS = bare soil).

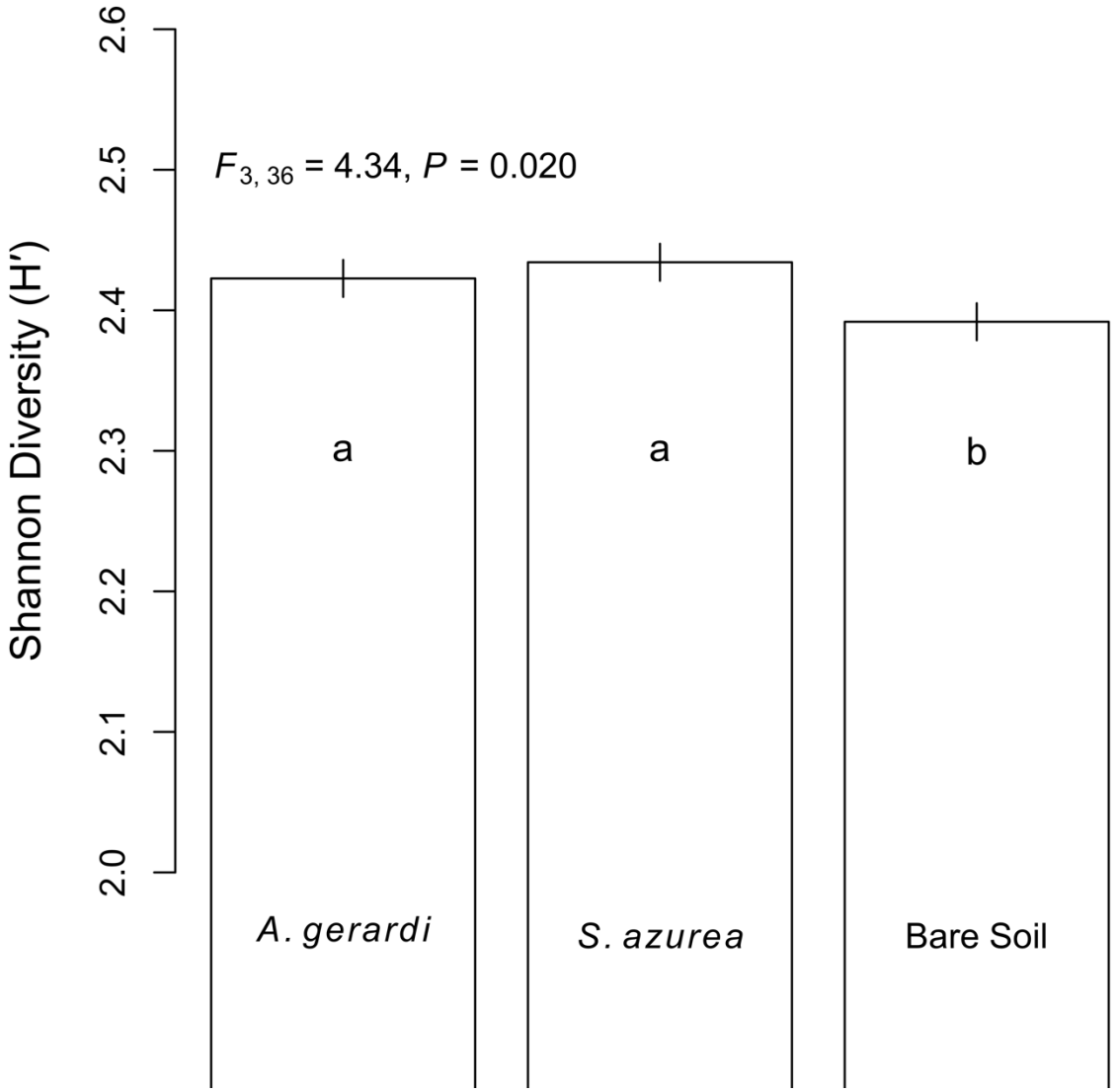

Figure 3.3. Shannon diversity of PLFA biomarkers in each plant treatment. Letters in bars represent significantly different groups. Abbreviations: AG = *Andropogon gerardii*, SA = *Salvia azurea*, and BS = bare soil. Total PLFA biomass is back-transformed from a log scale. Least squares means averaged over depth and nutrient availability treatments are shown.

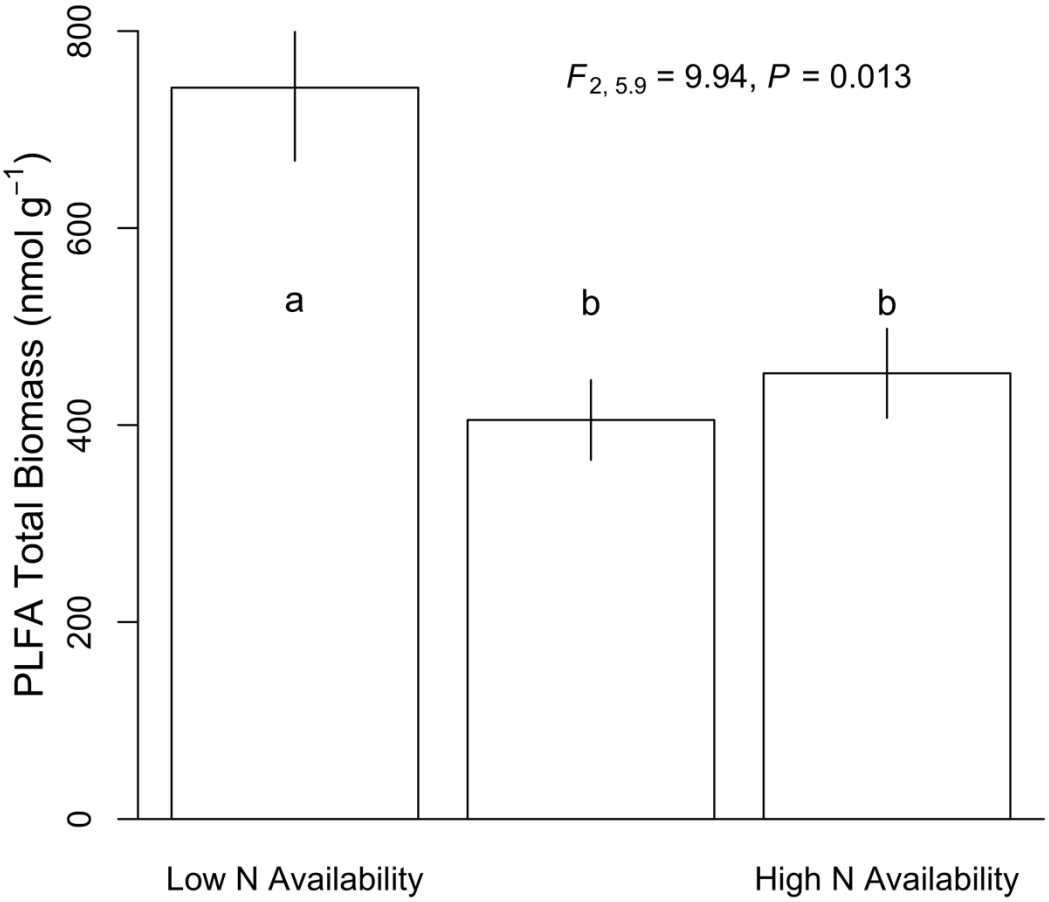

Figure 3.4. Total PLFA biomass among N availability treatments. Least-squares means estimates and standard errors are back transformed from a log scale.

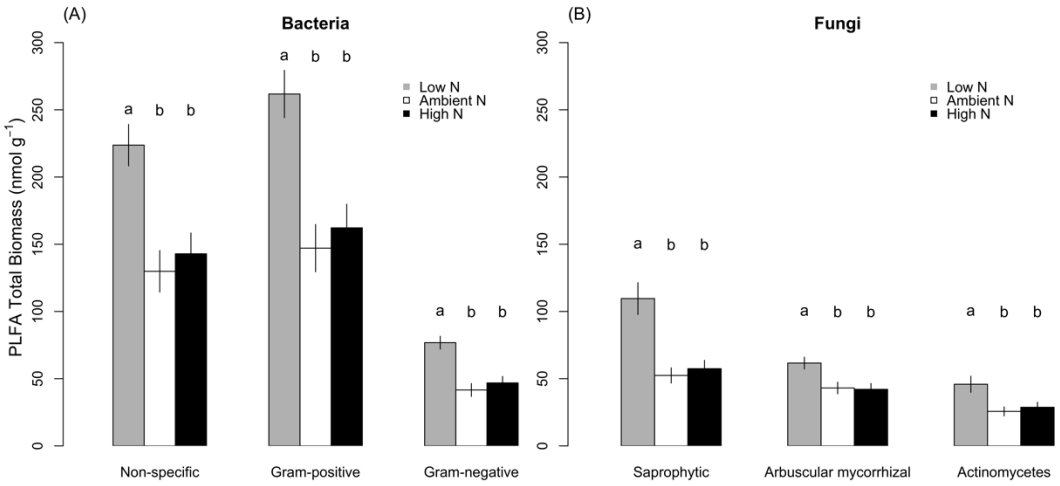

Figure 3.5. Biomass of (A) each bacterial PLFA group (B) each fungal PLFA group. Letters above bars represent differences among nutrient treatments. Bars represent least-squares means with error bars representing standard error. Saprophytic fungi and actinomycetes are back-transformed from a log scale.

CHAPTER 4

PLANT DIVERSITY INDIRECTLY DECREASES NITROUS OXIDE EMISSIONS FROM SOIL

Introduction

Agricultural soil is a major anthropogenic source of nitrous oxide (N_2O; Skiba et al. 1993, Zhang et al. 2014), a greenhouse gas that is 298x as potent as CO_2 (US EPA 2014). More N_2O is produced on a per mole basis during denitrification than nitrification, but anaerobic conditions are required. In agricultural soils, which are often drained and less likely to be anaerobic, nitrification can be the major source of N_2O (Skiba et al. 1993). A meta-analysis concluded that agricultural soils in reduced or no-till for >10 years produced less area-scaled direct nitrous oxide emissions as compared to conventional tillage (Kessel et al. 2013), suggesting less degraded soils produce less direct N_2O emissions. Prairie restoration from agricultural (row-crop) conditions has been shown to improve soil structure, increase microbial biomass and richness, and reduce nutrient availability (Baer et al. 2003, Bach et al. 2010, Baer et al. 2010). Prairie restoration can also increase plant diversity, as evenness and beta diversity levels of restored sites were similar or greater than reference prairies (Martin et al. 2005). However, it is not known whether plant diversity influences N_2O emissions from soil or the metabolic source of N_2O production.

Nitrogen cycling in soil predominately occurs through microbial transformations and plant uptake. Plants compete with soil microbes for nitrogen (Kaye and Hart 1997, Kuzyakov and Xu 2013, Liu et al. 2016). Nitrogen contained in the soil organic matter is depolymerized and N monomers are mineralized by a wide array of microbes to produce ammonium (NH_4^+; Schimel and Bennett 2004). Some NH_4^+ may volatize as ammonia gas (NH_3) under basic

conditions and some will be oxidized (nitrified) to nitrite (NO_2^-) and then to nitrate (NO_3^-). Nitrification can be an autotrophic or a heterotrophic process, depending on the soil microbial community (Evans 2007, van Groenigin et al. 2015). During nitrification, a small amount of NH_4^+ will be converted to N_2O. Nitrate is the more mobile form of nitrogen in many temperate ecosystems and can be taken up by plants. In the absence of O_2, NO_3^- can be used as an alternate electron acceptor and reduced to atmospheric nitrogen (N_2), a process known as denitrification. Complete denitrification is a stepwise reduction from nitrate to nitrite to nitrous oxide to dinitrogen. However, N_2O can be released during denitrification as some denitrifiers lack nitrous oxide reductase (Evans 2007, van Groenigen et al. 2015). Additionally, abiotic reduction of nitrite can occur to produce nitrous oxide. When nitrifiers produce nitrite that is then reduced it is termed nitrifier denitrification (van Groenigen et al. 2015). Nitrification and denitrification can occur simultaneously in soil due to heterogeneity in aerobic and anaerobic microsites within soil aggregates (Stolk et al. 2011).

The relative amount of denitrification compared to nitrification can influence the amount of N_2O produced and can be measured with stable isotopes. Isotopic composition of N in soil is difficult to measure in the field, but can be determined with well-designed lab experiments (Evans 2007). Most stable isotopes of N have an atomic weight of 14, but a small percent have an atomic weight of 15. Several biological processes discriminate against ^{15}N isotopes. Nitrogen fractionation observed in soil is typically expressed as $\delta^{15}N_{\text{substrate}} - \delta^{15}N_{\text{product}}$ (Evans 2007). Nitrous oxide produced during nitrification is a product of two reactions: the oxidation of NH_2OH with NOH as a precursor and reduction of NO_2^- by nitrite reductase. Nitrous oxide emissions produced during denitrification are an intermediate in the conversion of NO_3^- to N_2. Values of $\delta^{15}N$ can change with site and season, but a greater fractionation factor for nitrification

as compared to denitrification has been observed (Barford et al. 1999, Yoshida 1988). This means that N_2O with relatively low $\delta^{15}N$ values indicate more nitrification, while relatively high $\delta^{15}N$ represents more denitrification (Perez et al. 2000, 2001).

Nitrous oxide production from soil can be limited by organic carbon (an electron donor source; Tiedje 1984) and available nitrogen (Sotomayor and Rice 1995, Scott et al. in review Applied Soil Ecology), but the influence of plant diversity on emissions from soils has not been addressed. Plant diversity may indirectly influence N_2O emissions from soils by culturing microbial communities through litter inputs that alter N availability (Hunt et al. 1988, Gholz et al. 2000, Ayres et al. 2009). For example, exotic grasses have been demonstrated to promote ammonia-oxidizing bacteria and increase nitrification rates relative to native plant communities with, possibly due to proportionally more nitrate (as opposed to ammonium) assimilated by the exotic grass compared to the native grass (Hawkes et al. 2005). Lower diversity would be expected in invaded areas compared to uninvaded areas because the majority of invasive plant species that impacted plant richness and taxonomic diversity had negative effects, especially annual grasses (Pyšek et al. 2012). Plants that promote nitrifier populations may promote higher available nitrate in the soil leading to N_2O emissions from the soil from multiple pathways nitrification and denitrification. Alternatively, diverse communities may contain greater variation in root architecture than less diverse communities (e.g. dicots [Pagès 2014]) to result in more soil exploration by plants and uptake of N, leaving less available ammonium for nitrification and NO_3^- for denitrification. Greater plant N uptake is also consistent with studies demonstrating nitrogen use complementarity in plants (Ashton et al 2010, Johnson et al. 2016), including grasslands restored and managed for plant diversity (Klopf et al. 2017). A better understanding

of plant diversity effects on N_2O emissions and the source metabolic pathways may enable restoration practitioners to manage N_2O emissions from restored soils.

According to biodiversity-ecosystem function theory (BEF), plant diversity is predicted to promote ecosystem functioning through greater complementary in resource use (Tilman et al 2014). For example, diverse plant communities utilize more soil nitrate than less diverse plant communities (Tilman et al 2001, Ashton et al 2010, Johnson et al. 2016, Klopf et al. 2017). Our objective was to determine if plant diversity influenced nitrous oxide emissions and if so determine the mechanism. As such, I hypothesized that N_2O emissions from soil would be lower in diverse plant communities relative to less diverse communities. To test this hypothesis, I identified high and low plant diversity patches in restored prairie and measured potential N_2O emission from soil. I predicted N availability (indicated by extractable NH_4^+ and NO_3^-) and C availability (indicated by water extractable organic C [WEOC] and potential C mineralization) would be lower in diverse plant communities as compared to less diverse communities due to resource use complementarity by and less diverse organic matter inputs from plants. Lower nutrient availability would lead to less N transformations performed by the microbial community in the high-diversity plant community soils. I also tested if nitrous oxide emissions corresponded to abundance of a single species to ensure that there were true diversity effects.

Methods

Site Description

I quantified N_2O emissions at Nachusa Grasslands (Franklin Grove, IL, USA), a ~1600-hectare preserve of remnant grassland and woodland connected by restoration and managed by The Nature Conservancy, in early August 2017. Field sites were chosen from those identified and described in detail by Klopf et al. (2017). All sites were located in Lee and Ogle counties in

northern Illinois. Mean annual precipitation in this area (1985–2009), was 968 mm and mean monthly temperature was 9.3°C. Soils were similar and formed over glacial till with a loam texture (taxonomy in Table 4.1). All sites were managed regularly by prescribed burning (Table 4.1). *Bison bison* had access to one site (Site 3).

All sites were formerly cultivated and at the onset of restoration were sown with over 100 native species (Klopf et al. 2017). The fields were similar in age (10 to 12 years old; restored between 2005 and 2007) to reduce differences in N pools that change dynamically during restoration (Baer et al. 2002, Rosenzweig et al. 2016).

Sampling Design

An initial survey was conducted in July 2017 using 50 contiguous 0.25 m x 0.25 m (1/16 m^2) sampling frames near the center of each field along a temporary transect spanning 12.5 m along the length of the field. Percent cover was visually estimated for each vascular plant species rooted within the sampling frame. Initial survey results were analyzed with local quadrat variance, semivariogram, and quartile calculations and used to determine the appropriate sampling frame size and spatial arrangement (distance for statistical independence).

Appropriate sampling frame size was determined from the initial vegetation survey with three-term local quadrat variance (3TLQV; Hill 1973) analysis, which is less sensitive to global trends than two-term analogs, using Pattern Analysis, Spatial Statistics, and Geographic Exegesis version 2 (PASSaGE; Rosenberg and Anderson 2011) of the first axis scores from a principal component analysis (PCA), as suggested by Gibson & Greig-Smith (1986). In this analysis the first distinct peak indicated the scale of maximum variance. The average distance indicated by 3TLQV analysis was used as the length and width of the sampling frame for all sites in the final sampling. This analysis suggested the scale of maximum variance in PCA first axis scores was

1.3 m (Appendix J). A 1-m2 frame was used because this size frame is commonly used and is similar to the 3TQV result. A similar semivariogram analysis was conducted with the R package gstat (Pebesma 2004) to determine the distance between sampling frames of the same diversity level (Range), so that replications were spatially independent. The range was 18.1 m (Appendix K). Upper and lower quartiles were determined from Shannon diversity (H) calculations of each sampling frame within a field. These H values were used to determine high- and low-diversity plant treatments in sampling. The H cutoff values were < 0.89 for low diversity and > 1.33 for high diversity. Shannon diversity was displayed with a boxplot. Species richness (S), Pielou's evenness (J), and functional richness based on 8 *a priori* functional groups (warm-season graminoids, cool-season graminoids, annual and biennial forbs, ephemeral spring forbs, spring forbs, summer/fall forbs, legumes, and woody shrubs; Kindscher and Wells 1995) were also calculated for each frame and displayed boxplots.

Low-diversity (n = 6) and high-diversity (n = 6) patches were delineated within 5 restorations co-located at Nachusa Grasslands. Vegetation was surveyed in one frame per patch. In each field, I laid a 100 m transect and at a fixed distance apart (16.6 m), a sampling frame moved laterally (alternating at least 19 m from the main transect) until appropriate high- and low-diversity patches were encountered. This sampling method allowed for greater than 18.1 m between frames of the same treatment. Differences in secondary transects were accounted for by blocking (Fig. 4.1).

Response Variables

I removed ten 2 cm dia. cores to a depth of 10 cm from each sampling frame in early August. This sampling time was chosen because plants would be active. The soil cores were then composited for each frame and sieved (4 mm). While measurements were larger than the hotspot

scale, plot-based measurements likely reflect differences in the relative number of biogeochemical hotspots. An intact 5.5 cm dia. core was also taken from half of the frames within a diversity treatment to determine bulk density so that concentrations could be expressed on a per area basis.

Fresh, field moist soil was analyzed within one week of sampling for available inorganic N concentration (NH_4-N and NO_3-N). A subsample (~10 g) of sieved soil was extracted with 50 ml of 2 N KCl (Robertson et al. 1999a). The flasks were shaken for one hour then filtered using 0.4 μm HTTP Isopore membrane filters (Merk Millapore Ltd.). The filtrate was analyzed by colorimetry on an OI Analytical Flow Solution IV (OI Analytical Corp., College Station, TX, USA).

Water holding capacity of composited soil samples was determined by saturating a subsample (~20g) of fresh soil, then allowing soil to drain by gravity for 16 hours in a sealed cooler with 100% humidity (Robertson et al. 1999b). Water holding capacity was then calculated as the gravimetric water content of drained soils, determined by oven drying at 105° C. Bulk density of the soil was determined from drying the intact cores at 105° C.

To determine potential 1-day N_2O emissions under mixed aerobic and anaerobic conditions (Cheng et al. 2015), a subsample (~40 g dry equivalent weight) of the homogenized soil was placed in 250 ml flasks, adjusted to 40% water holding capacity then incubated at 23°C in the dark (flask housed within a mason jar fit with septa) for 1 d. A 12 ml headspace gas sample was injected into 12 ml gas vials that had been flushed with He gas and vented to approximately 1 atm of pressure. Natural abundance isotopic composition of N_2O emissions was then determined with gas chromatography-isotope ratio mass spectrometry (GC-IRMS) at the University of California Davis Stable Isotope Facility. Low $\delta^{15}N$-N_2O values indicate more

nitrification relative to denitrification (Perez et al. 200, 2001). Gas samples were purged from Exetainer 12 ml glass soda vials (Labco Limited, Lapeter, UK) through a double-needle sampler into a helium carrier stream (20 mL/min) and analyzed with a ThermoFinnigan GasBench + PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (Bremen, Germany).

Because differences in N_2O could also result from differences in microbial activity, I also measured soil respiration (C_{min}) using air-dried soils adjusted to 40% water holding capacity. Soil was placed in 150 ml Erlenmeyer flasks housed within mason jars fit with septa. Soil was incubated 7 d at 23° C after moisture adjustment. All containers were opened for 30 minutes after pre-incubation, then sealed and incubated for 7 days in dark at 23° C. Headspace gas was sampled at day 1 and 7 and analyzed for CO_2-C on a Shimadzu GC-8A gas chromatograph equipped with a thermoconductivity detector (Shimadzu Corp., Kyoto, Japan).

Because differences in N_2O could also result from differences in the availability of labile C, I measured water extractable organic carbon (WEOC; Bai et al. 2014). A subsample (~10 g) of air-dried soil was placed into glass centrifuge tubes with 40 ml of deionized water. Tubes were shaken for 1 hour, then centrifuged at 3600 rpm for 20 minutes. Effluent was then filtered using 0.4 μm HTTP Isopore Membrane Filters (Merk Millapore Ltd.). Filtrate was refrigerated until it could be analyzed with a total organic carbon analyzer (Shimadzu TOC-L, Shimadzu Corp., Kyoto, Japan).

Calculations and Statistical Analyses

Response variables were analyzed using mixed models with responses of Shannon diversity, species richness, Pielou's evenness, functional richness, N_2O emissions, extractable NH_4^+, extractable NO_3, $\delta^{15}N$-N_2O, soil respiration, and WEOC with a fixed effect of diversity

treatment and with secondary transect within restoration as a block effect. Z-transformed values of extractable NH_4^+, extractable NO_3^-, $\delta^{15}N\text{-}N_2O$, with a diversity treatment covariate were fixed predictors in a linear mixed model with N_2O emissions as a response and with restoration and secondary transect within restoration as a block effect (lme4 package of R, Bates et al. 2015). Backward model selection using a chi-squared test in the drop1 function produced candidate models, which were compared by Bayesian Information Criterion. Predictors of N_2O emissions from the most parsimonious model were then fit in a mixed model on the original scale. All mixed models were type III models with restricted maximum likelihood estimates and Satterthwaite approximations of degrees of freedom were used with lmerTest (Kuznetsova et al. 2016) to calculate *P* values. Data for this chapter are included in Appendix L.

Results

Sixty species were encountered in the final survey of all vegetation. One site (Site 3) had very high cover of a weedy species, *Trifolium pratense*, and two other sites (4 and 5) had high cover of the sedge, *Carex brevior*. The most frequently occurring species among all sites were *Sorghastrum nutans* (n = 32), *Coreopsis lanceolata* (n = 29), *Symphyotrichum ericoides* (n = 29), *Solidago missouriensis* (n = 26), *Monarda fistulosa* (n = 22), *Andropogon gerardii* (n = 19), *Oligoneuron rigidum* (n = 18), and *Solidago canadensis* (n = 18). The high and low diversity designations (H treatments) were effective. The high diversity treatment contained higher Shannon's diversity (Fig. 4.2 A), species richness (Fig. 4.2 B), species evenness (Fig. 4.2 C), and functional group richness (Fig. 4.2 D) than the low diversity treatment.

Nitrous oxide emissions were lower in the high-diversity patches as compared to the low-diversity patches (Fig. 4.3A). There were no differences for all other soil variables between high diversity and low diversity measurements (Fig. 4.3A–F). Isotopic signature with diversity

treatment as a covariate best explained N_2O emissions and was marginally significant (Fig. 4.4). There was a stronger effect of isotopic signature with the low diversity covariate ($t = 2.24$, $P = 0.031$) than isotopic signature with the high diversity covariate ($t = 0.18$, $P = 0.857$).

Discussion

My results suggest that plant taxonomic diversity affects ecosystem functioning. The results are consistent with my hypothesis that high-diversity plant communities would have lower N_2O emissions from soils relative to low-diversity plant communities, but variables that could explain this difference were similar between diversity treatments. Approximately two times as much N_2O was produced in soils with low plant diversity, on average at the plot scale (Fig. 4.3). I predicted lower N_2O emissions from more diverse communities would correspond with less N availability, water holding capacity, gravimetric water content, and denitrification relative to nitrification, but greater C availability and microbial activity that would immobilize N. Of these measurements, isotopic composition was the best indicator of N_2O emissions (Fig. 4.4). My results suggest that there is more nitrous oxide production from denitrification (high $\delta^{15}N\text{-}N_2O$) in low-diversity plant communities. While organic matter quality and management treatments can influence isotopic composition, this was accounted for with a random intercept model. Differences in nitrous oxide emissions between high and low plant diversity treatments were greatest in site 5 (Fig. 4.4).

Denitrification is related to plant functional diversity and its interaction with several variables: percent moisture, microbial biomass N, percent organic matter, and inorganic N (Sutton-Grier et al. 2011). High $\delta^{15}N\text{-}N_2O$ values in our study indicated greater N_2O emissions were from denitrification as opposed to nitrification. Furthermore, greater N_2O emissions occurred in the low diversity treat compared to high diversity treatment at $\delta^{15}N\text{-}N_2O$ values

indicative of denitrification (Perez et al. 2000, 2001). Similar water holding capacities and gravimetric water content of bulk density cores were observed and all soils were adjusted to 40 percent of water holding capacity in the lab.

My results suggest there are more N_2O emissions from denitrification hotspots (i.e. substantial emissions from small areas) in low-diversity plant communities (Fig. 4.4). This result is consistent with studies that demonstrated N_2O emissions are characterized by hotspots (Hill et al. 2000, Palta et al. 2014, Kravchenko et al. 2017, Loik et al. 2018). However, it remains unclear if this diversity effect on N_2O emissions is due to changes in microbial physiology, changes in microbial community composition, or changes in substrate availability over time. Changes in microbial physiology might be expected because diverse plant communities have more complex root architecture (Pagès 2014), which might promote soil aggregation such that there is faster oxygen diffusion into the soil (Doyle and MacLean 1958), limiting denitrification. Changes in microbial community composition might also be expected because a positive correlation of plant and soil microbial diversity has been demonstrated (Zak et al. 2003). Furthermore, several studies indicate that different plant species can have distinct soil microbial communities (reviewed by Sasse et al. 2018 and Hassani et al. 2018). Future work relating plant composition with functional genes relating to nitrite reductase could determine if composition of denitrifiers differs with plant diversity. Sequential measurements of available ammonium and nitrate pools might reveal if availability of substrates differ with plant diversity.

Availability of N and C can influence nitrous oxide production (Sotomayor and Rice 1995, Scott et al. in review Applied Soil Ecology). Nitrate use complementarity has been demonstrated in several studies (Tilman et al 2001, Ashton et al 2010, Johnson et al. 2016), which could lead to less available N in high diversity plant communities. However, I failed to

observe evidence of nitrate use complementarity, e.g. differences in amount of extractable NH_4^+ and NO_3^-. While these extractable pools represent initially available N pools at the onset of incubation, relative availability might have changed during the incubation. Isotopic composition of N suggests that nitrate became more available, because the signature was more consistent with denitrification rather than nitrification. Season can also influence nitrous oxide emissions. Sampling was performed in the summer, when an average of 67% of annual N_2O emissions occur from unfertilized soybean fields in this region (Bremner et al. 1980). This finding agrees with the recent findings that belowground resource partitioning alone cannot explain ecosystem functions including plant nutrient uptake (Jesh et al. 2018). Progressive N limitation, combined with an increase in labile C pools (microbial and mineralizable) that occurs during the first 10 years of grassland restoration (Baer et al. 2002, Rosenzweig et al. 2016) likely overrode a diversity effect on N availability. Klopf et al. (2017) found lower N availability in prairie restored and managed with more species using the same sites used in this study, but compared to prairie sown with less than 10 species and rarely managed by fire. Sites used in this study all were sown with more than 100 species and burned frequently. Frequent burning likely contributed to low N availability, as frequent burning causes lower net N mineralization (Blair 1997). Availability of C can also influence denitrification and therefore N_2O production, as organic C can be an important electron donor source (Tiedje 1984). However, the availability of C and microbial activity also did not differ between diversity treatments.

Aggregate structure has been shown to increase in recovery rate in high-diversity plant communities (Klopf et al. 2017), which could influence N_2O emissions through multiple mechanisms. Stable surface soil aggregates promote water infiltration (Franzluebbers 2002) so more anaerobic conditions would be expected in low diversity plant communities following

precipitation events. Soil aggregates can also physically protect soil organic matter from oxidation (reviewed by Six et al. 2004). Because there are more aggregates in high-diversity plant communities, which are a recalcitrant pool of organic matter (Scott et al. 2017), less nitrification and available NO_3^- would be expected. Less N_2O emissions would be expected if there were less nitrification and less available nitrate for denitrification in high-diversity plant communities. Extractable pools were used to represent the initial availability of N forms at the start of the incubation, but isotopic composition of N in N_2O might better reflect relative availability of ammonium and nitrate over the incubation period. It is unclear if plant uptake will be in stronger competition with nitrifiers or denitrifiers, because while some plant show a preference for ammonium over nitrate (Falkengren-Grerup and Lakkenborg-Kristensen 1994; Zhao et al. 2009) however sufficient evidence for general rules are lacking (Hewins and Hyatt 2009, Ashton et al. 2010, Boudsocq et al. 2012). While I did not measure soil aggregation, I would have expected to see aggregate effects reflected in extractable N pools.

The N_2O emissions I observed were low; N_2O emission rates over 16 d from grassland restorations in NE Kansas, USA on silt loam soils were as high as 0.36 g m^{-2} d^{-1} (Scott et al. in review Applied Soil Ecology). Low emission values are likely because of low water holding capacities due to sandier texture and short incubation times. Additionally I adjusted to only 40 percent water holding capacity and used sieved soils; the Kansas study was adjusted to 50 percent water holding capacity and used intact soil cores. I did this so that nitrification and denitrification could both occur, but nitrous oxide production would be maximized at ~60% water-filled pore space (Yoshinari 1993). My measurements likely do not reflect field emissions after precipitation events, but are useful for making comparisons between diversity treatments.

It is clear that managing for high-diversity plant communities can reduce N_2O emissions, likely by influencing microbial community composition, microbial physiology, or substrate availability. In addition to increasing biomass production (reviewed by Tilman et al. 2014, Chen et al. 2018), reducing nitrate leaching (Tilman et al 2001, Ashton et al 2010, Johnson et al. 2016), faster aggregate formation (Klopf et al. 2017), and more C sequestration (Chen et al. 2018), less N_2O production from soil is tangible ecosystem service that further justifying restoration and management of ecosystems for plant diversity.

Table 4.1. Site information. This information is presented because grazing, soil type, burn frequency, and restoration age can influence N isotopic composition in nitrous oxide emissions. Site was included as a random intercept in statistical models to account for variation.

Site	GPS Coordinate	Soil Type	Soil Taxonomy	Bison Grazing	Burn Frequency	Burned Last Year	Years Restored
1	41.867014, -89.357091	Jasper Loam	Mesic typic Arguidoll	No	Annual	Yes	10
2	41.866767, -89.358301	Jasper Loam & Martinsville silt loam	Mesic typic Arguidoll & Mesic typic Hapludalf	No	Annual	Yes	11
3	41.896570, -89.352700	Jasper Loam	Mesic typic Arguidoll	Yes	Biennial	Yes	12
4	41.906721, -89.335929	Waukee Loam	Mesic typic Hapludoll	No	Biennial	No	10

5	41.904505, -89.329443	Waukee Loam	Mesic typic Hapludoll	No	Biennial	No	11

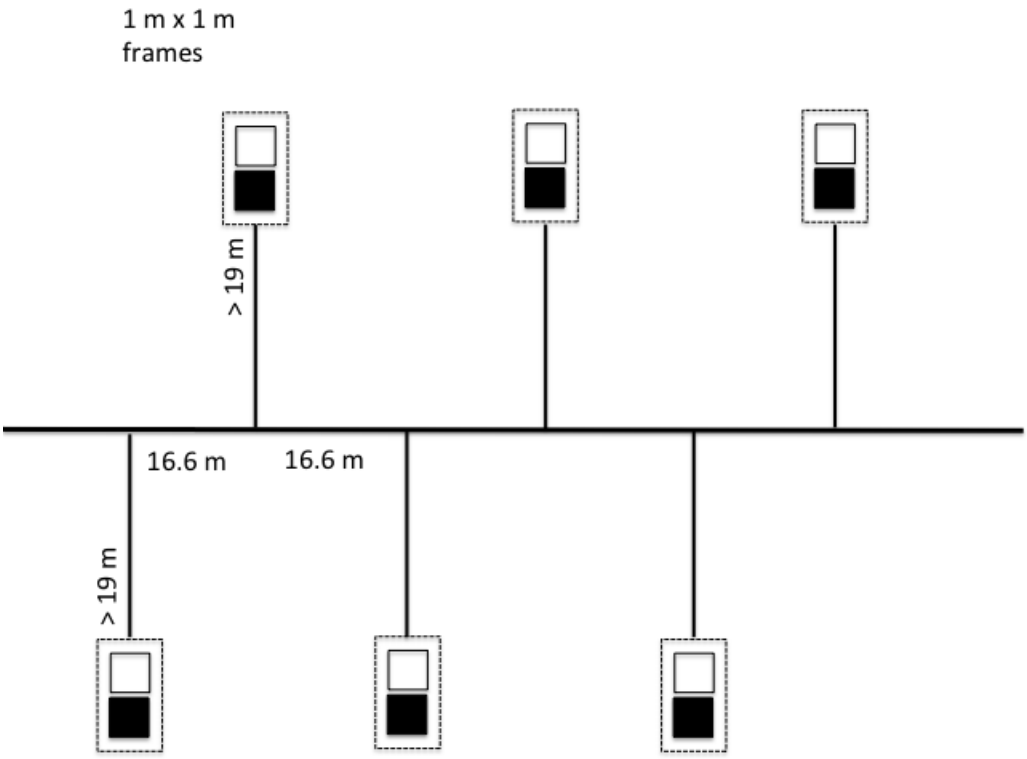

Figure 4.1. Design at one restoration site (block). A main transect with alternating secondary transects every 16.6 m was delineated. One high diversity and one low diversity frame were established at least 19 m from the main transect at each secondary transect. Frames on the same secondary transect were blocked within the restoration site block.

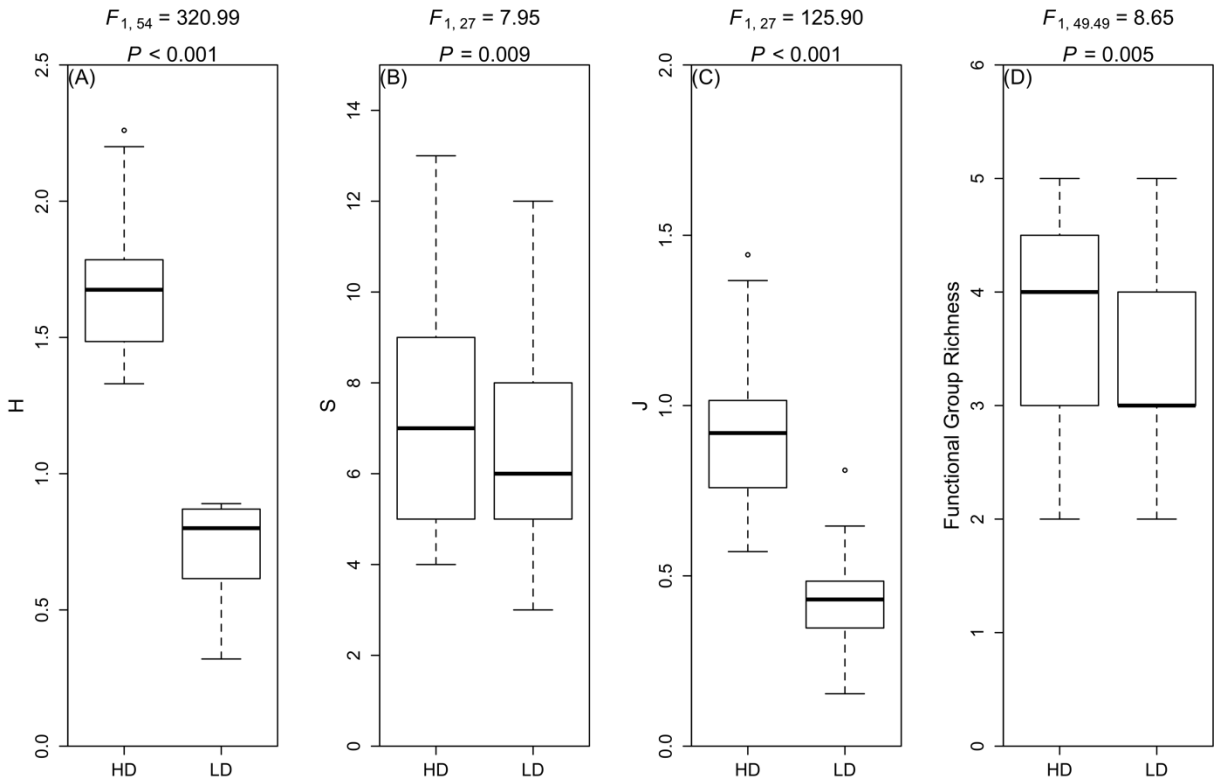

Figure 4.2. Boxplots of (A) Shannon diversity (H), (B) species richness (S), (C) Peilou's evenness (J), and (D) functional group richness. Statistics are from mixed models with a blocking effect of secondary transect within restoration site.

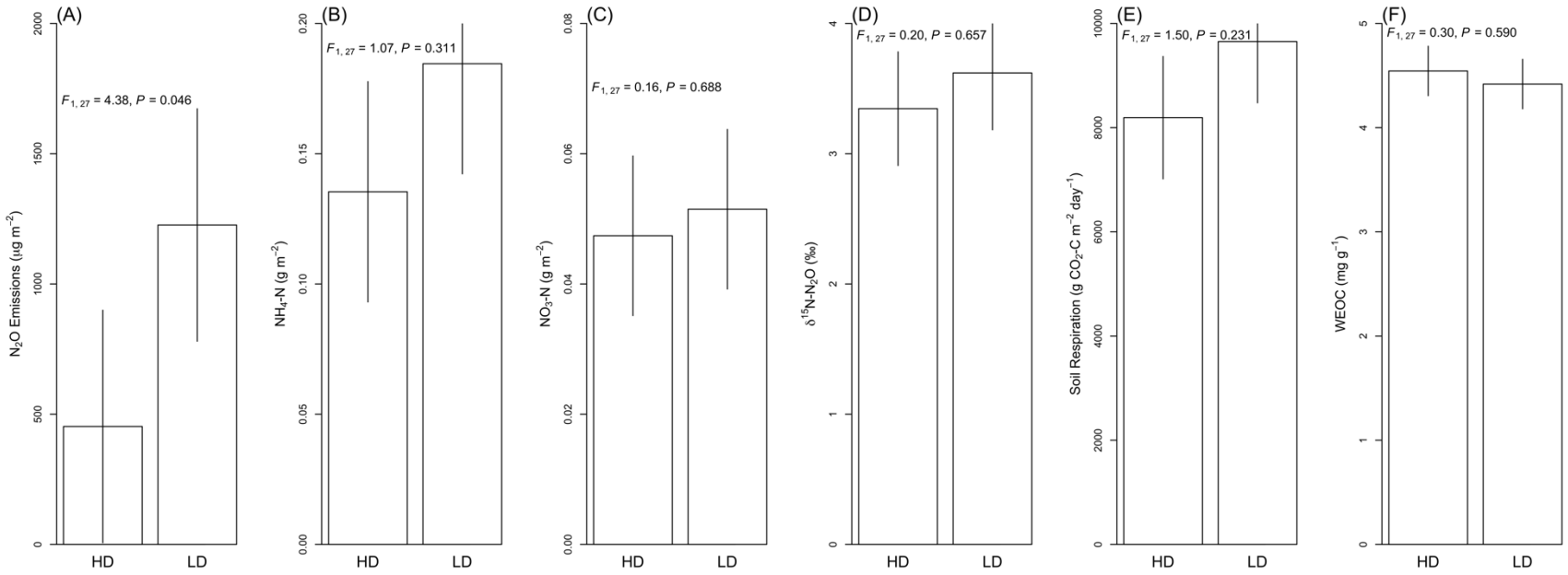

Figure 4.3. Least squares means estimates and associated standard errors of soil measurements in high-diversity and low-diversity plant patches; A) nitrous oxide emissions from a one day incubation, (B) extractable ammonium, (C) extractable nitrate, (D) isotopic composition of N_2O–N, (E) Soil respiration from a 10 day incubation of rehydrated soils, and (F) water extractable organic carbon. Statistics are from mixed models with a blocking effect of secondary transect within restoration site.

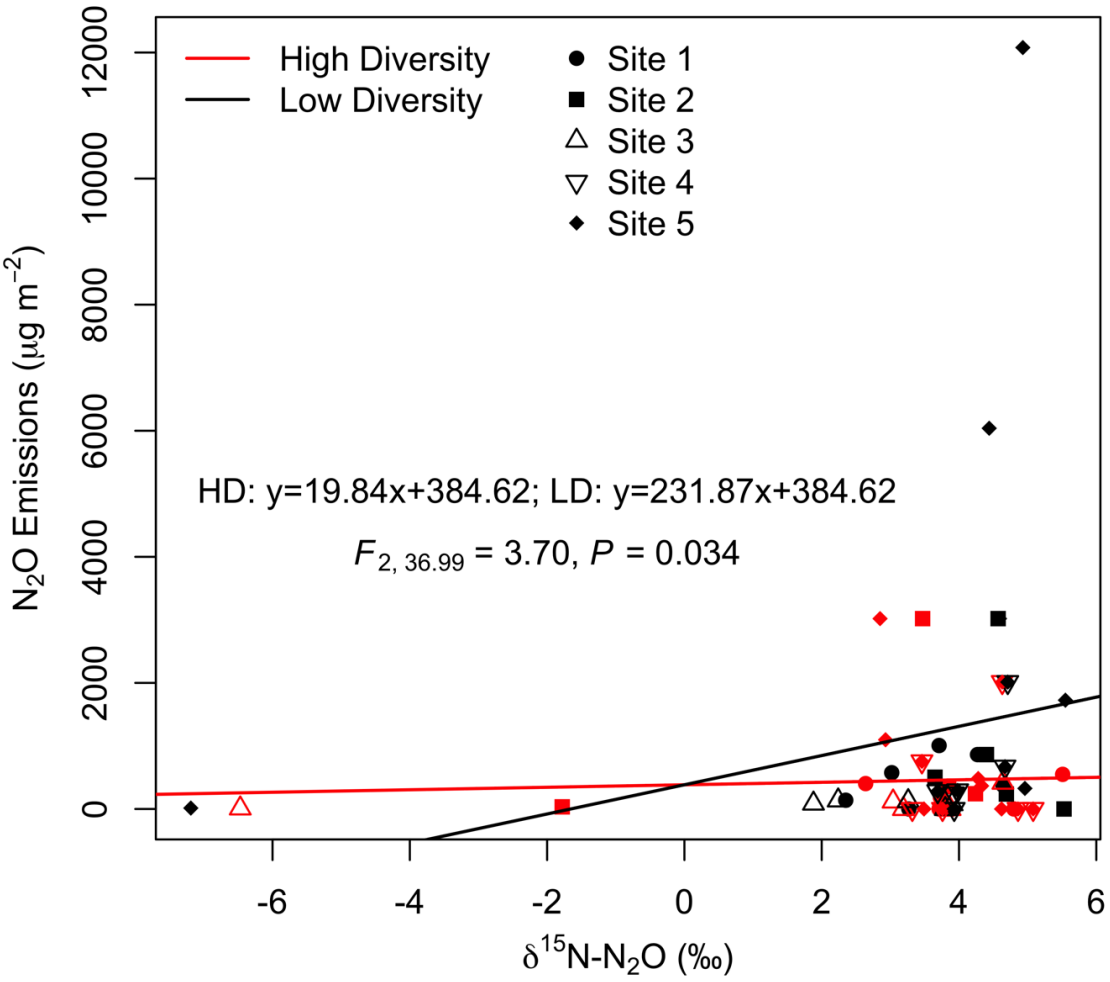

Figure 4.4. Nitrous oxide (N_2O) emissions from a 1d incubation predicted by isotopic signature of N from N_2O emissions with a plant diversity treatment covariate. Low $\delta^{15}N$-N_2O values indicate more nitrification relative to denitrification; high $\delta^{15}N$-N_2O values indicate more denitrification relative to nitrification (Perez et al. 2000, 2001). Levels of the covariate were high diversity (HD) and low diversity (LD).

CHAPTER 5

CONCLUSIONS

In chapter 2, I hypothesized that soil nitrate heterogeneity would increase initially with restoration age as plants begin to establish, and then would level off. This hypothesis is consistent with trends in variability of soil nitrate in a restoration chronosequence (Rosenzweig et al. 2016). I also hypothesized that available light heterogeneity would decrease as clonal grasses increase in dominance (Sluis 2002, Carter and Blair 2012, Baer et al. 2016). I also hypothesized heterogeneity of both soil nitrate and availability of light would be positively correlated with plant diversity and richness as predicted by the 'environmental heterogeneity hypothesis' (Ricklefs 1977). In contrast to my first hypothesis, nitrate heterogeneity did not increase with restoration age. Light heterogeneity did increase with restoration age, but slowly. Light heterogeneity was positively related to plant taxonomic diversity regardless of light availability, but high nitrate availability decreased the strength of the positive relationship of nitrate heterogeneity and plant taxonomic diversity.

Manipulating soil nitrate heterogeneity could enhance plant diversity and richness in agreement with the 'environmental heterogeneity hypothesis' (Ricklefs 1977). The results presented in Chapter 2 are consistent with this expectation, though cause and effect cannot be determined because this study was observational. High plant diversity could cause high levels of ecosystem functioning compared to low plant diversity according to biodiversity-ecosystem functioning (BEF) theory (Naeem and Wright 2003, Tilman et al. 2014). If plant diversity is a restoration goal, practices such as patchy application of a recalcitrant C source (e.g. sawdust or biochar) at the onset of restoration with subsequent patchy application of labile C sources (e.g.

sugars) and fertilizers should be used. If there is limited propagule supply, seed additions could realize the benefits of heterogeneity treatments (Baer et al. 2015).

In chapter 3, I hypothesized that plant species interacting with nutrient availability would have the greatest influence (the other treatment was soil depth) on microbial community composition. Nutrient availability can also have indirect effects through plants (Bardgett et al. 2003) on microbial community structure. Effects of plants on microbial communities are also well documented (Sasse et al. 2018, Hassani et al. 2018). Microbial PLFA biomarker diversity was highest in plant-influenced soils, as compared to bare soils. Microbial PLFA biomass was higher in low N (sucrose added) areas and this was consistent among all PLFA groups. If PLFA biomarker diversity can serve as a proxy for taxonomic diversity, then plant species present and soil nutrient availability could also influence soil microbial diversity and biomass. Reducing bare soil areas could enhance soil microbe PLFA diversity, as suggested in Chapter 3. High soil functional, and indirectly taxonomic diversity, could increase ecosystem functioning as compared to low functional diversity according to BEF theory (Naeem and Wright 2003, Tilman et al. 2014). If high soil microbe diversity is a restoration goal, high seed densities could maximize vegetation cover. Providing labile C could increase microbial biomass, as suggested in Chapter 3. High microbial biomass could contribute to ecosystem functions such as soil aggregation and C sequestration (Scott et al. 2017). If high microbial biomass is a restoration goal, C sources should be added to soils to reduce nutrient availability.

In chapter 4, I hypothesized that diverse plant patches would have less N_2O emissions from soil as compared to low-diversity patches (Tilman et al 2001, Ashton et al. 2010, Klopf et al. 2017). I found that there were higher nitrous oxide emissions form low-diversity plant stands and this was associated with a change in isotopic composition of N in N_2O emissions that is

associated with denitrification. This change in isotopic composition could reflect differences in relative amount of nitrification and denitrification in plant diversity treatments, possibly due to availability of substrate, oxygen, or nitrifiers. Creating high diversity plant stands could reduce nitrous oxide emissions from soils, as suggested in Chapter 4. This reduction in nitrous oxide emissions is due to low diversity plant communities being associated with the production of greater amounts of N_2O when denitrification is a dominant process. Nitrous oxide is a potent greenhouse gas (EPA 2014) that is increasing in atmospheric concentration with conventional agriculture (Skiba et al. 1993, Zhang et al. 2014). If reducing nitrous oxide emissions is a restoration goal, high diversity seed mixes with subsequent seed additions is a good practice.

The insights from this dissertation have clear implications for restoration practitioners, but the best practices depend on restoration goals. Many of the suggested practices are not mutually exclusive, but time and money can limit practices that are implemented. Restoration practitioners should consider stakeholder concerns to decide restoration goals with multi-criteria decision analysis (Convertino et al. 2013).

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APPENDICES

APPENDIX A

Chapter 2 spatial reference data of resin bag exchangeable nitrate from a 16 y-restored prairie located at Konza

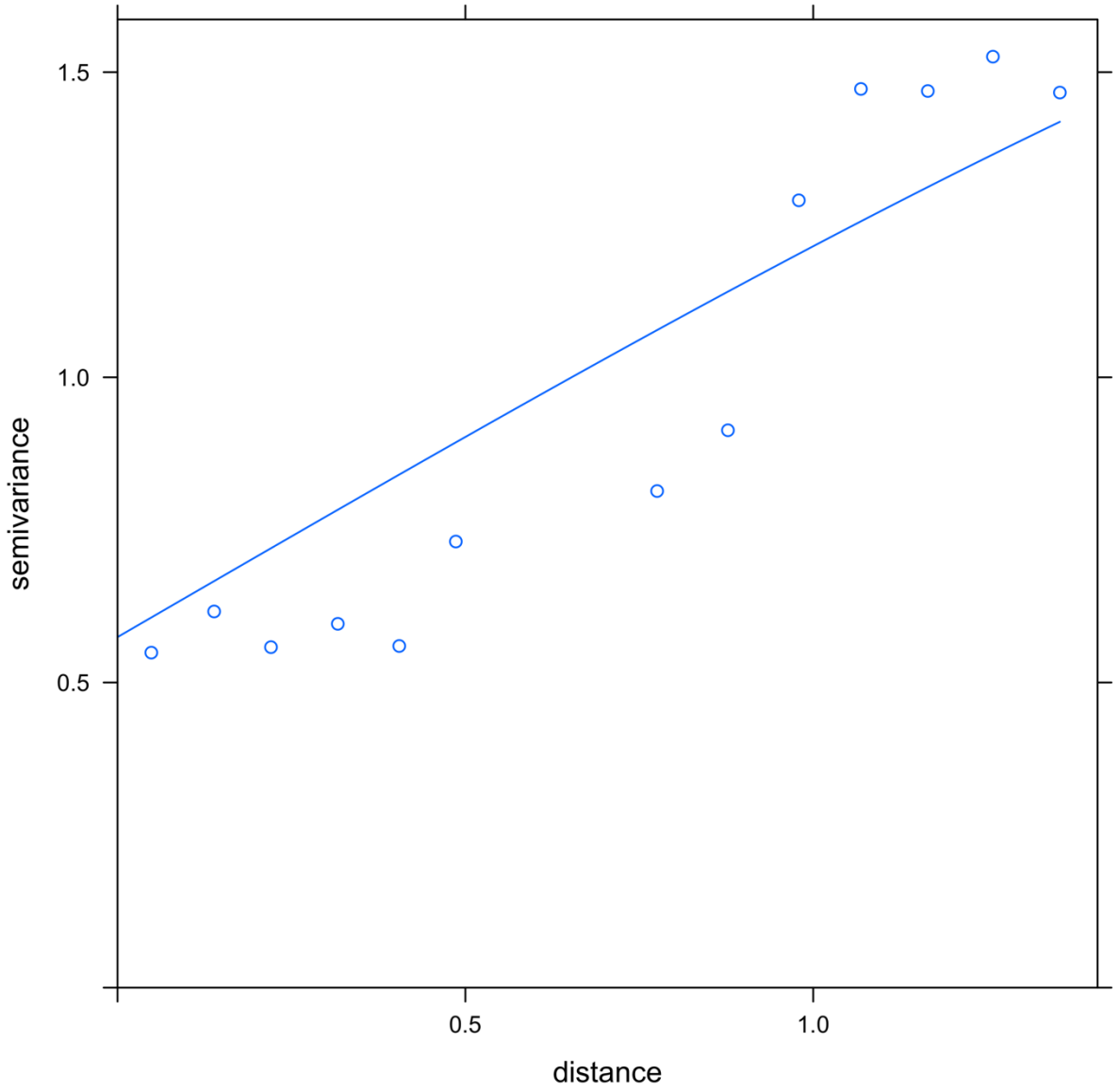
x distance	y distance	nitrate 2014	zX	zY	z nitrate 2014
25.25	0.75	2.035	0.313	-1.526	0.576
24.75	1.25	0.607	0.276	-1.494	-0.645
27.25	0.75	1.345	0.460	-1.526	-0.014
26.75	1.25	3.509	0.423	-1.494	1.836
29.25	0.75	2.847	0.607	-1.526	1.270
28.75	1.25	0.837	0.570	-1.494	-0.448
25.25	2.75	0.452	0.313	-1.400	-0.778
24.75	3.25	0.954	0.276	-1.368	-0.348
27.25	2.75	1.495	0.460	-1.400	0.114
26.75	3.25	1.062	0.423	-1.368	-0.256
29.25	2.75	0.595	0.607	-1.400	-0.655
28.75	3.25	1.395	0.570	-1.368	0.029
25.25	4.75	2.754	0.313	-1.274	1.191
24.75	5.25	1.346	0.276	-1.242	-0.013
27.25	4.75	1.125	0.460	-1.274	-0.202
26.75	5.25	0.614	0.423	-1.242	-0.639
29.25	4.75	1.397	0.607	-1.274	0.030
28.75	5.25	0.614	0.570	-1.242	-0.639
25.25	6.75	1.164	0.313	-1.148	-0.169
24.75	7.25	1.155	0.276	-1.117	-0.177
27.25	6.75	2.384	0.460	-1.148	0.874
26.75	7.25	2.785	0.423	-1.117	1.217
29.25	6.75	1.300	0.607	-1.148	-0.053
28.75	7.25	0.453	0.570	-1.117	-0.777
37.25	14.75	2.238	1.196	-0.645	0.750
36.75	15.25	0.301	1.159	-0.613	-0.907
39.25	14.75	1.072	1.343	-0.645	-0.247
38.75	15.25	1.881	1.306	-0.613	0.444
41.25	14.75	0.184	1.490	-0.645	-1.006
40.75	15.25	NA	1.453	-0.613	NA
37.25	16.75	1.383	1.196	-0.519	0.018
36.75	17.25	0.718	1.159	-0.488	-0.550
39.25	16.75	1.179	1.343	-0.519	-0.156
38.75	17.25	0.299	1.306	-0.488	-0.908
41.25	16.75	0.700	1.490	-0.519	-0.565
40.75	17.25	1.022	1.453	-0.488	-0.290
37.25	18.75	0.981	1.196	-0.393	-0.325

x distance	y distance	nitrate 2014	zX	zY	z nitrate 2014
36.75	19.25	1.280	1.159	-0.362	-0.069
39.25	18.75	1.865	1.343	-0.393	0.431
38.75	19.25	1.431	1.306	-0.362	0.060
41.25	18.75	1.091	1.490	-0.393	-0.231
40.75	19.25	0.776	1.453	-0.362	-0.500
37.25	20.75	0.943	1.196	-0.267	-0.358
36.75	21.25	2.527	1.159	-0.236	0.997
39.25	20.75	0.841	1.343	-0.267	-0.445
38.75	21.25	0.704	1.306	-0.236	-0.562
41.25	20.75	0.514	1.490	-0.267	-0.724
40.75	21.25	3.264	1.453	-0.236	1.627
1.25	28.75	0.652	-1.453	0.236	-0.607
0.75	29.25	0.164	-1.490	0.267	-1.024
3.25	28.75	0.307	-1.306	0.236	-0.902
2.75	29.25	0.121	-1.343	0.267	-1.061
5.25	28.75	0.355	-1.159	0.236	-0.860
4.75	29.25	0.118	-1.196	0.267	-1.063
1.25	30.75	0.144	-1.453	0.362	-1.041
0.75	31.25	0.330	-1.490	0.393	-0.882
3.25	30.75	1.538	-1.306	0.362	0.151
2.75	31.25	0.119	-1.343	0.393	-1.062
5.25	30.75	0.051	-1.159	0.362	-1.120
4.75	31.25	0.128	-1.196	0.393	-1.054
1.25	32.75	0.327	-1.453	0.488	-0.885
0.75	33.25	0.085	-1.490	0.519	-1.092
3.25	32.75	0.195	-1.306	0.488	-0.997
2.75	33.25	0.152	-1.343	0.519	-1.034
5.25	32.75	0.186	-1.159	0.488	-1.005
4.75	33.25	0.542	-1.196	0.519	-0.700
1.25	34.75	0.402	-1.453	0.613	-0.820
0.75	35.25	0.471	-1.490	0.645	-0.761
3.25	34.75	0.162	-1.306	0.613	-1.026
2.75	35.25	0.197	-1.343	0.645	-0.995
5.25	34.75	0.123	-1.159	0.613	-1.059
4.75	35.25	1.505	-1.196	0.645	0.123
13.25	42.75	1.448	-0.570	1.117	0.074
12.75	43.25	3.050	-0.607	1.148	1.444
15.25	42.75	1.978	-0.423	1.117	0.527
14.75	43.25	0.947	-0.460	1.148	-0.354

x distance	y distance	nitrate 2014	zX	zY	z nitrate 2014
17.25	42.75	1.778	-0.276	1.117	0.356
16.75	43.25	4.466	-0.313	1.148	2.655
13.25	44.75	1.814	-0.570	1.242	0.387
12.75	45.25	3.571	-0.607	1.274	1.890
15.25	44.75	6.006	-0.423	1.242	3.971
14.75	45.25	3.181	-0.460	1.274	1.556
17.25	44.75	3.472	-0.276	1.242	1.805
16.75	45.25	2.466	-0.313	1.274	0.944
13.25	46.75	2.970	-0.570	1.368	1.375
12.75	47.25	2.669	-0.607	1.400	1.118
15.25	46.75	1.914	-0.423	1.368	0.472
14.75	47.25	1.708	-0.460	1.400	0.296
17.25	46.75	2.255	-0.276	1.368	0.764
16.75	47.25	4.484	-0.313	1.400	2.669
13.25	48.75	0.535	-0.570	1.494	-0.707
12.75	49.25	0.634	-0.607	1.526	-0.622
15.25	48.75	1.061	-0.423	1.494	-0.257
14.75	49.25	2.995	-0.460	1.526	1.397
17.25	48.75	1.272	-0.276	1.494	-0.077
16.75	49.25	2.864	-0.313	1.526	1.285

APPENDIX B

Semivariogram analysis used in Ch. 2 to determine spacing between plots. Analysis was based on resin bag exchangeable nitrate data from a 16 y-restored prairie located at Konza. The presented graph was z-transformed. The untransformed range was 1.4. After back-transformation the range was 10 m



APPENDIX C

Plant diversity and soil heterogeneity data from Ch. 2. Abbreviations: cv = coefficient of variation, PAR = photosynthetically active radiation, S = species richness, H = Shannon diversity index, J = Pielou's evenness. Sampling was conducted in 0.25 m^2 quadrats; 24 quadrats were present within each 6 m x 8 m plot. Diversity metrics (S, H, and J) were reported on a per plot level

Sequence	Plot	Age	mean soil nitrate (µg)	cv soil nitrate	mean proportion of PAR available at soil surface	cv PAR availability	S	H	J
1	1	7	0.01	81.99	0.12	75.03	19.00	2.47	0.84
1	2	7	0.02	83.20	0.20	63.61	14.00	2.17	0.82
1	3	7	0.08	184.64	0.32	48.55	12.00	2.01	0.81
1	4	7	0.08	127.15	0.35	50.35	13.00	2.19	0.85
2	1	5	0.16	96.32	0.47	32.63	16.00	2.46	0.89
2	2	5	0.01	107.64	0.35	51.21	21.00	2.58	0.85
2	3	5	0.09	131.44	0.43	40.24	21.00	2.69	0.88
2	4	5	0.04	130.45	0.52	34.07	26.00	2.93	0.90
3	1	3	0.01	82.72	0.35	48.55	19.00	2.61	0.89
3	2	3	0.06	192.63	0.15	74.52	17.00	2.35	0.83
3	3	3	0.11	297.37	0.18	66.00	20.00	2.57	0.86
3	4	3	0.02	113.35	0.15	69.18	16.00	2.25	0.81
4	1	1	0.66	88.04	0.64	13.64	9.00	1.85	0.84
4	2	1	9.24	55.83	0.30	69.06	10.00	1.84	0.80
4	3	1	7.25	61.74	0.24	76.69	9.00	2.00	0.91
4	4	1	6.23	84.80	0.58	37.79	11.00	1.86	0.78
cultivated	1	0	1.94	57.98	NA	NA	NA	NA	NA
cultivated	2	0	5.63	86.69	NA	NA	NA	NA	NA
cultivated	3	0	6.55	68.69	NA	NA	NA	NA	NA
cultivated	4	0	3.17	74.34	NA	NA	NA	NA	NA

APPENDIX D

Frequency of species occurrence (per 20 1m^2 quadrats) within each plot from Ch. 2. Plot codes are the sequence number (youngest to oldest) followed by a plot number after the dash. Species identities are as follows: 1 = *Andropogon gerardii*, 2 = *Schizachyrium scoparium*, 3 = *Panicum virgatum*, 4 = *Sorghastrum nutans*, 5 = *Elymus canadensis*, 6 = *Bouteloua curtipendula*, 7 = *Baptisia bracteata*, 8 = *Baptisia australis*, 9 = *Asclepias verticillata*, 10 = *Asclepias viridis*, 11 = *Salvia azurea*, 12 = *Mirabilis nyctaginea*, 13 = *Silphium integrifolium*, 14 = unknown seedling J, 15 = *Lespedeza capitata*, 16 =*Desmodium illinoense*, 17 = *Kuhnia eupatorioides*, 18= *Tradescantia bracteata*, 19 = *Psoralea tenuiflora*, 20 = *Vicia americana*, 21 = unkown seedling I, 22 = *Mimosa nuttallii*, 23 = *Carex brevior*, 24 = *Desmanthus illinoensis*, 25 = *Helianthus pauciflorus*, 26 = *Liatris punctata*, 27 = *Oligoneuron rigidum*, 28 = *Vernonia fasciculate*, 29 = *Dalea multiflora*, 30 = *Dalea purpurea*, 31 = *Amorpha canescens*, 32 = *Rosa arkansana*, 33 = unknown seedling C, 34 = *Verbena stricta*, 35 = *Physalis pumila*, 36 = *Abutilon theophrasti*, 37 = *Ambrosia psilostachya*, 38 = *Setaria glauca*, 39 = *Chamaesyce maculata*, 40 = *Setaria faberi*, 41 = *Digitaria sanguinalis*, 42 = *Digitaria ciliaris*, 43 = *Eragrostis cilianensis*, 44 = *Solanum rostratum*, 45 = *Cenchrus longispinus*, 46 = *Lepidium densiflorum*, 47 = *Amaranthus rudis*, 48 = *Conyza canadensis*, 49 = *Galium aparine*, 50 = *Ambrosia artemisiifolia*, 51 = *Ulmus americana*, 52 = *Helianthus annuus*, 53 = *Solidago canadensis*, 54 = *Solanum ptycanthum*, 55 = *Cirsium discolor*, 56 = *Carex molesta*, 57 = *Carex blanda*, 58 = *Bromus tectorum*, 59 = *Hordeum pussilum*, 60= *Erigeron strigosus*, 61 = *Lonicera maackii*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1-1	0.19	0.09	0.00	0.06	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.07	0.00	0.00	0.00	0.00	0.00
1-2	0.28	0.20	0.00	0.09	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.01	0.00	0.08	0.00	0.00	0.00	0.00	0.00
1-3	0.28	0.18	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00
1-4	0.29	0.14	0.00	0.12	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.01	0.00	0.07	0.00	0.00	0.00	0.00	0.00
2-1	0.17	0.05	0.02	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.04	0.00	0.00	0.00	0.00	0.00
2-2	0.09	0.01	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-3	0.08	0.00	0.00	0.04	0.00	0.01	0.00	0.02	0.00	0.00	0.00	0.00	0.04	0.00	0.02	0.00	0.00	0.00	0.00	0.00
2-4	0.05	0.03	0.01	0.05	0.01	0.01	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00
3-1	0.20	0.04	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00
3-2	0.32	0.07	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.09	0.00	0.00	0.00	0.00	0.00
3-3	0.25	0.04	0.03	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.06	0.00	0.00	0.00	0.00	0.00
3-4	0.34	0.03	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.02	0.00	0.00	0.00	0.00	0.00
4-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
4-2	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4-3	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4-4	0.00	0.00	0.00	0.00	0.00	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1-1	0.00	0.00	0.00	0.10	0.19	0.03	0.03	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.05	0.00	0.00
1-2	0.00	0.00	0.00	0.04	0.12	0.03	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00
1-3	0.00	0.00	0.00	0.03	0.05	0.00	0.05	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
1-4	0.00	0.00	0.00	0.09	0.07	0.00	0.03	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-1	0.00	0.00	0.00	0.02	0.18	0.00	0.06	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.05	0.00
2-2	0.00	0.00	0.00	0.00	0.09	0.01	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.04	0.00	0.20	0.01	0.00
2-3	0.00	0.00	0.00	0.01	0.11	0.03	0.09	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.04	0.00	0.01
2-4	0.00	0.00	0.00	0.03	0.05	0.02	0.06	0.00	0.02	0.02	0.00	0.01	0.00	0.00	0.00	0.07	0.03	0.12	0.00	0.00
3-1	0.00	0.00	0.00	0.04	0.01	0.00	0.09	0.00	0.00	0.02	0.00	0.00	0.00	0.06	0.00	0.02	0.00	0.07	0.00	0.02
3-2	0.00	0.00	0.00	0.04	0.00	0.00	0.11	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00
3-3	0.00	0.00	0.00	0.07	0.00	0.00	0.12	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.04	0.00	0.00
3-4	0.00	0.00	0.00	0.02	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.07	0.00	0.00	0.00	0.00
4-1	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.26	0.14	0.04
4-2	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.22	0.00
4-3	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.11	0.00
4-4	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.03

	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61
1-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1-2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1-4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.12	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0.08	0.13	0.03	0.01	0.00	0.01	0.01	0.00	0.00	0.00
2-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.13	0.16	0.00	0.00	0.00	0.01	0.03	0.00	0.01	0.00
2-4	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.02	0.06	0.15	0.05	0.00	0.00	0.02	0.00	0.01	0.00	0.00	0.00
3-1	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.07	0.03	0.02	0.00	0.03	0.00	0.00	0.08	0.01	0.00	0.00
3-2	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.03	0.03	0.03	0.01	0.00	0.00	0.00	0.00	0.09	0.04	0.00	0.00
3-3	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.01	0.01	0.03	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.01
3-4	0.00	0.00	0.00	0.02	0.00	0.03	0.00	0.00	0.00	0.02	0.02	0.00	0.14	0.00	0.00	0.00	0.00	0.07	0.02	0.00	0.00
4-1	0.14	0.00	0.25	0.01	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4-2	0.16	0.01	0.24	0.00	0.00	0.21	0.05	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4-3	0.16	0.00	0.16	0.07	0.00	0.07	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4-4	0.02	0.02	0.20	0.00	0.00	0.25	0.10	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

APPENDIX E

Summary statistics from PERMANOVA of microbial community dissimilarity using concentrations of all PLFA biomarkers in response to treatments described in chapter 3. Abbreviations: Navail = nitrogen availability treatment and sp = plant species treatment

	Df	SumsOfSqs	MeanSqs	F.Model	R^2	P value
depth	1	0.0667	0.06674	1.7956	0.02011	0.53
Navail	2	0.8412	0.42058	11.3154	0.25345	0.53
sp	2	0.0355	0.01776	0.4777	0.0107	0.665
depth:Navail	2	0.0387	0.01934	0.5204	0.01166	0.53
depth:sp	2	0.0239	0.01193	0.3211	0.00719	0.837
Navail:sp	4	0.1473	0.03682	0.9905	0.04437	0.334
depth:Navail:sp	4	0.1585	0.03962	1.066	0.04775	0.283
Residuals	54	2.0071	0.03717	0.60477		
Total	71	3.3188	1			

APPENDIX F

Linear mixed model summary statistics for responses of Shannon diversity (H), richness (S), and Pielou evenness (J) to treatments described in chapter 3

Shannon Diversity	NumDF	DenDF	F.value	P value		**Richness**	NumDF	DenDF	F.value	P value
sp	3	36	4.342	0.02045		sp	3	36	2.28421	0.1164
Navail	2	5.938	1.9	0.23025		Navail	2	5.93	1.00772	0.42
depth	1	3.544	0.2429	0.65107		depth	1	3.598	0.08771	0.7834
sp:Navail	4	36	1.269	0.30013		sp:Navail	4	36	0.97368	0.434
sp:depth	2	36	1.2653	0.29439		sp:depth	2	36	1.54737	0.2266
Navail:depth	2	6.366	0.1922	0.82972		Navail:depth	2	6.413	0.3684	0.7056
sp:Navail:depth	4	36	0.7982	0.5343		sp:Navail:depth	4	36	0.45789	0.766

Pielou's Evenness	NumDF	DenDF	F.value	P value
sp	3	36	1.96867	0.1544
Navail	2	5.943	0.06833	0.9347
depth	1	3.53	0.01161	0.92
sp:Navail	4	36	1.34923	0.2708
sp:depth	2	36	1.40739	0.2579
Navail:depth	2	6.353	0.38607	0.6946
sp:Navail:depth	4	36	1.75779	0.1588

APPENDIX G

Linear mixed model summary statistics for responses of PLFA biomass for each major group for treatments described in chapter 3

Non-specific Bacteria	NumDF	DenDF	F.value	P value		**Gram-positive Bacteria**	NumDF	DenDF	F.value	P value
sp	3	36	0.2386	0.789		sp	3	36	0.0526	0.948866
Navail	2	5.899	10.382	0.01169		Navail	2	5.899	11.9939	0.008345
depth	1	3.848	1.597	0.27747		depth	1	3.848	2.1738	0.217109
sp:Navail	4	36	0.8654	0.49404		sp:Navail	4	36	1.1176	0.363428
sp:depth	2	36	0.1064	0.89937		sp:depth	2	36	0.0352	0.965429
Navail:depth	2	6.619	0.1007	0.90555		Navail:depth	2	6.619	0.0361	0.964756
sp:Navail:depth	4	36	1.6043	0.19436		sp:Navail:depth	4	36	1.4261	0.245179

Gram-negative Bacteria	NumDF	DenDF	F.value	P value		**Saprotrophic Fungi**	NumDF	DenDF	F.value	P value
sp	3	36	1.2654	0.294376		sp	3	36	1.96867	0.1544
Navail	2	5.899	14.931	0.004916		Navail	2	5.943	0.06833	0.9347
depth	1	3.848	0.6592	0.464063		depth	1	3.53	0.01161	0.92
sp:Navail	4	36	0.8754	0.488245		sp:Navail	4	36	1.34923	0.2708
sp:depth	2	36	0.2108	0.810913		sp:depth	2	36	1.40739	0.2579
Navail:depth	2	6.619	0.1406	0.871357		Navail:depth	2	6.353	0.38607	0.6946
sp:Navail:depth	4	36	1.7022	0.170912		sp:Navail:depth	4	36	1.75779	0.1588

Arbuscular Mycorrhizal Fungi	NumDF	DenDF	F.value	P value		**Actinomycetes**	NumDF	DenDF	F.value	P value
sp	3	36	0.9898	0.38154		sp	3	36	0.3745	0.69028
Navail	2	5.899	6.2225	0.03521		Navail	2	5.931	5.9415	0.03833
depth	1	3.848	2.2649	0.20953		depth	1	4.081	1.0306	0.36637
sp:Navail	4	36	1.0675	0.3868		sp:Navail	4	36	1.3552	0.26873
sp:depth	2	36	0.1898	0.82794		sp:depth	2	36	0.1661	0.84759
Navail:depth	2	6.619	0.6073	0.57258		Navail:depth	2	6.176	0.286	0.76071
sp:Navail:depth	4	36	0.7775	0.54714		sp:Navail:depth	4	36	1.3552	0.26872

Total PLFA	NumDF	DenDF	F.value	P value
sp	3	36	0.6203	0.5434
Navail	2	5.899	9.9428	0.0129
depth	1	3.848	2.7044	0.1782
sp:Navail	4	36	1.5083	0.2204
sp:depth	2	36	0.0037	0.9963
Navail:depth	2	6.619	0.2628	0.7766
sp:Navail:depth	4	36	1.1658	0.3421

APPENDIX H

Data from Ch. 3. Abbreviations: Navail = nitrogen availability treatment, sp = species treatment, HS = horizontal strip block, VS = vertical strip block, NS = non-specific bacteria biomass, GPB = Gram-positive bacteria biomass, GNB = Gram-negative bacteria, SapF = saprotrophic fungi, AMF = arbuscular mycorrhizal fungi biomass, Actino = actinomycetes biomass, Total = total PLFA biomass, S = PLFA biomarker richness, H = PLFA biomarker Shannon diversity, J = PLFA biomarker Pielou's evenness. All biomarker concentrations are expressed in ng/g dry soil

plot	subplot	depth	Navail	sp	HS	VS	NS	GPB	GNB	SapF	AMF	Actino	Total	S	H	J
1	7	deep	ambient	AG	1001	1003	116.24	140.16	45.42	63.31	44.76	22.69	432.58	15	2.39	0.88
1	7	deep	ambient	SA	1001	1003	69.08	72.25	28.38	39.48	27.39	12.21	248.77	17	2.47	0.87
1	7	deep	ambient	BS	1001	1003	107.81	128.71	33.62	56.30	36.09	19.64	382.17	15	2.37	0.87
1	8	deep	high	AG	1002	1003	206.43	261.56	66.10	87.82	85.78	47.67	755.35	14	2.35	0.89
1	8	deep	high	SA	1002	1003	122.92	137.99	50.11	54.17	40.98	24.62	430.80	17	2.48	0.88
1	8	deep	high	BS	1002	1003	71.12	92.27	22.77	24.18	22.77	16.72	249.83	14	2.33	0.88
1	9	deep	low	AG	1003	1003	251.56	252.68	81.07	141.09	64.80	42.62	833.82	17	2.45	0.87
1	9	deep	low	SA	1003	1003	135.49	151.60	49.55	77.82	36.69	28.66	479.82	17	2.49	0.88
1	9	deep	low	BS	1003	1003	234.35	261.43	68.86	125.39	52.64	43.78	786.45	14	2.35	0.89
1	10	shallow	ambient	AG	1001	1004	99.31	113.89	37.24	45.43	40.80	18.88	355.56	17	2.45	0.87
1	10	shallow	ambient	SA	1001	1004	121.73	130.94	41.67	64.96	39.12	30.67	429.08	17	2.49	0.88
1	10	shallow	ambient	BS	1001	1004	136.04	155.12	37.08	61.92	49.60	28.98	468.74	15	2.38	0.88
1	5	shallow	high	AG	1002	1002	242.97	297.02	80.21	102.00	91.08	46.08	859.35	15	2.40	0.89
1	5	shallow	high	SA	1002	1002	212.58	275.89	61.90	67.95	74.07	47.88	740.28	15	2.35	0.87
1	5	shallow	high	BS	1002	1002	113.80	130.68	35.35	59.87	36.28	23.42	399.40	17	2.46	0.87
1	6	shallow	low	AG	1003	1002	172.54	175.65	58.19	102.28	43.27	33.89	585.81	17	2.46	0.87
1	6	shallow	low	SA	1003	1002	264.47	307.27	86.66	132.27	71.56	55.45	917.68	16	2.45	0.88

plot	subplot	depth	Navail	sp	HS	VS	NS	GPB	GNB	SapF	AMF	Actino	Total	S	H	J
1	6	shallow	low	BS	1003	1002	303.59	331.86	84.90	156.29	55.70	64.97	997.30	17	2.43	0.86
6	7	deep	low	AG	6001	6003	392.64	427.09	146.89	226.77	95.60	67.28	1356.28	17	2.44	0.86
6	7	deep	low	SA	6001	6003	247.80	283.41	89.33	122.83	85.81	44.17	873.34	17	2.43	0.86
6	7	deep	low	BS	6001	6003	117.39	135.36	43.38	62.88	28.74	22.64	410.38	17	2.47	0.87
6	8	deep	high	AG	6002	6003	85.77	108.31	36.71	37.70	22.61	19.36	310.45	17	2.47	0.87
6	8	deep	high	SA	6002	6003	100.82	113.01	38.94	35.83	27.58	22.77	338.95	17	2.48	0.88
6	8	deep	high	BS	6002	6003	56.07	62.75	15.97	18.78	16.26	12.67	182.49	15	2.36	0.87
6	3	deep	ambient	AG	6003	6001	93.47	85.39	29.25	52.96	26.19	15.97	303.23	17	2.45	0.86
6	3	deep	ambient	SA	6003	6001	250.82	267.27	72.47	107.26	79.60	53.99	831.42	15	2.39	0.88
6	3	deep	ambient	BS	6003	6001	200.93	247.16	57.83	74.04	68.13	43.52	691.61	14	2.34	0.89
6	10	shallow	low	AG	6001	6004	164.11	198.30	61.06	82.47	49.70	37.39	593.03	17	2.49	0.88
6	10	shallow	low	SA	6001	6004	129.72	144.45	38.22	67.71	36.69	23.92	440.71	16	2.45	0.88
6	10	shallow	low	BS	6001	6004	183.12	218.98	66.76	81.92	49.99	32.90	633.66	17	2.44	0.86
6	11	shallow	high	AG	6002	6004	185.21	216.76	73.81	84.53	78.05	29.17	667.53	16	2.43	0.88
6	11	shallow	high	SA	6002	6004	93.24	108.50	27.78	42.41	32.05	14.93	318.90	14	2.38	0.90
6	11	shallow	high	BS	6002	6004	152.21	155.58	60.65	60.20	53.62	23.32	505.58	17	2.43	0.86
6	12	shallow	ambient	AG	6003	6004	118.22	145.51	40.26	48.10	47.06	30.38	429.53	15	2.37	0.88
6	12	shallow	ambient	SA	6003	6004	84.09	94.84	23.23	37.47	31.16	15.22	286.02	15	2.37	0.88
6	12	shallow	ambient	BS	6003	6004	84.05	86.98	22.17	30.91	34.72	12.84	271.67	16	2.36	0.85
10	1	deep	low	AG	10001	10001	117.92	158.10	46.06	63.69	43.40	31.73	460.89	15	2.42	0.89
10	1	deep	low	SA	10001	10001	226.05	265.71	93.51	105.34	82.00	58.98	831.59	15	2.37	0.87
10	1	deep	low	BS	10001	10001	202.45	234.93	67.61	109.78	55.91	46.00	716.68	16	2.45	0.88
10	2	deep	high	AG	10002	10001	159.38	182.54	40.02	68.06	0.00	70.37	520.37	13	2.20	0.86

plot	subplot	depth	Navail	sp	HS	VS	NS	GPB	GNB	SapF	AMF	Actino	Total	S	H	J
10	2	deep	high	SA	10002	10001	178.59	184.94	73.13	87.25	52.03	38.50	614.44	15	2.46	0.91
10	2	deep	high	BS	10002	10001	243.94	260.34	69.18	77.79	56.37	46.45	754.07	15	2.34	0.86
10	3	deep	ambient	AG	10003	10001	139.71	137.82	54.69	70.98	51.72	26.63	481.54	17	2.47	0.87
10	3	deep	ambient	SA	10003	10001	17.03	18.06	5.28	8.89	7.07	3.71	60.04	15	2.35	0.87
10	3	deep	ambient	BS	10003	10001	128.21	123.04	43.03	46.20	42.44	24.12	407.05	16	2.43	0.88
10	10	shallow	low	AG	10001	10004	102.56	124.88	30.95	76.02	25.04	23.79	383.25	15	2.42	0.89
10	10	shallow	low	SA	10001	10004	312.37	442.75	112.18	143.73	108.28	89.53	1208.83	14	2.37	0.90
10	10	shallow	low	BS	10001	10004	277.70	358.97	80.33	126.13	73.11	66.33	982.58	14	2.35	0.89
10	11	shallow	high	AG	10002	10004	293.43	217.18	85.77	367.27	57.43	39.38	1060.46	16	2.36	0.85
10	11	shallow	high	SA	10002	10004	108.10	126.41	46.92	38.29	32.61	25.03	377.36	17	2.46	0.87
10	11	shallow	high	BS	10002	10004	191.60	236.44	39.43	64.14	42.81	38.18	612.59	14	2.32	0.88
10	6	shallow	ambient	AG	10003	10002	219.84	240.62	74.63	106.61	76.21	44.36	762.27	16	2.44	0.88
10	6	shallow	ambient	SA	10003	10002	150.46	157.33	54.72	61.83	57.83	34.73	516.90	16	2.44	0.88
10	6	shallow	ambient	BS	10003	10002	118.33	135.61	31.38	48.67	36.58	28.51	399.08	14	2.34	0.89
15	1	deep	high	AG	15001	15001	74.95	79.48	23.25	39.11	31.13	16.34	264.26	16	2.41	0.87
15	1	deep	high	SA	15001	15001	106.90	132.92	38.48	52.09	32.65	27.69	390.73	15	2.44	0.90
15	1	deep	high	BS	15001	15001	97.08	122.58	36.69	41.81	29.81	24.37	352.34	17	2.48	0.87
15	2	deep	low	AG	15002	15001	156.55	183.36	57.88	71.35	46.30	35.48	550.92	17	2.46	0.87
15	2	deep	low	SA	15002	15001	286.46	322.47	106.68	148.41	81.16	56.53	1001.72	17	2.51	0.89
15	2	deep	low	BS	15002	15001	195.80	244.27	63.10	107.01	60.60	60.83	731.62	15	2.42	0.89
15	3	deep	ambient	AG	15003	15001	105.29	129.43	34.26	45.02	46.79	24.01	384.79	15	2.40	0.88
15	3	deep	ambient	SA	15003	15001	88.16	106.89	37.33	29.44	35.90	20.46	318.18	17	2.46	0.87
15	3	deep	ambient	BS	15003	15001	144.54	167.34	30.67	47.80	0.00	67.98	458.33	13	2.30	0.90

plot	subplot	depth	Navail	sp	HS	VS	NS	GPB	GNB	SapF	AMF	Actino	Total	S	H	J
15	10	shallow	high	AG	15001	15004	98.93	111.31	34.19	54.99	28.69	21.10	349.20	17	2.48	0.88
15	10	shallow	high	SA	15001	15004	133.34	149.05	45.20	65.26	34.76	31.91	459.51	16	2.46	0.89
15	10	shallow	high	BS	15001	15004	100.91	129.41	22.21	50.08	30.91	35.96	369.48	14	2.36	0.89
15	11	shallow	low	AG	15002	15004	297.70	346.07	99.31	144.12	78.66	60.08	1025.95	16	2.44	0.88
15	11	shallow	low	SA	15002	15004	281.02	351.05	110.80	134.49	78.45	67.72	1023.53	15	2.43	0.90
15	11	shallow	low	BS	15002	15004	316.45	361.71	100.08	173.12	74.74	70.34	1096.45	17	2.46	0.87
15	12	shallow	ambient	AG	15003	15004	235.40	283.50	74.53	125.88	71.20	60.80	851.32	17	2.47	0.87
15	12	shallow	ambient	SA	15003	15004	109.83	129.44	35.13	51.90	36.08	32.37	394.74	16	2.44	0.88
15	12	shallow	ambient	BS	15003	15004	177.89	233.01	54.06	78.91	47.36	49.55	640.79	15	2.43	0.90

APPENDIX I

PLFA biomass of each biomarker in each subplot from Ch. 3. All biomarker concentrations are expressed in ng/g dry soil

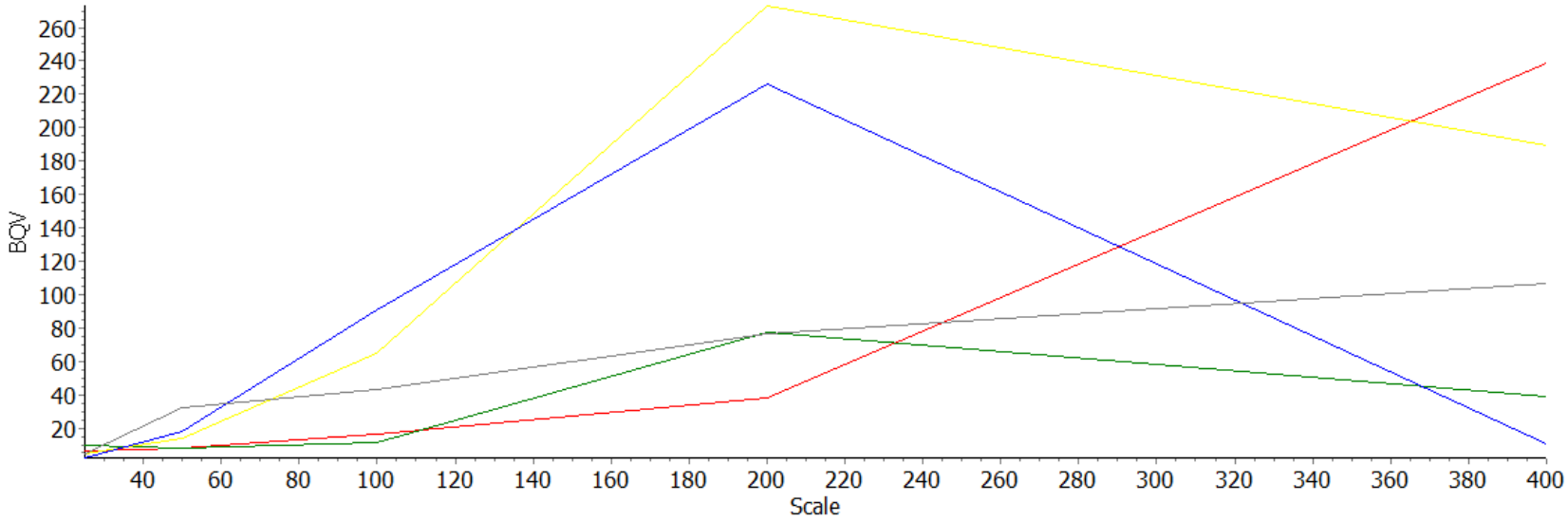
	NSB_C 14:0	GPB_i-C15:0	GPB_a-C15:0	NSB_C 15:0	GPB_i-C16:0	NSB_C 16:0	GNB_C 16:1_9	ACT_10 Me C16:0	AMF_C 16:1_11 cis	GPB_i-C17:0	NSB_C 17:0	GNB_cy17:0	NSB_C 18:0	SAP_C 18:1_9 cis	SAP_C 18:2 _9,12	GNB_cy19:0	GNB_2-OH C16:0
1-7AG	5.89	60.66	37.91	2.74	27.56	93.94	30.1	22.69	44.76	14.03	0	9.25	13.67	44.36	18.95	0	6.07
1-7SA	3.12	31.07	18.19	2	14.67	53.67	15.38	12.21	27.39	8.32	1.37	3.58	8.92	26.25	13.23	5.53	3.88
1-7BS	5.16	57.17	32.05	1.68	25.07	84.27	28.6	19.64	36.09	14.43	2.66	5.01	14.03	36.85	19.45	0	0
1-8AG	9.02	109.53	70.45	5.32	55.03	168.62	47.92	47.67	85.78	26.56	0	18.18	23.47	63.44	24.38	0	0
1-8SA	5.24	56.69	35.93	3.83	27.95	94.83	25.76	24.62	40.98	17.43	2.11	10.78	16.91	34.58	19.59	8.79	4.79
1-8BS	3.7	40.49	26.69	2.13	15.2	57.04	16.41	16.72	22.77	9.89	0	6.36	8.25	19.18	5	0	0
1-9AG	16.06	92.68	68.01	7.97	68.34	189.07	49.96	42.62	64.8	23.65	5.38	15.22	33.08	103.12	37.97	6.98	8.91
1-9SA	7.3	58.87	36.79	5.21	37.2	100.36	28.53	28.66	36.69	18.75	3.47	9.04	19.16	56.96	20.87	6.51	5.47
1-9BS	14.94	92.97	77.89	7.15	62.34	186.43	51.93	43.78	52.64	28.22	0	16.93	25.83	98.69	26.71	0	0
1-10AG	4.74	49.46	30.3	3.83	22.27	74.75	21.21	18.88	40.8	11.86	2.2	8.59	13.79	33.77	11.66	2.29	5.15
1-10SA	4.67	52.5	31.81	3.89	28.21	91.83	23.19	30.67	39.12	18.42	3.85	7.81	17.49	42.13	22.82	5.4	5.27
1-10BS	7.48	65.83	40.45	2.95	31.63	106.24	28.46	28.98	49.6	17.2	2.24	8.62	17.13	39.82	22.1	0	0
1-5AG	12.4	121.56	76.6	6.87	65.64	183.2	53.21	46.08	91.08	33.22	0	15.15	40.51	77.82	24.18	0	11.84
1-5SA	12.48	113.19	79.53	5.85	50.55	168.73	43.69	47.88	74.07	32.63	0	13.67	25.53	57.01	10.94	4.55	0
1-5BS	5.93	54.35	33.98	4.08	27.74	84.9	20.69	23.42	36.28	14.6	2.86	6.91	16.03	42.38	17.49	2.22	5.54
1-6AG	11.23	68.82	41.46	5.76	46.44	129.75	35.31	33.89	43.27	18.92	3.17	10.39	22.63	73.29	28.98	2.68	9.81
1-6SA	17.48	131.13	69.11	10.25	78	194.34	58.09	55.45	71.56	29.03	10.03	19.93	32.36	96.29	35.98	8.63	0
1-6BS	20.43	116.85	76.61	9.17	100.5	222.01	54.95	64.97	55.7	37.9	7.59	19.65	44.4	126.28	30.01	5.03	5.27
6-7AG	24.12	152.97	134.97	13.04	99.17	294.38	97.4	67.28	95.6	39.98	7.33	30.7	53.77	182.96	43.82	8.14	10.64
6-7SA	16.69	96.8	88.49	7.83	71.77	189.98	64.05	44.17	85.81	26.35	4.54	13.21	28.76	92.5	30.33	4.27	7.81
6-7BS	7.76	53.76	35.75	4.67	32.08	86.4	26.7	22.64	28.74	13.77	2.68	8.55	15.88	49.47	13.41	3.37	4.76
6-8AG	4.74	48.75	28	2.75	20.37	65.42	20.09	19.36	22.61	11.19	1.63	8.5	11.23	25.05	12.65	3.54	4.58
6-8SA	9.54	49.43	30.77	2.94	20.06	73.42	22.4	22.77	27.58	12.76	1.99	8.54	12.93	24.37	11.46	3.96	4.04
6-8BS	2.6	28.06	17.12	1.66	10.98	43.76	11.12	12.67	16.26	6.59	1.12	4.85	6.93	14.56	4.21	0	0
6-3AG	3.98	35.82	21.21	2.99	18.53	72.88	15.94	15.97	26.19	9.82	1.85	4.08	11.77	31.52	21.43	2.58	6.64

	NSB_C 14:0	GPB_i-C15:0	GPB_a-C15:0	NSB_C 15:0	GPB_i-C16:0	NSB_C 16:0	GNB_C 16:1_9	ACT_10 Me C16:0	AMF_C 16:1_11 cis	GPB_i-C17:0	NSB_C 17:0	GNB_cy17:0	NSB_C 18:0	SAP_C 18:1_9 cis	SAP_C 18:2 _9,12	GNB_cy19:0	GNB_2-OH C16:0
6-3SA	13.25	115.09	68.94	6.51	54.31	195.29	49.67	53.99	79.6	28.93	0	14.01	35.77	69.51	37.75	0	8.8
6-3BS	9.65	113.3	59.12	6.54	43.83	157.88	47.72	43.52	68.13	30.92	0	10.11	26.87	49.96	24.08	0	0
6-10AG	10.04	72.16	58.13	5.44	46.49	120.97	35.56	37.39	49.7	21.51	4.13	13.57	23.54	59.88	22.59	4.43	7.5
6-10SA	9.3	51.03	40.09	5.32	38.87	96.26	25.4	23.92	36.69	14.46	2.9	6.4	15.93	46.53	21.18	6.42	0
6-10BS	12.14	79.99	63.81	5.85	53.5	137.54	41.9	32.9	49.99	21.67	3.57	16.33	24.02	66.19	15.73	3.59	4.94
6-11AG	14.19	93.09	52.46	6.73	49.31	144.34	45.44	29.17	78.05	21.9	0	10.92	19.96	59.47	25.06	3.74	13.72
6-11SA	4.28	43.57	27.8	3.25	20.97	73.37	20.57	14.93	32.05	16.15	0	7.21	12.35	26.77	15.64	0	0
6-11BS	8.37	71.47	35.95	5.57	32.16	120.65	33.48	23.32	53.62	16	2.56	13.16	15.05	44.02	16.18	4.4	9.61
6-12AG	4.94	64.39	39.46	3.82	25.97	95.32	27.04	30.38	47.06	15.69	0	9.97	14.15	34.48	13.63	3.25	0
6-12SA	3.62	41.87	25.57	3.42	16.12	66.88	16.97	15.22	31.16	11.29	0	3.52	10.16	22.22	15.25	2.73	0
6-12BS	3.74	37.33	23.37	2.12	17.51	65.71	15.86	12.84	34.72	8.77	1.74	3.99	10.74	21.73	9.18	2.32	0
10-1AG	7.89	66.95	41.33	3.84	32.27	91.71	31.75	31.73	43.4	17.54	0	10.87	14.48	42.64	21.05	0	3.44
10-1SA	11.63	123.86	79.75	8.37	47.93	179.68	65.91	58.98	82	14.17	0	17.45	26.38	83.26	22.07	10.15	0
10-1BS	13.19	84.83	73.87	7.16	54.98	150.82	45.23	46	55.91	21.25	4.14	14.16	27.15	79.36	30.42	8.22	0
10-2AG	6.16	80.25	49.79	3.87	33	134.77	29.03	70.37	0	19.5	0	10.99	14.57	52.65	15.41	0	0
10-2SA	8.82	75.81	51.08	6.64	35.43	137.46	40.59	38.5	52.03	22.63	0	12.53	25.66	47.96	39.29	20	0
10-2BS	12.99	112.4	72.11	7.21	48.3	195.01	43.77	46.45	56.37	27.54	0	20.32	28.73	62.99	14.8	5.09	0
10-3AG	6.53	60.06	33.24	3.99	28.05	107.59	32.15	26.63	51.72	16.48	3.54	11.09	18.05	40.72	30.26	5.92	5.53
10-3SA	4.38	47.73	25.9	2.09	20.56	75.85	24.76	20.22	41.69	11.62	0	7.73	13.11	27.67	6.93	1.72	0
10-3BS	6.55	53.65	30.34	4.86	25.52	99.96	23.8	24.12	42.44	13.53	0	7.7	16.83	29.66	16.54	4.35	7.18
10-10AG	4.51	47.04	34.01	2.67	31.77	81.77	15.69	23.79	25.04	12.06	0	5.68	13.61	34.82	41.2	9.58	0
10-10SA	20.19	173.34	124.57	10.56	100.92	238.7	85.42	89.53	108.28	43.92	0	26.76	42.93	121.09	22.64	0	0
10-10BS	17.3	146.44	99.23	9.46	84.92	208.19	61.19	66.33	73.11	28.38	0	19.14	42.76	108.9	17.23	0	0
10-11AG	11.42	92.58	59.08	6.09	42.77	177.35	47.55	39.38	57.43	22.75	0	12.66	98.57	215.04	152.24	13.3	12.27
10-11SA	4.56	54.2	32.87	2.44	24.77	84.07	26.52	25.03	32.61	14.57	2.7	9.15	14.33	26.8	11.49	6.43	4.81

	NSB_C 14:0	GPB_i-C15:0	GPB_a-C15:0	NSB_C 15:0	GPB_i-C16:0	NSB_C 16:0	GNB_C 16:1_9	ACT_10 Me C16:0	AMF_C 16:1_11 cis	GPB_i-C17:0	NSB_C 17:0	GNB_cy17:0	NSB_C 18:0	SAP_C 18:1_9 cis	SAP_C 18:2 _9,12	GNB_cy19:0	GNB_2-OH C16:0
10-11BS	9.1	99.88	60.53	6.29	48.5	151.82	29.37	38.18	42.81	27.54	0	10.05	24.39	47.22	16.92	0	0
10-6AG	10.6	105.44	57.06	10.5	49.35	171.53	42.5	44.36	76.21	28.78	0	10.65	27.21	64.96	41.65	5.91	15.57
10-6SA	9.42	68.74	39.99	4.52	30.34	117.04	31.9	34.73	57.83	18.25	4.63	12.52	14.85	41.02	20.8	10.3	0
10-6BS	4.52	60.54	36.03	2.98	23.13	96.62	22.54	28.51	36.58	15.9	0	8.84	14.21	30.55	18.12	0	0
15-1AG	3.13	32.95	21.14	2.16	16.89	60.28	14.74	16.34	31.13	8.49	0	4.11	9.37	20.26	18.85	1.97	2.43
15-1SA	4.81	52.98	35.11	3.52	29.23	81.99	23.79	27.69	32.65	15.59	0	7.2	16.58	32.81	19.29	7.49	0
15-1BS	5.22	51.55	31.67	3.29	25.74	73.52	19.76	24.37	29.81	13.62	2.23	9.73	12.82	28.3	13.5	3.7	3.5
15-2AG	9.81	73.14	46.35	5.6	44.48	116.36	37.36	35.48	46.3	19.39	3.62	12.69	21.16	55.49	15.86	4.56	3.28
15-2SA	17.41	121.31	84.78	11.16	82.12	209.1	57.98	56.53	81.16	34.25	9.43	22.7	39.36	101.71	46.71	15.13	10.87
15-2BS	9.92	96.25	61.01	6.78	52.01	160.53	37.38	60.83	60.6	35	0	13.68	18.56	68.46	38.55	12.05	0
15-3AG	6.37	51.27	32.87	2.51	30.19	82.16	23.94	24.01	46.79	15.09	0	5.06	14.25	32.23	12.79	0	5.25
15-3SA	4.3	46.48	27.56	2.87	20.79	66.8	21.75	20.46	35.9	12.06	2.32	7.86	11.87	22.54	6.9	3.12	4.6
15-3BS	6.94	71.16	44.81	27.03	33.88	95.46	22.64	67.98	0	17.49	0	8.03	15.11	33.68	14.12	0	0
15-10AG	5.05	43.08	29.01	3.74	26.21	76.21	18.57	21.1	28.69	13	2.36	6.11	11.58	34.05	20.94	4.46	5.04
15-10SA	7.4	59.28	37.46	4.11	32.83	104.38	22.35	31.91	34.76	19.49	0	9.51	17.45	39.97	25.29	6.34	6.99
15-10BS	4.81	47.49	34.48	4.07	27.37	81.5	18.88	35.96	30.91	20.06	0	3.34	10.54	36.7	13.38	0	0
15-11AG	19.41	133.7	95.04	6.68	81.47	222.7	64.23	60.08	78.66	35.86	8.74	26.89	40.18	99.97	44.15	8.2	0
15-11SA	19.52	137.53	93.85	10.87	82.34	215.06	74.75	67.72	78.45	37.33	0	20.99	35.57	100.51	33.98	15.06	0
15-11BS	20.01	137.62	98.25	11.48	89.62	231.18	56.47	70.34	74.74	36.21	7.53	22.34	46.24	137.16	35.96	11.66	9.61
15-12AG	13.3	109.87	68.09	7.7	63.14	182.18	45.03	60.8	71.2	42.41	4.25	13.49	27.96	79.59	46.29	8.9	7.11
15-12SA	5.61	50.57	34.29	3.58	26.3	86.11	22.22	32.37	36.08	18.28	0	6.78	14.53	34.64	17.25	3.12	3.01
15-12BS	10.61	86.05	58.21	7.67	61.73	131.76	35.83	49.55	47.36	27.02	0	9.76	27.85	56.43	22.48	8.47	0

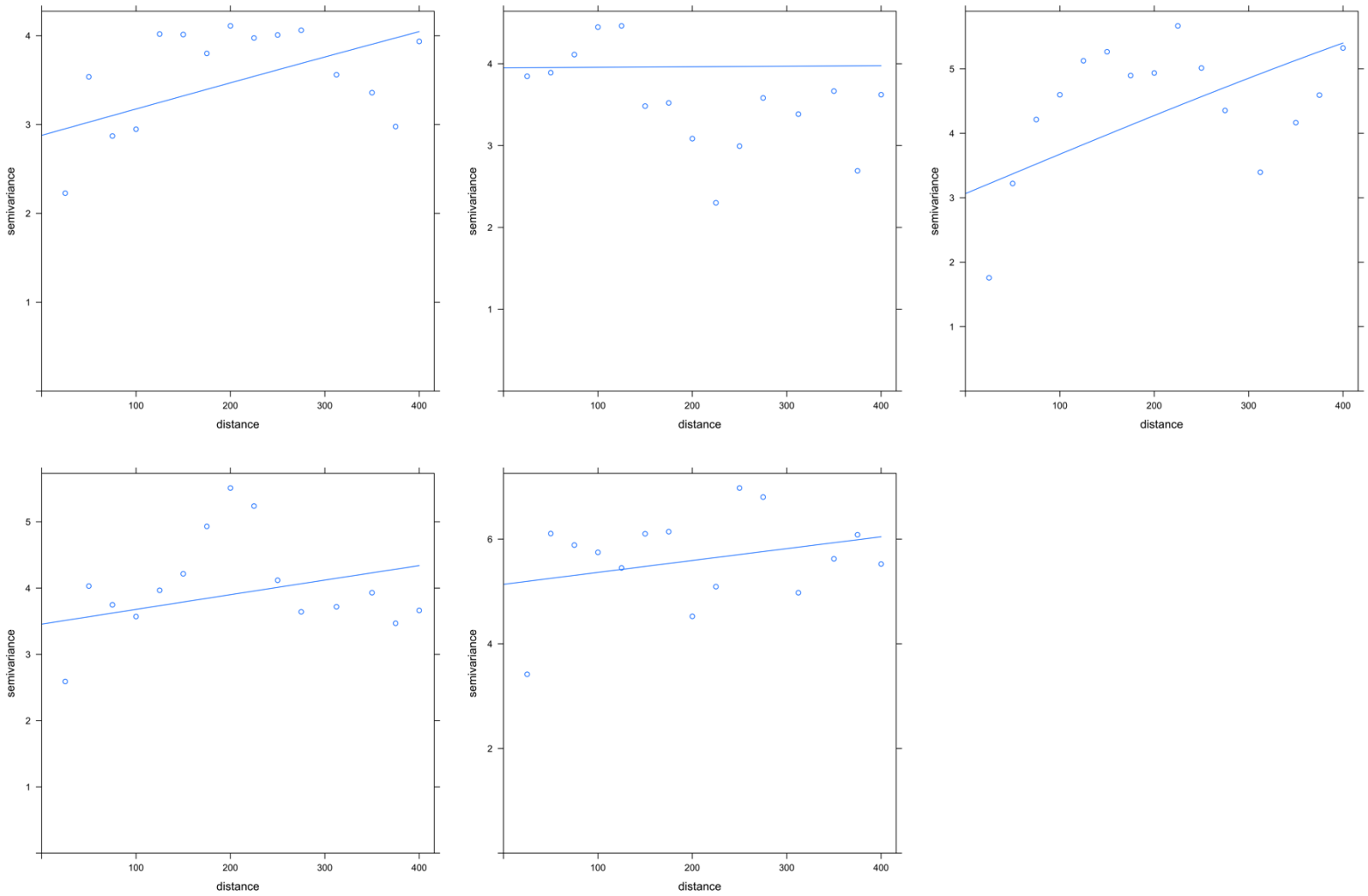
APPENDIX J

Three-term local quadrat variance (3TLQV) analyses for first axis of principal component analysis scores used to justify sampling frame size in Ch. 4. The scale on the x-axis is in cm. First peak represents the distance of maximum variance in plant community composition. The average of all sites was 1.3 m



APPENDIX K

Spherical semivariogram analyses for first axis of principal component analysis scores used to justify spacing between sampling frames in Ch. 4. The ranges were Site 1 = 1809.6 cm, Site 2 = 77305.1 cm, Site 3 = 1068.6 cm, Site 4 = 2482.5 cm, and Site 5 = 3822.0 cm. The average range was 18.1 m



APPENDIX L

Data from Ch. 4. Abbreviations: HD = high plant diversity, LD = low plant diversity, rep = secondary transect block, Diversity= Shannon diversity, S = species richness, J = Pielou's evenness, NO3 = 2N KCl extracted NO_3^-–N (g m^{-2}), NH4 = 2N KCl extracted NH_4^+– N (g m^{-2}), Next = 2N extracted inorganic N (g m^{-2}), N2O = nitrous oxide emissions from 1 d incubation (µg m^{-2}), d15N = $\delta^{15}N$–N_2O (atmosphere reference), soilR = soil respiration rate (g m^{-2} d^{-1}), WEOC = water extractable organic carbon (mg g $^{-1}$). Diversity metrics were assessed on a 1 m^2 scale

Site	Treatment	rep	Diversity	S	J	NO3	NH4	Next	N2O	d15N	soilR	WEOC
1	HD	1	1.48	13	0.58	0.03	0.08	0.11	0.00	3.81	8870.49	5.42
1	LD	1	0.89	11	0.37	0.02	0.05	0.06	575.17	3.02	4489.46	5.13
1	HD	2	1.79	10	0.78	0.01	0.04	0.05	549.02	5.51	1960.21	6.78
1	LD	2	0.87	9	0.40	0.23	0.12	0.35	138.83	2.35	8476.58	3.66
1	HD	3	1.56	8	0.75	0.03	0.09	0.12	180.28	3.73	11167.38	5.25
1	LD	3	0.82	9	0.37	0.02	0.06	0.08	377.45	4.64	12070.48	5.14
1	HD	4	1.52	5	0.94	0.06	0.12	0.17	0.00	4.80	9294.33	3.82
1	LD	4	0.83	5	0.52	0.03	0.09	0.12	1006.54	3.71	14242.85	4.51
1	HD	5	1.33	4	0.96	0.10	0.16	0.26	402.62	2.64	4675.62	3.77
1	LD	5	0.75	6	0.42	0.09	0.21	0.30	0.00	3.27	6183.28	3.48
1	HD	6	1.61	5	1.00	0.05	0.18	0.23	0.00	3.71	-9168.23	4.04
1	LD	6	0.77	5	0.48	0.07	0.16	0.23	862.75	4.27	12337.80	5.58
2	HD	1	1.37	11	0.57	0.15	0.04	0.19	33.83	-1.78	10396.00	3.30
2	LD	1	0.84	12	0.34	0.08	0.04	0.12	862.75	4.40	11123.39	3.28
2	HD	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	LD	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	HD	3	1.48	10	0.64	0.03	0.07	0.09	3019.63	3.47	11938.58	5.76
2	LD	3	0.88	7	0.45	0.03	0.04	0.07	3019.63	4.57	5255.51	2.86
2	HD	4	1.67	8	0.80	0.13	0.13	0.26	355.25	3.85	4616.93	4.14
2	LD	4	0.84	7	0.43	0.11	0.16	0.27	503.27	3.65	6299.94	3.71
2	HD	5	1.99	7	1.02	0.06	0.11	0.17	241.57	4.24	2883.25	3.49
2	LD	5	0.59	3	0.54	0.07	0.24	0.31	236.83	4.69	10745.03	3.80

Site	Treatment	rep	Diversity	S	J	NO3	NH4	Next	N2O	d15N	soilR	WEOC
2	HD	6	1.66	6	0.93	0.12	0.19	0.31	0.00	3.82	3894.86	3.71
2	LD	6	0.89	5	0.55	0.06	0.29	0.35	0.00	5.53	5459.79	4.40
3	HD	1	1.77	4	1.28	0.02	0.14	0.16	118.42	3.04	11498.88	3.45
3	LD	1	0.85	5	0.53	0.00	0.13	0.14	76.45	3.90	8013.37	4.31
3	HD	2	1.75	8	0.84	0.01	0.13	0.14	0.00	3.87	898.42	4.79
3	LD	2	0.84	6	0.47	0.02	0.16	0.19	135.71	2.24	4319.20	3.52
3	HD	3	1.96	7	1.01	0.02	0.22	0.24	416.50	4.64	5086.19	4.16
3	LD	3	0.32	8	0.15	0.01	0.17	0.19	0.00	3.80	8086.42	3.73
3	HD	4	1.76	10	0.76	0.08	0.17	0.25	8.70	-6.47	7414.42	4.12
3	LD	4	0.56	8	0.27	0.05	0.21	0.26	85.06	1.88	9302.76	3.79
3	HD	5	1.35	6	0.75	0.01	0.23	0.24	0.00	3.19	10166.04	5.43
3	LD	5	0.52	4	0.38	0.03	0.13	0.16	124.52	3.26	14273.02	5.16
3	HD	6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	LD	6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	HD	1	2.26	6	1.26	0.10	0.10	0.20	0.00	3.32	9631.81	4.60
4	LD	1	0.87	6	0.49	0.07	0.10	0.17	246.50	3.92	4853.84	4.33
4	HD	2	2.2	5	1.37	0.03	0.11	0.14	0.00	5.08	7679.11	4.43
4	LD	2	0.42	5	0.26	0.02	0.15	0.17	280.90	3.69	13255.39	4.48
4	HD	3	2.1	10	0.91	0.01	0.07	0.08	754.91	3.46	10164.39	4.10
4	LD	3	0.78	9	0.35	0.03	0.13	0.15	294.60	3.98	7932.62	4.58
4	HD	4	1.78	9	0.81	0.04	0.12	0.17	0.00	4.86	14405.83	4.25
4	LD	4	0.89	7	0.46	0.03	0.06	0.09	0.00	3.93	13153.21	3.87
4	HD	5	1.73	5	1.07	0.10	0.06	0.16	0.00	3.76	6114.34	4.58
4	LD	5	0.89	3	0.81	0.13	0.08	0.21	671.03	4.67	7162.24	4.87
4	HD	6	2	4	1.44	0.04	0.12	0.16	2013.09	4.63	14639.47	4.80
4	LD	6	0.67	4	0.48	0.04	0.08	0.12	2013.09	4.71	8730.16	4.52
5	HD	1	1.49	4	1.07	0.02	0.16	0.18	0.00	3.49	12206.45	5.51
5	LD	1	0.71	3	0.65	0.01	0.14	0.15	1725.50	5.55	15069.59	5.22
5	HD	2	1.34	9	0.61	0.03	0.21	0.24	0.00	4.62	15598.86	5.37

Site	Treatment	rep	Diversity	S	J	NO3	NH4	Next	N2O	d15N	soilR	WEOC
5	LD	2	0.87	7	0.45	0.14	1.52	1.66	14.31	-7.19	28146.24	9.16
5	HD	3	1.68	9	0.76	0.01	0.19	0.20	483.14	4.28	13986.10	4.81
5	LD	3	0.64	10	0.28	0.01	0.14	0.15	3019.63	4.59	2063.24	4.03
5	HD	4	1.43	8	0.69	0.02	0.17	0.19	3019.63	2.85	9431.46	4.86
5	LD	4	0.53	6	0.30	0.03	0.15	0.18	12078.53	4.93	9140.95	4.44
5	HD	5	1.72	6	0.96	0.02	0.23	0.24	1098.05	2.93	8239.93	4.78
5	LD	5	0.47	4	0.34	0.01	0.19	0.20	6039.26	4.44	6126.62	4.63
5	HD	6	1.54	5	0.96	0.00	0.16	0.17	366.02	4.33	12894.73	4.20
5	LD	6	0.77	6	0.43	0.00	0.17	0.17	326.45	4.96	15154.53	4.01

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