12-1-2013

Regional-climate and Local-microbial Controls on Ecosystem Processes During Grassland Restoration

Meredith Lynne Mendola
Southern Illinois University Carbondale, mmendola123@gmail.com

REGIONAL-CLIMATE AND LOCAL-MICROBIAL CONTROLS ON ECOSYSTEM PROCESSES DURING GRASSLAND RESTORATION

By Meredith Lynne Mendola

B.S. Southern Illinois University, 2008

A Thesis
Submitted in Partial Fulfillment of the Requirements for the
Master of Science Degree
Department of Plant Biology

In the Graduate School
Southern Illinois University
December 2013

THESIS APPROVAL

REGIONAL-CLIMATE AND LOCAL-MICROBIAL CONTROLS ON ECOSYSTEM PROCESSES DURING GRASSLAND RESTORATION

By

Meredith Lynne Mendola

A Thesis Submitted in Partial

Fulfillment of the Requirements

for the Degree of

Master of Science

in the field of Plant Biology

Approved by:

Dr. Sara G. Baer, Chair

Dr. Brian Klubek

Dr. Dale Vitt

Graduate School
Southern Illinois University Carbondale
October 31, 2013

AN ABSTRACT OF THE THESIS OF

MEREDITH L MENDOLA, for the Master of Science degree in PLANT BIOLOGY, presented on October 31st, 2013, at Southern Illinois University Carbondale.

TITLE: REGIONAL-CLIMATE AND LOCAL-MICROBIAL CONTROLS ON ECOSYSTEM PROCESSES DURING GRASSLAND RESTORATION

MAJOR PROFESSOR: Dr. Sara G. Baer

Root productivity likely has consequences for the composition, activity, and recovery of soil microbial populations and the belowground processes mediated by these organisms. In tallgrass prairie, ecotypic variation potentially exists in response to a strong precipitation gradient across the Great Plains. Thus, ecotypic variation within a species may differentially affect belowground net primary productivity (BNPP), the associated soil microbial community, and may scale up to affect ecosystem processes. The goals of this study were to elucidate: (1) whether ecotype, environment, or an ecotype by environment interaction regulate BNPP of a dominant species (*Andropogon gerardii*) collected from and reciprocally planted in common gardens across a precipitation gradient, and (2) whether variation in BNPP scales to affect microbial biomass and ecosystem processes. I quantified root biomass, BNPP (using root ingrowth bags), soil microbial biomass, and nutrient mineralization rates in root-ingrowth cores below six population sources of *A. gerardii* (2 Illinois, 2 eastern Kansas, and 2 central Kansas) established in southern Illinois, eastern Kansas, and central Kansas. An ecotype effect was found on above and belowground net primary productivity, but these findings did not translate to soil response variables.

Microbial populations themselves may affect the productivity and composition of prairie species. In a second study, soil ecological knowledge (SEK) was tested by applying a native prairie soil slurry amendment to restoration plots to determine efficacy of this method as a restoration practice. The goals of this two year study were to elucidate: (1) whether a slurry

amendment of prairie soil would increase above and belowground productivity and belowground ecosystem processes in a prairie restoration, and (2) to evaluate whether differences in plant diversity will scale to affect belowground productivity and ecosystem processes. I quantified aboveground net primary productivity (ANPP) and species composition, as well as root biomass, belowground net primary productivity (BNPP), soil microbial biomass, and nutrient mineralization rates in root-ingrowth cores installed in treated and control plots. A treatment effect was noted on root biomass and total PLFA biomass; however, there was no treatment effect on cover, ANPP, or soil microbial processes. Though the soil microbial community did represent native prairie soil, there was poor establishment of prairie plant species. These factors may be due to the limited time available for data collection and the lack of precipitation in the second growing season. Longer studies may be necessary to fully examine the effects of soil slurry amendments as restoration tools.

DEDICATION

For Mom and Dad

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Sara Baer, for her continued guidance in research and writing, and for sharing her vast library of knowledge with me. I am also indebted to the other members of my committee, Dr. Brian Klubek and Dr. Dale Vitt, for their guidance and feedback. I have learned so much more through this process than I had ever thought possible. I especially thank my many labmates for their help with field work, lab work, data collection, friendship, and laughter: Rachel Goad, Ben Wodika, Jason Willand, Ryan Klopf, Dana Carpenter, Kim Elsenbroek, Juliette Donatelli, Evan McCrea, Amanda Rothert, Jake Jeffries, Mike Beck, and Nick Lelonek. My husband, Nathan Rothwell, lent his keen eye to edit this work, as well as a great deal of support and cheerleading when I needed it most.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

In 1700, half of the terrestrial biomes on Earth were considered wild, with the majority of the remainder in a seminatural state and very little land area used for agriculture and human settlements (Ellis *et al*. 2010). In 2000, the majority of terrestrial Earth was dedicated to agriculture with less than 20% of land in seminatural or wild states (Ellis *et al*. 2010). Tallgrass prairie existed in the most mesic area of the central North American grassland, once covering approximately 68 million hectares in an area called the 'prairie peninsula' (Transeau 1935). The nineteenth century saw the greatest conversion of grasslands to agriculture in this area (Ellis *et al*. 2010). The restoration of biomes aims to overcome factors that restrict ecosystem development to create resilient interconnected ecosystems that provide goods and services to humanity and nature (Hobbs 2007). Grasslands provide ecosystem services through provisioning (food and fiber), regulating (air and water quality), habitat (migratory animals), and cultural (aesthetic and spiritual) means (Millenium Ecosystem Assessment 2005). Plant-microbe-soil nutrient transformations may increase these ecosystems services (Jackson *et al.* 2008). Dominant species likely exhibit intraspecific variation in traits, which has implications for ecosystem functioning (Grime 1998). This may be highly relevant to tallgrass prairie restoration, where the dominant grasses drive the recovery of root systems, microbial communities, and carbon accrual in soil (Baer *et al.* 2010).

Local and Regional Drivers of Belowground Ecosystem Processes

Aboveground plant biomass has been correlated with variations in autotrophic and heterotrophic respiration (Flanagan & Johnson 2005), however, the majority of plant biomass in some

ecosystems resides belowground, i.e., tallgrass prairie (Gill *et al.* 2002; Milchunas & Lauenroth 2001), creating an expansive interface with diverse resources. Belowground biomass is important for plant acquisition of nutrients to sustain production and the energy provisioned to soil microorganisms from root turnover regulates whole-ecosystem biogeochemical cycles (Schlesinger 1997). Roots absorb water and nutrients from the soil and transport these to the stem for storage, growth, and use in the synthesis of hormones for plant growth and reproduction (Wild 1988). Belowground net primary productivity (BNPP) provides the majority of organic matter inputs to the rhizosphere soil to stimulate microbial activity and transformations of limiting nutrients to plants (Craine *et al*. 2003; Rice *et al.* 1998; Knops *et al.* 2002).

Hiltner first used the word "rhizosphere" in 1904 to describe the region of soil directly influenced by roots. Rhizosphere soil represents the site of highest microbial respiration resulting from labile root exudates and turnover (Raich & Tufekcioglu 2000). Thus, soil respiration, a measure of biological activity, results from the combined release of CO_2 from roots and microbial decomposition of organic matter (Raich & Schlesinger 1992). In mesic grasslands, which contain more plant biomass belowground than aboveground and an extensive network of fine roots, soil in the surface 20 cm (where 80% of belowground biomass resides) is essentially all rhizosphere soil (Parton & Risser 1980; Rice *et al*. 1998).

In addition to roots, soil microorganisms influence biogeochemical cycling in ecosystems. The soil microbiota play an important role in regulating plant productivity, as both fungal and bacterial symbionts are responsible for the acquisition of limiting water and mineral nutrients (Smith & Read 1997). Ecosystem functions such as nitrogen and carbon transformations and soil formation are also mediated by soil microorganisms (Tiedje 1988; Kowalchuk & Stephen 2001; Hogberg *et al.* 2001; Rillig & Mummey 2006).

Vesicular arbuscular mycorrhizal fungi (AMF) are the most common belowground microbial symbionts with plants and associated with roots of approximately 80% of terrestrial plant species (Smith & Read 1997). Vesicular arbuscular mycorrhizal fungi are associated with many tallgrass prairie species, including the dominant grass, *Andropogon gerardii* Vitman (Hetrick *et al.* 1987). These associations may enhance plant productivity by up to 100% (Anderson *et al.* 1994; van der Heijden *et al.* 1998a; Vogelsang *et al.* 2006) with a 7 to 70 fold increase in seedling biomass of *A. gerardii* in the presence of AMF (Hetrick *et al.* 1989). These mycorrhizae enhance protection from parasites and herbivory in the rhizosphere, improve growth with increased access to water, and increase soil exploration and uptake of phosphorus and other nutrients (Hayman 1983).

Nitrogen is the limiting nutrient to plant productivity in many ecosystems (Hetrick *et al.* 1989). Plants need nitrogen in order to build proteins, enzymes, and genetic material, but many species are unable to fix atmospheric N into organic forms (Chapin 1980). Many plants rely on the activities of nitrogen-fixing bacteria to transform biologically unavailable N_2 into biologically reactive forms either through symbiotic associations with N-fixing microorganisms (Wild 1988) or by free-living nitrogen fixing microorganisms (Smith & Read 1997). One study found that twenty percent of all organic nitrogen that is acquired annually by vegetation is contributed by N-fixing bacterial symbionts (van der Heijden *et al.* 2006a). The intrasystem N cycle, that is the supply of N from mineralization of organic matter by microorganisms, provides most of the N for plant growth (Schlesinger 1997).

Variation in root productivity can result from variation within species (intraspecific) or between species (interspecific), and likely has consequences for the activity and composition of

soil microbial communities and ecosystem processes. *Andropogon gerardii* (big bluestem) is responsible for the majority of the aboveground net primary productivity in tallgrass prairie (Weaver 1965, Risser *et al.* 1981) and this species exhibits intraspecific variation (or ecotypic variation) in response to climate (McMillan 1959). This species ranges from Canada in the north to central Mexico in the south and widely across the North American continent from east to west (USDA). The C_4 photosynthetic pathway allows this species to produce the greatest biomass in the warm summer months (Gould & Shaw 1983) with roots extending more than 1 m into the soil to access deep resources during drought to sustain productivity (Albertson & Weaver 1944) in grassland restoration. Thus, at the regional scale in this species, ecotypic variation may have effects on belowground net primary productivity. At a local scale, however, the variation among species (overall diversity) and composition of the soil microbial community likely modulates belowground net primary productivity and resultant ecosystem processes (Figure 1.1).

Objectives and Hypotheses

Two field studies were used to address regional (interaction between precipitation and ecotypic variation of a dominant species) and local (soil microbial) controls on ecosystem processes with relevance and direct application to restoration, respectively. The same response variables of belowground net primary productivity, microbial biomass, soil respiration and net N mineralization potentials, were used in each study to: (1) evaluate the effect of precipitation and ecotypic variation of a dominant species on ecosystem processes; and (2) elucidate whether local microbial communities encourage diversity with consequence ecosystem processes. These objectives were used to test the corresponding hypotheses ($H_{1\text{-}2}$).

H_{1a}: Genes (ecotypic variation corresponding to population sources) and the environment

interact to affect ecosystem processes above and belowground. Specifically, I predicted each population source will have greater BNPP when planted at a site with higher rates of precipitation, but have highest productivity in their home environment, resulting in a gene by environment interaction.

H_{1b}: Ecotypic variation in root traits will differentially affect ecosystem processes. Specifically, I predicted the ecotype with the greatest BNPP will support the largest microbial biomass, resulting in higher potential carbon mineralization rates.

H_{2a}: Soil microbial amendments will promote establishment of a more diverse prairie community. Specifically, I predicted that soil amended with the microbial community from native prairie will result in more diverse restored prairie.

H_{2b}: Higher plant diversity will increase ANPP and belowground net primary productivity (BNPP) and ecosystem processes. Specifically, I predicted that plant diversity in restored prairie treated with a soil amendment would increase above and belowground biomass. I also predicted that greater amounts of roots (and therefore root exudates) would support a larger microbial population and increase soil respiration.

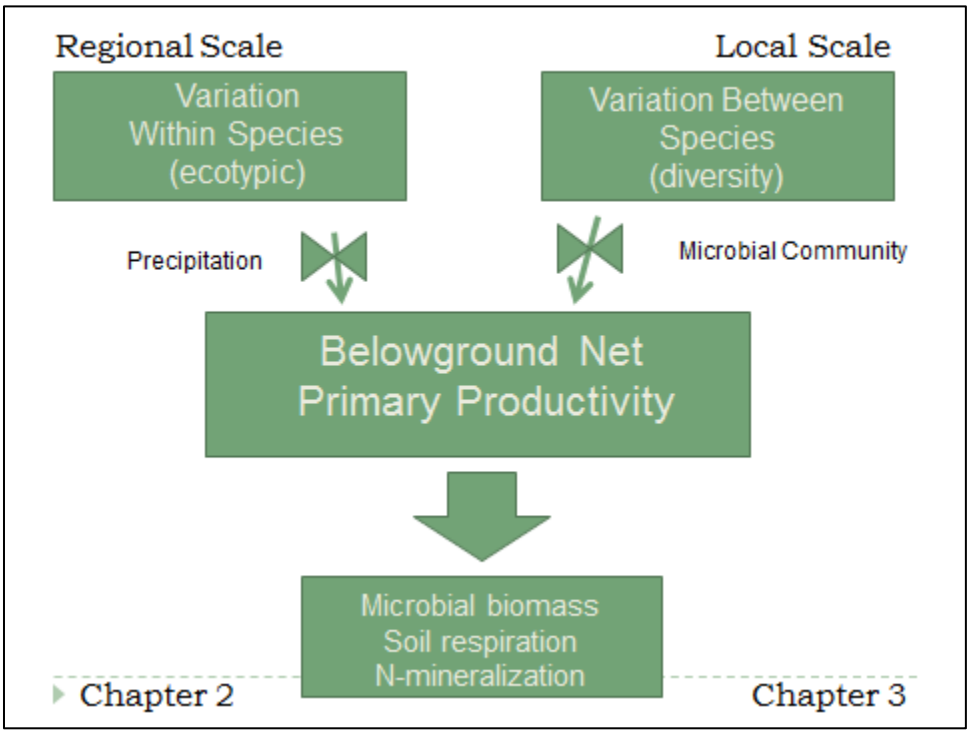


Figure 1.1. Conceptual model showing relationship between intraspecific variation and interspecific variation and how these affect BNPP and ecosystem processes.

CHAPTER 2

ECOTYPIC VARIATION IN ROOT BIOMASS AND BELOWGROUND NET PRIMARY PRODUCTIVITY OF *ANDROPOGON GERARDII*: IMPLICATIONS FOR RESTORATION

Introduction

Plant populations may become locally adapted if certain genotypes are favored in local environmental conditions (Turesson 1922). This may potentially create ecotypes, populations distinguished by a composite of variations in traits over space (Lowry 2012). Interactions between genes and the environment may result in a phenotype with positive fitness effects for an individual in their local environment, but negative or neutral effects in a foreign environment (Whitham *et al*. 2005). Further, intraspecific variation in a dominant species may result in community heritability, the tendency of genetically similar individuals to support similar communities of organisms and ecosystem processes, known as the 'extended phenotype' concept (Dawkins 1982). For example, genetic differences among individual plants of *Populus* species have been shown to alter associated herbivore communities, belowground microbial communities, and ecosystem processes (i.e., decomposition) in response to variation in the quality (chemistry) of foliar tissue and litter (Crutsinger *et al*. 2006; Whitham *et al*. 2006; Schweitzer *et al.* 2011). The dominant grass species in tallgrass prairie, *Andropogon gerardii*, exhibits genetic (Gray 2012) and phenotypic variation (Olson *et al*. 2013, Johnson *et al*. unpublished data) to result in putative ecotypes across an east-west precipitation gradient. Thus, at the regional scale, ecotypic variation within this species has potential to affect ecosystem processes (e.g., above and belowground net primary productivity) and associated soil microbial biomass and belowground processes regulated by the size and activity of the microbial biomass.

The climate of the central US grassland is highly variable, both spatially and temporally (Borchert 1950). Precipitation ranges from 200 mm/yr in Colorado to >1200 mm/yr in the Illinois (Lauenroth *et al*. 1999) and aboveground net primary productivity (ANPP) is responsive to precipitation across spatial scales that correspond to varying precipitation (Sala *et al*. 1988; Lauenroth & Sala 1992; Knapp & Smith 2001). Precipitation can also be highly variable between years within the tallgrass prairie and ANPP is responsive to this temporal (interannual) variation in precipitation. In a long-term study at the Konza Prairie Long Term Ecological Research site, grass ANPP was highly correlated (r^2=0.79) with soil moisture over a 20 year time period (Briggs & Knapp 1995). There is less information about the responsiveness of belowground net primary productivity (BNPP) to variability in precipitation, but seasonal changes in precipitation have a corollary relationship with BNPP (Milchunas & Lauenroth 2001), and drought conditions can reduce total root length and live root biomass (Hayes & Seastedt 1987).

Experiments assessing gene (or ecotype) by environment interactions provide insight into how genomes cope with changing environmental conditions (Hodgins-Davis & Townsend 2009). Studying organisms subjected to natural climatic conditions and in new environments is needed to elucidate whether phenotypic variation is under genetic control and to what degree climatic and edaphic factors regulate differentiation (Turesson 1922). Further, documenting whether phenotypic variation exists and the degree of phenotypic response to environmental variation (phenotypic plasticity or ecological amplitude) is needed to provide insight into which population sources (ecotypes) will be best suited for establishment and persistence in ecological restorations (Falk *et al*. 2006) under current and changing environmental conditions (Harris *et al*. 2006).

Dominant species can strongly influence the aboveground structure of a community (Usher 1966) and contribute greatly to overall plant biomass (Grime 1998). In tallgrass prairie, C_4 grasses comprise >80% of the above ground net primary productivity (Parton & Risser 1980) and modulate ecosystem functioning (Smith & Knapp 2003). I used a reciprocal common garden approach to: (1) determine whether ecotype, environment, or an ecotype-by-environment interaction regulates ANPP and BNPP of the dominant prairie grass species, *Andropogon gerardii*; and (2) elucidate the extent to which ecotypic variation in the productivity of this species extends to affect microbial biomass and microbially-mediated processes in the rhizophere associated with individual plants originating from different population sources within three regions across a precipitation gradient. I predicted that productivity measures would exhibit an interaction between ecotype and environment ('site'), in that geographically separate source populations within a region ('population sources') and local ecotypes from a region ('regional ecotypes') would have greater ANPP and BNPP when planted at a site with higher precipitation when compared to sites with lower precipitation, but populations and regional ecotypes would also exhibit the highest biomass and productivity at the site closest to the population's origin (i.e., 'home site advantage'). Similarly, I predicted microbial biomass and mediated processes (i.e., carbon and nitrogen mineralization) would differ by site due to different soil properties, but would respond to local and regional ecotypic variation in productivity within a site. In recognition of microbial dependency on root turnover, I predicted that the ecotype with the greatest BNPP would support the largest microbial biomass, resulting in higher carbon (C) mineralization rate, but lower net nitrogen (N) mineralization rate due to greater microbial demand for N in response to higher C inputs and larger microbial biomass.

Methods

Study Sites

The common gardens were established in three regions where source populations of *A. gerardii* were collected: central Kansas (CKS), eastern Kansas (EKS) and southern Illinois (SIL) (Figure 2.1). The CKS site was located in Hays, Kansas at the Agricultural Research Extension owned and maintained by Kansas State University (Ellis County, 38°50'N, 99°19'W). Average annual rainfall has been 582 mm, based on a 50 year record (NOAA 2012). Soil at the site was classified as a Harney silt-loam (Fine, smectic, mesic Typic Argustoll) (NRCS 2010). Average monthly temperature and precipitation for the 2011 growing season (May through August) were 25°C and 73 mm respectively (NOAA 2012).

The EKS site was located at the USDA Plant Materials Center in Manhattan, Kansas (Riley County, 39°08'N, 96°38'). This area has received an annual average of 871 mm of precipitation according to a 50 year record (NOAA 2012). Soil at the site was a Belvue silt-loam, characterized as Coarse-silty, mixed, superactive, nonacid mesic Typic Udifluvent (NRCS 2010). Average monthly temperature and precipitation for the 2011 growing season (May through August) were 24°C and 99 mm respectively (NOAA 2012).

The SIL site was located in Carbondale, Illinois at the Agricultural Research Center owned by Southern Illinois University (Jackson County, 37°41'N, 89°14'W). Average annual precipitation of approximately 1167 mm per year is based on a 50 year record (NOAA 2012). Soil was characterized as a Stoy silt loam, which is a fine-silty, mixed, superactive, mesic,

Fragiaquic Hapludalf. Average monthly temperature and precipitation for the 2011 growing season (May through August) were 24º C and 137 mm respectively (NOAA 2012).

Experimental Design

In fall of 2008, seed of *A. gerardii* was collected from 12 independent populations representing three putative ecotypes sourced from central KS, eastern KS, and southern Illinois. Populations where seed of *A. gerardii* was collected were within 80 km radius of each common garden site. Seeds were collected by hand from remnant prairies in central KS, eastern KS, and southern IL (4 populations per region). Seeds were germinated and grown in a greenhouse during the summer of 2009 at Kansas State University and transplanted to the common gardens in early August of 2009. Each common garden site was established according to a randomized complete block design that consisted of 10 blocks. Each block contained 12 *Andropogon gerardii* plants, one from each population collection source, spaced approximately 0.5 m apart within a matrix of black landscaping plastic in order to discourage the growth of other species. Two randomly selected populations of *A. gerardii* from each collection region (Table 2.1) in 5 of the 10 blocks (n=90; n=30 per site; and n=5 per population source at each site) were used for this study. All belowground response variables were also measured in soil containing no plants to serve as a root-free control.

Soil Properties at Each Site

Soil texture, pH, cation exchange capacity (CEC) and phosphorus availability were determined from composited soil samples to 20 cm in depth at the KSU Soil Testing Lab to characterize the soil at each site (Table 2.2). Plant available P was determined following the Bray extraction method (1945), where 2 grams of 2 mm sieved and air-dried soil were extracted with 20 mL of 0.025 N HCl + 0.03 N NH_4F for one minute on a shaker and filtered immediately through a 0.4 μm filter. Phosphate-P was determined on a Flow IV Autoanalyzer (OI Analytical, College Station, TX). Soil moisture at each site was measured twice weekly on a percent water by volume basis using an EC-20 soil moisture probe (Decagon Devices, Washington, USA).

Belowground response to ecotype and environment

I measured root biomass, BNPP, soil microbial biomass, C mineralization potential, and potentially mineralizable nitrogen below each of the 6 population sources of *A. gerardii* at each site. Root ingrowth bags allow measurement of root growth over a known time period (Neill 1992) and have been used to measure root production of many species in various ecosystems (Steingrobe *et al*. 2001; Lukac & Godbold 2010; Meier & Leuschner 2008; Johnson & Matchett 2001). In May 2011, one soil core (5.5 cm dia. x 20 cm deep) was taken 25 cm from the central tiller of each plant at each site and five cores were taken beneath the landscape fabric to serve as a root-free comparison. After all cores were removed, the soil from each sample was homogenized through a 4 mm sieve and roots were hand-picked from the soil and placed in coin envelopes. A fiberglass (1 mm x 1 mm mesh screen) ingrowth bag was placed in each soil core

extraction hole (Johnson & Matchett 2001) and the root-free soil was placed into the ingrowth bag and returned to the same location from which it was extracted. Root ingrowth bags remained in the field for 16 weeks. In September 2011, root ingrowth bags were removed by cutting into the soil around each root bag. Following retrieval, roots were clipped at the fiberglass surface of the root ingrowth bag. Roots harvested from the initial extraction of soil cores were used to quantify standing root biomass, and roots that grew into the root ingrowth bags were used to determine BNPP.

In the laboratory, roots were hand-picked from the soil contained within each ingrowth bag. All roots were washed with deionized water, dried at 55^{o}C for 7 days, and weighed to determine BNPP. Percent C and N of root tissue were measured from a 50 µg sample of root tissue dried at 55°C for 7 days, ground to a fine powder in a ball mill (Spex CertiPrep, Metuchen, New Jersey, USA), and combusted on a Flash 1112 CN Analyzer (CE Elantech Corp., Lakewood, New Jersey, USA).

After roots were picked, the root-free soil from each bag was homogenized through a 4 mm mesh sieve. A 100 g subsample was used to determine gravimetric water content (dried at 105^{o}C), potential C and N mineralization rates (C_{MIN} and N_{MIN}), and microbial biomass C and N (MBC and MBN). All soil samples were adjusted to 50% water holding capacity before soil analyses. Microbial biomass C and N were determined using the chloroform fumigation incubation technique described by Jenkinson & Powlson (1976) as modified by Voroney & Paul (1984). Four 10 g samples from each soil core were pre-incubated in 165 mL serum bottles for 5 days at 23^{o}C. Afterwards, half of these samples were fumigated with chloroform in a vacuum desiccator for 18 hours. Following fumigation, samples were evacuated for eight 3 minute intervals, during which time all non-fumigated samples were aerated. All serum bottles were

then sealed and incubated for 10 days at 23°C. After the incubation period, CO_2-C was measured by analyzing 0.5 mL sample of headspace gas on a GC-8A gas chromatograph with a thermoconductivity detector (Shimadzu, Kyoto, Japan). Microbial biomass carbon (MBC) was determined by calculating the difference between fumigated and non-fumigated samples divided by a decomposition constant (K_c) of 0.4 (Voroney & Paul 1984). Potential CO_2-C mineralization rate was determined from the non-fumigated samples. Mineralized carbon ($\mu g\ C\ g^{-1}$) from the two replicates was averaged over the incubation period.

Following headspace gas measurements, soil in each serum bottle was extracted for inorganic N to determine MBN. Inorganic N concentrations were determined by adding 50 mL of 2 N KCl to each serum bottle. Samples were agitated for 1 hour at 200 rpm, and then filtered through 0.4 μm polycarbonate membranes. The filtrates were analyzed colorimetrically for adsorbance (Keeney & Nelson 1982) of NH_4-N and NO_3-N on a Flow IV Autoanalyzer (OI Analytical, College Station, TX). Microbial biomass N was calculated from differences between fumigated and non-fumigated NH_4-N + NO_3-N on a per g soil basis divided by a decomposition constant (k_n=0.6).

Potential net N mineralization (N_{MIN}) rate was determined using the aerobic laboratory incubation procedure (Robertson *et al*. 1999; Baer *et al*. 2002). A 10 g soil sample was extracted for inorganic N ($N_{initial}$) according to the methods described for MBN. The non-fumigated soil used to determine MBN was used as the 'final' measurement of inorganic N (N_{final}) for each soil core. The difference between the final and initial concentrations of inorganic N (NH_4-N + NO_3-N) divided by the number of incubation days [($N_{final} - N_{initial}$)/d] was used to calculate net N mineralization rate.

Aboveground biomass

Each plant was clipped to ~2 cm above the soil surface at the time root-ingrowth bags were retrieved, which also coincided with peak plant biomass. Each plant was dried at 55˚C and weighed.

Statistical Analyses

Aboveground biomass, root biomass, BNPP and N storage in roots were analyzed according to a randomized complete block (RCB) design using the mixed model procedure in SAS (SAS Institute 2009) to test for main effects and interactions between site and population source. Contrast and estimate statements were used to compare regional ecotypes (ecotypes pooled by CKS, EKS, and SIL) within a site and each regional ecotype response across sites. Block was assigned as a random effect. Microbial biomass and potential C and N mineralization rates were analyzed according to a RCB design by site for population source and regional ecotype effects (relative to root-free soil controls) due to differences in soil texture among sites. Pearson's correlation procedure was used to examine relationships between root and soil response variables.

Results

Aboveground Biomass

Aboveground biomass varied among population sources across all sites (population source main effect: $F_{5,68} = 4.26$, $P = 0.002$, Figure 2.2A). This main effect was largely due to consistently higher biomass of *A. gerardii* plants from '12M' prairie in southern IL relative to all Kansas population sources across all sites. When population sources were combined by region, a regional ecotype effect ($F_{2,74} = 5.77$, $P = 0.0047$) and site by regional ecotype interaction ($F_{4,74} = 2.58$, $P = 0.0443$, Figure 2.2B) were revealed, showing that site had an effect on productivity of regional ecotypes, with all regional ecotypes exhibiting greater aboveground biomass at sites with higher precipitation.

Root Biomass, BNPP, and Quality

Root biomass varied among population sources across all sites (population source main effect: $F_{5,67} = 18.91$, $P < 0.001$) and root biomass corresponded to the geographic gradient of ecotype origin, with populations from environments with greater precipitation exhibiting greater root biomass. The SIL population sources ('12M' and 'DES') exhibited higher root biomass than the central KS population sources ('REL' and 'CDB'), with eastern KS population sources ('TAL' and 'KNZ') generally intermediate of western KS and southern IL populations (Figure 2.2C). When population sources were combined by region, root biomass was affected by an interaction between site and regional ecotype ($F_{4,73} = 2.85$, $P = 0.030$, Figure 2.2D). This interaction resulted from significantly higher aboveground biomass of the SIL regional ecotypes relative to both KS regional ecotypes in Carbondale and Manhattan, which was not maintained in Hays, KS, where all regional ecotypes produced similar aboveground biomass.

Belowground NPP showed the same pattern as root biomass, as root biomass was strongly correlated with BNPP (r=0.67, P<0.001). There was a main effect of population source ($F_{5, 68}$ = 17.64, P<0.001, Figure 2.2E) resulting from significantly higher BNPP of *A. gerardii* originating from the two SIL populations relative to the KS populations at all sites. Similar to root biomass, there was an interaction between site and regional ecotype ($F_{4, 74}$ = 3.77, P=0.010, Figure 2.2F) on BNPP. Aboveground biomass of plants was positively correlated with root biomass (r=0.51, P <0.001) and BNPP (r=0.43, P<0.001).

The quality of roots, as indexed by C:N ratio of BNPP, varied among sites ($F_{2,71}$ = 21.11, P < 0.001, Figure 2.3A). Across all population sources, roots produced in Hays, KS contained lower C:N ratios than plants growing in Manhattan, KS or Carbondale, IL. There were no differences among population sources, regional ecotypes, or interaction between site and population source for root C:N.

Quantity of N belowground in root biomass, however, exhibited a main effect of population source ($F_{5,72}$=11.82, P<0.0001, Figure 2.4C) resulting from higher N storage in roots of plants from De Soto and 12 Mile Prairie across all sites. Subsequently, the SIL population sources had higher N storage in roots across the climate gradient when grouped by regional ecotype ($F_{2,81}$=22.49, P<0.0001, Figure 2.4B).

Microbial C and N Pools and Potential Fluxes

All soil response variables showed an effect of site (Table 2.3). There were no effects of population source across all sites on any soil response variable, or effect of regional ecotype at each site. There were few differences in the soil response variables among population sources or

regional ecotypes within each site. Only in Manhattan, KS, was population source effect ($F=2.23_{5,20}$, $P=0.091$, $\alpha = 0.10$, Figure 2.5) seen in potential net N mineralization rate, with one southern IL and one central KS source with the greatest values compared to the other population sources.

Using the root free controls to determine the relative change in response variables, there was an effect of site on carbon mineralization ($F=34.89_{2,66}$, $P<0.0001$), potentially mineralizable nitrogen ($F=11.43_{2,67}$, $P=<0.0001$), and microbial biomass nitrogen ($F=19.35_{2,68}$, $P<0.0001$), but not on microbial biomass carbon. The Hays, KS, site consistently produced the greatest rates of C_{MIN}, N_{MIN}, and MBN; and the Manhattan, KS, site consistently produced an order of magnitude lower rates of these variables. There was a weak population source effect on C_{MIN} across all sites found ($F=1.9_{6,66}$, $P=0.093$) resulting from one southern IL source and one central KS source with the highest rates of C_{MIN}. Across all sites and at each site there was no effect of regional ecotype on any of the response variables. At each site there was no effect of population source on any soil response variable (Table 2.3).

Due to limited significant effects of population sources on the soil microbial responses, correlations between these variables and root parameters to explore the degree to which belowground plant properties explained variation in soil microbial biomass and mediated processes, was not fruitful. There were no significant correlations between root biomass and BNPP with microbial biomass (C or N), potential C_{MIN} rates, and net N_{MIN} rates ($P > 0.05$).

Discussion

This study represents the first to examine intraspecific variation in belowground attributes of

putative ecotypes of *A. gerardii* where ecotypic variation was more pronounced in root biomass and productivity than aboveground biomass at the end of the growing season. Across the west-east climate gradient, the southern Illinois population sources and regional ecotype exhibited the greatest aboveground biomass (only due to one population source), root biomass, and BNPP relative to the Kansas populations and regional ecotypes. My prediction that *A. gerardii* biomass (above and belowground) would exhibit an interaction between population source and site resulting from increasing biomass across the precipitation gradient with sources producing the most biomass in the common garden closest to the population origin ('home site advantage' [Hufford and Mazer 2003]) was not realized for all sources, only the southern Illinois ones. Above- and belowground biomass of KS sources (populations or putative regional ecotypes) did not vary across the precipitation gradient, whereas the SIL regional ecotype increased in above and belowground biomass across the gradient and exhibited a 'home site advantage.' Means of each regional source were highest in its region of origin relative to the other sites, with central Kansas ecotypes having the greatest biomass in Hays, eastern Kansas ecotypes having the greatest biomass in Manhattan, and Illinois ecotypes having the greatest biomass in Carbondale, but these results were not significant.

The plants studied in this experiment represented a subset of a large collaborative effort to assess phenotypic variation in numerous traits of *A. gerardii* across the precipitation gradient of the Great Plains. Ecotypic variation has been documented in establishment (Johnson *et al. submitted*) and leaf morphology (Olsen *et al. in press*) of this species, with the central KS ecotype exhibiting greatest variation from the southern Illinois ecotype. Others have found significant interaction between site and population source in tissue chemistry, i.e., glucan, lignin, and hydrogen contents, and the aboveground C content of all sources increasing from west to

east (Zhang *et al.* 2012). The carbon content of bio-oil produced was highest in the Illinois ecotypes across all sites (Gan *et al.* 2012), which may be explained by the higher aboveground biomass produced by southern Illinois sources. The patterns of increasing biomass from west to east was only evident for the southern Illinois ecotype in this study, but root biomass was consistent with the pattern in bio-oil production by Gan *et al*. (2012), highest in the southern Illinois ecotype across all sites.

The southern Illinois sources showed nearly 300% more N stored in roots across all sites due to higher belowground biomass and no variation in root C:N ratio, which would indicate variation in nutrient acquisition or use. Although root biomass was lowest in Hays, KS, there was more nitrogen stored belowground resulting from lower C:N ratio of roots among all sources at this site relative to the more eastern sites. This likely resulted from higher soil organic matter pool and presumably more fertile conditions associated in Hays, KS, also indicated by the highest microbial biomass and soil respiration (i.e., potential C-mineralization rates) at this site. The central KS source studied in this experiment has also been shown to exhibit higher leaf N than the eastern KS and southern IL ecotypes (B. R. Maricle, *unpublished data*).

In grasslands, soil moisture content directly affects metabolic rates of plants and the soil microflora (Flanagan & Johnson 2005), with consequences for soil organic matter storage (O'Brien *et al*. 2010). Moisture influences the quantity and quality of plant substrate available to microbes through root exudates and plant litter (Garcia 1992; Flanagan & Johnson 2005). Variations in vegetation type can affect quality and quantity of organic matter received by soil biota, which may scale to affect ecosystem processes including soil respiration (Raich & Tufekcioglu 2000). Flanagan & Johnson (2005) found an increase in both plant biomass and rates of total ecosystem respiration with a five-fold increase in precipitation. In addition, soil

respiration rates were found to increase linearly with mean annual precipitation along an Oklahoma grassland precipitation gradient (Zhou *et al*. 2009). In my experiment, the site with the greatest precipitation did not have the highest soil respiration potential, but the site that received the least amount of precipitation during the growing season as well as the entire year, showed the highest microbial biomass and C mineralization potential, despite the lowest plant biomass. The discrepancy in my results with Zhou *et al*. (2009) can be attributed to highly contrasting soils in the common gardens, which affect clay content and CEC. For example, the lowest values for all microbial response variables occurred in Manhattan, Kansas, where CEC was lower and the sand content was 40%, which generally provides little refugia for soil microbiota.

Ecotypic variation within a species can differentially affect belowground resources and the associated soil microbial community with consequences for ecosystem processes. Soil microorganisms have been shown to respond to variation in resources as affected by intraspecific variation in a dominant species (i.e., *Populus*), thus differentially effecting processes such as nutrient cycling to include microbial biomass nitrogen (Schweitzer *et al.* 2008). Variation in litter chemistry of *Quercus* had a large effect on almost all ecosystem responses measured (Madritch & Hunter 2002). Short term experiments in forests have also found variation in soil community characteristics between genotypes of *Betula* (Kasurinen *et al*. 2005). My results did not conform to the phenomenon of population sources differentially affecting belowground microbial biomass or microbially-mediated ecosystem processes. I attributed this to the lack of strong variation among population sources on secondary compounds known to affect litter decomposition (e.g., tannins, C:N ratio), which was responsible for variation in ecosystem processes in other studies (Schweitzer *et al.* 2008). There was significant variation among

population sources in the size and activity of the associated soil microbial biomass resulting from two population sources (one eastern KS and one southern IL) with very high levels of potential C-mineralization rates and microbial biomass carbon. Soil associated with these population sources also had low rates of potentially net mineralizable nitrogen, indicating increased microbial demand. These results, however, appear to be independent of root biomass and root C:N ratio among the individual ecotypes or regional sources. Root traits not measured in this study such as root architecture (Klopf & Baer 2011), turnover (Lynch *et al.* 2013), or rhizosphere priming (Shahzad 2012) could explain variation in soil microbial biomass and potential respiration rates among population sources of *A. gerardii*.

A variety of genotypes within or among populations can result in higher probability for a species to adapt to changing environmental conditions, e.g., climate, disturbances, and disease (Falk *et al*. 2006). In previous transplant experiments (McMillan 1959), *A. gerardii* was one of seven species that exhibited varying flowering times when transplanted in two locations across an east-west gradient, and also revealed varying heights of flowering culms when transplanted within a north-south gradient. These findings indicate that intraspecific variation in biomass production may lead to some ecotypes outperforming others. This phenotypic variation could allow for greater success in migrating across the landscape, if resource-capture results in higher seed production and viability.

In conclusion, there is increasing evidence for ecotypic variation in the dominant species of the tallgrass prairie ecosystem and this is the first study to document ecotypic variation belowground. Intraspecific variation in biomass production – above and belowground – may lead to some ecotypes outperforming others and the disproportionate utilization of resources in restorations. This knowledge will assist practitioners in selecting ecotypes best suited to achieve

restoration goals (i.e., cover vs. diversity). It should be recognized that genetic mixing between foreign and local ecotypes can occur with the potential for altered hybrid fitness (Hufford & Mazer 2003) and unknown potential to adapt to changing environmental conditions (McKay *et al.* 2005).

Table 2.1. Locations of remnant prairies where *Andropogon gerardii* seeds were collected to establish the reciprocal gardens.

Collection Site	**Abbreviation**	**County**	**Latitude (N)**	**Longitude (W)**	**Elevation (m)**
Central Kansas	**CKS**				
Relic Prairie	REL	Ellis	38.85	99.37	630.6
Cedar Bluffs Reserve	CDB	Trego	38.75	99.77	652.9
Eastern Kansas	**EKS**				
Konza Prairie	KNZ	Riley/Geary	39.08	96.6	346.6
Tallgrass Prairie	TAL	Chase	38.42	96.55	381.0
Southern Illinois	**SIL**				
De Soto	DES	Jackson	37.85	89.23	167.6
Twelve Mile	12M	Marion	38.77	88.83	189.0

Table 2.2. Common garden study site climate and soil characteristics. Annual and growing season (May-Aug 2011; coinciding with root ingrowth bag incubation) was summarized from NOAA weather stations.

	Central Kansas (Hays, KS)	Eastern Kansas (Manhattan, KS)	Southern Illinois (Carbondale, IL)
County, State	Ellis, KS	Riley, KS	Jackson, IL
Latitude	38˚51’	39˚08’	37˚41’
Longitude	99˚19’	96˚38’	89˚14’
2011 Total Precipitation Jan-Dec (mm)	511.4	900	1778.1
2011 Average Temperature Jan-Dec (˚C)	13.6	12.7	13.6
Growing Season Precipitation (mm)	292.9	397.9	550.8
Growing Season Temperature (˚C)	25.4	24.7	24.0
% Soil Moisture Jan-Dec	7	12	13
Soil Type	Silt loam	Silt loam	Silt loam
Soil Order	Mollisol	Entisol	Alfisol
Cation Exchange Capacity (meq/100g)	25.1	8.5	13.7
%C ± SE	1.88± 0.039	0.71 ± 0.011	2.67 ± 0.179
%N ± SE	0.17 ± 0.004	0.06 ± 0.001	0.21 ± 0.007
% sand, silt, clay	22, 59, 20	41, 51, 8	8, 79, 14
$PO_4^{=}$(ug g^{-1})	8.3	29.3	85.6
pH	7.5	5.9	4.9

Table 2.3. Mean (± standard error) absolute soil response and difference from control soil for each ecotype at each common garden site in Hays, KS, Manhattan, KS, and Carbondale, IL. Abbreviations indicate the following statistical results: SC = effect of site before subtracting control; S = effect of site after subtracting control; and P = population source main effect (shown in Figure 2.6). Means accompanied by the same letter were not significantly different ($\alpha = 0.10$); upper and lower case letters compare SC and S effects, respectively; letters x-z indicate population source differences at particular sites. An asterisk indicates the difference from the control was significantly different from zero (i.e., a change in process relative to no plants). Abbreviations are as follows: CMIN= carbon mineralization, NMIN= potential net nitrogen mineralization, MBN=microbial biomass nitrogen, MBC=microbial biomass carbon. CKS=central Kansas, EKS=eastern Kansas, SIL=southern Illinois. Prairie populations abbreviated accordingly: CDB=Cedar Bluff, REL=Relic, KNZ=Konza, TAL=Tallgrass, DES=DeSoto, 12M=Twelve Mile Prairie.

	REGIONAL ECOTYPE:	CKS		EKS		SIL			Site Means
	POPULATION SOURCE:	CDB	REL	KNZ	TAL	DES	12M	Control	
C_{min} (ug C g^{-1} d^{-1})$^{S, SC}$	Hays, KS	38.5 ± 1.5	41.8 ± 0.9	40.3 ± 2.4	42.8 ± 2.6	43.9 ± 2.2	34.3 ± 3.3	29.1 ± 3.7	40.3 ± 1.3^{A}
	Source – Control	9.4 ± 1.5*	12.7 ± 0.9*	11.3 ± 2.4*	13.8 ± 2.6*	14.9 ± 2.2*	9.7 ± 3.3*	⋯	12.1 ± 1.1^{a}
	Manhattan, KS	3.8 ± 1.0	2.4 ± 0.6	3.2 ± 0.8	3.4 ± 1.1	3.3 ± 1.3	4.5 ± 1.1	3.0 ± 0.8	3.4 ±0.4^{B}
	Source – Control	2.9 ± 1.1	0.6 ± 0.1	1.5 ± 0.5	2.5 ± 2.0	2.4 ± 2.4	2.5 ± 0.5	⋯	2.1 ± 0.4^{b}

	Carbondale, IL	18.9 ± 2.2	19.0 ± 1.7	23.4 ± 3.3	29.4 ± 6.6	26.3 ± 5.2	21.2 ± 2.6	10.7 ± 0.2	23.1 ±1.6[C]
	Source – Control	8.2 ± 2.2*	8.3 ± 1.7*	12.7 ± 3.3*	18.6 ± 6.6*	15.6 ± 5.2*	10.5 ± 2.6*	⋯	12.4 ± 1.6[a]
MBC (ug C g^{-1})[SC]	Hays, KS	437 ± 54	451 ± 10	441 ± 18	469 ± 23	491 ± 28	364 ± 32	321 ± 31	442 ± 18[A]
	Source – Control	115 ± 54	130 ± 10	120 ± 18	148 ± 23	170 ± 28	107 ± 17	⋯	70 ± 21
	Manhattan, KS	41 ± 9	27 ± 7	34 ± 8	38 ± 11	34 ± 12	47 ± 12	35 ± 11	37 ± 4[B]
	Source – Control	28 ± 9	4 ± 2	13 ± 5	28 ± 13	45 ± <0.1	22 ± 6	⋯	20 ± 4
	Carbondale, IL	211 ± 21	209 ± 16	256 ±32	317 ± 65	280 ± 49	225.8 ± 12.4	119 ± 2	251 ± 16[C]
	Source-Control	92 ± 21	90 ± 16	138 ± 32	198 ± 65	162 ± 49	107 ± 12	⋯	62 ± 17
MBN (ug N g^{-1})[S, SC]	Hays, KS	48.5 ± 4.5	41.9 ± 4.4	46.2 ± 3.8	48.3 ± 4.9	50.9 ± 4.7	42.9 ± 1.8	42.2 ± 3.4	46.4 ± 1.6[A]
	Source − Control	10.8 ± 6.4	5.6 ± 3.0	8.2 ± 4.9	13.2 ± 3.3	10.9 ± 5.3*	4.4 ± 2.1	⋯	9.2 ± 1.8[a]
	Manhattan, KS	4.5 ± 1.2	6.4 ± 1.4	5.3 ± 1.3	8.7 ± 0.3	5.1 ± 1.0	6.2 ± 1.1	5.7 ± 1.5	6.0 ± 0.5[B]
	Source – Control	-	3.7 ± 1.5	2.3 ± 1.3	4.1 ± 0.3*	1.8 ± 1.2	2.6 ± 0.9	⋯	3.0 ± 0.4[a]
	Carbondale, IL	30.4 ± 1.0	26.5 ± 1.9	28.9 ± 2.7	26.9 ± 3.1	31.9 ± 1.9	32.2 ± 1.9	17.5 ± 1.9	29.5 ± 0.9[C]
	Source - Control	12.9 ± 1.0*	8.9 ± 1.9*	11.4 ± 2.7*	9.4 ± 3.1*	14.5 ± 1.9*	14.8 ± 1.9*	⋯	12.0 ± 0.9[b]

N_{min} (ug N g^{-1} d^{-1})^{S,SC}	Hays, KS	0.6 ± 0.1	1.0 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.8 ± 0.0	0.7 ± 0.1	0.7 ± 0.1^{A}
	Source − Control	0.0 ± 0.0	0.7 ± 0.3	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	…	0.2 ± 0.1^{a}
	Manhattan, KS^{P}	0.5 ± 0.1^{y}	0.4 ± 0.0^{xy}	0.3 ± 0.0^{xy}	0.3 ± 0.0^{x}	0.5 ± 0.1^{y}	0.3 ± 0.0^{x}	0.5 ± 0.1	0.4 ± 0.0^{B}
	Source – Control	…	…	…	…	…	…	…	…
	Carbondale, IL	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.0^{C}
	Source - Control	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	…	0.2 ± 0.0^{b}

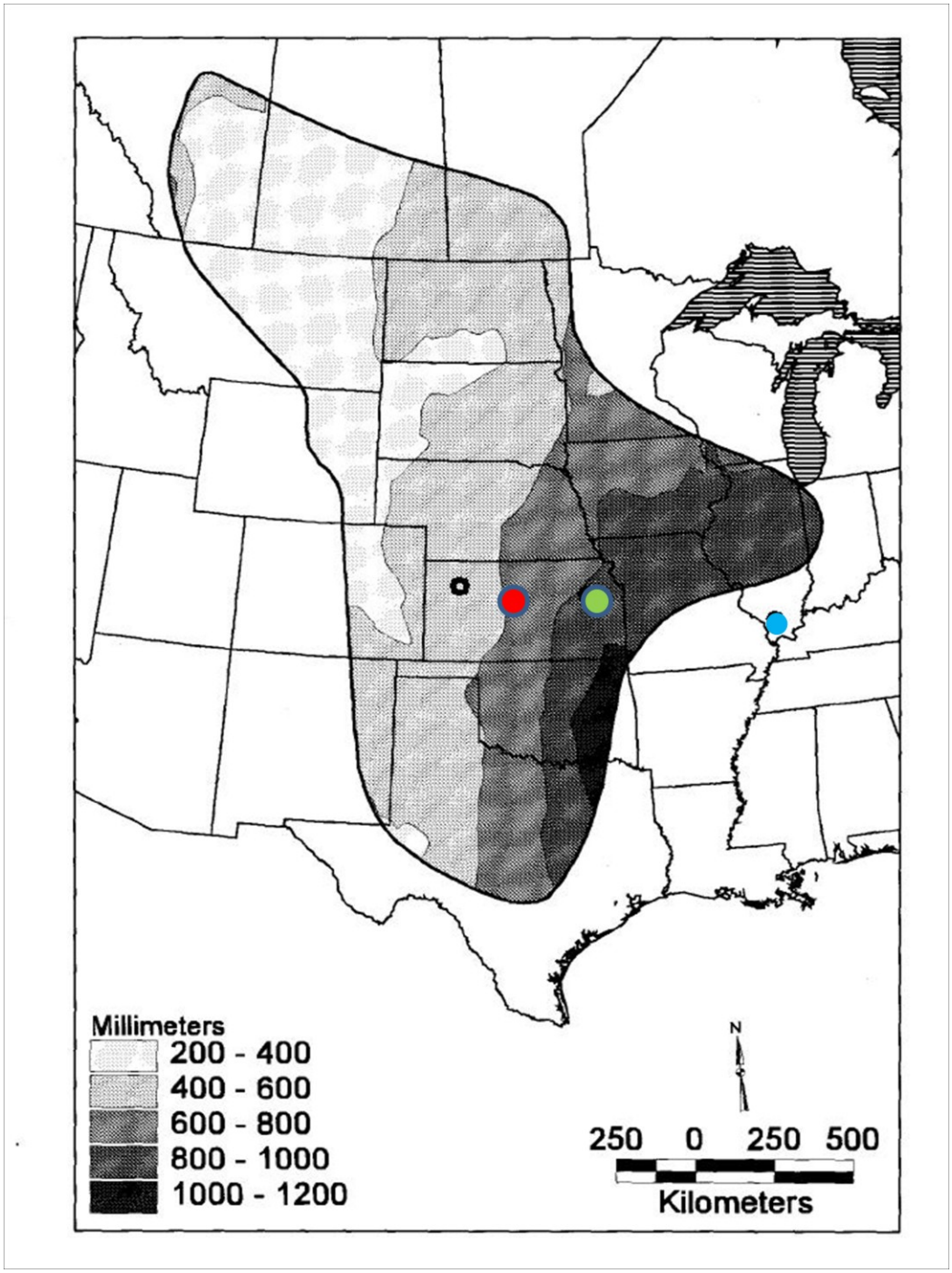


Figure 2.1. Map of common garden locations across the North American precipitation gradient. Red dot indicates Hays, KS. Green dot indicates Manhattan, KS. Blue dot indicates Carbondale, IL. Adapted from Lauenroth *et al.* 1999.

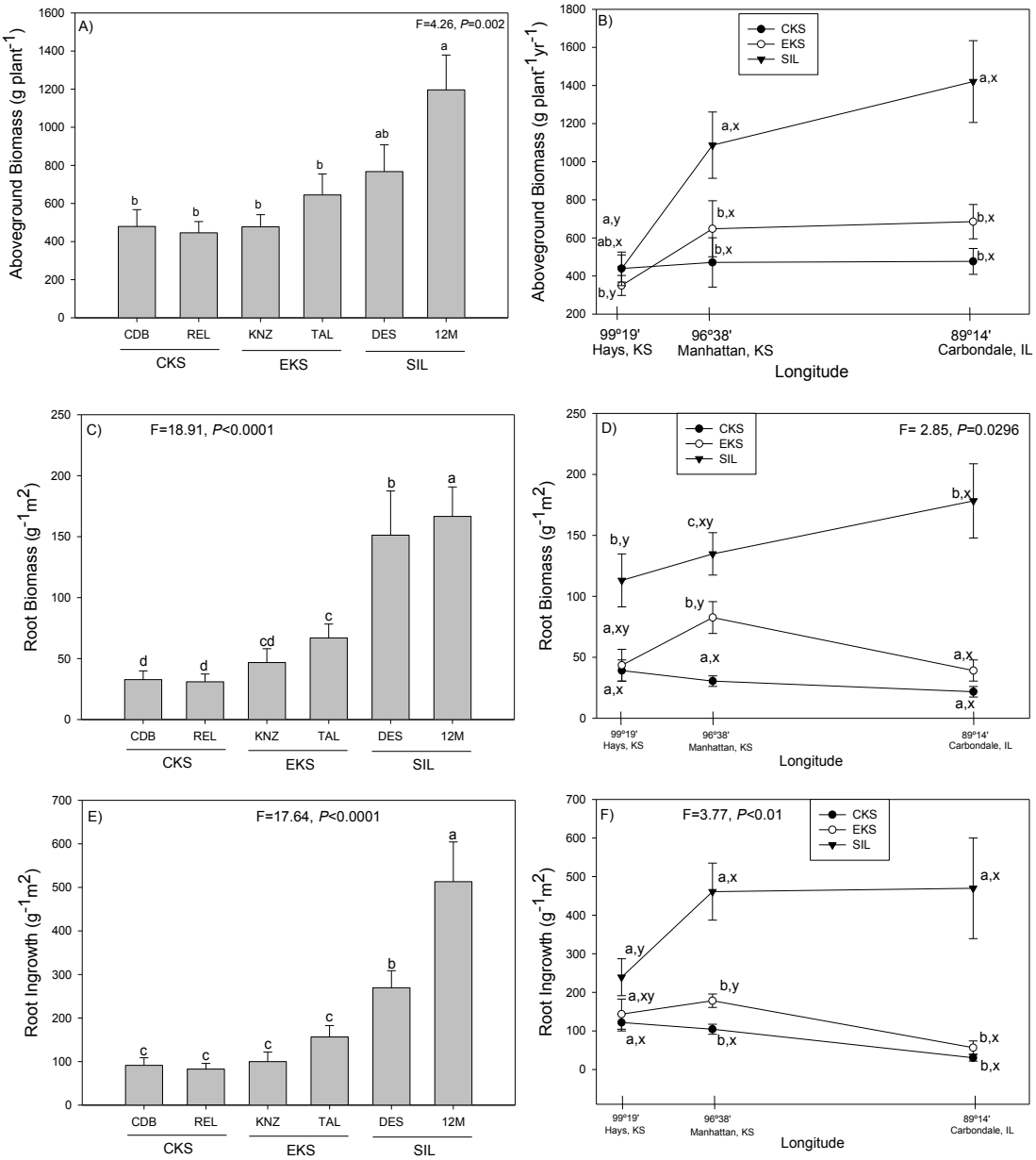


Figure 2.2. Mean (± standard error [SE]) aboveground biomass (whole plant) of *A. gerardii* (A) population sources across all sites and (B) regional ecotypes by site. In panel A, means accompanied by the same letter were not significantly different (α=0.05). In panel B, letters *a-c* indicate differences among regional ecotypes within a site and letters *x-z* indicate differences among sites within a regional ecotype. Mean (± SE) root biomass to a depth of 20 cm (C) among population sources across all sites and (D) among regional ecotypes by site; and average BNPP (E) among the population sources across all sites and (F) among regional ecotypes by site. Means accompanied by the same letter were not significantly different (α=0.05). Ecotypes abbreviated as follows: CKS=central Kansas, EKS=eastern Kansas, and SIL=southern Illinois. Prairie populations abbreviated as follows: CDB=Cedar Bluff, REL=Relic, KNZ=Konza, TAL=Tallgrass, DES=DeSoto, 12M=Twelve Mile Prairie.

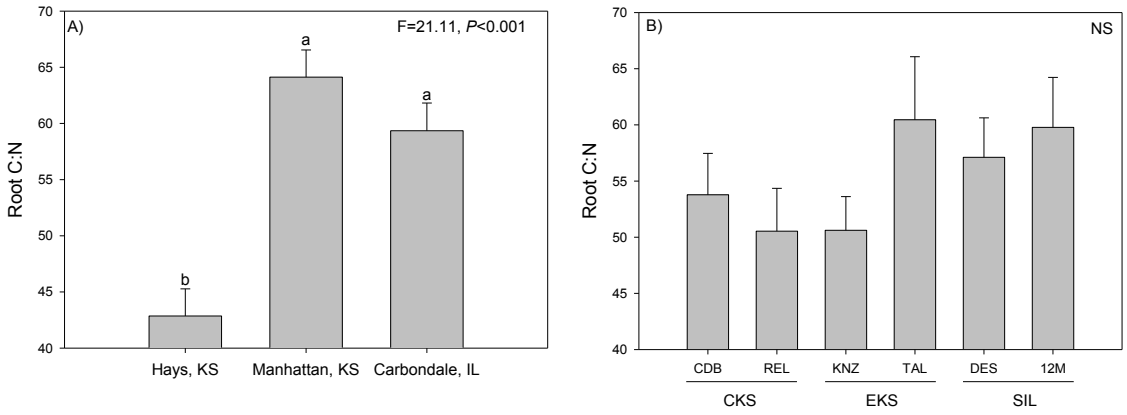


Figure 2.3. Average (± standard error) ratio of C:N in root tissue (A) at each site across all population sources and (B) among population sources across all sites. Means accompanied by the same letter were not significantly different (α=0.05; NS = not significantly different). Sites indicated by the name of town closest to where the field site was located. Prairie population abbreviations are as follows: CDB=Cedar Bluff, REL=Relic, KNZ=Konza, TAL=Tallgrass, DES=DeSoto, 12M=Twelve Mile Prairie. Populations are grouped within ecotypes (CKS = central Kansas; EKS = eastern Kansas, and SIL = southern Illinois), indicated by a line in Panel B.

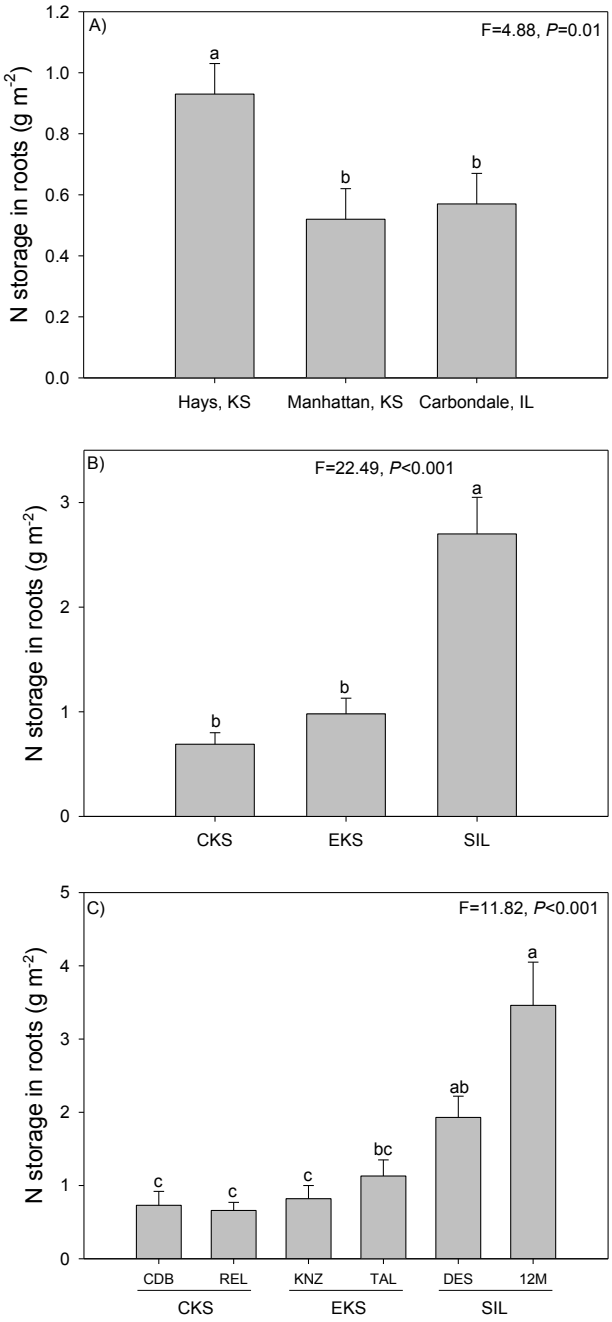


Figure 2.4. Average (± standard error) quantity of N in root tissue to a depth of 20 cm (A) at each site across all sources, (B) all regional ecotypes across all sites, and (C) all population sources across all sites. Means accompanied by the same letter were not significantly different (α=0.05). Ecotypes abbreviated accordingly: CKS=central Kansas, EKS=eastern Kansas, SIL=southern Illinois. Prairie population abbreviations are as follows: CDB=Cedar Bluff, REL=Relic, KNZ=Konza, TAL=Tallgrass, DES=DeSoto, 12M=Twelve Mile Prairie.

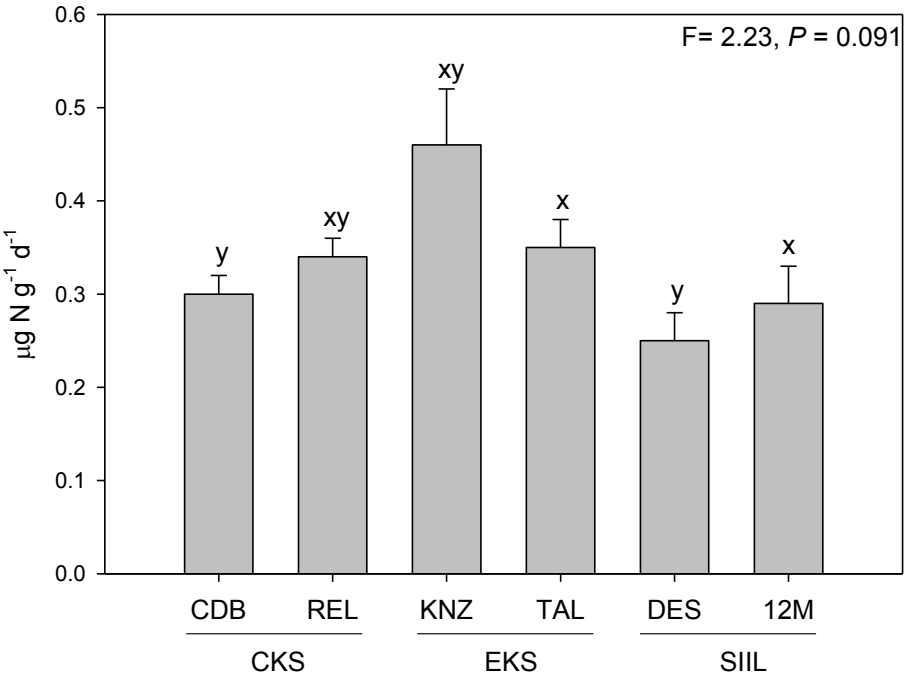


Figure 2.5. Population source effect on N_{MIN} at Manhattan, KS, site. Means (± standard error) accompanied by the same letter were not significantly different (α=0.10). Ecotypes abbreviated accordingly: CKS=central Kansas, EKS=eastern Kansas, SIL=southern Illinois. Prairie population abbreviations are as follows: CDB=Cedar Bluff, REL=Relic, KNZ=Konza, TAL=Tallgrass, DES=DeSoto, 12M=Twelve Mile Prairie.

CHAPTER 3

EFFECTIVENESS OF SOIL AMENDMENT ON PROMOTING ABOVEGROUND DIVERSITY AND ECOSYSTEM PROCESSES

Introduction

Microorganisms mediate biogeochemical cycling in ecosystems, and in doing so, play an important role in regulating plant productivity, as both fungal and bacterial symbionts are responsible for the acquisition of limiting water and mineral nutrients (Smith & Read 1997). Ecosystem functions such as nitrogen and carbon transformations and soil formation are also mediated by soil microorganisms (Tiedje 1988; Kowalchuk & Stephen 2001; Hogberg *et al*. 2001; Rillig & Mummey 2006).

Vesicular arbuscular mycorrhizal fungi (AMF) are the most common belowground symbionts with plants and are associated with roots of approximately 80% of terrestrial plant species (Smith & Read 1997). These mycorrhizae increase soil exploration and uptake of phosphorus and other nutrients, improve growth with increased access to water, and enhance protection from parasites and herbivory in the rhizosphere (Hayman 1983). Arbuscular mycorrhizal fungi (AMF) are associated with many tallgrass prairie species, including the dominant grass, *Andropogon gerardii* Vitman (Hetrick *et al*. 1987). These associations may enhance plant productivity by up to 100% (Anderson *et al.* 1994; van der Heijden *et al*. 1998a; Vogelsang *et al*. 2006) with a 7 to 70 fold increase in seedling biomass of *A. gerardii* in the presence of AMF (Hetrick *et al*. 1989).

The soil microbial community may be a key factor in restoration of diverse tallgrass prairie communities, as the soil microbial community has been shown to affect plant community diversity and composition (Fitzsimons & Miller 2010). In North American old fields, European grasslands, and tallgrass prairies, mycorrhizal fungal diversity was significantly correlated with root biomass, plant biodiversity, productivity, and variability (van der Heijden *et al.* 1998a & 1998b; Vogelsang *et al*. 2006). In addition to fungi, bacteria are also critical. Rhizobia isolated from dune grasslands were used as inoculum and led to greater productivity in native plants, greater nitrogen availability, and increased community evenness (van der Heijden *et al*. 2006a).

Researchers have used this knowledge to attempt to manipulate systems through soil amendments. Soil inoculum treatments have been effective in restoration of various ecosystems. Cyanobacterial inoculation in a desert system was shown to restore biological soil crusts thereby leading to the colonization of mosses and eventually higher plants, indicating that this practice may further restore the ecological system (Wang *et al*. 2009). In prairies, inoculum treatments contributed to the decrease in cover of non-native species while native species cover increased (Rowe *et al*. 2009). Prairie soil inoculation may support the growth of prairie species, as remnant prairie soil can serve to infect native grasses with AMF and has been shown to increase growth of *S. sudanese* (Kemery & Dana 1995). If these previous restoration studies saw improvement in colonization and cover of native species with microbial amendments, then perhaps this positive response aboveground would scale to affect belowground productivity and ecosystem processes.

I used experimental restoration plots to elucidate: (1) whether a slurry amendment of prairie soil would increase above and belowground productivity and belowground ecosystem processes in a prairie restoration, and (2) to evaluate whether differences in plant diversity will

scale to affect belowground productivity and ecosystem processes. I predicted that soil microbial amendments will promote establishment of a more diverse prairie community consisting of greater composition and aboveground net primary productivity (ANPP) in planted grasses and forbs than control plots. I also predicted that higher plant diversity will increase ANPP and belowground net primary productivity (BNPP), thus providing increased root exudates that will support a larger microbial population and increase soil respiration. Furthermore, plots amended with soil slurry will have greater rates of carbon mineralization and higher microbial biomass carbon and nitrogen. Lastly, there will be higher diversity and richness in PLFA biomarkers in soil taken from the amended plots versus the control plots.

Methods

Study Site

A restoration experiment was established at the Agronomy Research Center at Southern Illinois University (37°41'N, 89°14'W) in spring of 2010. The average annual temperature of the region is 14.7°C (maximum: 20.1°C, minimum: 7.4°C), and average annual precipitation is approximately 1198 mm based on a 29 year record from the Carbondale Southeast Wastewater Treatment Plant, Jackson County, IL (NOAA 2010). Soil at the site is characterized as a Stoy silt loam, which is a Fine-silty, mixed, superactive, mesic, Fragiaquic Hapludalf with topsoil comprised of silt loam (0-0.25 m) and subsoil (0.25-1.3 m) comprised of silt clay loam (Herman *et al.* 1975). Average monthly temperature and precipitation for the 2010 growing season (May – August) were 22˚C and 354 mm respectively (NOAA 2012). Average monthly temperature and

precipitation for the 2011 growing season (May – August) were 24˚C and 137 mm respectively (NOAA 2012).

Inoculum soil was obtained from Twelve Mile Prairie, a railroad prairie remnant located just north of Kinmundy, Illinois on IL Route 37 (38°46'41.28"N, 88°50'23.04"W). Soils at this site are characterized as Reading silt-loam, Fine-silty, mixed, superactive, mesic Pachic Argiudolls (NRCS 2010). The area receives an average of 900 mm of rainfall per year according to a 29 year record from the Bondville National Atmospheric Deposition Program Station in Champaign County, IL.

Experimental Design

The former agricultural field site was prepared via roto-tilling and application of a 2% glyphosate solution two weeks prior to seeding and slurry application. Twelve 2 x 2 m plots were each seeded with a 20/80 mix of grasses and forbs totaling 600 seeds per m^2 (Table 3.1). Using a completely randomized design, six plots were amended with remnant prairie soil slurry and six plots served as the control (Figure 3.1).

The soil slurry was a 1:1 mixture of soil (originating from Twelve Mile Prairie) to deionized water passed through a series of sieves (1 mm, 500 µm, 250 µm) and applied to field plots with clean hand-held herbicide sprayers in June of 2010. The mixture was comprised of 19.3 kg of soil and an equal amount of deionized water. Each treatment plot received 3.8 L of the slurry mix while control plots received the corresponding amount of water in lieu of the slurry mixture. This application rate of the inoculum slurry was based on previous field (Rowe *et al.* 2009) and greenhouse (Hetrick *et al*. 1987; Kemery & Dana 1995) studies that have

addressed the role of soil microbial communities on plant growth and diversity. Following addition of the inoculum slurry, seeds were hand broadcast in each plot and lightly raked to incorporate the seed and slurry (or water) into the soil. The plots were overseeded in early May of 2011 prior to the second growing season using the same seed mixture.

Plant Responses

Species composition in each plot was determined by visually estimating percent cover of all species in a central 0.25 m^2 quadrat at the end of the first growing season in 2010, and in May and September of 2011. The maximum cover value from sampling each year was used to calculate Shannon's diversity index and cover of specific groups (planted grasses, planted forbs, volunteer grasses, volunteer forbs, total volunteer, total planted).

Aboveground net primary productivity (ANPP) was determined by harvesting plants from one 0.1 m^2 quadrat and drying biomass at 55°C. Plants were sorted into categories of planted and volunteer grasses and forbs.

Belowground net primary production (BNPP) was measured in the second year of restoration. In May of 2011, 2 soil cores (5.5 cm dia. x 20 cm deep) were removed from each plot. The soil was then sieved (4 mm) and roots were hand-picked and placed in coin envelopes. A fiberglass (1 mm x 1 mm mesh screen) ingrowth bag was placed in each soil core extraction hole (Johnson & Matchett 2001) and de-rooted soil was returned to the same location from which it was extracted. Root ingrowth bags remained in the field for sixteen weeks, at which time root bags were removed by cutting soil around each root bag. Following extraction, roots were snipped at the root bag-soil interface. Roots harvested from the initial extraction of soil

cores were used to quantify standing root biomass and roots that grew into the root ingrowth bags harvested in September were used to determine BNPP. At retrieval, the soil was sieved (2 mm), and roots removed. Roots were washed, dried at 55°C for 7 days, weighed, then ground and analyzed for %C and %N through combustion analysis on a Flash 1112 CN Analyzer (CE Elantech Corp., Lakewood, New Jersey, USA).

Soil Responses

One week after slurry application, and again 12 months later, twelve cores of 5 cm in depth were taken from each plot and composited by plot. Soil was stored at 4˚C and sieved (2 mm). Approximately 30 g was frozen immediately for phospholipid fatty acid (PLFA) analyses. PLFAs differentiate microbial groups based on membrane structures found within the soil microbial community. These membrane differences are analogous to phylogenetic differences, allowing for an assessment of the proportion of different phylogenetic groups present within the soil (Bossio *et al.* 1998). Samples were sent for PLFA determination at Oklahoma State University (G. T. Wilson).

A 100 g subsample was used for determination of gravimetric water content (dried at 105^{o}C), potential carbon mineralization rate (C_{MIN}), microbial biomass carbon and nitrogen (MBC and MBN), and potential net N mineralization rates (N_{MIN}). Microbial biomass carbon and nitrogen were determined using the chloroform fumigation incubation technique described by Jenkinson & Powlson (1976) as modified by Voroney & Paul (1984). Four 10 g samples from each soil core were pre-incubated in 165 mL serum bottles for 5 days at 23^{o}C. Afterwards, half of these samples were fumigated with amylene stabilized chloroform in a vacuum desiccator

for 18 hours. Following fumigation, samples were evacuated for eight 3 minute intervals to remove chloroform. All non-fumigated samples were aerated while fumigated samples were evacuated. All serum bottles were then sealed and incubated for 10 days at 23^{o}C. After the incubation period, CO_2-C was measured by analyzing 0.5 mL sample of headspace gas on a GC-8A gas chromatograph (Shimadzu, Kyoto, Japan). Microbial biomass carbon (MBC) was determined by calculating the difference between fumigated and non-fumigated samples as divided by a decomposition constant (K_c) of 0.4 (Voroney & Paul 1984). Potential CO_2-C mineralization rates were determined from the non-fumigated samples. Mineralized carbon (μg/g dry soil) from the two replicates was averaged over the incubation period.

Following headspace gas measurements, soil in each serum bottle was extracted for inorganic N to determine MBN. Inorganic N concentrations were determined by adding 50 mL of 2 N KCl, by shaking solutions for 1 hour at 200 rpm, then filtering samples through a 0.4 μm membrane. The filtrates were analyzed colorimetrically for adsorbance (Keeney & Nelson 1982) of NH_4-N and NO_3-N on a Flow IV Autoanalyzer (OI Analytical, College Station, TX). The difference in NH_4-N + NO_3-N on a per g soil basis between fumigated and non-fumigated samples) were divided by a decomposition constant (K_n=0.6) to obtain MBN.

Potential net N mineralization rate was determined (2011 only) using the aerobic laboratory incubation procedure (Robertson *et al*. 1999), as used by Baer *et al*. (2002). Following retrieval of soil cores and removal of roots, a 10 g soil sample was extracted for inorganic N ($N_{initial}$) according to the methods described for MBN. The non-fumigated soil used to determine MBN was used as the final measurement of inorganic N (N_{final}) for each soil core. The difference between the final and initial concentrations of inorganic N (NH_4-N + NO_3-N) was divided by the number of incubation days [($N_{final} - N_{initial}$)/d] to calculate net N mineralization

rate (Robertson *et al.* 1999). Soil obtained from the root ingrowth bags was also used to measure MBC, MBN, C_{MIN} and N_{MIN} using the same methods as described above to determine if a relationship existed between root mass and soil processes.

In addition, the composite soil samples were used to measure soil pH and available P in order to characterize the soil. To determine pH, approximately 5 g of air dried soil was mixed with 5 mL of deionized water and left to settle for 15 minutes. After this time, the mixture was swirled once more and pH measured with an Accumet Basic AB15 pH Meter (Fisher Scientific, USA). Plant available P was determined following the Bray extraction method (1945), where 2 grams of 2 mm sieved and air-dried soil were extracted with 20 mL of 0.025 N HCl + 0.03 N NH_4F for one minute on a shaker and filtered immediately through a 0.4 µm filter. Phosphate-P was determined on a Flow IV Autoanalyzer (OI Analytical, College Station, TX).

Soil from root ingrowth bags was used to determine C_{MIN}, N_{MIN}, MBC, and MBN using the methods described above for composite soil.

In July of 2010 and 2011, two ion exchange resin bags were buried in each plot to obtain an index of relative inorganic N availability (Binkley & Matson 1983). In October of 2010 and 2011, bags were retrieved and extracted with 75 mL of 2N KCl after shaking for one hour at 200 rpm, filtered through a 0.4 µm filter membrane, and analyzed for NH_4-N and NO_3-N on a Flow IV Solution Autoanalyzer (O.I. Corporation, College Station, Texas, USA).

Statistical Analyses

All above and belowground response variables measured in both 2010 and 2011 were analyzed according to a completely randomized design with repeated measures using a mixed model procedure in SAS (SAS Inst. 2009) in order to test for effects of the amendment (treatment),

time, and interaction between amendment and time. Belowground response variables quantified only in 2011 were analyzed according to a completely randomized design using a mixed model procedure in SAS in order to test for main effect of amendment. Correlation analyses were performed to determine dependence of microbial biomass and C_{MIN} on root production. Biomass of total gram positive, total gram negative, total AMF, total saprophytic fungi, and total PLFA biomarkers were analyzed according to a completely randomized design in SAS (SAS Inst. 2009) in order to test for effects of the amendment (treatment).

Composition and biomass of 12 PLFA biomarkers in soil sources were analyzed using non-metric multidimensional scaling (NMDS) with PRIMER-e v.6 software (Clarke & Gorley 2006). Ordinations were run in Decoda (Minchin 2011) on post-experiment control and treatment soils, and pre-experiment amendment soils. All ordinations were performed using Bray-Curtis dissimilarity on untransformed data. Dissimilarity values were permuted with a maximum iteration limit of 100. Analysis of Similarity (ANOSIM) was also run to determine whether soil sources supported different microbial communities.

Results

Plant Responses

The composition of the plant community changed significantly over time for control and amended soil. There was an effect of year, showing an increase, on: total cover within all plots ($F=99.62_{1,20}$, $P<0.0001$), cover of all volunteer species ($F=50.44_{1,20}$, $P<0.0001$), cover of all forb species ($F=66.11_{1,20}$, $P<0.0001$), cover of all planted species ($F=175.95_{1,20}$, $P<0.0001$), total

richness ($F=347.26_{1,20}$, $P<0.0001$), and total diversity ($F=169.09_{1,20}$, $P<0.0001$). Greater diversity in the second year of both treated and control plots showed that with time more species were able to colonize the area. The soil amendment had a significant effect on cover of volunteer species ($F=4.82_{1,20}$, $P=0.0400$) and total cover of all species ($F=3.12_{1,20}$, $P=0.0924$). There were no significant effects of soil amendment on diversity or richness (Table 3.2).

There was an effect of year ($F=11.12_{1,20}$, $P=0.0033$) on total ANPP within all plots, with significantly more biomass produced in the second year of study. There was also an effect of year on productivity of all volunteer species ($F=7.27_{1,20}$, $P=0.0139$), and volunteer forbs ($F=26.30_{1,20}$, $P<0.0001$), showing an increase in biomass of both groups within treated and control plots over time. There was an effect of year on productivity of total planted species ($F=15.62_{1,20}$, $P=0.0008$), and planted forbs ($F=13.88_{1,20}$, $P=0.0013$), but the establishment was still poor in comparison to volunteer species within treated and control plots. Volunteer grasses showed an effect of year ($F=4.87_{1,20}$, $P=0.0391$), with biomass numbers decreasing in the second year of restoration (Table 3.2).

Roots collected from cores (5.5 cm x 20 cm) taken in May 2011 differed between the amended and control soil ($F=3.70_{1,10}$, $P=0.0833$, $\alpha < 0.1$) with nearly double the amount of roots in plots treated with the slurry amendment (Table 3.3, Figure 3.2). Variability of root biomass in the amended soil, however, led to a weak significant result. This pattern was not reflected in BNPP collected in September 2011, potentially due to the abiotic constraint of limited precipitation during the 2011 growing season. There was no effect of treatment on C:N in roots collected in either May or September.

Soil responses

There was no effect of amendment on MBC, MBN, C_{MIN}, or N_{MIN} from soil within the ingrowth cores. Further, there was no effect of amendment, year, or an interaction of amendment and year on plant available phosphorous, pH, MBC, MBN, C_{MIN}, or N_{MIN} in composite soil (0-5 cm) taken from the plots. All values were generally higher in the second year, though not significant, likely due to the overall increase in root material available to microbiota (Table 3.3).

Inorganic N collected on resin bags was lower in year two than year one ($F=2247.38_{1,10}$, $P<0.0001$), but was not affected by soil amendment. Inorganic N collected on the resin bags decreased more than five hundred per cent. This is likely due to the increase in plants taking up greater amounts of nitrogen (Figure 3.3).

Phospholipid Fatty Acids (PFLAs)

Phospholipid fatty acids (PLFAs) were compared among the treatments within the experimental plots and the soil obtained from the prairie used as the inoculum. There were no significant differences between control soil, treated soil, and inoculum soil in total gram positive bacteria and total gram negative bacteria (Figure 3.4, Table 3.4). There was a treatment effect on total AMF ($F=3.31_{2,7}$, $P=0.09$, Figure 3.5A), total saprophytic fungi ($F=6.98_{2,7}$, $P=0.02$, Figure 3.5B), and total PLFA biomass ($F=3.45_{2,7}$, $P=0.09$, Figure 3.5C) with both the control and treated soil having greater biomass of these PLFA biomarkers, relative to the inoculum soil.

Composition of PLFAs did not differ among the control soil in the field experiment, amended field soil, and inoculum soil. Results of the NMDS ordination gave 2D configuration

stress values (Stress = 0.04) in 98 of the 100 iterations. The ordination of soil inoculum, and both 2010 and 2011 treated and control soils were plotted on a 2D graph (Figure 3.6). ANOSIM was used to determine whether soil sources supported different microbial communities, showing that all microbial communities were similar to each other in overall composition (Figure 3.7).

Discussion

One goal of restoring cultivated systems is establishing perennial species, but there are efforts to restore biological and physical complexity to better represent historic or extant systems. This may require more soil ecological knowledge (SEK) in the context of plant-soil feedback (Baer *et al*. 2012). Amendments to soil are an example of applying SEK to achieve specific restoration goals. For example, carbon additions (such as sugar and sawdust) to soil can modulate floristic diversity in newly established prairie (Baer *et al*. 2003) and reduce the cover of invasive species (DeCrappeo 2010). Inoculum of two cyanobacteria strains have been shown to promote the establishment of biological soil crusts in deserts (Wang *et al*. 2009). Whole soil additions (Kemery & Dana 1995) paired with mycorrhizal inoculum (Rowe *et al*. 2009) have aided in reducing unwanted species in prairies. These examples of successful application of SEK in a restoration helped guide this study, which aimed reintroduce the soil microbial community from prairie that had never been cultivated to soil at the onset of a prairie restoration to promote plant diversity and ecosystem functioning.

For many response variables, results failed to reject the null hypothesis (H_0: $\mu_{treatment} = \mu_{control}$), but two potentially important aspects of restoration were affected by the amendment. Although species composition was largely unaffected by the addition of the native prairie soil

slurry, the amendment increased the cover of volunteer species and increased belowground biomass. Higher root biomass in the amended soil, however, did not differentially influence microbial biomass and composition, or belowground ecosystem processes (i.e., BNPP, N_{MIN}, C_{MIN}).

There are several potential reasons why the slurry amendment did not affect plant diversity, microbial communities, and belowground ecosystem processes. First, although diversity and richness increased in the second year of restoration, there was poor establishment of seeded prairie plant species during both years, with volunteer species comprising the majority of biomass and cover. Thus, competition with weeds may be a more significant biotic factor affecting the cover and diversity of seeded species in the first few years of restoration. Though there was an increase in the cover and productivity of prairie species, a higher proportion of prairie species was expected to feed-back and promote a microbial community representative of native prairie (Hetrick *et al.* 1989). Second, there was half as much precipitation in the second growing season relative to the first growing season, giving way to abnormally dry portions of the growing season for southern Illinois (US Drought Monitor). Tissue C:N ratios, cover patterns, and productivity may be affected by environmental limitations including precipitation (Vinton & Burke 1997). Water limitation can slow the recovery rate of soil processes and may have affected quantity of root biomass available to microbial populations (Hayes & Seastedt 1987). Third, high levels of nutrients left behind from fertilizer use in this previously agricultural field could alter competitive relationships among soil microorganisms. An order of magnitude difference in phosphate levels between the experimental plot soils and the remnant prairie soil could have altered communities to favor ones adapted for agricultural conditions or specific plant functional groups (Egerton-Warburton *et al*. 2007). Fourth, I cannot say that the amendment

resulted in a soil microbial community that represented native prairie soil due the much higher amounts of PLFA biomarkers in the experimental soils. For example, total biomass of AMF, saprophytic fungi, and total PLFA biomarkers were higher in both the treated and control plots in comparison to the inoculum soil obtained from a remnant prairie, yet, the opposite was expected. However, the microbial community compositions of the three soil sources were similar. This analysis indicates the agricultural soil community was functionally not very degraded, which was not expected based on PLFA analyses of cultivated soil at the onset of other tallgrass prairie restorations from cultivated conditions (Bach *et al*. 2011).

Ecological restoration can provide valuable tests of our understanding about ecosystems and failures demonstrate when knowledge is incomplete. "Setbacks" in an attempted replacement of *Phalaris arundinacea* with native sedge species informed investigators about the growth of this invasive species and how to better approach management of this monoculture in the future (Healy & Zedler 2010). A review of 30 invasive plant eradication projects in the Galapagos showed that only four were successful and lack of success was attributed to inadequate resources or cooperation from land owners (Gardener *et al*. 2010). This study shows that a greater understanding of SEK is necessary in order to determine if a soil amendment can serve as a useful tool in grassland restoration practices. Thus, setbacks and failures in restoration can serve as part of a necessary foundation for subsequent success (Gardener *et al*. 2010; Hobbs 2009).

Table 3.1. List of species seeded in the soil amendment experiment. Nomenclature follows USDA Plants Database (USDA & NRCS 2010).

C_3 GRASSES	C_4 GRASSES	FORBS	LEGUMES
Koeleria macrantha (Ledeb.) Schult.	*Andropogon gerardii* Vitman	*Parthenium integrifolium* L.	*Lespedeza capitata* Michx.
Elymus Canadensis L.	*Schizachyrium scoparium* (Michx.) Nash.	*Eryngium aquaticum* L.	*Dalea candida* Michx. ex Willd.
	Sorghastrum nutans (L.) Nash	*Allium stellatum* Fraser ex Ker Gawl.	*Baptisia alba* (L.) Vent.
	Bouteloua curtipendula (Michx.) Torr.	*Silphium terebinthinaceum* Jacq.	*Astragalus Canadensis* L.
		Tradescantia ohiensis Raf.	*Amorpha canescens* Pursh
		Ruellia humilis Nutt.	*Desmodium illinoense* A. Gray
		Monarda fistulosa L.	*Dalea purpurea* Vent.
		Penstemon digitalis Nutt. ex Sims	
		Asclepias tuberosa L.	
		Echinacea purpurea (L.) Moench	
		Oenothera macrocarpa Nutt.	
		Ratibida pinnata (Vent.) Barnhart	
		Vernonia fasciculate Michx.	
		Liatris pycnostachya Michx.	
		Oligoneuron rigidum (L.) Small	

Table 3.2. Effects of year and treatment on cover and aboveground net primary productivity (ANPP) in Slurry and Control plots in 2010 and 2011. Highly significant treatment effect indicated with two asterisks ($\alpha = 0.05$); one asterisk indicates significant treatment effect ($\alpha = 0.10$); Y indicates a significant year effect ($\alpha = 0.05$). There were no significant interactions between treatment and year.

YEAR:	2010		2011	
TREATMENT:	**SLURRY**	**CONTROL**	**SLURRY**	**CONTROL**
Cover (%)				
Forbs^{Y}	40.0 ± 14.9	28.2 ± 13.1	140.2 ± 13.1	128.7 ± 6.8
Grasses	72.8 ± 9.8	70 ± 7	69.3 ± 9.0	62.0 ± 8.5
Planted Species^{Y}	0.3 ± 0.2	1.5 ± 0.6	37.3 ± 8.7	38.3 ± 10.0
Volunteer Species^{**,Y}	112.5 ± 5.8	96.7 ± 8.6	172.2 ± 10.0	152.3 ± 7.5
Total^{*,Y}	112.8 ± 5.9	98.2 ± 8.2	209.5 ± 15.0	66.7 ± 3.8
Total Richness^{Y}	4.0 ± 0.6	3.7 ± 0.3	20.2 ± 0.8	20.7 ± 1.1
Total Diversity^{Y}	0.6 ± 0.1	0.5 ± 0.1	2.2 ± 0.1	2.3 ± 0.1
ANPP (g m^{-2} y^{-1})				
Volunteer Grasses^{Y}	422.6 ± 67.9	326.3 ± 33.4	279.0 ± 72.6	232.3 ± 37.0
Volunteer Forbs^{Y}	101.3 ± 74.9	90.8 ± 56.0	332.4 ± 30.8	540.7 ± 128.5
Volunteer Species^{Y}	261.9 ± 68.3	208.6 ± 47.2	305.7 ± 38.5	386.5 ± 78.9
Planted Grasses^{Y}	0	0	39.6 ± 34.5	5.5 ± 3.4
Planted Forbs^{Y}	0	0.3 ± 0.3	20.9 ± 9.4	13.5 ± 5.3
Planted Species^{Y}	0	0.2 ± 0.1	30.2 ± 17.3	9.5 ± 3.2
Total^{Y}	523.9 ± 73.6	417.5 ± 61.0	671.8 ± 72.3	792.1 ± 100.9

Table 3.3. Root biomass and soil response in plot composite and root ingrowth bag soil from the slurry and control plots each year. An asterisk indicated a significant treatment effect as shown in Figure 3.2 ($\alpha = 0.10$). There were no significant effects of year or interactions between treatment and year on composite soils.

YEAR:	2010		2011		2011	
DEPTH:	0-5 cm				0-20 cm	
SOURCE:	COMPOSITE				INGROWTH BAGS	
TREATMENT:	SLURRY	CONTROL	SLURRY	CONTROL	SLURRY	CONTROL
Response Variables						
pH	5.5 ± 0.1	5.7 ± 0.1	5.5 ± 0.0	5.6 ± 0.0	…	…
Phosphate (ug g^{-1} PO_4)	66.4 ± 2.1	64.6 ± 3.2	67.3 ± 3.5	62.4 ± 2.0	…	…
MBC (ug C g^{-1})	348.5 ± 25.6	333.6 ± 22.1	351.5 ± 31.5	397.9 ± 50.5	426.2 ± 25.2	423.7 ± 35.6
C_{MIN} (ug C g^{-1} d^{-1})	31.7 ± 2.3	30.3 ± 2.0	32.0 ± 2.9	36.2 ± 4.6	38.7 ± 2.3	38.5 ± 3.2
MBN (ug N g^{-1})	53.2 ± 2.8	58.9 ±2.6	72.0 ± 3.2	74.6 ± 3.2	38.6 ± 2.4	40.2 ± 3.3
N_{MIN} (ug N g^{-1} d^{-1})	…	…	1.05 ± 0.1	1.01 ± 0.1	0	0
Root Biomass (g m^{-2}) *	…	…	…	…	80.2 ± 16.6	48.1 ± 9.0
C:N	…	…	…	…	38.1 ± 2.1	39.3 ± 3.0
BNPP (g m^{-2} growing $season^{-1}$)	…	…	…	…	81.4 ± 10.7	75.9 ± 11.9
C:N	…	…	…	…	38.9 ± 3.4	37.1 ± 3.0

Table 3.4. Mean biomass (nmol/g) of 12 PLFA biomarkers of various phylogenetic groups in inoculum, slurry, and control soils in both 2010 and 2011. Differences in 2011 samples noted in Figure 3.4.

TREATMENT:	INOCULUM	SLURRY		CONTROL	
YEAR:	…	2010	2011	2010	2011
PLFA marker					
Gram+					
i-C15:0	9.30 ± 0.18	15.24 ± 1.63	16.58 ± 1.47	15.09 ± 0.86	16.16 ± 2.81
a-C15:0	3.41 ± 0.08	5.54 ± 0.68	4.42 ± 1.51	5.66 ± 0.43	2.46 ± 1.42
i-C16:0	4.14 ± 0.12	4.76 ± 0.59	5.72 ± 0.53	4.8 ± 0.30	5.62 ± 0.92
1-C17:0	4.36 ± 0.44	5.49 ± 0.46	5.80 ± 0.49	5.37 ± 0.28	5.82 ± 0.84
Total Gram+	21.25 ± 0.46	31.02 ± 3.35	32.52 ± 1.72	30.91 ± 1.85	30.07 ± 3.73
Gram-					
C16:1_9	3.33 ± 0.13	4.97 ± 0.68	6.57 ± 0.79	5.15 ± 0.45	6.71 ± 1.27
C17:0 Δ 9,10	1.21 ± 0.06	3.61 ± 0.53	3.83 ± 0.41	3.68 ± 0.22	3.81 ± 0.73
2-OH C16:0	0	0	0	0	0
C18:1_9 trans	0	0	0	0	0
cyC19:0_Δ 9,10	4.72 ± 4.39	5.83 ± 0.67	6.73 ± 0.53	5.74 ± 0.44	6.57 ± 1.24
Total Gram-	9.09 ± 0.23	14.41 ± 1.88	17.13 ± 1.73	14.57 ± 1.06	17.09 ± 3.25
A.M.F.					
C16:1_11	2.84 ± 0.12	3.92 ± 0.48	6.01 ± 0.57	3.92 ± 0.22	5.83 ± 1.01
Sap Fungi					
C18:12_9,12	1.69 ± 0.13	2.17 ± 0.26	1.71 ± 0.99	1.88 ± 0.66	2.24 ± 0.77
C18:1_9cis	3.64 ± 0.01	7.59 ± 1.18	10.44 ± 1.20	7.65 ± 0.78	10.07 ± 1.97
Total Sap Fungi	5.33 ± 0.14	9.76 ± 1.43	12.15 ± 1.29	9.53 ± 1.01	12.31 ± 1.23

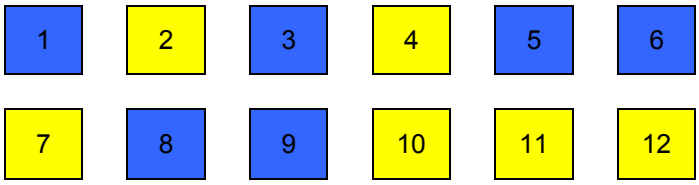


Figure 3.1. Randomized complete block design of experimental field plots. Plots are 2 by 2 m^2 with 1 m spacing. Blue squares indicate plots treated with slurry amendment. Yellow squares indicate control plots.

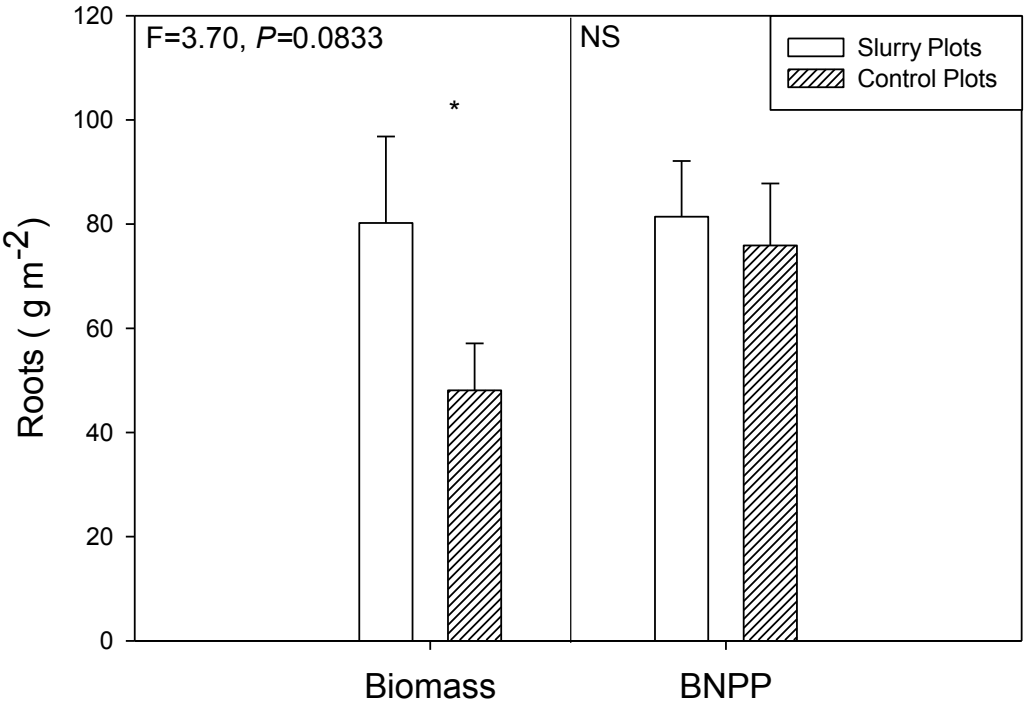


Figure 3.2. Effect of treatment (left) on root biomass in the Slurry versus the Control plots.

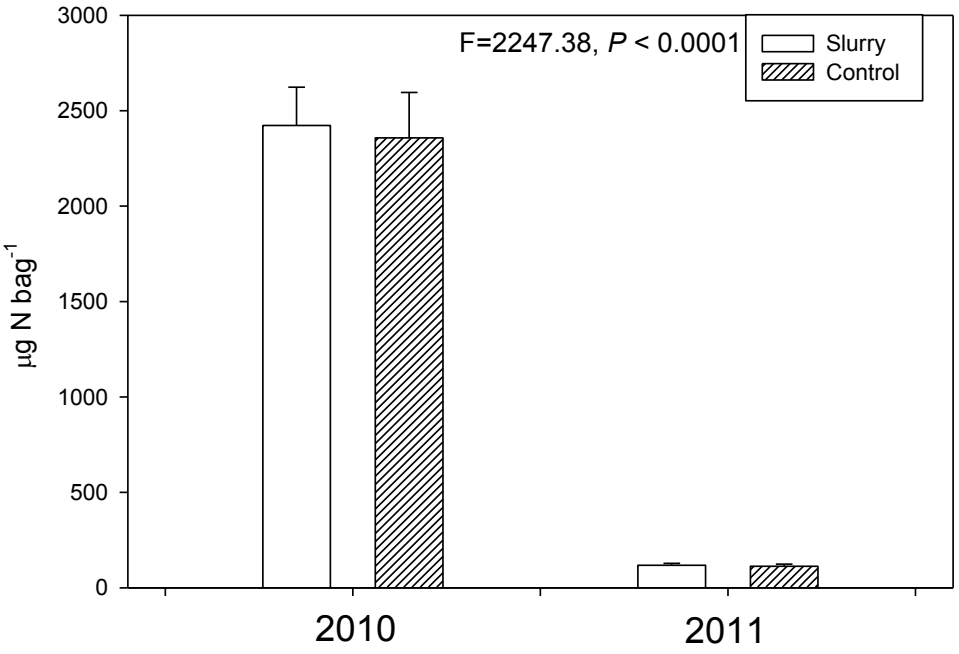


Figure 3.3. Effect of year on total inorganic nitrogen in resin bags installed in slurry and control plots.

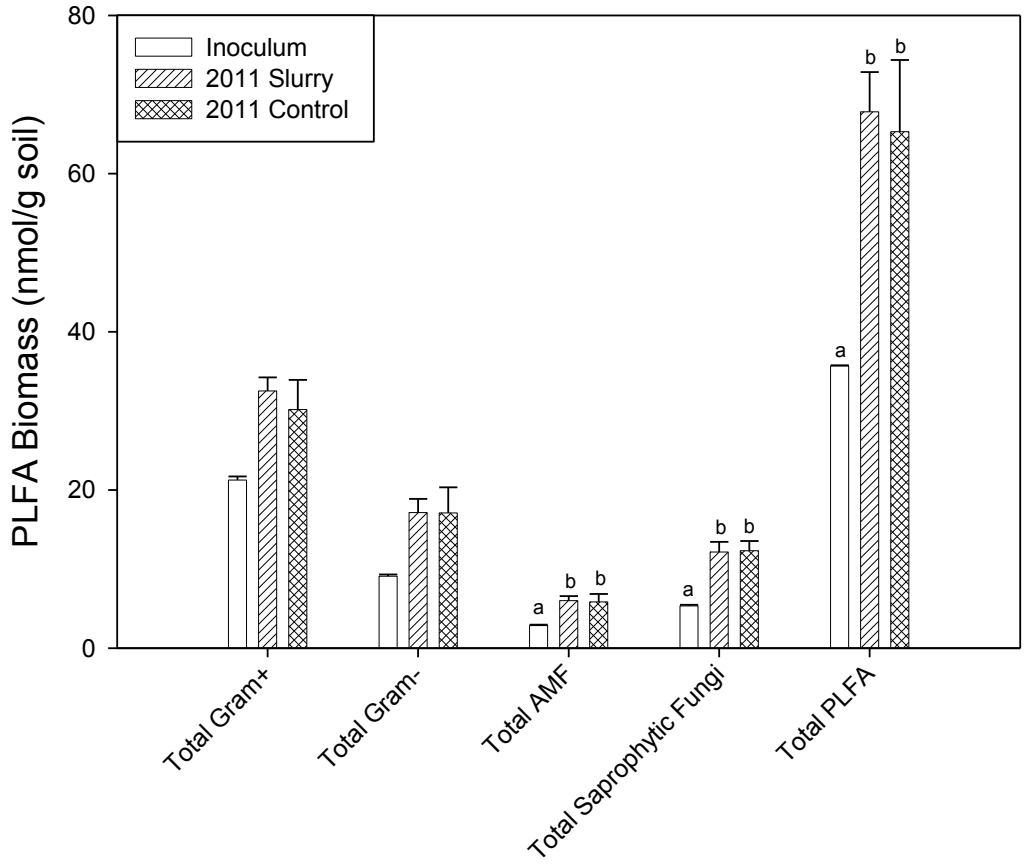


Figure 3.4. Phospholipid fatty acid (PLFA) biomass of major phylogenetic groups in original inoculum soil and final soil from slurry and control plots in 2011. Means accompanied by the same letter were not significantly different (α=0.10) within phylogenetic groups. Gram+ is gram positive bacteria. Gram- is gram negative bacteria. AMF=arbuscular mycorrhizal fungi.

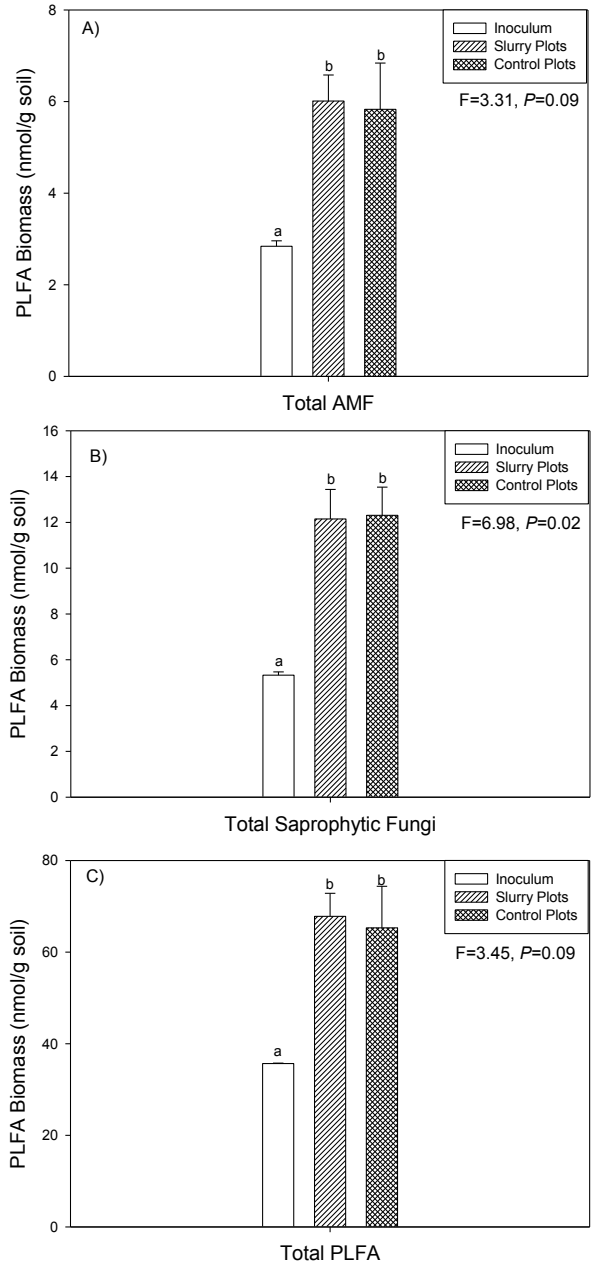
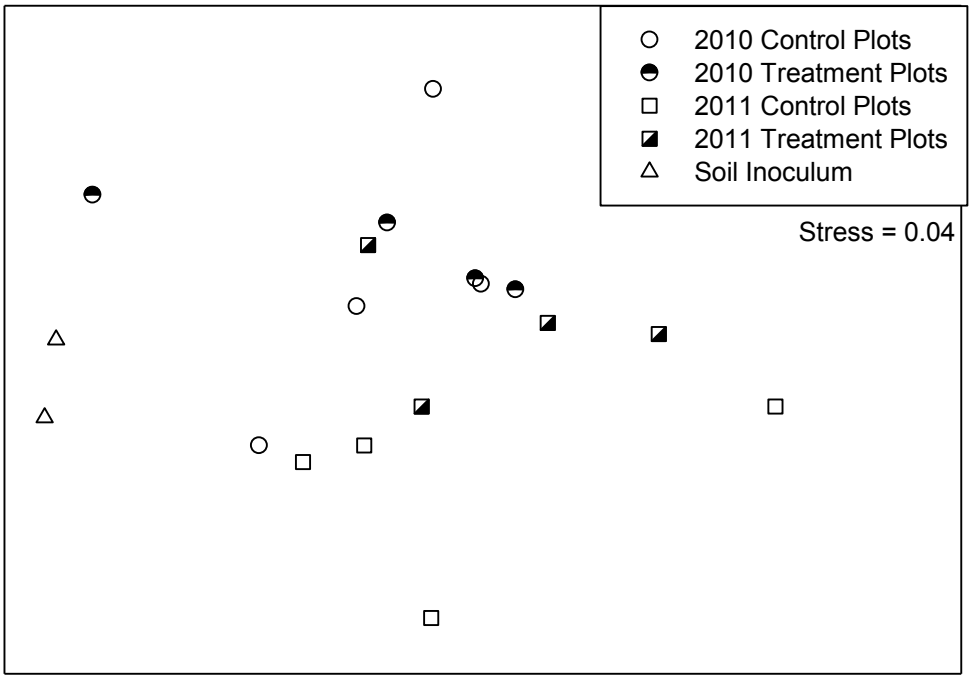


Figure 3.5. Significant difference in (A) total arbuscular mycorrhizal fungi (AMF), (B) total saprophytic fungi, and (C) total phospholipid fatty acid (PLFA) biomarkers between inoculum soil and both slurry and control plot soils in 2011. Means accompanied by the same letter were not significantly different (α=0.10).



Overall ANOSIM	**R = -0.1117, *P* = 0.8616**
Control vs Treatment	R = -0.0578, *P* = 0.7152
Control vs Inoculum	R = -0.2629, *P* = 1.0000
Treatment vs Inoculum	R = -0.1983, *P* = 1.0000

Figure 3.6. PLFA NMDS and ANOSIM analysis showing relationships between microbial communities in the soil inoculum, treatment and control plots.

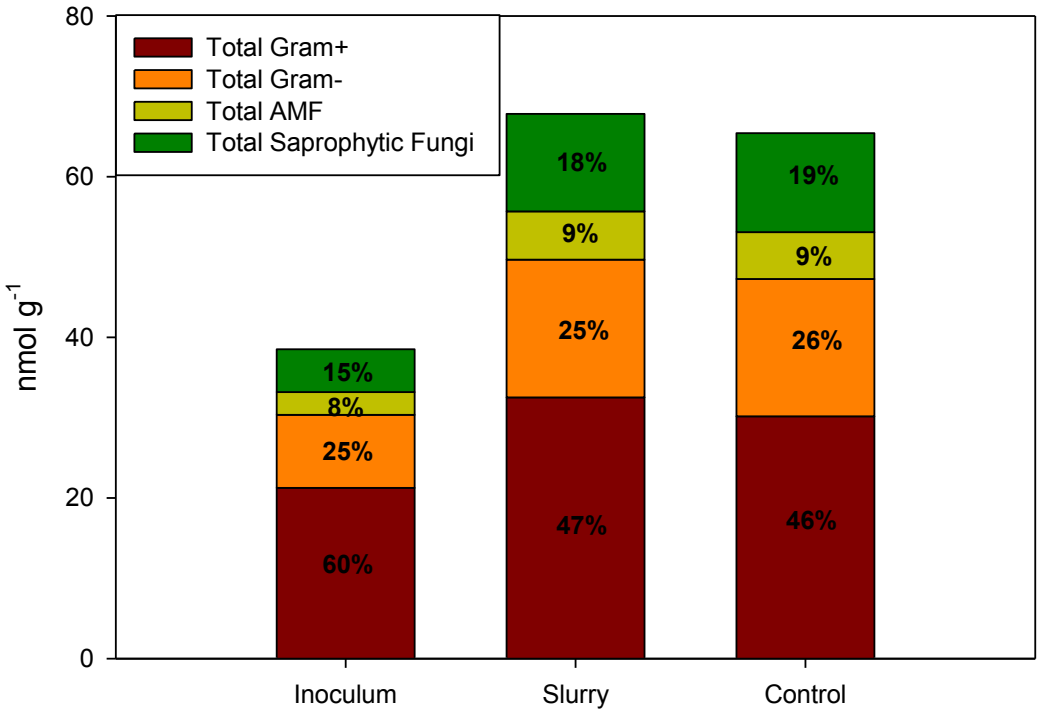


Figure 3.7. Per cent composition of major phospholipid fatty acid (PLFA) phylogenetic groups within the entire PLFA population of each soil source. Microbial group abbreviations are as follow: Gram+ = Gram positive bacteria; Gram− = Gram negative bacteria; and AMF = arbuscular mycorrhizal fungi.

CHAPTER 4

CONCLUSION

Through these two field studies, I addressed the role of regional (interaction between precipitation and ecotypic variation of a dominant species) and local (soil microbial) factors on the restoration of ecosystem processes.

Ecotypic variation may be exhibited in intraspecific differences in dominant prairie grasses, which has been noted in various studies. In previous transplant experiments (McMillan 1959), *A. gerardii* was one of seven species that exhibited varying flowering times when transplanted in two locations across an east-west gradient, and also revealed varying heights of flowering culms when transplanted within a north-south gradient. Ecotypic variation has been documented in establishment (Johnson *et al*. *submitted*) and leaf morphology (Olsen *et al. in press*) of this species. Others have found significant interaction between site and population source in tissue chemistry (Zhang *et al.* 2012). These findings indicate that intraspecific variation in biomass production (above- and belowground) may lead to some ecotypes outperforming others if non-local sources are used in restoration. This phenotypic variation could allow for greater success in migrating across the landscape, particularly if resource-capture results in higher reproductive output (i.e., seed production). These studies indicate an increasing amount of evidence for ecotypic variation in the dominant species of the tallgrass prairie ecosystem, and this is the first study to document ecotypic variation belowground.

Intraspecific variation in biomass production – above and belowground – may lead to disproportionate utilization of resources among ecotypes in restorations. This knowledge will assist practitioners in selecting ecotypes best suited to achieve restoration goals (i.e., cover vs.

diversity). Local adaptation may be more important later in restorations as plants reproduce and experience greater competition from maturing vegetation (Rice & Knapp 2008). When determining restoration goals and objectives, population seed sources should be considered as the resultant community structure may affect function during restoration.

Belowground net primary productivity (BNPP) provides the majority of organic matter inputs to the soil to stimulate microbial activity and transformations of limiting nutrients to plants (Craine *et al.* 2003; Rice *et al.* 1998; Knops *et al.* 2002). Restoring dominant species in degraded environments drives the recovery of soil C and N stocks, particularly in grasslands (Baer *et al.* 2010). Soil respiration, a measure of biological activity, results from the combined release of CO_2 from roots and microbial decomposition of organic matter (Raich & Schlesinger 1992). Though this study did see ecotypic variation in root biomass, this variation did not differentially influence belowground ecosystem processes which may be due to the plants being newly established (2 years).

In addition to roots, soil microorganisms influence biogeochemical cycling in ecosystems. The soil microbiota play an important role in regulating plant productivity, as both fungal and bacterial symbionts are responsible for the acquisition of limiting water and mineral nutrients (Smith & Read 1997). Ecosystem functions such as nitrogen and carbon transformations and soil formation are also mediated by soil microorganisms (Tiedje 1988; Kowalchuk & Stephen 2001; Hogberg *et al.* 2001; Rillig & Mummey 2006).

Arbuscular mycorrhizal fungi (AMF) have symbiotic associations with 80% of all terrestrial plants (Smith & Read 1997), many of which are prairie species (Hetrick *et al.* 1989). Symbiotic (Wild 1988) as well as free living (Smith & Read 1997) N_2 fixing microorganisms are associated with prairie plants. Previous studies saw success with reestablishment of native

species through microbial inoculations (Wang *et al*. 2009; Rowe *et al*. 2009). My second study took into account this soil ecological knowledge, expecting to observe more prairie species and greater diversity in plots amended with prairie soil treatment. By providing a community seeded with prairie species, the resultant treated soil was expected to promote a plant and microbial community more closely resembling that of a remnant prairie. Though this was not realized, studies such as this can serve to obtain a greater understanding of systems (Hobbs 2009) and to inform future restoration practices. A greater understanding of SEK is necessary in order to determine if a soil amendment can serve as a useful tool in grassland restoration practices.

REFERENCES

Albertson, F.W. & Weaver, J.E. (1944) Nature and degree of recovery of grassland from the great drought of 1933 to 1940. *Ecological Monographs.* **14**, 393-479.

Anderson, R.C., Hetrick, B.A.D., & Wilson, G.W.T. (1994) Mycorrhizal dependence of Andropogon gerardii and Schizachyrium scoparium in two prairie soils. *Am. Mid. Nat*. **132**, 366-376.

Bach, E.M., Baer, S.G., and Six, J. (2011) Plant and Soil Responses to High and Low Diversity Grassland Restoration Practices. *Environmental Management,* **49,** 412–424.

Baer, S.G., Kitchen, D.J., Blair, J.M, and Rice, C.W. (2002) Changes in Ecosystem Structure and Function along a Chronosequence of Restored Grasslands. *Ecological Applications,* **12**, 1688-1701.

Baer, S.G., Blair, J. M., Collins, S. L., and Knapp, A.M. (2003) Soil resources regulate productivity and diversity in newly established prairie. *Ecology,* **84**, 724-735.

Baer, S.G., Meyer, C.K., Bach, E.M., Klopf, R.P., & Six, J. (2010) Contrasting ecosystem recovery on two soil textures: implications for carbon mitigation and grassland conservation. *Ecosphere*. 1: doi: 10.1890/ES10-00004.1 art5.

Baer, S.G., L. Heneghan, and V. Eviner. (2012) Applying soil ecological knowledge to restore ecosystem services. Page 377-393. In D. Wall (eds.) Soil Ecology and Ecosystem Services. Oxford University Press. Oxford, UK.

Binkley, D. and P. Matson. (1983) Ion exchange resin bag method for assessing available forest soil nitrogen. *Soil Sci. Soc. Am. J.* **47**, 1050-1052.

Borchert, J.R. (1950) The climate of the central North American grassland. *Anls. Assn. Amer. Geog.* **40,** 1-39.

Bossio, D.A., Scow, K.M., Gunapala, N. & Graham, K.J. (1998) Determinants of soil microbial communities: Effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. *Microbial Ecology,* **36,** 1-12.

Bray, R. H. & Kurtz, L. T. (1945) Determination of total, organic, and available forms of phosphorus in soils. *Soil Science,* **59,** 39-45.

Briggs, J.M. & Knapp, A.K. (1995) Interannual variability in primary production in tallgrass prairie: climate, soil moisture, topographic position, and fire as determinants of aboveground biomass. *Amer. J. Bot.***82,** 1024-1030.

Chapin, F.S. (1980) The mineral nutrition of wild plants. *Annu. Rev. Ecol. Syst*. **11**, 233-260.

Clarke, KR, Gorley, RN, (2006). PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth.

Craine, J.M., Wedin, D.A., Chapin III, F.S., & Reich, P.B. (2003) The dependence of root system properties on root system biomass of 10 North American grassland species. *Plant and Soil.* **250,** 39-47.

Crutsinger, GM., Collins, MD., Fordyce, JA., Gompert, Z., Nice, CC., & Sanders, NJ. (2006) Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science*. **313**, 966-968.

Dawkins, R. (1982) The extended phenotype: the gene as the unit of selection. W.H. Freeman & Company, Oxford & San Francisco.

DeCrappeo, N.M. (2010) Soil community dynamics in sagebrush and cheatgrass-invaded ecosystems of the northern Great Basin. Ph.D. dissertation. Oregon State University, Corvallis, Oregon, USA.

Egerton-Warburton, L.M., Johnson, N.C., & Allen, E.B. (2007) Mycorrhizal community dynamics following nitrogen fertilization: a cross-site test in five grasslands. *Ecological Monographs*. **77**, 527-544.

Ellis, E. C., Klein Goldewijk, K., Siebert, S., Lightman, D. and Ramankutty, N. (2010) Anthropogenic transformation of the biomes, 1700 to 2000. *Global Ecology and Biogeography*, **19**: 589–606.

Falk, D.A., C.M. Richards, A.M. Montalvo, and E.E. Knapp. (2006) Population and ecological genetics in restoration ecology. Pages 14-41, in D.A. Falk, M.A. Palmer, and J.B. Zedler (editors), Foundations of Restoration Ecology. Island Press, Washington, USA.

Fitzsimons, M.S. & Miller, R.M. (2010) The importance of soil microorganisms for maintainting diverse plant communities in tallgrass prairie. *Amer. J. Bot.* **97,** 1-7.

Flanagan, L.B., & Johnson, B.G. (2005) Interacting effects of temperature, soil moisture and plant biomass production on ecosystem respiration in a northern temperate grassland. *Agri. For. Meteorology.* **130,** 237-253.

Gan, J., et al (2012) Hydrothermal conversion of big bluestem for bio-oil production: the effect of ecotype and planting location. *Bioresource Technology*. **116**, 413-420.

Garcia, F.O. (1992) Carbon and nitrogen dynamics and microbial ecology in tallgrass prairie. Ph.D. dissertation. Kansas State University, Manhattan, Kansas, USA.

Gardener, M. R., Atkinson, R. and Rentería, J. L. (2010), Eradications and People: Lessons from the Plant Eradication Program in Galapagos. *Restoration Ecology*. **18**: 20–29.

Gill, R.A., Kelly, R.H., Parton, W.J., Day, K.A., Jackson, R.B., et al. (2002) Using simple environmental variables to estimate below-ground productivity in grasslands. *Global Ecology and Biogeography.* **11**, 79-86.

Gould, F.W. and R.B. Shaw. (1983) Grass systematics. 2nd ed. Texas A&M Univ. Press, College Station, Tx.

Gray, M.M. (2012) Genomic differentiation of big bluestem (Andropogon gerardii) along the Great Plains' environmental gradient. Master's Thesis. Kansas State University, Manhattan, Kansas, USA.

Grime, J.P. (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *J. Ecology.* **86,** 902-910.

Harris, J.A., Hobbs, R.J., Higgs, E., & Aronson, J. (2006) Ecological restoration and global climate change. *Restoration Ecology*. **14**, 170-176.

Hayes, D.C., & Seastedt, T.R. (1987) Root dynamics of tallgrass prairie in wet and dry years. *Can. J. Bot.* **65**, 787-791.

Hayman, D.S. (1983) The physiology of vesicular-arbuscular endomycorrhizal symbiosis. *Can. J. Bot*. **61,** 944-963.

Healy, M. T. and Zedler, J. B. (2010), Set-backs in Replacing *Phalaris arundinacea* Monotypes with Sedge Meadow Vegetation. *Restoration Ecology*, **18**: 155–164.

Herman, R. J., Miles, C. C., Currie, B. E. & Dungan, L. A. (1975) Soil survey of Jackson County, Illnois. *Agricultural Experiment Station Soil Report 106*. University of Illinois.

Hetrick, B.A.D., Kitt, D.G, & Wilson, G.W.T. (1987) Mycorrhizal dependence and growth habit of warm-season and cool-season tallgrass prairie plants. *Can. J. Bot*. **66**, 1376-1380.

Hetrick, B.A.D., Wilson, G.W.T., and Owensby, C.E. (1989) Influence of mycorrhizal fungi and fertilization on big bluestem seedling biomass. *Journal Range Management*. **42**, 213-216.

Hobbs, R. J. (2007), Setting Effective and Realistic Restoration Goals: Key Directions for Research. *Restoration Ecology*, **15**: 354–357.

Hobbs, R. (2009), Looking for the Silver Lining: Making the Most of Failure. *Restoration Ecology*, **17**: 1–3.

Hodgins-Davis, A. & Townsend, J.P. (2009) Evolving gene expression: from G to E to G x E. *Trends in Ecology and Evolution.* **24,**649-658.

Hogberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Hogberg, M.N., Nyberg, G., Ottosson-Lofvenius, M., & Read, D.J. (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature,* **411**, 789-792.

Hufford, K. M. & Mazer, S. J. (2003) Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends in Ecology Evolution,* **18,** 147-155.

Jackson, L.E., Burger, M., & Cavagnaro, T.R. (2008) Roots, Nitrogen Transformations,and Ecosystem Services. *Annu. Rev. Plant Biol*. **59**, 41–63.

Jenkinson, D. S. & Powlson, D. S. (1976) Effects of biocidal treatments on metabolism in soil. 1. Fumigation with chloroform. *Soil Biology & Biochemistry,* **8,** 167-177.

Johnson, L.C., & Matchett, J.R. (2001) Fire and grazing regulate belowground processes in tallgrass prairie. *Ecology.* **82**, 3377-3389.

Kasurinen, A., Keinanen, M.M., Kaipainen, S., Nilsson, L.O., Vapaavuori, E., Kontro, M.H., & Holopainen, T. (2005) Belowground response of silver birch trees exposed to elevated CO_2 and O_3 for three growing seasons. *Global Change Biology.* **11,** 1167-1179.

Keeney, D.R. & D.W. Nelson. (1982) Nitrogen - inorganic forms. p. 643-687. In: A.L. Page, et al. (ed.). Methods of Soil Analysis: Part 2. Agronomy Monogr. 9. 2nd ed. ASA and SSSA, Madison, WI.

Kemery, R.D. & Dana, M.N. (1995) Prairie remnant soil as a source of mycorrhizal inoculum. *HortScience.* **30,** 1015-1016.

Klopf, R.P. & Baer, S.G. (2011) Root dynamics of cultivar and non-cultivar population sources of two dominant grasses during initial establishment of tallgrass prairie. *Restoration Ecology,* **19,** 112-117.

Knapp, A.K. & Smith, M.D. (2001) Variation among Biomes in Temporal Dynamics of Aboveground Primary Production. *Science.* **291,** 481-484.

Knops, J.M.H., Bradley, K.L., & Wedin, D.A. (2002) Mechanisms of plant species impacts on ecosystem nitrogen cycling. *Ecology Letters* **5**, 454-466.

Kowalchuk, G.A. & Stephen, J.R. (2001) Ammonia-oxidizing bacteria: a model for molecular microbial ecology. *Ann. Rev. Microbiol*. **55**, 485-529.

Lauenroth, W.K. & Sala, O.E. (1992) Long-term forage production of North American shortgrass stepp. *Ecological Applications*. **2**, 397-403.

Lauenroth, W.K., Burke, I. & Gutmann, M.N. (1999) The structure and function of ecosystems in the central North American grassland region. *Great Plains Research,* **9,** 223-259.

Lukac, M., & Godbold, D.L. (2010) Fine root biomass and turnover in southern taiga estimated by root inclusion nets. *Plant Soil*. **331**, 505-513.

Lynch, D.J., Matamala, R., Iversen, C.M., Norby, R.J. & Gonzalez-Meler, M.A. (2013) Stored carbon partly fuels fine-root respiration but is not used for production of new fine roots. *New Phytologist.* **199,** 420–430.

Madritch, M.D. & Hunter, M.D. (2002) Phenotypic Diversity Influences Ecosystem Functioning in an Oak Sandhills Community. *Ecology*, **83**, 2084-2090.

McKay, J. K., Christian, C. E., Harrison, S., & Rice, K. J. (2005) "How local is local?"- A review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology.* **13,** 432-440.

McMillan, C. (1959) The role of ecotypic variation in the distribution of the central grassland of North America. *Ecol. Monogr.* **29**: 285-308.

Meier, I.C., & Leuschner, C. (2008) Belowground drought response of European beech; fine root biomass and carbon partitioning in 14 mature stands across a precipitation gradient. *Global Change Biology.* **14**, 2081-2095.

Milchunas, D.G. & Lauenroth, W.K. (2001) Belowground Primary Production by Carbon Isotope Decay and Longterm Root Biomass Dynamics. *Ecosystems.* **4,** 139-150.

Millennium Ecosystem Assessment. (2005). Ecosystems and Human Well-being: Biodiversity Synthesis. World Resources Institute, Washington, DC.

Neill, C. (1992) Comparisons of soil coring and ingrowth methods for measuring belowground production. *Ecology.* **73**, 1918-1921.

NOAA (2012) U.S. Climate Normals. National Climatic Data Center, US Department of Commerce.

NRCS (2010) Web Soil Survey. United States Department of Agriculture.

O'Brien, S.L., Jastrow, J.D., Grimley, D.A., & Gonzalez-Meler, M.A. (2010) Moisture and vegetation controls on decadal-scale accrualof soil organic carbon and total nitrogen in restored grasslands. *Global Change Biology*. **16**, 2573–2588.

Olsen, J.T., Caudle, K.L., Johnson, L.C., Baer, S.G., & Maricle, B.R. (2013) Environmental and genetic variation in leaf anatomy among populations of *Andropogon gerardii* (*Poaceae*) along a precipitation gradient. *In press*.

Parton, W.J. & Risser, P.G. (1980) Impact of Management Practices on the Tallgrass Prairie. *Oecologia*, **46**, 223-234.

Raich, J.W. & Schlesinger, W.H. (1992) The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus*. **44B**, 81-99.

Raich, J.W. & Tufekcioglu, A. (2000) Vegetation and soil respiration: correlations and controls. *Biogeochemistry.* **48,** 71-90.

Rice, C.W., Todd, T.C., Blair, J.M., Seastedt, T.R., Ramundo, R.A., & Wilson, G.W.T. (1998) Belowground Biology and Processes *in* Grassland Dynamics: Long-Term Ecological Research in Tallgrass Prairie. Eds Knapp, A.K., Briggs, J.M., Hartnett, D.C., & Collins, S.L. Oxford University Press: New York.

Rice, K.J. & Knapp, E.E. (2008) Effects of competition and life history stage on the expression of local adaptation in two native bunchgrasses. *Restoration Ecology,* **16,** 12-23.

Rillig, M.C. & Mummey, D.L. (2006) Mycorrhizas and soil structure. *New Phytologist*. **171**, 41-53.

Risser, P.G., Birney, E.C., Blocker, H.D., May, S.W., Parton, W.J. & Weins, J.A. (1981) The True Prairie Ecosystem. US/IBP Synthesis Series Number 16. Hutchinson, Stroudsburg, PA, USA.

Robertson, G.P., Coleman, D.C., Bledsoe, C.S., & Sollins, P. (1999) Standard soil methods for long-term ecological research. Oxford University Press: New York.

Rowe, H.I., Brown, C.S., & Paschke, M.W. (2009) The influence of soil inoculums and nitrogen availability on restoration of high-elevation steppe communities invaded by Bromus tectorum. *Restoration Ecology*. **17**, 686-694.

Sala, O.E., Parton, W.J., Joyce, L.A., Lauenroth, W.K. (1988) Primary production of the central grassland region of the United States. *Ecology*. **69**, 40-45.

SAS (2009) SAS Institute Publication. SAS Institute Inc., Cary, NC.

Schlesinger, W.H. (1997) *Biogeochemistry: An Analysis of Global Change*. Academic Press, San Diego, CA.

Schweitzer, J.A., Bailey, J.K., Fischer, D.G., LeRoy, C.J., Lonsdorf, E.V., Whitham, T.G., & Hart, S.C. (2008) Plant-soil-microorganism interactions: heritable relationship between plant genotype and associated soil microorganisms. *Ecology.* **89,** 773-781.

Schweitzer, J.A., Fischer, D.G., et al. (2011) Forest gene diversity is correlated with the composition and function of soil microbial communities. *Popul Ecol*. **53**, 35-46.

Shahzad, T., Chenu, C., Repinçay, C., Mougin, C., Ollier, J.L., & Fontaine, S. (2012) Plant clipping decelerates the mineralization of recalcitrant soil organic matter under multiple grassland species. *Soil Biology and Biochemistry*. **51**, 73–80.

Smith, M.D., & Knapp, A.K. (2003) Dominant species maintain ecosystem function with non-random species loss. *Ecology Letters.* **6,** 509-517.

Smith, S.E. & Read, D.J. (1997) Mycorrhizal symbiosis. Academic Press, San Diego, California.

Steingrobe, B., Schmid, H., & Claassen, N. (2001) The use of the ingrowth core method for measuring root production of arable crops – influence of soil and root disturbance during installation of the bags on root ingrowth into the cores. *European Journal of Agronomy.* **15**, 143-151.

Tiedje, J.M. (1988). Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In: Biology of Anaerobic Microorganims (ed. Sehnder, A.J.B.). Wiley, New York. Pp. 179-244.

Transeau, E.N. (1935) The prairie peninsula. *Ecology,* **16,** 423-437.

Turesson, G. (1922) The species and variety as ecological units. *Hereditas.* **3**, 100-113.

USDA & NRCS (2010) The PLANTS Database. National Plant Data Center, Baton Rouge, LA, 70874-4490 USA.

US Drought Monitor (2011) National Drought Mitigation Center, University of Nebraska, Lincoln, NE, 68583–0988 USA.

Usher, G. (1966) A Dictionary of Botany. Constable & Co Ltd: London.

van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutogolis, P., Streitwolf-Engel, R., Boller, T. et al. (1998a) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature.* **396,** 69-72.

van der Heijden, M. G.A., Boller, T., Weimken, A., & Sanders, I.R. (1998b) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology.* **79**, 2082-2091.

van der Heijden, M.G.A., Bakker, R., Verwaal, J., Scheublin, T.R., Rutten, M., van Logtestijn, R. et al. (2006a) Symbiotic bacteria as a determinant of plant community structure and plant productivity in a dune grassland. *FEMS Microb. Ecol.* **56,** 178-187.

Vinton, M.A. & Burke, I.C. (1997) Contingent effects of plant species on soils along a regional moisture gradient in the Great Plains. *Oecologia*. **110,** 393-402.

Vogelsang, K.M., Reynolds, H.L., & Bever, J.D. (2006) Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. *New Phytologist.* **172,** 554-562.

Voroney, R. P. & Paul, E. A. (1984) Determination of K_C and K_N in situ for calibration of the chloroform fumigation incubation method. *Soil Biology & Biochemistry,* **16,** 9-14.

Wang, W., Liu, Y., Li, D., Hu, C., & Rao, B. (2009) Feasibility of cyanobacterial inoculation for biological soil crusts formation in desert area. *Soil Biology & Biochemistry*. **41**, 926-929.

Weaver, J.E. (1965) Native vegetation of Nebraska. University of Nebraska Press: Lincoln.

Whitham T.G., Bailey J.K., Schweitzer J.A. et al. (2006) A framework for community and ecosystem genetics: from genes to ecosystems. *Nat Rev Gen* **7**:510–523.

Whitham, T.G., et al. (2005) All effects of a gene on the world: extended phenotypes, feedbacks, and multi-level selection. *Ecoscience*. **12**, 5-7.

Wild, A. ed. (1988) Russell's Soil Conditions and Plant Growth. Eleventh Ed. Longman Group, UK.

Zhang, K., et al (2012) Chemical and elemental composition of big bluestem as affected by ecotype and planting location along the precipitation gradient of the Great Plains. *Industrial Crops and Products*. **40**, 210-218.

Zhou, X., Talley, M., & Luo, Y. (2009) Biomass, litter, and soil respiration along a precipitation gradient in southern Great Plains, USA. *Ecosystems.* **12**, 1369-1380.

APPENDICES

APPENDIX A: All response variables for population sources in Carbondale, IL, 2011. Root biomass and BNPP weight in grams taken from a 5.5 cm diameter x 20 cm deep core. Region and population source abbreviations follow Table 2.1. Abbreviations are as followings: MBC = microbial biomass carbon (μg g^{-1}); CMIN = potential carbon mineralization rate (μg g^{-1} d^{-1}); NMIN = potential net nitrogen mineralization rate (μg g^{-1} d^{-1}); MBN = microbial biomass nitrogen (μg g^{-1}); ANPP = aboveground net primary productivity (g $plant^{-1}$); BNPP = belowground net primary productivity (g 5.5 x 20 cm $core^{-1}$).

								Root Biomass			BNPP		
Block	**Region**	**Population**	**MBC**	**CMIN**	**NMIN**	**MBN**	**ANPP**	**Weight**	**%C**	**%N**	**Weight**	**%C**	**%N**
11	CKS	CDB	331.484	23.677	0.324	33.129	830	0.045	42.84	0.48	0.080	43.58	0.55
12	CKS	CDB	249.414	24.941	0.799	29.816	400	0.023	46.22	1.11	0.027	39.70	0.83
13	CKS	CDB	171.039	17.104	0.795	32.002	335	0.040	43.27	0.81	0.070	38.47	0.65
14	CKS	CDB	158.987	14.453	0.412	27.603	434	0.143	38.79	0.93	0.126	38.11	0.68
15	CKS	CDB	143.428	14.343	0.510	29.201	508	0.003	37.40	0.00	0.000	35.33	0.00
11	SIL	DES	251.667	17.976	0.467	27.615	1994	0.471	39.35	1.17	1.092	41.60	0.90
12	SIL	DES	240.102	24.010	0.702	32.294	938	0.404	42.23	1.54	0.682	43.97	0.58
13	SIL	DES	404.683	40.468	0.487	34.382	1038	0.396	34.09	0.84	0.579	36.89	0.52
14	SIL	DES	157.492	14.317	0.673	27.687	546	0.069	38.98	1.00	0.130	38.99	0.69
15	SIL	DES	348.034	34.803	0.656	37.826	847	1.402	34.29	1.12	1.246	40.31	0.68
11	EKS	KNZ	299.973	21.427	0.432	20.510	761	0.014	46.00	0.00	0.009	35.11	1.21
12	EKS	KNZ	300.876	30.088	0.754	30.075	685	0.046	34.12	0.60	0.039	42.34	0.68
13	EKS	KNZ	295.433	29.543	0.542	37.166	659	0.112	35.92	1.54	0.034	42.04	0.57
14	EKS	KNZ	239.490	21.772	0.457	29.424	457	0.066	43.24	1.90	0.215	41.33	0.89
15	EKS	KNZ	145.506	14.551	0.491	27.167	199	0.011	3.61	0.06	0.014	41.81	0.71
11	CKS	REL	267.141	19.082	0.474	30.139	425	0.020	44.19	0.77	0.102	35.30	0.60
12	CKS	REL	184.633	18.463	0.489	24.854	402	0.088	41.71	0.67	0.137	26.57	0.70
13	CKS	REL	245.345	24.535	0.873	24.539	514	0.048	40.35	0.72	0.013	40.59	0.77
14	CKS	REL	189.414	17.219	0.557	31.762	105	0.010	42.14	1.25	0.019	39.79	0.81
15	CKS	REL	159.143	15.914	0.409	20.936	814	0.086	40.61	1.34	0.218	33.93	0.93
11	EKS	TAL	317.059	22.647	0.379	26.356	988	0.154	44.39	1.21	0.247	37.66	0.76
12	EKS	TAL	262.728	26.273	0.663	29.250	1057	0.189	42.44	1.04	0.182	37.69	0.67
13	EKS	TAL	195.896	19.590	0.458	17.782	517	0.166	35.94	1.29	0.019	43.47	0.34
14	EKS	TAL	283.002	25.727	0.762	24.614	1050	0.127	37.91	1.34	0.374	38.12	0.73
15	EKS	TAL	527.060	52.706	0.548	36.522	478	0.047	40.98	1.07	0.221	38.05	0.66
11	SIL	12M	188.517	13.466	0.423	27.027	2520	0.242	40.43	0.51	3.508	44.26	0.65
12	SIL	12M	238.269	23.827	0.832	36.263	1465	0.242	36.11	1.01	0.160	34.38	0.76
13	SIL	12M	238.925	23.893	0.659	30.289	1964	0.867	39.20	1.09	0.910	39.70	0.43
14	SIL	12M	.	.	0.588	37.527	2094	0.591	38.44	1.61	1.494	38.80	0.63
15	SIL	12M	237.651	23.765	0.535	30.109	799	0.465	36.11	1.20	1.008	40.55	0.69
11	SIL	Control	161.592	11.542	0.167	19.816	.	.	.	.	.	.	.
12	SIL	Control	110.899	11.090	0.658	10.564	.	.	.	.	.	.	.
13	SIL	Control	104.826	10.483	0.393	18.056	.	.	.	.	.	.	.
14	SIL	Control	111.674	10.152	0.559	21.655	.	.	.	.	.	.	.
15	SIL	Control	104.556	10.456	0.596	17.234	.	.	.	.	.	.	.

APPENDIX B: All response variables for population sources in Hays, KS, 2011. Root biomass and BNPP weight in grams taken from a 5.5 cm diameter x 20 cm deep core. Region and population source abbreviations follow Table 2.1. Abbreviations are as followings: MBC = microbial biomass carbon (μg g^{-1}); CMIN = potential carbon mineralization rate (μg g^{-1} d^{-1}); NMIN = potential net nitrogen mineralization rate (μg g^{-1} d^{-1}); MBN = microbial biomass nitrogen (μg g^{-1}); ANPP = aboveground net primary productivity (g $plant^{-1}$); BNPP = belowground net primary productivity (g 5.5 x 20 cm $core^{-1}$).

								Root Biomass			BNPP		
Block	**Region**	**Population**	**MBC**	**CMIN**	**NMIN**	**MBN**	**ANPP**	**Weight**	**%C**	**%N**	**Weight**	**%C**	**%N**
11	CKS	CDB	596.13	42.58	0.25	65.64	813	0.218	42.25	1.26	0.541	41.40	1.02
12	CKS	CDB	356.45	35.65	0.52	48.99	274	0.056	41.87	1.98	0.112	39.90	0.91
13	CKS	CDB	384.89	38.49	0.71	42.18	258	0.037	39.34	1.50	0.082	41.78	0.92
14	CKS	CDB	408.73	37.16	0.69	41.06	376	0.046	38.60	0.79	0.475	41.66	1.04
15	CKS	CDB	.	.	0.58	44.42	599	0.178	37.80	1.51	0.421	42.36	0.99
11	SIL	DES	753.01	53.79	0.61	66.31	440	0.129	42.54	1.79	0.761	42.28	0.78
12	SIL	DES	457.26	45.73	0.65	57.18	748	0.493	42.04	1.70	0.380	41.65	1.03
13	SIL	DES	351.59	35.16	0.55	45.04	163	0.271	39.15	2.09	0.082	44.23	1.29
14	SIL	DES	492.00	44.73	0.71	44.07	242	0.066	37.99	1.59	0.691	40.30	0.97
15	SIL	DES	403.32	40.33	1.00	41.96	441	0.173	38.32	1.81	0.071	43.36	1.02
11	EKS	KNZ	536.54	38.32	0.53	60.02	289	0.039	42.05	1.46	0.126	40.94	1.19
12	EKS	KNZ	451.14	45.11	0.76	47.73	389	0.423	38.86	1.53	0.657	40.84	0.87
13	EKS	KNZ	395.74	39.57	0.83	39.75	638	0.041	41.45	0.84	0.073	38.81	0.93
14	EKS	KNZ	370.28	33.66	0.72	39.76	62	0.047	41.28	1.18	0.121	43.32	0.99
15	EKS	KNZ	450.20	45.02	0.44	43.65	528	0.120	41.57	1.42	0.553	39.00	0.87
11	CKS	REL	429.61	30.69	0.65	47.87	638	0.068	42.03	1.21	0.238	38.61	0.97
12	CKS	REL	453.64	45.36	0.93	53.08	547	0.236	39.86	1.92	0.483	41.81	0.89
13	CKS	REL	468.92	46.89	1.86	26.92	188	0.039	40.10	1.65	0.135	39.43	0.94
14	CKS	REL	475.57	43.23	1.19	42.66	571	0.023	44.40	1.18	0.197	41.61	1.05
15	CKS	REL	427.32	42.73	0.65	39.24	131	0.018	38.85	0.86	0.145	37.59	1.04
11	EKS	TAL	581.13	41.51	0.53	58.27	254	0.101	40.15	1.35	0.576	38.12	0.85
12	EKS	TAL	470.46	47.05	1.01	48.81	449	0.098	42.67	1.46	0.292	42.26	0.92
13	EKS	TAL	491.03	49.10	0.39	59.19	229	0.034	39.45	1.96	0.072	39.58	0.69
14	EKS	TAL	429.46	39.04	0.39	33.13	405	0.093	38.06	1.50	0.811	40.83	0.96
15	EKS	TAL	374.82	37.48	0.83	41.85	260	0.067	36.25	1.23	0.062	43.16	1.03
11	SIL	12M	234.07	16.72	0.74	41.29	82	0.120	39.16	1.97	0.191	42.16	1.02
12	SIL	12M	300.67	30.07	0.92	44.63	935	0.570	41.00	1.56	0.995	40.32	0.88
13	SIL	12M	471.46	47.15	0.79	48.74	385	0.327	41.11	1.52	0.977	41.50	0.96
14	SIL	12M	399.20	36.29	0.75	41.92	701	0.091	37.97	2.08	0.471	39.61	0.96
15	SIL	12M	414.33	41.43	0.68	37.93	240	0.393	40.69	1.74	0.980	41.49	1.04
11	CKS	Control	322.58	23.04	0.58	42.11	.	.	.	.	.	.	.
12	CKS	Control	244.06	24.41	0.95	34.55	.	.	.	.	.	.	.
13	CKS	Control	395.50	39.55	0.45	54.22	.	.	.	.	.	.	.
14	CKS	Control	322.53	29.32	0.68	37.37	.	.	.	.	.	.	.
15	CKS	Control	.	.	0.71	42.92	.	.	.	.	.	.	.

APPENDIX C: All response variables for population sources in Manhattan, KS, 2011. Root biomass and BNPP weight in grams taken from a 5.5 cm diameter x 20 cm deep core. Region and population source abbreviations follow Table 2.1. Abbreviations are as followings: MBC = microbial biomass carbon (μg g^{-1}); CMIN = potential carbon mineralization rate (μg g^{-1} d^{-1}); NMIN = potential net nitrogen mineralization rate (μg g-1 d-1); MBN = microbial biomass nitrogen (μg g^{-1}); ANPP = aboveground net primary productivity (g $plant^{-1}$); BNPP = belowground net primary productivity (g 5.5 x 20 cm $core^{-1}$).

								Root Biomass			BNPP		
Block	**Region**	**Population**	**MBC**	**CMIN**	**NMIN**	**MBN**	**ANPP**	**Weight**	**%C**	**%N**	**Weight**	**%C**	**%N**
11	CKS	CDB	23.840	1.589	0.290	7.240	210	0.036	19.91	0.5	0.133	45.56	0.77
12	CKS	CDB	27.955	2.795	0.343	4.403	378	0.055	37.64	1.0	0.163	33.55	0.64
13	CKS	CDB	71.496	7.150	0.421	3.060	1464	0.145	31.29	1.0	0.535	34.77	0.39
14	CKS	CDB	53.455	4.860	0.600	0.824	147	0.055	37.70	1.2	0.251	29.11	0.62
15	CKS	CDB	28.216	2.822	0.642	7.181	161	0.051	31.23	0.9	0.153	38.10	0.75
11	SIL	DES	5.066	0.338	0.441	2.293	561	0.271	41.41	1.2	0.859	41.90	0.61
12	SIL	DES	24.258	2.426	0.379	8.607	1899	0.400	35.76	1.2	0.640	41.86	0.60
13	SIL	DES	25.041	2.504	0.405	4.916	531	0.159	34.83	1.0	1.044	42.03	0.55
14	SIL	DES	34.063	3.097	0.361	5.367	272	0.209	31.18	1.1	0.422	39.73	0.62
15	SIL	DES	79.416	7.942	0.729	4.346	850	0.305	35.82	0.7	0.649	41.50	0.72
11	EKS	KNZ	16.843	1.123	0.304	5.822	791	0.141	39.87	1.5	0.359	43.61	0.89
12	EKS	KNZ	36.603	3.660	0.413	3.915	186	0.181	39.33	1.7	0.229	41.12	0.77
13	EKS	KNZ	13.824	1.382	0.365	5.344	544	0.110	40.99	1.0	0.397	39.30	0.66
14	EKS	KNZ	51.792	4.708	0.391	1.868	181	0.132	33.57	1.2	0.300	38.27	0.62
15	EKS	KNZ	52.992	5.299	0.261	9.455	790	0.130	33.25	1.0	0.332	40.08	0.74
11	CKS	REL	41.871	2.791	0.225	8.416	523	0.059	32.83	1.5	0.168	44.42	0.81
12	CKS	REL	12.158	1.216	0.391	4.382	138	0.116	37.94	0.8	0.271	37.73	0.51
13	CKS	REL	35.703	3.570	0.373	3.015	370	0.077	36.24	0.8	0.212	41.99	0.48
14	CKS	REL	8.845	0.804	0.380	5.465	507	0.065	39.42	0.9	0.383	37.05	0.61
15	CKS	REL	37.664	3.766	0.393	10.809	815	0.115	39.20	0.8	0.180	28.23	0.67
11	EKS	TAL	49.749	3.317	0.355	9.471	709	0.107	37.13	1.2	0.415	31.84	0.65
12	EKS	TAL	20.207	2.021	0.378	8.705	282	0.258	41.32	1.0	0.452	40.53	0.56
13	EKS	TAL	24.905	2.491	0.263	7.943	980	0.400	30.77	0.6	0.675	37.58	0.50
14	EKS	TAL	16.563	1.506	0.382	9.200	318	0.157	39.77	1.7	0.606	40.86	0.72
15	EKS	TAL	76.110	7.611	0.116	8.066	1699	0.312	37.35	1.3	0.416	39.33	0.49
11	SIL	12M	4.651	0.332	0.308	2.632	1665	0.490	38.91	1.7	1.550	42.50	0.69
12	SIL	12M	44.903	4.490	0.328	6.324	1422	0.601	42.37	1.3	2.127	41.25	0.54
13	SIL	12M	48.874	4.887	0.225	6.235	1028	0.290	33.97	1.3	1.683	39.29	0.48
14	SIL	12M	72.464	6.588	0.369	6.092	1638	0.138	34.65	1.5	1.207	42.09	0.51
15	SIL	12M	61.586	6.159	0.295	9.795	1002	0.322	36.78	1.2	0.490	42.53	0.73
11	EKS	Control	65.952	4.397	0.318	7.978	.	.	.	.	.	.	.
12	EKS	Control	21.370	2.137	0.295	2.425	.	.	.	.	.	.	.
13	EKS	Control	28.155	2.816	0.180	3.129	.	.	.	.	.	.	.
14	EKS	Control	4.883	0.444	0.173	4.675	.	.	.	.	.	.	.
15	EKS	Control	52.450	5.245	0.316	10.399	.	.	.	.	.	.	.

APPENDIX D: Per cent soil moisture (SM) and water holding capacity (WHC) in control soils at each site used to prepare soil for lab incubations.

SITE	%SM at 100% WHC	50% WHC
Carbondale	48.37	24.19
Carbondale	48.06	24.03
Carbondale	46.66	23.33
Carbondale	51.22	25.61
Carbondale	47.99	23.99
Manhattan	30.58	15.29
Manhattan	29.07	14.53
Manhattan	32.05	16.02
Manhattan	35.20	17.60
Manhattan	30.99	15.49
Hays	55.81	27.91
Hays	52.66	26.33
Hays	56.29	28.15
Hays	51.74	25.87
Hays	53.29	26.64

APPENDIX E: Composite soil (0-5 cm) response variables measured in the slurry experiment (corresponding to Chapter 3) in 2010. Bray phosphate ($PO_4^{=}$) reported in $\mu g\ g^{-1}$ soil. Treatments indicated as follows: TREAT = plots treated with slurry amendment and CTRL = control plots. *All units follow appendix A.

TRT	YEAR	PLOT	REP	NMIN	MBN	MBC	CMIN	pH	PO4
TREAT	2010	1	1	…	61.84	161.57	14.69	5.37	69.61
TREAT	2010	1	2	…	58.70	367.86	33.44	5.12	…
CTRL	2010	2	1	…	53.59	329.43	29.95	5.27	74.25
CTRL	2010	2	2	…	52.41	400.17	36.38	5.39	…
TREAT	2010	3	1	…	62.77	369.46	33.59	5.35	70.35
TREAT	2010	3	2	…	59.46	305.00	27.73	5.79	…
CTRL	2010	4	1	…	63.10	287.14	26.10	5.32	71.36
CTRL	2010	4	2	…	54.75	383.93	34.90	5.81	…
TREAT	2010	5	1	…	41.31	304.77	27.71	5.16	73.42
TREAT	2010	5	2	…	57.92	359.69	32.70	5.76	…
TREAT	2010	6	1	…	40.66	270.14	24.56	5.08	66.21
TREAT	2010	6	2	…	32.30	338.30	30.75	5.72	…
CTRL	2010	7	1	…	79.79	186.35	16.94	5.67	66.72
CTRL	2010	7	2	…	69.94	489.61	44.51	5.91	…
TREAT	2010	8	1	…	53.30	334.62	30.42	5.66	62.04
TREAT	2010	8	2	…	52.75	468.04	42.55	5.81	…
TREAT	2010	9	1	…	58.06	403.04	36.64	5.74	56.90
TREAT	2010	9	2	…	58.88	499.88	45.44	5.77	…
CTRL	2010	10	1	…	51.55	322.40	29.31	5.74	58.31
CTRL	2010	10	2	…	58.02	340.95	31.00	5.79	…
CTRL	2010	11	1	…	50.81	307.82	27.98	5.78	58.96
CTRL	2010	11	2	…	64.31	381.70	34.70	5.84	…
CTRL	2010	12	1	…	52.68	310.17	28.20	5.77	57.81
CTRL	2010	12	2	…	55.65	260.67	23.70	5.77	…

APPENDIX F: Composite soil (0-5 cm) response variables measured in the slurry experiment (corresponding to Chapter 3) in 2011. Bray phosphate ($PO_4^{=}$) reported in $\mu g\ g^{-1}$ soil. Treatments indicated as follows: TREAT = plots treated with slurry amendment and CTRL = control plots. *All units follow appendix A.

TRT	YEAR	PLOT	REP	NMIN	MBN	MBC	CMIN	pH	PO4
TREAT	2011	1	1	1.58	77.94	298.78	27.16	5.45	67.71
TREAT	2011	1	2	1.49	80.32	227.44	20.68	5.49	…
CTRL	2011	2	1	1.35	76.94	325.24	29.57	5.51	68.36
CTRL	2011	2	2	1.35	77.49	535.63	48.69	5.52	…
TREAT	2011	3	1	1.02	85.92	436.32	39.67	5.62	71.10
TREAT	2011	3	2	1.11	79.86	251.90	22.90	5.62	…
CTRL	2011	4	1	0.74	86.39	645.27	58.66	5.63	64.41
CTRL	2011	4	2	0.85	76.05	104.66	9.51	5.62	…
TREAT	2011	5	1	0.99	58.14	295.15	26.83	5.42	74.63
TREAT	2011	5	2	0.75	60.59	323.26	29.39	5.42	…
TREAT	2011	6	1	0.68	56.36	479.88	43.63	5.61	72.42
TREAT	2011	6	2	0.58	55.80	295.55	26.87	5.30	…
CTRL	2011	7	1	1.57	91.42	585.66	53.24	5.68	65.84
CTRL	2011	7	2	1.56	89.95	209.05	19.00	5.67	…
TREAT	2011	8	1	1.09	77.78	498.89	45.35	5.54	58.13
TREAT	2011	8	2	1.08	80.52	453.05	41.19	5.52	…
TREAT	2011	9	1	1.28	70.15	189.15	17.20	5.51	59.68
TREAT	2011	9	2	0.99	80.41	469.06	42.64	5.51	…
CTRL	2011	10	1	0.93	72.95	444.27	40.39	5.53	57.37
CTRL	2011	10	2	0.93	68.25	401.20	36.47	5.51	…
CTRL	2011	11	1	0.61	69.97	426.42	38.77	5.50	59.71
CTRL	2011	11	2	0.60	68.03	581.08	52.83	5.50	…
CTRL	2011	12	1	0.77	64.19	354.98	32.27	5.49	58.61
CTRL	2011	12	2	0.86	53.33	160.86	14.62	5.48	…

APPENDIX G: Response variables associated with root ingrowth bags measured in the slurry experiment (corresponding to Chapter 3) in 2011. Bray phosphate ($PO_4^{=}$) reported in $\mu g\ g^{-1}$ soil. Treatments indicated as follows: TREAT = plots treated with slurry amendment and CTRL = control plots. *All units follow appendix A.

			Root Biomass		BNPP		BNPP Associated Soil Response Variables			
TRT	PLOT	REP	C:N	Weight	C:N	Weight	NMIN	MBN	MBC	CMIN
TREAT	1	1	30.32	0.103	36.10	0.191	(0.09)	45.37	541.61	49.24
TREAT	1	2	27.94	0.085	30.85	0.128	(0.01)	48.89	390.59	35.51
CTRL	2	1	43.68	0.167	58.95	0.167	0.05	47.11	435.81	39.62
CTRL	2	2	47.33	0.046	35.62	0.269	(0.08)	49.66	589.65	53.60
TREAT	3	1	43.38	0.135	38.28	0.191	0.12	42.56	450.18	40.93
TREAT	3	2	42.10	0.303	52.19	0.393	(0.06)	32.13	321.04	29.19
CTRL	4	1	29.69	0.277	41.29	0.076	(0.00)	51.76	649.97	59.09
CTRL	4	2	61.47	0.161	25.93	0.165	0.16	37.39	368.14	33.47
TREAT	5	1	42.26	0.434	33.22	0.164	0.03	31.31	394.01	35.82
TREAT	5	2	25.47	0.120	21.83	0.087	0.06	47.83	570.25	51.84
TREAT	6	1	40.15	0.052	52.94	0.115	(0.09)	23.75	332.97	30.27
TREAT	6	2	38.88	0.438	49.03	0.108	(0.03)	36.30	295.14	26.83
CTRL	7	1	34.54	0.067	29.15	0.400	(0.00)	53.82	506.42	46.04
CTRL	7	2	26.09	0.044	33.63	0.213	(0.04)	53.10	536.72	48.79
TREAT	8	1	35.66	0.154	27.96	0.187	(0.04)	39.53	452.58	41.14
TREAT	8	2	49.06	0.156	36.16	0.266	(0.20)	34.73	483.09	43.92
TREAT	9	1	45.56	0.133	28.40	0.160	0.01	49.71	493.93	44.90
TREAT	9	2	36.43	0.101	59.23	0.256	0.05	31.24	388.80	35.35
CTRL	10	1	42.45	0.076	55.94	0.094	(0.20)	23.34	361.99	32.91
CTRL	10	2	31.93	0.061	36.32	0.251	0.01	36.43	388.48	35.32
CTRL	11	1	41.99	0.170	37.54	0.123	(0.05)	34.47	326.61	29.69
CTRL	11	2	29.33	0.121	31.53	0.092	(0.06)	43.51	367.79	33.44
CTRL	12	1	33.95	0.099	31.71	0.126	(0.05)	30.80	324.33	29.48
CTRL	12	2	48.86	0.039	28.02	0.117	(0.02)	21.02	228.23	20.75

APPENDIX H: Phospholipid fatty acid concentrations (PLFA; nmol/g soil) in the slurry experiment (corresponding to Chapter 3) conducted in 2010 and 2011. Treatments indicated as follows: TREAT = plots treated with slurry amendment and CTRL = control plots.

Year	Plot	Trt	i-C15:0	a-C15:0	i-C16:0	i-C17:0	C16:1_9	C17:0 Δ 9,10	2-OH C16:0	C18:1_9 trans	cyC19:0_ Δ 9,10	SF C18:2 _9,12	SF C18:1_9cis	Total Gram+	Total Gram-	Total AMF	Total SAP Fungi	Total PLFA
2010	2	CTRL	13.13	4.57	4.03	4.80	4.05	3.09	0.00	0.00	4.99	3.09	6.32	26.53	12.14	3.34	9.42	51.42
2010	7	CTRL	16.61	6.47	5.35	5.54	6.27	4.16	0.00	0.00	6.63	2.32	9.91	33.96	17.05	4.34	12.23	67.59
2010	11	CTRL	14.15	5.35	4.61	5.06	5.12	3.60	0.00	0.00	4.98	2.09	7.05	29.18	13.70	3.82	9.14	55.84
2010	12	CTRL	16.46	6.23	5.21	6.08	5.16	3.89	0.00	0.00	6.34	0.00	7.33	33.98	15.39	4.19	7.33	60.90
2010	3	TRT	17.90	6.50	5.73	6.01	6.12	4.51	0.00	0.00	7.14	2.84	9.75	36.14	17.77	4.61	12.60	71.12
2010	5	TRT	10.56	3.54	3.10	4.11	3.15	2.19	0.00	0.00	4.03	1.58	4.45	21.30	9.37	2.51	6.03	39.21
2010	8	TRT	16.83	6.26	5.43	6.08	5.89	4.33	0.00	0.00	6.48	2.26	8.99	34.60	16.69	4.50	11.25	67.04
2010	9	TRT	15.66	5.86	4.77	5.74	4.72	3.40	0.00	0.00	5.69	2.01	7.16	32.03	13.82	4.04	9.17	59.06
2011	2	CTRL	12.61	4.77	4.37	4.71	4.85	2.76	0.00	0.00	4.72	2.80	7.37	26.47	12.33	4.28	10.17	53.25
2011	7	CTRL	24.51	0.00	8.31	8.33	10.42	5.96	0.00	0.00	10.16	0.00	15.84	41.14	26.54	8.74	15.84	92.26
2011	11	CTRL	14.57	0.00	5.33	5.16	6.28	3.53	0.00	0.00	6.28	2.70	9.26	25.06	16.09	5.56	11.96	58.67
2011	12	CTRL	12.97	5.06	4.48	5.08	5.31	3.00	0.00	0.00	5.12	3.46	7.80	27.59	13.42	4.73	11.27	57.01
2011	3	TRT	20.09	0.00	7.10	7.18	8.48	4.85	0.00	0.00	7.98	0.00	13.69	34.37	21.31	7.44	13.69	76.81
2011	5	TRT	14.66	5.58	5.29	4.98	5.52	3.38	0.00	0.00	6.05	3.43	8.76	30.52	14.95	5.10	12.19	62.76
2011	8	TRT	17.85	6.83	5.89	5.79	7.21	4.11	0.00	0.00	7.25	3.40	10.81	36.36	18.57	6.44	14.21	75.58
2011	9	TRT	13.73	5.28	4.59	5.25	5.06	2.98	0.00	0.00	5.66	0.00	8.50	28.85	13.70	5.06	8.50	56.11
		INOC	9.49	3.37	4.02	3.92	3.45	1.15	0.00	0.00	4.72	1.82	3.65	20.80	9.33	2.95	5.47	38.55
		INOC	9.12	3.53	4.26	4.80	3.20	1.26	0.00	0.00	4.39	1.56	3.63	21.71	8.86	2.72	5.19	38.48

APPENDIX J: Aboveground biomass (g m^{-2}) in treatment and control plots of the slurry experiment collected from a 0.10 m^2 frame. Treatments indicated as follows: TREAT = plots treated with slurry amendment and CTRL = control plots.

			PLANTED			VOLUNTEER			
Trt	Plot	Year	Grasses	Forbs	Total	Grasses	Forbs	Total	Total Biomass
TREAT	1	2010	…	…	…	250.6	460.5	711.1	711.1
CTRL	2	2010	…	…	…	350.8	280.1	630.9	630.9
TREAT	3	2010	…	…	…	592.9	…	592.9	592.9
CTRL	4	2010	…	…	…	390.4	5.6	396.0	396.0
TREAT	5	2010	…	0.2	0.2	209.5	6.5	216.0	216.4
TREAT	6	2010	…	0.2	0.2	476.3	132.5	608.8	609.2
CTRL	7	2010	…	0.1	0.1	268.2	255.0	523.2	523.4
TREAT	8	2010	…	…	…	599.4	8.2	607.6	607.6
TREAT	9	2010	…	…	…	406.8	0.1	406.9	406.9
CTRL	10	2010	…	…	…	187.3	2.8	190.1	190.1
CTRL	11	2010	…	1.8	1.8	370.0	1.4	371.4	375.0
CTRL	12	2010	…	…	…	391.2	0.1	391.3	391.3
TREAT	1	2011	…	30.5	30.5	325.4	360.7	686.1	747.1
CTRL	2	2011	…	19.0	19.0	345.3	252.6	597.9	635.9
TREAT	3	2011	…	…	…	545.8	428.9	974.7	974.7
CTRL	4	2011	…	11.2	11.2	219.1	509.9	729.0	751.4
TREAT	5	2011	26.9	56.0	82.9	103.3	248.3	351.6	517.4
TREAT	6	2011	210.5	34.2	244.7	72.8	361.0	433.8	923.2
CTRL	7	2011	…	34.7	34.7	320.5	439.4	759.9	829.3
TREAT	8	2011	…	0.7	0.7	245.5	361.0	606.5	607.9
TREAT	9	2011	…	3.9	3.9	381.0	234.5	615.5	623.3
CTRL	10	2011	4.2	15.8	20.0	235.0	384.2	619.2	659.2
CTRL	11	2011	7.3	0.5	7.8	170.1	504.9	675.0	690.6
CTRL	12	2011	21.6	…	21.6	104.0	1153.2	1257.2	1300.4

APPENDIX K: Percent cover of planted species, volunteer species, grasses, and fobs, as well as richness and diversity in a 0.25 m^2 quadrat in the treatment and control plots of the slurry experiment in 2010 and 2011. Treatments indicated as follows: TREAT = plots treated with slurry amendment and CTRL = control plots.

TRT	PLOT	YEAR	Total	Planted	Volunteer	Grass	Forb	Richness	Diversity
TREAT	1	2010	123	1	122	50	73	5	0.94
TREAT	1	2011	213	31	182	35	178	19	2.38
CTRL	2	2010	104	1	103	95	9	4	0.38
CTRL	2	2011	177	31	146	70	107	20	2.21
TREAT	3	2010	109	1	108	102	7	5	0.52
TREAT	3	2011	185	17	168	82	103	24	2.31
CTRL	4	2010	131	0	131	45	86	3	0.68
CTRL	4	2011	187	16	171	74	113	19	2.28
TREAT	5	2010	121	0	121	45	76	3	0.70
TREAT	5	2011	169	16	153	61	108	20	1.95
TREAT	6	2010	130	0	130	60	70	3	1.06
TREAT	6	2011	224	69	155	80	144	20	2.05
CTRL	7	2010	106	0	106	60	46	3	0.73
CTRL	7	2011	173	23	150	22	151	18	2.05
TREAT	8	2010	103	0	103	90	13	6	0.58
TREAT	8	2011	273	56	217	98	175	18	2.13
TREAT	9	2010	91	0	91	90	1	2	0.06
TREAT	9	2011	193	35	158	60	133	20	2.51
CTRL	10	2010	89	4	85	75	14	3	0.53
CTRL	10	2011	198	22	176	61	137	23	2.54
CTRL	11	2010	84	2	82	80	4	4	0.24
CTRL	11	2011	202	75	127	64	138	19	2.44
CTRL	12	2010	75	2	73	65	10	5	0.52
CTRL	12	2011	207	63	144	81	126	25	2.39

VITA

Graduate School
Southern Illinois University

Meredith Lynne Mendola

mmendola123@gmail.com

Southern Illinois University Carbondale
Bachelor of Science, Forestry, May 2008

Special Honors and Awards:

Graduate Dean's Fellow, Graduate School, Southern Illinois University, 2011-2012
University Women's Professional Development Travel Grant, Southern Illinois University, 2012

Thesis Title:
Regional-climate and local-microbial controls on ecosystem processes during grassland restoration

Major Professor: Sara G. Baer