

**DISSERTATION**

**BIOGEOCHEMICAL RESPONSE OF U.S. GREAT PLAINS GRASSLANDS  
TO REGIONAL AND INTERANNUAL VARIABILITY IN PRECIPITATION**

**Submitted by**

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY REBECCA LYNNE MCCULLEY ENTITLED BIOGEOCHEMICAL RESPONSE OF U.S. GREAT PLAINS GRASSLANDS TO REGIONAL AND INTERANNUAL VARIABILITY IN PRECIPITATION BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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## **ABSTRACT OF DISSERTATION**

### **BIOGEOCHEMICAL RESPONSE OF U.S. GREAT PLAINS GRASSLANDS TO REGIONAL AND INTERANNUAL VARIABILITY IN PRECIPITATION**

Current climate change scenarios predict increasing variability in both the amount and timing of rainfall for the Great Plains region of North America. In this region, aboveground production is tightly linked to both long-term average and interannual precipitation patterns, suggesting that future changes in climate may have significant consequences for grassland ecosystem function. However, aboveground production accounts for only ~50% of the carbon input into these ecosystems, and little is known about the belowground production response or biogeochemical consequences of interannual variability in precipitation. Biogeochemical processes, such as nitrogen mineralization, determine the amount of resources available for plant growth and have shown sensitivity to alterations in water availability. Thus, interannual variability in precipitation is likely to have direct and indirect effects on plant production by influencing water availability and by altering biogeochemical processes.

In this dissertation, I address the influence of regional, seasonal, and interannual variability in precipitation on nitrogen (N) and carbon (C) cycling and microbial biomass and community composition in grassland ecosystems of the

Great Plains. At 5 sites spanning a 500 mm mean annual precipitation gradient and encompassing, from west to east, shortgrass steppe, mixed grass prairie, and tallgrass prairie plant community types, I measured monthly *in situ* net N mineralization and soil respiration rates and annual above- and belowground net primary production and litter decomposition rates during the 1999-2001 time period. To quantify variability in the microbial biomass and community composition I analyzed the phospholipid fatty acid content of soil samples taken in October 2000 and June 2001 from these 5 sites.

Carbon cycling rates and microbial biomass increased from semi-arid shortgrass steppe to sub-humid tallgrass prairie. At each site, C cycling rates were responsive to interannual variability in precipitation and this responsiveness varied across grassland community types. There were no significant regional, seasonal, or interannual trends in N cycling rates. Microbial biomass was larger during the growing season than in the fall, and microbial community composition was different for each of the 3 grassland types but was not significantly different across landscapes (uplands or lowlands) or between seasons at any of the sites.

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## TABLE OF CONTENTS

Chapter	Page
I. Introduction	1
II. <i>In situ</i> net nitrogen mineralization response to regional and interannual variation in precipitation across the Central Great Plains of North America	12
III. Regional and interannual variability in carbon cycling across the Central Great Plains of North America	45
IV. Microbial community composition across the Central Great Plains of North America: landscape versus regional variability	94
V. Summary and Conclusions	142
VI. Appendix I – Soil pedon descriptions for the sites	146

## CHAPTER I: INTRODUCTION

Variability in ecological processes exists across spatial scales from landscapes to regions, and over seasonal, interannual, and decadal temporal scales. In terms of both space and time, ecologists recognize that scale matters when interpreting data (Schneider 2001) and that the application of traditional space-for-time substitutions can result in erroneous predictions concerning ecosystem response to projected scenarios such as climate change. A primary example of the problem of space-for-time substitution has been reported for the grasslands of the Great Plains of North America (Lauenroth and Sala 1992). Here, a regional analysis has shown that mean annual precipitation (MAP) is highly correlated with aboveground net primary production (Figure 1.1). This relationship is not surprising given that these grasslands characteristically have lower annual precipitation than annual potential evapotranspiration rates and experience water stress at some point during the year (Borchert 1950, Lauenroth and Burke 1995). However, analyses of long-term temporal data from both western semi-arid shortgrass steppe (Lauenroth and Sala 1992) and eastern sub-humid tallgrass prairie (Knapp et al. 1998) have shown that the response of aboveground production to changes in precipitation among years at specific



locations within the region is more buffered than the relationship across sites, based on long-term average conditions suggests (Figure 1.1). Thus, utilizing the regional production versus MAP regression relationships to predict grassland production for any specific site in a given year would lead to over-estimates in wet years and under-estimates in dry years.

What causes this buffered site-level aboveground production response to interannual variability in precipitation? While the answer to this question is not definitive, it seems likely that other types of constraints on plant production (such as vegetative, species-level production responses, nutrient limitations, or other abiotic limitations such as temperature or light) interact to result in this observed interannual response (Knapp and Seastedt 1986, Lauenroth and Sala 1992, Burke et al. 1997, Knapp et al. 1998, Paruelo et al. 1999).

Grasslands are known to be primarily water and N limited (Hooper and Johnson 1999). Strong increasing mean annual precipitation trends from west to east across the Great Plains suggest that water limitation decreases from semi-arid shortgrass steppe to sub-humid tallgrass prairie (Burke et al. 1991, Lauenroth and Burke 1995). Soil organic matter, aboveground net primary production, litter C:N ratios, nitrogen use efficiency, and litter % lignin also increase from west to east across this region (Burke et al. 1989, Ojima et al. 1994, Epstein et al. 1997, Murphy et al. *In press*). Based on these patterns, a current biogeochemical model predicts that the relative importance of nutrient constraints, primarily nitrogen, on plant production increases from western

semi-arid shortgrass steppe to eastern sub-humid tallgrass prairie (Figure 1.2) (Ojima et al. 1994, Burke et al. 1997).

The response of plant production to interannual variability in precipitation is governed by the relative limitations of water and nitrogen across the time-scale in question. The dramatic regional climatic and biogeochemical patterns reflect long-term average conditions and belie the complexity of year-to-year variability and consequent interactions of these controlling parameters on plant production. However, the regional trends in the relative limitation of water and N to plant production (Figure 1.2) suggest that mixed grass prairie communities occurring in the middle of the range of both limiting factors should experience the lowest combined level of constraint on net primary production in any given year and may be more responsive to interannual variability in precipitation than the communities existing at either end of these limitation gradients (Paruelo et al. 1999). Additionally, mixed grass prairie contains species common to both shortgrass steppe and tallgrass prairie (Coupland 1992), and may therefore experience less community-level vegetative constraints on production. Paruelo et al. (1999), in a study of the interannual response of aboveground production of the Great Plains grasslands, found that mixed grass prairie communities with mean annual precipitation values ca. 475 mm experienced the largest interannual variability in production. From this, we can conclude that all grassland community types in the Great Plains region do not respond similarly to interannual variability in precipitation.

While the aboveground response to interannual variability in precipitation has been well documented, little is known about the belowground response of these grassland ecosystems. Given that belowground production in grasslands is characteristically equal to or 2-3 times more than aboveground production (Sims and Singh 1978, Hayes and Seastedt 1987, Milchunas and Lauenroth 1992, Rice et al. 1998, Milchunas and Lauenroth 2001) and most of the biogeochemical processes controlling nitrogen limitation experienced at individual sites occurs belowground, our understanding of grassland carbon and nitrogen cycling responses to interannual variability in precipitation is limited.

A recent analysis of long-term data has indicated that grasslands are more responsive to interannual variability in precipitation than many different biome types, thereby suggesting grasslands may be “ecological bellwethers” of climatic change (Knapp and Smith 2001). However, within the Great Plains, grassland types vary in their responsiveness to interannual variability in precipitation. In addition, current climate change scenarios for the Great Plains region predict decreasing summer precipitation, increasing summer temperatures, and increasing variability in both the amounts and timing of rainfall (Easterling 1990, Houghton et al. 1990, Karl et al. 1991). These projections, coupled with the recent interest in the carbon sequestration potential (Conant et al. 2001) of grassland ecosystems, suggest that a more complete understanding of grassland ecosystem response to variability in precipitation is needed.

The primary objective of my dissertation is to address the roles of regional, seasonal and interannual precipitation patterns on the nitrogen and carbon cycling of shortgrass steppe, mixed grass prairie and tallgrass prairie. To accomplish this objective, I established 5 sites spanning a 500 mm regional mean annual precipitation gradient across the Central Great Plains region of North America (Figure 1.3). I measured a variety of grassland ecosystem processes in 1999, 2000, and 2001. Chapter II specifically addresses the regional, seasonal and interannual trends in net nitrogen mineralization and plant available nitrogen from these different grassland community types. Chapter III presents data on the regional trends in carbon input and output fluxes, as well as addressing the interannual carbon cycling responsiveness of the different grassland types. Based on observed differences in nitrogen and carbon cycling across the grassland community types presented in Chapters II and III, Chapter IV describes and quantifies the spatial (landscape versus regional) and temporal (seasonal) variability associated with the microbial communities that control these biogeochemical processes in the different grassland types. I specifically address whether microbial community structure is primarily driven by landscape, regional, or seasonal scale processes. In Chapter V, I summarize the findings and present the main conclusions from my dissertation.

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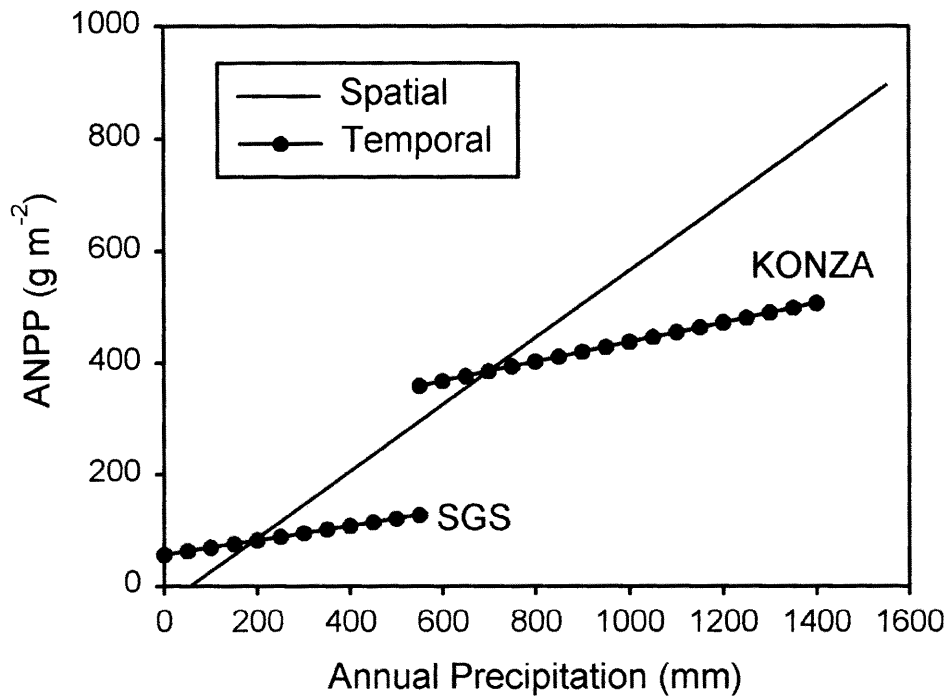
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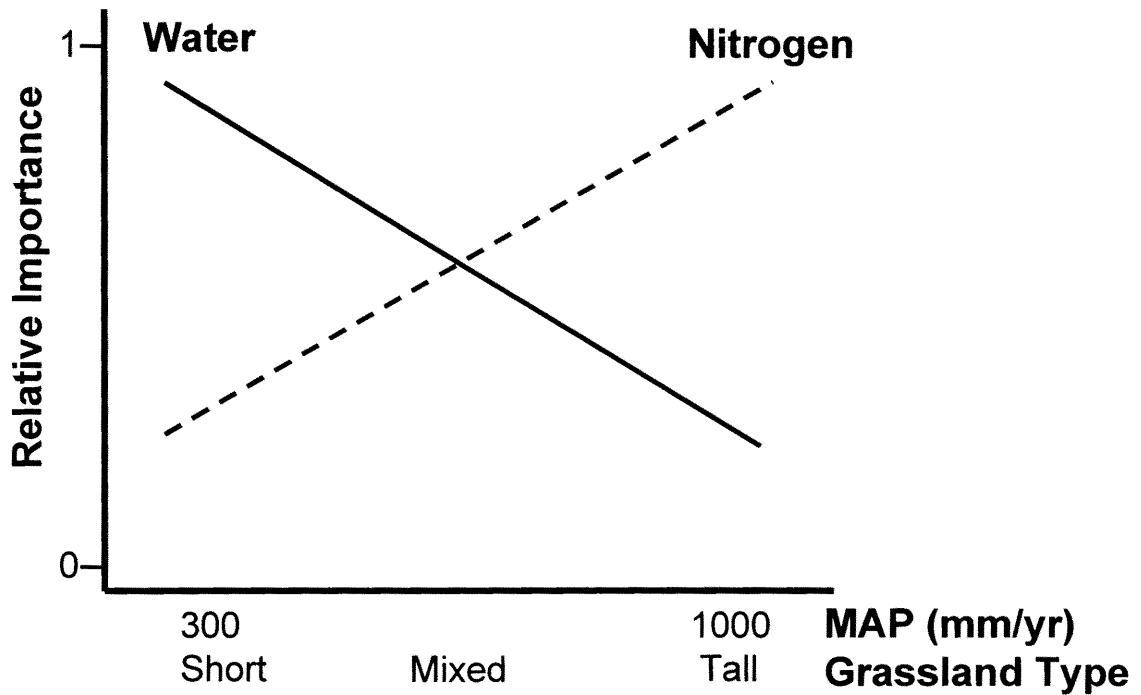
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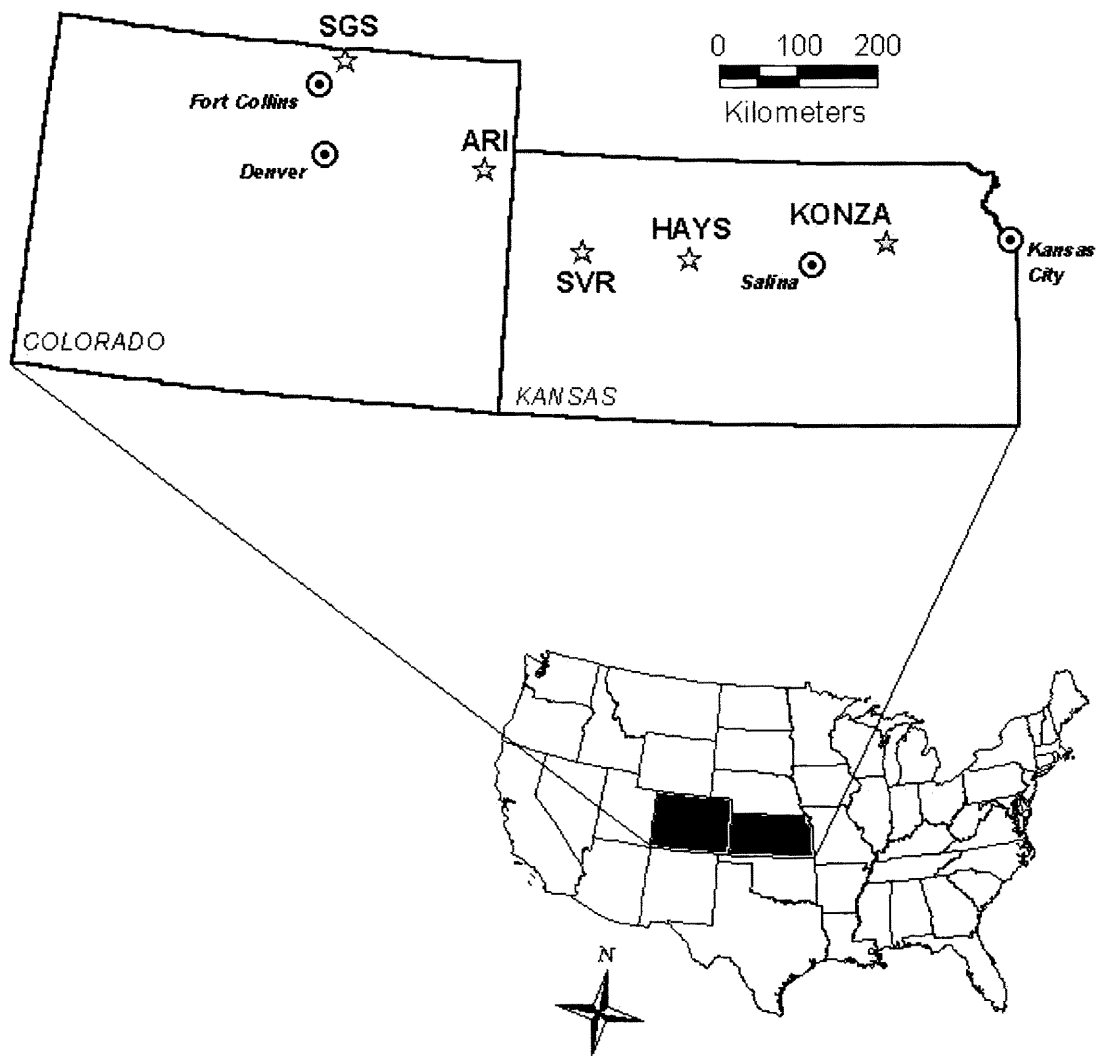


**Figure 1.1** - The spatial regional relationship of mean annual precipitation versus average aboveground net primary production (ANPP) is from Sala et al. (1988). The temporal relationships for both Long-Term Ecological Research sites (Shortgrass Steppe - SGS and Konza Prairie Natural Research Area - KONZA) included in this study were derived from long-term ANPP and actual yearly precipitation data from each of the sites (from Lauenroth and Sala 1992 and Knapp *et al.* 1998 for SGS and KONZA, respectively).





**Figure 1.2** - A conceptual diagram of the relative importance of water (solid line) and nitrogen (dashed line) in limiting plant production across the mean annual precipitation gradient (MAP) from shortgrass steppe (Short) to mixed grass (Mixed) and tallgrass (Tall) prairies.



**Figure 1.3** - The geographic location within the United States of the five sites used in this project. The site abbreviations correspond with Table 1 in Chapters II-IV.

## **CHAPTER II: IN SITU NET NITROGEN MINERALIZATION RESPONSE TO REGIONAL AND INTERANNUAL VARIATION IN PRECIPITATION ACROSS THE CENTRAL GREAT PLAINS OF NORTH AMERICA**

### **Introduction:**

Across the Central Great Plains region, large gradients in climatic conditions, soil organic carbon, soil nitrogen, microbial biomass and plant production exist (Burke et al. 1989, Zak et al. 1994, Epstein et al. 1996, Lauenroth et al. 1999). Current biogeochemical conceptual and simulation models predict that in situ net nitrogen (N) mineralization rates should mirror these general gradients and increase from western semiarid shortgrass steppe to eastern subhumid tallgrass prairie (Burke et al. 1997). However, while in situ net N mineralization rates have been measured at the Long-Term Ecological Research sites associated with the shortgrass steppe and tallgrass prairie at both ends of this regional gradient (Blair 1997, Turner et al. 1997, Hook and Burke 2000), the methods employed have varied (either covered or uncovered cores were used) as have the year of measurement and the associated climatic conditions. Additionally, no data exist for the southern mixed grass prairie that occurs in the middle of the semi-arid to sub-humid gradient. Therefore, our ability to make comparisons of in situ net N mineralization rates across this region is somewhat limited.

Grassland ecosystems are generally considered to be co-limited by water and nitrogen availability (Hooper and Johnson 1999). Indeed, water availability is thought to strongly govern rates of microbial activity and therefore N turnover and availability in these ecosystems frequented by drought (Schimel and Parton 1986). Future climate change scenarios for the Great Plains region predict alterations in the seasonal distribution and total annual quantities of precipitation (Easterling 1990, Houghton et al. 1990, Karl et al. 1991). Such changes could have significant impacts in grasslands where regional studies have shown important ecosystem processes, such as aboveground net primary production (ANPP) and net N mineralization, are tightly coupled to mean annual precipitation (Sala et al. 1988, Burke et al. 1997). A recent analysis of long-term data found evidence that aboveground net primary production in grassland ecosystems is more responsive to interannual variability in precipitation than other biome types (Knapp and Smith 2001), suggesting that grasslands may be the first biome type to show a response to climatic change. However, both ANPP and modeled rates of net N mineralization for these grassland ecosystems have been shown to be less sensitive to interannual variability at specific sites over time than the spatial relationship derived from mean annual precipitation across the region suggest (Sala et al. 1988, Lauenroth and Sala 1992, Burke et al. 1997). Mechanisms responsible for the different regional and interannual responses to alterations in precipitation have not been identified but are most likely related to either vegetational or biogeochemical constraints present at specific sites across the region (Paruelo et al. 1999).

Given the projected changes in water availability, the known co-limitation by water and nitrogen commonly experienced in these grasslands, and the relatively few data currently available to evaluate seasonal, interannual, and regional trends in net N mineralization across the Great Plains, I initiated an *in situ* net N mineralization study across a 500 mm mean annual precipitation gradient from semi-arid shortgrass steppe to sub-humid tallgrass prairie. Specifically, I addressed the following questions:

1. Are seasonal patterns in net N mineralization similar across grassland types?
2. Do rates of net N mineralization vary predictably with interannual changes in precipitation and are these interannual responses similar across grassland types?
3. Is there evidence to support modeling results that show a positive, linear increase in net N mineralization across the Great Plains?

## **Methods:**

### *Study Area*

I conducted this study at five grassland sites spanning a 800 kilometer transect from eastern Colorado to central-eastern Kansas (Table 2.1). All sites are native grassland managed with moderate levels of cattle grazing and have not been previously cultivated, as evidenced by detailed site histories and well-developed soil profiles that lack a plow layer. The two westernmost sites, the Shortgrass Steppe Long-term Ecological Research site (SGS) and a Nature

Conservancy owned ranch near the Arickaree River in central eastern Colorado (ARI), are typical shortgrass steppe plant communities, dominated by *Buchloe dactyloides* and *Bouteloua gracilis* (Lauenroth and Milchunas 1992). Smokey Valley Ranch (SVR), also owned by The Nature Conservancy, is located just north of the Smokey River in central western Kansas and has a mixture of both shortgrass steppe and mixed grass prairie vegetation. In central Kansas, a southern mixed grass prairie owned and managed by Ft. Hays State University (HAYS) is dominated by plants typically found in this grassland community type, *Schizachyrium scoparium* and *Bouteloua curtipendula* (Coupland 1992). The most eastern site, the Konza Prairie Biological Station (KONZA), is owned by The Nature Conservancy, managed by Kansas State University, and is also a Long-term Ecological Research site. KONZA is a tallgrass prairie with dominant plant species being *Andropogon gerardii* and *Sorghastrum nutans* (Silletti and Knapp 2000). KONZA is the only one of my sites where management includes fire. My plots at this site are located in a watershed that is burned once every four years. The plots were burned in April 1996 and 2000.

Both mean annual temperature and precipitation increase from west to east across the transect (Table 2.1). During site selection, I attempted to minimize soil textural differences between sites. Monthly precipitation and air temperature data were obtained from on-site meteorological stations at KONZA and SGS. For the other sites, data from the nearest meteorological station associated with either the National Climatic Data Center or the Colorado

Agricultural Meteorological Network were used. At each site, 4 permanent plots (4m x 4m) on a level upland were fenced to exclude cattle in late June of 1999.

#### *In situ Net Nitrogen Mineralization*

I used the uncovered intact core incubation technique (Raison et al. 1987, Hook and Burke 1995) to estimate *in situ* net N mineralization. Within each of the 4 permanent plots, 2 adjacent soil cores (4.8 cm internal diameter, 15 cm long) were removed. I immediately placed one core sample in a plastic bag and stored it on ice until initial N extraction took place. The second core was removed in an aluminum sleeve, the bottom 2.5 cm of soil were removed, and a nylon mesh bag containing ~25 g mixed cation and anion exchange resins was inserted to adsorb ions leaching through the core during the field incubation (Binkley and Matson 1983). I re-inserted the intact, uncovered core back into the ground. I collected the field incubation cores 28-32 days later.

Collected soils were sieved through a 2 mm screen prior to extracting inorganic N. Due to the high clay content of the soil at KONZA, 2 mm sieving was not always possible for these soils (when soils were either overly wet or dry). I quantified the effect of sieving on the extractable N content by extracting both sieved and un-sieved soil samples over the course of the study. Sieving had no effect on extractable  $\text{NO}_3\text{-N}$ , but did increase the amount of  $\text{NH}_4\text{-N}$  extracted. I used the regression equation of the significant relationship between extractable  $\text{NH}_4\text{-N}$  in sieved and un-sieved soil samples to correct  $\text{NH}_4\text{-N}$  concentrations from KONZA in the months when sieving was not possible. I

extracted 25 g soil subsamples and individual resin bags in 50 mL of 2.0 M KCl-phenyl mercuric acetate (5 ppm). All extractions occurred within 8 hours of field soil collection. The  $\text{NH}_4$  and  $\text{NO}_3+\text{NO}_2$  concentrations of the extracts were determined colorimetrically on an Alpkem Autoanalyzer (Perstorp Analytical, Silver Springs, MD). I calculated net N mineralization as the change in  $\text{NH}_4$  plus  $\text{NO}_3+\text{NO}_2$  between the initial and field incubated soil samples plus the inorganic N found on exchange resins.

I estimated *in situ* net N mineralization monthly from July-September in 1999 and from May-September in 2000. Previous work from the semi-arid shortgrass steppe has shown that presence and absence of plants can have significant effects on measured biogeochemical parameters (Hook et al. 1991), and that this effect varies across this regional transect (Vinton and Burke 1997). Therefore, in 1999, I sampled net N mineralization directly under stems/crowns of plants and in open, interspaces between plants in each plot at all sites (2 locations (between and under plants) x 4 plots, n=8 per site and month). Statistical analyses of these data indicated that there were no differences in *in situ* net N mineralization rates from these two locations (between and under plants). Consequently, I reduced and randomized the sampling for 2000 (no location subsampling – only 1 incubation per plot, n=4 per site and month).

#### *Soil Moisture, C and N content, and Bulk Density*

On the same day that *in situ* net N mineralization incubations were started, I collected additional soil cores (same dimensions as those for



incubations) for determining soil moisture content. Soil was removed from the corer, thoroughly mixed, and a sub-sample was placed in a tin can and sealed with electrical tape until the sample could be oven-dried. I weighed samples, dried them in a 110 °C oven for 3 days, and re-weighed for gravimetric soil moisture determination. Using the gravimetric soil moisture and bulk density data, soil water-filled pore space was calculated according to (Linn and Doran 1984).

I analyzed the August 1999 initial soil samples for carbon and nitrogen content (15 cm depth). Sieved soils were air-dried, ground in a ball mill, acid-washed to remove inorganic C, and analyzed on a LECO CHN-1000 analyzer (St. Joseph, MI) for organic C and total N. I used air-dried, sieved sub-samples for texture analysis utilizing the hydrometer method (Gee and Bauder 1986). HAYS soils contained significant quantities of calcium carbonate; therefore, soil organic C was determined via the loss-on-ignition method (Nelson and Sommers 1996). I collected additional soil cores of the same dimensions in August 1999 for bulk density determination (1 core per plot).

#### *Aboveground Net Primary Production and N content*

I estimated aboveground net primary production (ANPP) by clipping 10, 0.5 m<sup>2</sup> quadrats in August, the month of peak standing biomass, from additional exclosure cages placed randomly around the permanent fenced plots. Biomass was sorted at the time of clipping to remove previous year's standing dead from current year's alive and dead material and into grass and forb components.

Biomass samples were dried at 50°C for a week, weighed, and then ground in a Wiley mill. Ground samples were well-mixed and sub-sampled for ash and C and N determination. I ground this subsample yet more finely in a ball grinder, and a portion was analyzed for total C and N content on the LECO CHN-1000 analyzer mentioned previously. I combusted an additional portion of the ballground sample in a muffle furnace at 500°C for 16 hours to estimate percent ash content. The nitrogen content of the aboveground production (ANPP-N) is presented on an ash-free basis.

I calculated the newly available plant N values (Table 5) by using the ANPP-N values in conjunction with average root:shoot ratios and estimates of the percent of ANPP-N translocated from shoots to roots at the end of the growing season for each of the 5 sites (determined from a concurrent study (McCulley 2002). The ANPP-N and root:shoot ratios allowed calculation of the amount of N stored in net primary production (NPP-N). Newly available plant N is the difference between NPP-N and the amount of N translocated during senescence.

### *Statistics*

Given my interest in determining seasonal patterns, interannual relationships, and regional trends in net N mineralization rates, I analyzed the data using both ANOVA and regression analyses in the SAS (version 8) statistical software program (SAS 1989). I used a significance level of 0.05 for all analyses. Because my net N mineralization and water-filled pore space soil

moisture datasets have 3 months of data in 1999 and 5 months in 2000, I ran an ANOVA investigating the site and month main effects on each year's data separately, incorporating all months in a repeated measures design. In addition, I ran the common months for the 2 years of data (July-September) together in a repeated measures ANOVA addressing the significance of the site, month, and year main effects. I also summed the common months of net N mineralization data to one value for each plot, site and year measured. I then analyzed these 3-month total net N mineralization values with an ANOVA for site and year main effects. Similarly, I analyzed the aboveground net primary production N content (ANPP-N) and the total amount of inorganic N captured in the resin bags over the common 3-month collection period in an ANOVA for site and year main effects. I evaluated relationships between monthly climatic and soil moisture parameters for both years of monthly net N mineralization data using linear regression analyses. I also used regression to evaluate the relationships between the 3-month total net N mineralization data and climatic, edaphic, and biotic parameters that varied between the two measurement years and across the regional transect. I transformed all data as needed to fit the normality assumptions of the applied statistical test.

## **Results & Discussion:**

*Are seasonal patterns in net N mineralization similar across grassland types?*

In general, the rates of monthly *in situ* net N mineralization that I estimated in this study were low (between  $-0.4$  and  $0.5 \mu\text{g N g soil}^{-1} \text{ day}^{-1}$ ) but

within the range of other previously reported grassland *in situ* net N mineralization data (Wedin and Tilman 1990, Ruess and Seagle 1994, Turner et al. 1997, Frank and Groffman 1998, Johnson and Matchett 2001). All statistical analyses showed a highly significant 'site\* month' interaction term (Figure 2.1, Table 2.2), indicating that seasonal monthly net N mineralization trends differed across the sites and grassland community types sampled in this study. This result differs somewhat from previous work in the shortgrass steppe and tallgrass prairie and from biogeochemical Century modeling predictions. All of these studies have shown seasonal peaks in net N mineralization rates in May and June (Blair 1997, Turner et al. 1997, Hook and Burke 2000, Kelly et al. 2000). In general, the site exhibiting the most seasonal variability in net N mineralization rates was KONZA and July was the month with the largest spatial variability in net N mineralization rates across the region (as represented by the range of values across sites within a month) for both years of the study.

Seasonal patterns in net N mineralization, a microbial-mediated process, are thought to reflect variation in climatic conditions and substrate quality and quantity over the course of the year. My data indicate air temperature trends across months were similar for all sites, peaking in July and August in both years; however, patterns in monthly precipitation were highly variable across sites and between years (Figure 2.1). KONZA was generally the wettest site (Figure 2.1), but interpretation of the water-filled pore space data is difficult as all site, month, and year interaction terms were significant (Table 2.2). The apparent lack of a strong seasonal net N mineralization trend across the region

may be related to confounding water availability and temperature conditions across months and sites.

The seasonal relationship between monthly net N mineralization rates and climatic variables was explored further using regression analyses. Regressions run on monthly net N mineralization and monthly precipitation, temperature, and the water-filled pore space soil moisture data for both years at each site separately indicate that at the two semi-arid shortgrass steppe sites (SGS and ARI), monthly net N mineralization was significantly related to monthly precipitation. However, there was no significant relationship between the mixed grass prairie sites (SVR and HAYS) and these climatic parameters, and at the tallgrass prairie site, KONZA, monthly net N mineralization was negatively related to monthly temperatures but showed no relationship with precipitation (Table 2.3). These results are consistent with the general knowledge that shortgrass steppe is more water-limited than tallgrass prairie and that therefore, ecosystem processes in this grassland type are more sensitive to variability in precipitation and resulting water availability (Burke et al. 1998), although other studies addressing the role of water availability and precipitation on net N mineralization in the shortgrass steppe have found no relationship (Schimel and Parton 1986, Hook and Burke 2000). The negative relationship found in my data between monthly air temperature and net N mineralization at Konza is somewhat counter-intuitive given that increased temperatures are predicted to stimulate net N mineralization and decomposition processes in general (Burke et al. 1997).

While these regression relationships support the idea that climate plays some role in determining seasonal net N mineralization rates, particularly at the dry and wet end of the regional gradient, the proportion of variability accounted for in all significant monthly regression models was low ( $R^2 < 0.2$ , Table 3). The low predictive capability of the climate regression models coupled with the observed general lack of a major monthly seasonal net N mineralization trend suggests that monthly net N mineralization rates in this grassland region are the result of complex interactions among the controlling parameters measured here and perhaps other additional parameters not measured, such as substrate quantity and quality for microbial activity.

#### *Interannual and Regional Variability in Net N Mineralization*

I hypothesized that interannual variability in precipitation would be tightly coupled to growing season net N mineralization rates at all sites across the regional gradient. My hypothesis was based on the observations that grasslands are characterized by frequent periods of drought occurring at seasonal, interannual, and decadal time-scales (Borchert 1950, Lauenroth and Burke 1995) and that tight relationships between water-availability and ecosystem processes, such as net primary productivity, have been repeatedly substantiated in these ecosystems (Sala et al. 1988, Lauenroth and Sala 1992, Knapp et al. 2001). All 5 sites received more precipitation in 1999 during the July-September growing season than in 2000, and in support of my hypothesis, 2000 total net N mineralization values were lower than those measured in 1999

('year' p-value=0.005), except for ARI, where the reverse was true, hence the significant 'site\*year' interaction term (Figure 2.2, Table 2.4). While these overall interannual trends suggest a positive relationship between water-availability and net N mineralization, regressions indicated that KONZA was the only site with a significant relationship between the total July-September net N mineralization and the average July-September water-filled pore space (Table 2.3). However, since KONZA was burned in April 2000 as part of a traditional 4-year fire return interval management plan and studies from tallgrass prairie have shown that burning previously unburned sites reduces net N mineralization (Blair 1997), it is possible that the reduction in net N mineralization measured in this study in 2000 at KONZA could be a combined result of the fire as well as reflecting the more than 100 mm decrease in precipitation between the two measured growing seasons. Therefore, while my data do indicate significant differences between the 1999 and 2000 growing season net N mineralization rates, the lack of a relationship between growing season net N mineralization and precipitation at majority of the sites and the low proportion of variance explained by 'year' in the statistical model is small ( $R^2=0.14$ ) suggests that net N mineralization is less sensitive to interannual variability in precipitation than I originally hypothesized.

Regionally, mean annual precipitation, aboveground net primary production, and the total amount of N contained in aboveground production increase from semi-arid shortgrass steppe to subhumid tallgrass prairie (Figure 2.2, Table 2.4). These trends suggest that N availability to plants increases in a

similar fashion. In these grassland systems, N is made available to plants primarily via net N mineralization; therefore, I hypothesized that N mineralization rates should increase from shortgrass steppe to tallgrass prairie. However, contrary to this hypothesis, my July-September total net N mineralization data do not increase in such a manner (Figure 2.2). In fact, no significant 'site' main effect was found for either the two years of July-September total net N mineralization (Table 2.4) or the more seasonally comprehensive May-September total net N mineralization data available for the year 2000 only (Table 2.5). In 1999, means separation tests on the significant 'site' main effect (Figure 2.1) indicated that rates of monthly net N mineralization were higher at KONZA than the other sites, but the trend was not consistent across years (as indicated by the significant 'site\*year' interaction term, Table 2.2).

This observed lack of a regional trend in net N mineralization rates was surprising. To further explore potential regional relationships between net N mineralization rates and average climatic and edaphic conditions in my data, I ran regression analyses on each year's July-September net N mineralization for all sites together. In 1999, mean annual precipitation and soil organic carbon explained 35% of the variance in net N mineralization values, but in 2000, clay content alone explained 34% of the variance (Table 2.3). These results concur to some extent with a previous meta-analysis of data collected from many different ecosystem types that found that net N mineralization increased with increasing mean annual precipitation ( $R^2=0.71$ ) and temperature ( $R^2=0.29$ ,



Rustad et al. 2001). However, temperate forest literature has reported that litter lignin:N ratios explain significantly more of the variation ( $R^2=0.74$ ) in net N mineralization rates than climatic variables (Scott and Binkley 1997). To address the relationship of litter quality and net N mineralization rates and in an attempt to explain as much variation in the dataset as possible, I ran a regression with the net N mineralization data and all interannual and regional parameters, including ANPP-N and above- and belowground lignin:N ratios in one model (Table 2.3). Contrary to the temperate forest findings, no relationship between net N mineralization and ANPP-N and/or above- and belowground lignin:N ratios were found. These results are similar to the one site grassland dataset analyzed in by Scott and Binkley (1997). My complete regression model explained only 27% of the variance in net N mineralization rates. Of the 3 significant parameters (total July-September precipitation, soil organic C, and soil water-filled pore space) only total July-September precipitation had a significant relationship with net N mineralization when run in the model alone (Figure 2.3), explaining only 8% of the variance.

These regression results are similar to those found in a larger grassland regional study of shorter duration (Barrett et al. *In Press*). Both studies report low  $R^2$  values for the regression models and indistinct and confounding seasonal and regional relationships between net N mineralization and climatic as well as vegetation parameters. Taken together, these studies suggest that grassland regional net N mineralization relationships may not be particularly robust or predictable.

### *Comparison to Modeling Results*

The lack of significant differences in net N mineralization across the various grassland plant communities studied here directly contradicts modeling predictions across this region. CENTURY, a biogeochemical model developed and validated for the Great Plains (Parton et al. 1987), predicts large ( $>4 \text{ g N m}^{-2} \text{ year}^{-1}$ ) linear increases in net N mineralization as grassland community type changes from shortgrass steppe to tallgrass prairie (Burke et al. 1997). My field-derived net N mineralization numbers are systematically lower than CENTURY predictions, but are within the range of other previously reported *in situ* net N mineralization values for both the shortgrass steppe and tallgrass prairie sites (Table 5). Discrepancies between CENTURY modeled and field-derived values are common (Kelly et al. 2000) and could be the result of several potential factors. First, *in situ* cores sever roots, which prevent active plant N uptake and might enhance net N mineralization estimates. Conversely, severed roots could act as a C source for microbial metabolism and increase immobilization of N. Finally, *in situ* cores often experience altered soil moisture content and potentially temperature depending on the type of material the sleeve is made of, the ambient conditions, and soil texture (Hook and Burke 1995). Model predictions do not have the problems associated with altering immobilization potential and soil microclimate.

My estimates of 'plant available N' (Table 2.5) are closer to the CENTURY model predictions than the field-derived estimates, but are in

general still significantly lower than the model predictions. The difference between the newly available plant N estimates and the May-September net N mineralization rates represents what Barrett et al. (*in press*) called the “N deficit,” i.e. the amount of N measured in plant production that was not measured via *in situ* net N mineralization techniques. Barrett et al. (*in press*) reported that N deficit increased from shortgrass steppe to tallgrass prairie. This general N deficit trend suggests that N immobilization potential increases from shortgrass steppe to tallgrass prairie, which is consistent with results from an <sup>15</sup>N lab incubation experiment across a soil organic carbon gradient in the Great Plains (Barrett and Burke 2000). I found no general increase in the N deficit across the region (Table 2.5); however, the total growing season N trapped in the resin bags decreased from shortgrass steppe to tallgrass prairie, despite increasing precipitation and therefore increasing potential N leaching losses through the soil cores (Figure 2.4). This result suggests that immobilization of N at KONZA keeps N from moving through the soil cores with available water and becoming trapped in the resin.

My data suggest that trends of net N mineralization across the region and between years at specific sites are subtle and possibly do not reflect patterns of plant available N due to complex interactions between gross N mineralization and immobilization. Immobilization is a process dependent on the size and activity of the microbial biomass, the availability of a readily mineralizable substrate pool, and the climatic conditions (Zak et al. 1990, Kaye and Hart 1997), all of which vary seasonally, interannually, and regionally

across broad climatic and vegetation gradients, such as the gradient in this study (Zak et al. 1994, McCulley 2002). Interpretation of *in situ* net N mineralization rates or gaining a better quantified estimate of the amount of N internally cycled in these grassland ecosystems will remain difficult without a better understanding of the seasonal, interannual, and regional patterns of gross N mineralization and immobilization processes.

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**Table 2.1:** Characteristics of 5 sites spanning a precipitation gradient in the Great Plains (SGS – the Shortgrass Steppe Long-Term Ecological Research (LTER) site, ARI – the Arickaree River Ranch, a shortgrass steppe site owned by The Nature Conservancy (TNC), SVR – the Smokey Valley River Ranch, a mixed grass/shortgrass steppe site also owned by TNC, HAYS – the Hays Range Area, a mixed grass prairie owned by Ft. Hays State University, KONZA – the Konza Prairie Research Natural Area and LTER site, MAP = mean annual precipitation, MAT = mean annual temperature, NPP = net primary production). All soil parameters are from the top 15 cm. Values are averages  $\pm$  standard deviations. Different letters indicate significant differences between means within rows ( $p$ -value  $\leq 0.05$ ). Statistical results were the same on soil carbon and nitrogen pools (expressed on a  $\text{g m}^{-2}$  basis) as the concentrations reported here.

	<b>SGS</b> Shortgrass Steppe - LTER	<b>ARI</b> Arickaree - TNC	<b>SVR</b> Smokey Valley - TNC	<b>Hays</b> Ft. Hays State University	<b>KONZA</b> Konza Prairie - LTER
<b>Climate &amp; Vegetation:</b>					
Latitude	40° 52'	39° 45'	39° 54'	38° 53'	39° 06'
Longitude	104° 41'	102° 30'	100° 58'	99° 23'	96° 32'
MAP (mm)	345	450	506	578	835
MAT (°C)	8.5	10.3	11.6	11.9	13
Vegetation Type	shortgrass steppe	shortgrass steppe	mixed grass	mixed grass	tallgrass
<b>Soils:</b>					
Textural Class	Clay loam	Sandy loam	Silt loam	Loam	Silty clay loam
% Sand	40 <sup>b</sup>	56 <sup>a</sup>	24 <sup>c</sup>	39 <sup>b</sup>	18 <sup>d</sup>
% Clay	30 <sup>b</sup>	18 <sup>d</sup>	25 <sup>c</sup>	24 <sup>c</sup>	38 <sup>a</sup>
Bulk Density ( $\text{g cm}^{-3}$ )	0.90 $\pm$ 0.10 <sup>b</sup>	1.17 $\pm$ 0.06 <sup>a</sup>	0.98 $\pm$ 0.09 <sup>b</sup>	1.05 $\pm$ 0.12 <sup>ab</sup>	1.01 $\pm$ 0.13 <sup>b</sup>
pH <sup>§</sup>	7.6	7.4	7.3	7.4	6.9

	<b>SGS</b> Shortgrass Steppe - LTER	<b>ARI</b> Arickaree - TNC	<b>SVR</b> Smokey Valley - TNC	<b>Hays</b> Ft. Hays State University	<b>Konza</b> Konza Prairie - LTER
Organic C (mg C g soil <sup>-1</sup> )	17.27 ± 1.72 <sup>c</sup>	8.74 ± 1.47 <sup>d</sup>	18.50 ± 1.40 <sup>c</sup>	34.31 ± 5.30 <sup>a</sup>	26.76 ± 3.76 <sup>b</sup>
Total N (mg N g soil <sup>-1</sup> )	1.95 ± 0.14 <sup>c</sup>	1.20 ± 0.11 <sup>d</sup>	1.89 ± 0.14 <sup>c</sup>	3.20 ± 0.67 <sup>a</sup>	2.70 ± 0.33 <sup>b</sup>
Soil C:N	8.87 ± 0.63 <sup>a</sup>	7.27 ± 0.98 <sup>a</sup>	9.78 ± 0.77 <sup>a</sup>	10.71 ± 0.61 <sup>a</sup>	9.92 ± 0.88 <sup>a</sup>

<sup>s</sup> - E.F. Kelly (unpublished data).

**Table 2.2:** Analysis of variance statistics (ANOVA) for the common months across both years (July, August, and September for 1999 and 2000) of *in situ* net nitrogen mineralization and water-filled pore space (Wfps) soil moisture data from the 5 sites spanning the regional precipitation gradient. Partial  $R^2$  are given for all significant ( $p$ -value $<0.05$ ) main effects and interactions.

	Net Nmin			Wfps		
	<i>F</i>	<i>P-value</i>	$R^2$	<i>F</i>	<i>P-value</i>	$R^2$
<b>Site</b>	1.56	0.2262	---	28.77	<0.0001	0.886
<b>Month</b>	1.89	0.1548	---	295.53	<0.0001	0.189
<b>Site*Month</b>	5.02	<0.0001	0.194	58.96	<0.0001	0.151
<b>Year</b>	10.69	0.0014	0.242	342.62	<0.0001	0.844
<b>Site*Year</b>	4.79	0.0012	0.424	12.13	0.0001	0.120
<b>Month*Year</b>	0.09	0.9156	---	434.91	<0.0001	0.279
<b>Site*Month*Year</b>	5.81	<0.0001	0.222	133.43	<0.0001	0.342

**Table 2.3:** In order to evaluate the relationships between net nitrogen mineralization (Nmin) values and abiotic variables and other site-associated characteristics, we ran regression analyses on the *monthly* Nmin rates with abiotic variables that varied on a monthly time-step (precipitation - monPPT, air temperature - monTemp, and water-filled pore space) for each site separately. Interannual regressions were run on July-September total net nitrogen mineralization data and July-September average water-filled pore space (Wfps) for both years by site. Regional regressions were run by year on July-September total net nitrogen mineralization data and variables that varied at the 'site' or regional level: % sand, % silt, % clay (%Clay), soil organic carbon (SOC), total soil nitrogen, bulk density, soil C:N ratio, mean annual precipitation (MAP), mean annual temperature, and the July-September total precipitation (PPT) and average temperature for the actual year of measurement. A regression incorporating both the interannual and regional variability was run on the July-September total net nitrogen mineralization and all interannual and regional variables, as well as aboveground net primary production nitrogen content (ANPP-N) and above- and belowground lignin:N ratios for both years. All models and variables shown are significant at the  $p < 0.05$  level (N.S. indicates no significant relationship between N mineralization and covariates).

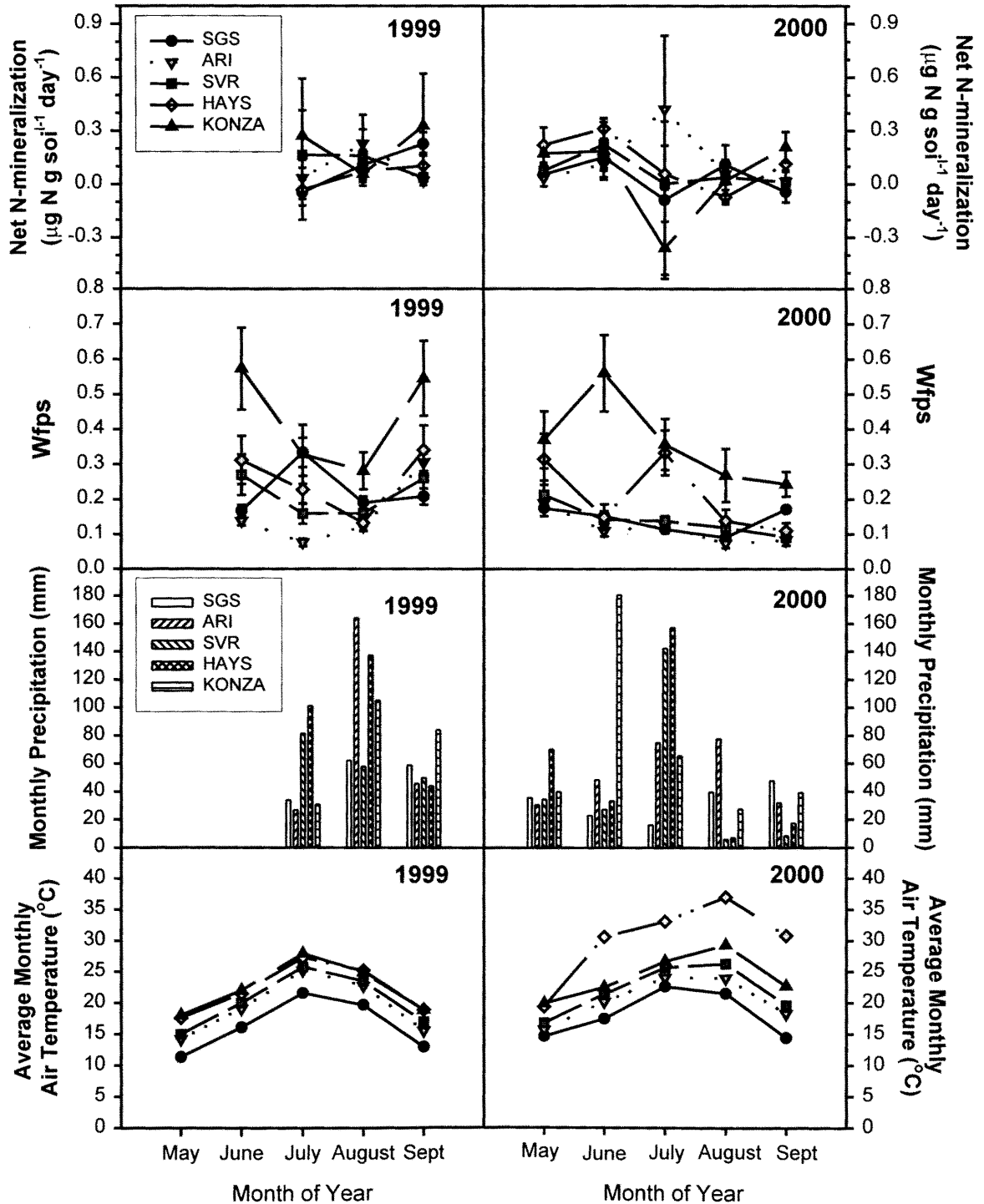
<b><i>Regression Equations</i></b>	<b><i>R<sup>2</sup></i></b>
<b>Monthly</b>	
SGS: $N_{min} = -0.111 + 0.004(monPPT)$	0.147
ARI: $N_{min} = 0.005 + 0.002(monPPT)$	0.181
SVR:	N.S.
HAYS:	N.S.
KONZA: $N_{min} = 0.739 - 0.025(monTemp)$	0.110
<b>Interannual</b>	
SGS:	N.S.
ARI:	N.S.
SVR:	N.S.
HAYS:	N.S.
KONZA: $N_{min} = 0.57 + 4.26(Wfps)$	0.444
<b>Regional</b>	
1999: $N_{min} = 1.209 + 0.001(MAP) - 0.0001(SOC)$	0.351
2000: $N_{min} = 4.399 - 0.146(\%Clay)$	0.344
<b>Interannual &amp; Regional</b>	
$N_{min} = 1.576 + 0.025(PPT) - 0.0001(SOC) + 1.27(Wfps)$	0.267

**Table 2.4:** ANOVA between years (1999 and 2000) and across the 5 sites (SGS, ARI, SVR, HAYS, and KONZA) for the July-September 3 month total *in situ* net nitrogen mineralization (Net Nmin), the nitrogen stored in aboveground net primary production at peak standing biomass (ANPP-N), and the total inorganic nitrogen captured in the resin bags (Resin-N). Partial R<sup>2</sup> are given for all significant (p-value<0.05) main effects and interactions.

	Net Nmin			ANPP-N			Resin-N		
	<i>F</i>	<i>P-value</i>	<i>R</i> <sup>2</sup>	<i>F</i>	<i>P-value</i>	<i>R</i> <sup>2</sup>	<i>F</i>	<i>P-value</i>	<i>R</i> <sup>2</sup>
<b>Site</b>	2.13	0.1174	---	66.89	<0.0001	0.848	32.75	<0.0001	0.896
<b>Year</b>	8.96	0.0050	0.141	75.59	<0.0001	0.420	42.16	<0.0001	0.375
<b>Site*Year</b>	4.90	0.0030	0.321	16.02	<0.0001	0.320	8.79	<0.0001	0.313

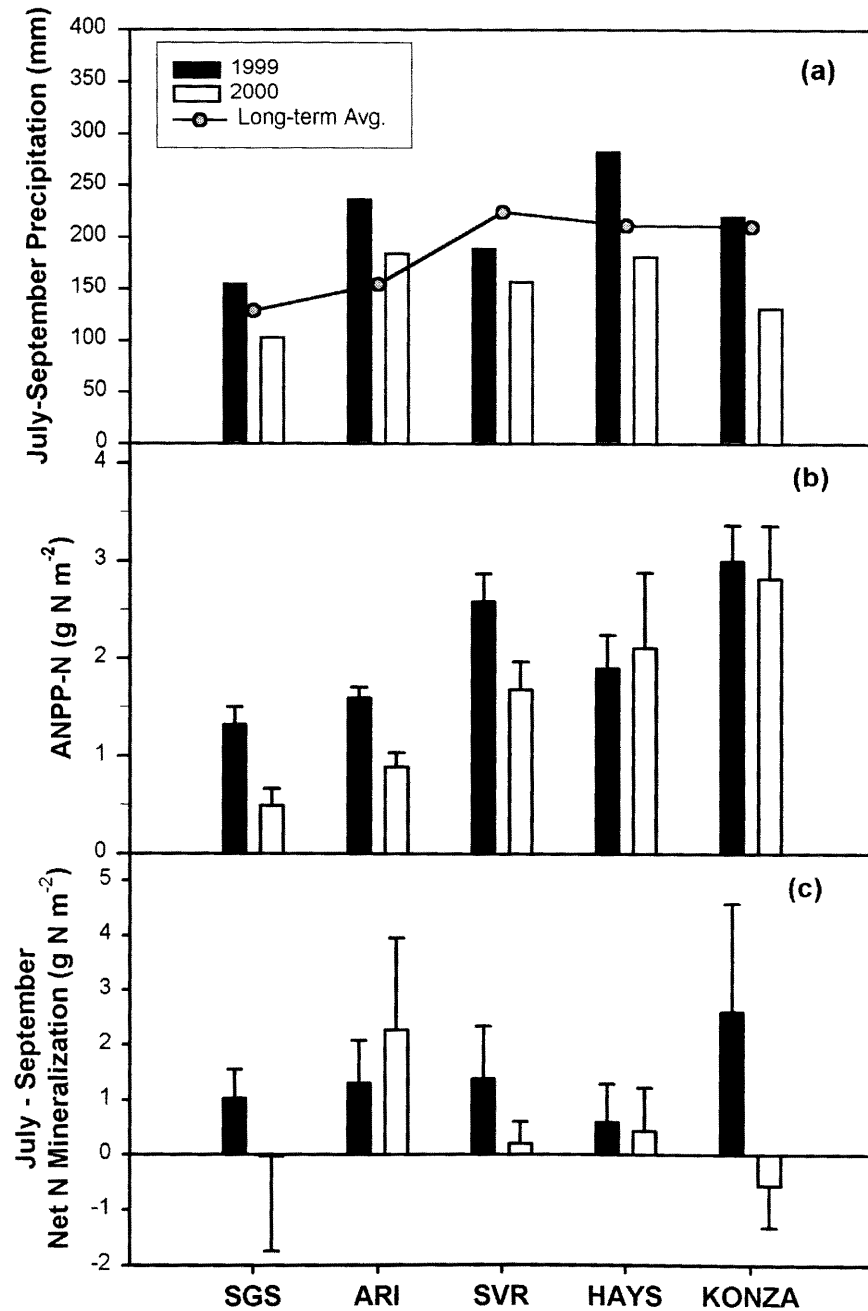
**Table 2.5:** Comparison of this study's 5 month total *in situ* net N mineralization rates (May-September of 2000, g N m<sup>-2</sup>) and those from other field studies conducted at the same sites or grassland community types, as well as, biogeochemical model (Century) net N mineralization predictions for the Central Great Plains region. The mean annual precipitation for the 5 sites of this study were used with the Burke et al. (1997) regression relationships for net nitrogen mineralization rates derived from Century to generate values specific to the individual sites. Results from this study are reported as averages  $\pm$  standard deviation. The range of values reported in the literature is presented. Methods for determining *in situ* net N mineralization, the length of the field incubations, and the duration of measurements throughout the year varied from study to study. Back calculations of annual plant available N were based on aboveground production, nitrogen content, root:shoot ratios, and N translocation data collected in a concurrent study (McCulley 2002).

Reference	Shortgrass Steppe		Mixed Grass Prairie		Tallgrass Prairie
	SGS	ARI	SVR	HAYS	KONZA
<b><i>In situ</i> Net N Mineralization</b>					
This study	0.71 $\pm$ 1.91	2.93 $\pm$ 2.07	1.36 $\pm$ 0.63	2.64 $\pm$ 1.42	0.86 $\pm$ 0.63
Ojima et al. (1994)	----	----	----	----	0.71 – 2.65
Turner et al. (1997)	----	----	----	----	0.5 – 2.0
Blair (1997)	----	----	----	----	1.0 – 4.0
Wedin and Tilman (1990)	----	----	----	----	1.0 – 6.0
Schimel et al. (1985)	3.0 – 5.5	----	----	----	----
Hook and Burke (2000)	1.5 – 9.1	----	----	----	----
<b>Century Model Predictions</b>					
Burke et al. (1997)	3.18	4.24	4.80	5.53	8.13
Ojima et al. (1994)	----	----	----	----	3.2 – 4.6
<b>New Annual Plant Available N</b>					
This study	1.64	2.21	3.26	2.84	3.93
<b>N Deficit (Plant Available N – Net N min.)</b>					
This study	-0.93	+0.72	-1.90	-0.20	-3.07

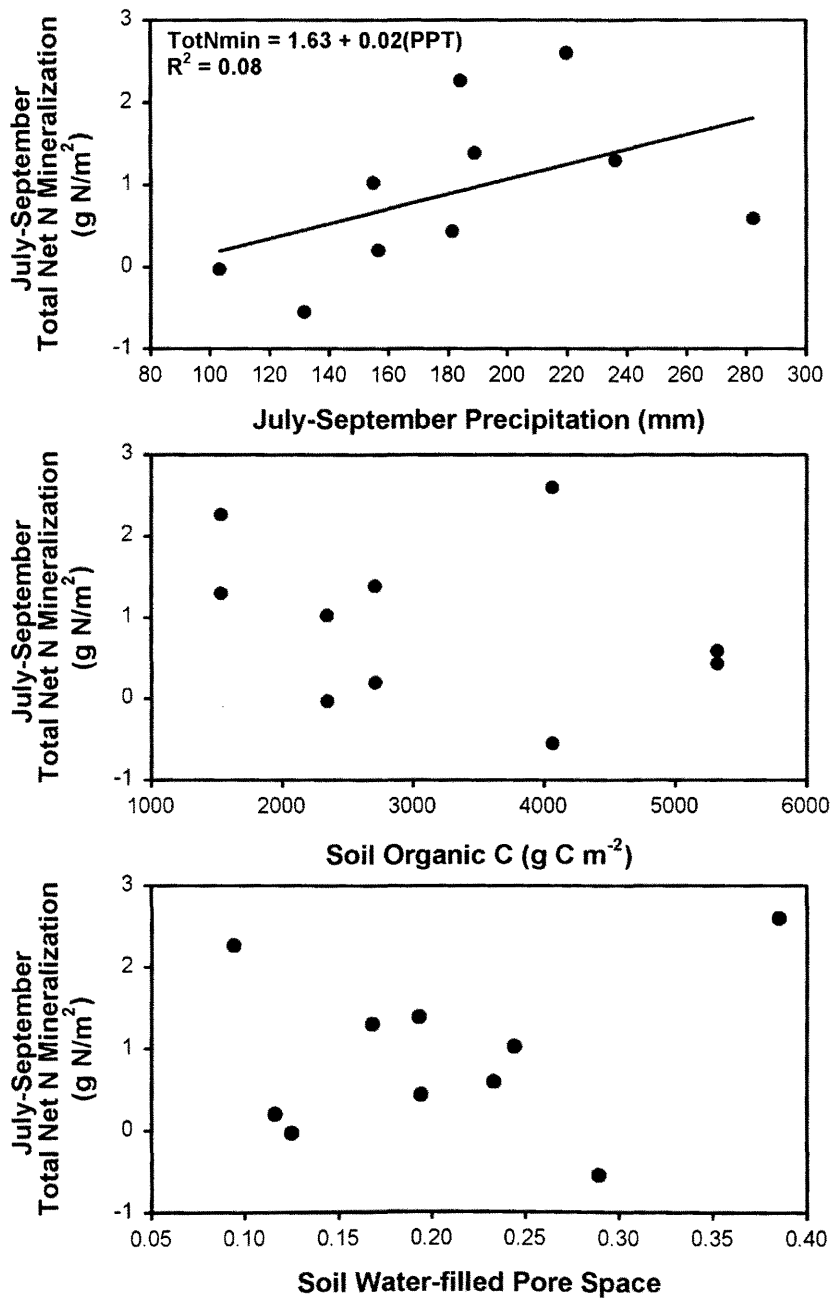


**Figure 2.1** - Mean  $\pm$  1 S.D. monthly net nitrogen (N) mineralization, water-filled pore space (data points represent only one day during the month), and monthly precipitation and air temperature. Repeated measures ANOVA on the monthly net N mineralization data indicated that for 1999, the main effect 'site' was significant ( $p$ -value $<$ 0.01, partial  $R^2=0.533$ ) but 'month' was not significant. For 2000 monthly net N mineralization data, 'site' was not significant, but 'month' was ( $p$ -value $<$ 0.001, partial  $R^2=0.137$ ). The 'site\*month' interaction term was significant for both years ( $p$ -value $<$ 0.0001 for both years).

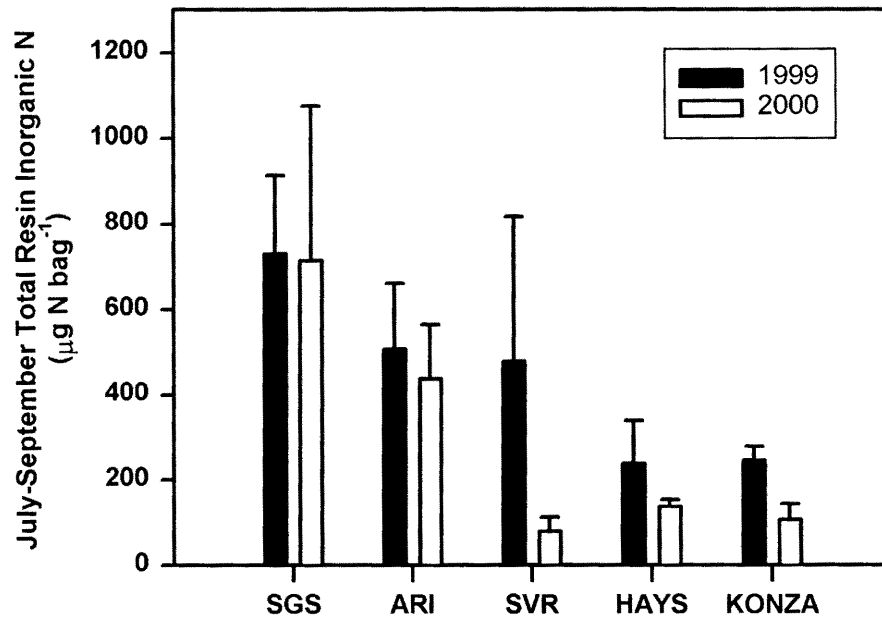




**Figure 2.2** - (a) July-September total precipitation for the two years of the study and the long-term average for the same time period at each of the sites. (b) Aboveground net primary production nitrogen content (ANPP-N), and the (c) July-September total net N mineralization rates for all sites and both years of the study (all values are means  $\pm 1$  S.D.).



**Figure 2.3** - July-September total net N mineralization for both years and all sites versus the 3 significant covariates (soil water-filled pore space, soil organic carbon, and July-September total precipitation) from the interannual and regional regression. No significant relationships were found when soil water-filled pore space and soil organic carbon were run in singly in the model.



**Figure 2.4** - Mean  $\pm$  1 S.D. of the total inorganic N captured in the resin bags at the bottom of the incubation cores over the July-September time period for both 1999 and 2000.

## **CHAPTER III: REGIONAL AND INTERANNUAL VARIABILITY IN CARBON CYCLING ACROSS THE CENTRAL GREAT PLAINS OF NORTH AMERICA**

### **Introduction:**

Due to the unprecedented rate of increase in atmospheric carbon dioxide concentrations, public and scientific attention has been drawn to the potential capability of managing grassland ecosystems to actively sequester carbon from the atmosphere into relatively stable soil organic C pools (Lal et al. 1998, Conant et al. 2001). Alterations in the size of the soil carbon pool at a specific location are determined by the relative changes in the inputs (above- and belowground net primary production) and outputs (decomposition of plant material and soil organic matter) of carbon over yearly and longer time-scales. In grasslands, all of these ecosystem carbon fluxes are strongly influenced by precipitation and water-availability (Hayes and Seastedt 1987, Sala et al. 1988, Knapp et al. 2001, Epstein et al. 2002); therefore, potential soil carbon sequestration rates in grasslands are likely to be highly dependent on current and future climatic predictions.

Water-limitation and high interannual variability in precipitation are characteristics of grasslands world-wide as well as in the Great Plains region (Borchert 1950, Ripley 1992, Lauenroth and Burke 1995). Recent reports suggest that potential global climate change is likely to result in decreased

summer precipitation, increased summer temperatures, and increased variability in both the amounts and timing of rainfall in the Great Plains region (Easterling 1990, Houghton et al. 1990, Karl et al. 1991). How will these predicted climatic changes influence grassland ecosystem function in this region? Many studies have shown aboveground net primary production is tightly linked to precipitation over both regional spatial gradients, for instance from shortgrass steppe to tallgrass prairie, and through time with interannual variability at specific sites (Sala et al. 1988, Lauenroth and Sala 1992, Knapp et al. 1998a). Such trends suggest that grasslands should be relatively responsive to alterations in the precipitation regime due to climate change. Indeed, a recent study comparing the interannual variability in aboveground primary production across many different biomes found that grasslands and old fields experience the greatest interannual variability in this carbon input flux and may be the most responsive biome to future climatic changes (Knapp and Smith 2001).

However, aboveground primary production represents only ~1/2 or less of the net primary production of grassland systems (Sims and Singh 1978, Milchunas and Lauenroth 2001), and in terms of potential carbon sequestration, it is likely that belowground production is a more important source of soil carbon than aboveground production in these ecosystems. Due to methodological difficulties, few estimates of belowground carbon inputs exist for the Great Plains region and even fewer of these estimates were made at multiple sites across the region during the same time period and utilizing the same technique.

One such study, Sims and Singh (1978), found that root production was higher in shortgrass steppe communities than either mixed grass or tallgrass prairie and that root production decreased with increasing mean annual temperature; this climatic factor was also used in a global grassland model of belowground production (Gill et al. 2002). Results from tallgrass prairie indicate that root growth and production decline during drought (Hayes and Seastedt 1987), but long-term annual root biomass data from the shortgrass steppe suggest that roots are less temporally dynamic and responsive to interannual variability in precipitation than the aboveground components (Milchunas and Lauenroth 2001). Thus, contrary to the well-quantified aboveground production response to interannual variability in precipitation, the response of belowground production is relatively unknown.

Similarly, field-derived estimates of carbon output fluxes via decomposition of aboveground and belowground plant material and soil organic matter are lacking at a regional scale across the Great Plains. Epstein et al. (2002) recently used steady state assumptions to evaluate soil organic matter decomposition patterns across this region and found that mean annual precipitation and soil texture had greater explanatory power on decomposition rates than mean annual temperature. The authors interpreted this result as indicative of the overall water-limitations generally imposed on these grassland systems, and a larger-scale global analysis has also shown decomposition rates to be strongly influenced by water availability and temperature (Meentemeyer 1978). Both of these studies focused on long-term mean annual

climatic conditions. Little is known regarding the response of decomposition of above- and belowground plant material and soil organic to interannual variability in precipitation. Decomposition is a microbially-mediated process, and as such, it is generally hypothesized to decrease in times of water limitation. Understanding the interannual response of decomposition, the primary flux of carbon from these ecosystems, to variability in precipitation is critical to assessing the carbon sequestration potential of these grasslands under changing climatic conditions.

Given the predictions for climatic change and the lack of data on the interannual response of the carbon input and output fluxes governing changes in the soil carbon pool, I initiated a biogeochemical study at 5 sites, representing shortgrass steppe, mixed grass prairie, and tallgrass prairie, occurring across a 500 mm mean annual precipitation gradient in the Central Great Plains. I asked the following questions:

1. Do carbon input and output fluxes have similar trends across a spatial, regional-scale gradient in precipitation?
2. Are these input and output fluxes similarly sensitive to interannual variability in precipitation?
3. Does carbon flux sensitivity to interannual precipitation vary across grassland community types?

## Methods:

### *Study Area*

I conducted this study at five grassland sites spanning a 800 kilometer transect from eastern Colorado to eastern Kansas (Table 3.1). All sites are native grassland managed with moderate levels of cattle grazing and have not been previously cultivated, as evidenced by detailed site histories and well-developed soil profiles that lack a plow layer. The two westernmost sites (SGS, the Shortgrass Steppe Long-term Ecological Research site and ARI, a ranch near the Arickaree River in central eastern Colorado owned by The Nature Conservancy) are typical shortgrass steppe plant communities, dominated by *Buchloe dactyloides* and *Bouteloua gracilis* (Association 1986, Lauenroth and Milchunas 1992). Smokey Valley Ranch (SVR), also owned by The Nature Conservancy, has a mixture of both shortgrass steppe and mixed grass prairie vegetation. In central Kansas, the HAYS site is owned and managed by Ft. Hays State University and is dominated by plants typically found in southern mixed grass prairie, *Schizachyrium scoparium* and *Bouteloua curtipendula* (Coupland 1992). The Konza Prairie Biological Station (KONZA), is owned by The Nature Conservancy, managed by Kansas State University, and is also a Long-term Ecological Research site. KONZA is a tallgrass prairie with dominant plant species *Andropogon gerardii* and *Sorghastrum nutans* (Silletti and Knapp 2000). KONZA is the only site where typical grassland management includes fire. My plots at this site were located in a watershed



that burns once every four years. The plots were burned in April 1996 and 2000.

Both mean annual temperature and precipitation increase from west to east across the transect (Table 3.1). During site selection, we attempted to minimize soil textural differences between sites (soil taxonomy and other characteristics in Table 3.1). At each site, 4 permanent plots (4m x 4m) on a level upland were fenced to exclude cattle in late June of 1999.

### *Net Primary Production*

I estimated aboveground net primary production (ANPP) at each site by clipping 10, 0.5 m<sup>2</sup> quadrats in August 2000 and 2001, the month of peak standing biomass, from exclosure cages placed randomly around the permanently fenced plots in early May of the same year. Biomass was sorted at the time of clipping to remove previous year's standing dead from current year's alive and dead material and into grass and forb components. Biomass samples were dried at 50°C for a week, weighed, and ground in a Wiley mill. We sub-sampled ground samples for ash and C and N determination. Sub-samples were ball ground and a portion of each was analyzed for total C and N content on a LECO CHN-1000 analyzer (St. Joseph, MI). An additional portion of the ball-ground samples was combusted in a muffle furnace at 500°C for 5 hours for percent ash content. The carbon content of the aboveground production is presented on an ash-free basis (ANPP-C).

I obtained belowground net primary production (BNPP) estimates using a modified root in-growth technique (Jordan and Escalante 1980, Neill 1992, Lauenroth 2000). During the first 2 weeks of May 2000 and 2001, a bucket augur was used to excavate 30 cm deep cylindrical holes from 2 locations (directly under the crown material of an individual plant and in the interspace between individuals) in each of the 4 permanently fenced plots (8 root in-growth holes per site and year). The HAYS site occurs on relatively shallow soils with underlying limestone bedrock; therefore, root in-growth cores were installed as deeply as possible at this site (between 18 and 25 cm). I refilled the holes with a root-free sandy loam soil collected at the Shortgrass Steppe LTER (Ascalon soil series with <0.0003% roots after 2 mm sieving, 0.48% C and 0.04% N, and 83.2% sand and 7.1% clay). Screens with small stakes were placed over the root in-growth cores to prevent rodent disturbance. I collected root in-growth samples the following October. Collection consisted of driving a smaller diameter soil core (4.8 cm internal diameter, 18 cm long) in the center of the root in-growth hole and placing all of extracted sample into paper bags. Two depth increments were collected (0-15 cm and 16-30 cm).

I dried the collected samples at 50°C for a week to prevent decomposition occurring during the time between collection and processing (at most one month). Dried samples were placed in buckets, washed and mixed with tap water. Floating roots were decanted into a 0.147 mm mesh sieve. This flotation procedure was repeated at least 3 times per sample or until no more visible roots floated to the surface. Material collected in the 0.147 mm

sieve was thoroughly washed with tap water, dried at 50°C for 4 days, hand picked to remove any material clearly not root-derived, and weighed. Due to the low amounts of root biomass collected in 2000, I combined depth increments for each sample, ground the combined sample in a Wiley mill, and combusted 0.20 g of the sample in a muffle furnace at 500°C for 5 hours to obtain percent ash content. Some combined samples did not have 0.20 g of material. In such cases, all of the sample was used in the ash determination. For samples with 0.1 g root material remaining after the ashing procedure, I obtained %C and %N concentrations by running the samples on the LECO CHN-1000 analyzer mentioned previously. It is common for root samples to have high ash content (>50%)(Bohm 1979), and soil-corrections have been derived to take into account the quantity of soil contamination during the calculation of root %C and %N (Hunt et al. 1999). My samples, even after extensive washing, had high ash content (35-80% ash); therefore, I employed the Hunt et al. (1999) soil-correction equations with the known root-free soil %C and %N values in our calculation of root %C and %N. I eliminated root samples with >70% ash contamination from the root %C and %N data pool. Averaged root %C and %N values by site and year were used for missing samples, and these values were multiplied by the ash-corrected belowground primary production values obtained from the total root sample weight and core size to obtain the belowground primary production carbon values presented here (BNPP-C in  $\text{g C m}^{-2} \text{ yr}^{-1}$ ).

Root in-growth techniques are known to incorporate artifacts ranging from enhanced root proliferation due to soil disturbance and altered nutrient availability and growing conditions in the root-free soil to possible underestimation of belowground production resulting from concurrent growth and death of roots within the cores (Neill 1992, Lauenroth 2000). Each of the BNPP methods possible has artifacts (Lauenroth 2000). Due to the regional-scale of this project, the root in-growth technique was the most logistically feasible. I recognize that by using non-native soil in my root in-growth cores I may have accentuated the methodological problems associated with altered nutrient availability and growing conditions. The Ascalon root-free soil was more coarse-textured and had lower %C and N values than the native soil at all sites. Thus, the in-growth cores probably had reduced nutrient availability compared to the native soils, but also, the cores potentially had different water availability due to the textural differences. The fact that the relative differences between the Ascalon root-free soil and the native soil varied by site requires that the site differences in belowground net primary production estimates reported in this paper be interpreted with caution; however, interannual differences in belowground net primary production at individual sites should reflect the response of this variable to altered climatic conditions between years as methodology remained the same for both 2000 and 2001.

I summed above- and belowground net primary production values to estimate total net primary production (NPP) at each of the sites. Because the above- and belowground production measurements were not experimentally

paired (an  $n=10$  for ANPP and  $n=8$  for BNPP) and because the BNPP-C values were considerably more variable than the ANPP-C values, I used the average ANPP-C value per site and year in the summation for NPP-C.

### *Soil Respiration, Temperature, and Moisture*

I measured soil respiration with an Environmental Gas Monitor (EGM version 1; PP Systems, Haverhill, MA), a continuous flow infrared gas analyzer. The EGM was placed on polyvinylchloride collars (10.3 cm internal diameter) inserted 2.5 cm into the ground at the onset of the growing season (May 2000 & 2001). Collars were inserted into the soil at least 12 hours before the first measurement for the year was taken. At each site, I placed 2 collars within each of the 4 permanently fenced plots: one collar was located in interspaces between plants ('between' location), and the second collar was placed directly on top of an individual crown ('under' location,  $n=8$  collars per site). Previous work has shown such microsite location differences can have significant effects on mineralizable carbon pools, an indicator of microbial activity, in semi-arid ecosystems (Burke et al. 1999). Collars were left in place for the entire growing season due to the limited space available inside the permanent plots, and green aboveground plant material was clipped and removed from within the collars at least one hour before respiration measurements occurred every month. I recognize that leaving the collars in place over the growing season and repeated clipping on the vegetation inside in the collars most likely introduced some artifacts into our data. Collars can become loose as repeated wetting and

drying cycles occur over the growing season. Loose collars can inflate soil respiration rates by collar movement increasing soil pore CO<sub>2</sub> efflux when the EGM is set in place (Norman et al. 1992). Conversely, clipping aboveground plant material has been shown to reduce soil respiration rates by ~20% (Bremer et al. 1998, Craine et al. 1999). While my data includes these types of artifacts, methodology was the same across all years and sites, and therefore, trends in the data are due to real differences in soil respiration rates and not methodological artifacts.

Soil respiration measurements required <2 minutes per reading, depending on the respiration rate. Measurements were taken one day a month between 10:00 to 17:00 hours at each of the sites over the course of the growing season (May-October for 2000 and 2001). Given the regional-scale of this project, it was not possible to sample all sites on the same day. I took diurnal soil respiration measurements (measurements occurred at 04:00, 10:00, 14:30, and 18:00 hours) in June 2000 and August 2001. My one-time point mid-day monthly readings were well-correlated with the average diurnal soil respiration flux (data not shown, but  $R^2=0.915$ ,  $p\text{-value}<0.0001$ , and the slope of the regression line was not significantly different from 1) and therefore, appear to adequately represent daily soil respiration fluxes at our site. I scaled the one-time point mid-day readings to monthly values by multiplying the instantaneous rate (in  $\text{mg CO}_2\text{-C m}^{-2} \text{ hr}^{-1}$ ) by the number of hours in the day and the number of days in the month. The growing season total soil respiration is the sum of all measured months (May-October for 2000 and 2001).

I measured soil temperature concurrently with soil respiration. Measurements consisted of inserting a soil temperature probe 3 cm into the soil less than 10 cm away from the soil respiration collar, allowing 2 minutes of equilibration and recording the temperature. Due to the paired nature of the soil temperature and respiration measurements, a microsite location main effect (between and under plants) was incorporated into the soil temperature dataset.

I collected gravimetric soil moisture samples on the same day the soil temperature and respiration measurements were made. A soil core (4.8 cm internal diameter, 15 cm long) was taken from random locations immediately adjacent but outside of the permanently fenced plots (due to the destructive nature of this type of sampling and the limited area inside the plots). Soil was removed from the corer, thoroughly mixed, and a sub-sample was placed in a tin can and sealed with electrical tape until the sample could be oven-dried. Samples were weighed, dried in a 110 °C oven for 3 days, and re-weighed for gravimetric soil moisture determination. Using the gravimetric soil moisture and bulk density data (obtained from additional soil cores of the same dimensions collected in August 1999 (n=1 core per plot)), soil water-filled pore space was calculated according to (Linn and Doran 1984).

### *Litter Decomposition*

I measured aboveground litter decomposition rates on two types of litter: a “common” litter (*Bouteloua gracilis* leaves and stems) and a “dominant” litter (consisting of leaves and stems of the dominant plant species at only SGS and

KONZA). All of the *Bouteloua gracilis* material for the “common” litterbags was collected in June 1999 from an exposed slope on sandy loam soils at the Shortgrass Steppe LTER. Because the SGS site is also dominated by *Bouteloua gracilis*, this plant material also functioned as the “dominant” plant material for this site. Plants were excavated, shaken to remove attached soil, and dried at 50°C for 4 days. *Bouteloua gracilis* leaves and stems were cut from crowns and roots and stored in a large bucket. Once enough material was collected to construct the necessary bags, the leaves and stems were thoroughly mixed. I placed 3.0 g of *Bouteloua gracilis* material ( $\pm 0.01$  g) inside 10x10 cm<sup>2</sup> litterbags constructed of 1.4 mm<sup>2</sup> fiberglass-nylon mesh. I heat-sealed the open side of the litterbag.

The common *Bouteloua gracilis* litterbags allowed me to evaluate regional climatic effects on decomposition; however, due to concurrent changes in plant species and litter quality across the transect (Table 1), these common litterbag decomposition rates are unlikely to be representative of the decomposition occurring on the dominant plant species at the mixed grass and tallgrass sites. Therefore, I also made litterbags of the “dominant” vegetation (*Andropogon gerardii*) from KONZA, the tallgrass prairie site. This dominant plant material was clipped in late June 1999 from an annually burned watershed at KONZA. Due to the height of the plant material at KONZA, I had to clip the vegetation into smaller pieces before mixing. Otherwise, the dominant litterbags were constructed in the same manner as the common litterbags.



Litterbags (n=4-6 per site, type (common or dominant), and year) were placed randomly on the soil surface with at least one bag inside each of the 4 permanently fenced plots at each site. Screens were placed over the bags to prevent removal by rodents. Litterbags were placed in the field at all sites in May 2000 and 2001 and collected in October of the same year. Extra litterbags of both types of litter (5-7 bags each) were carried on the litterbag distribution trips every year to quantify how much litter was lost from the bags in transport and the initial litter %C and %N data. Although significant changes in the initial litter %C and %N did occur over the years of the study, they were not large (<0.5% decrease in C and <0.2% decrease in N) or systematic over time, and may therefore simply be reflecting the variability within the initial plant material collected.

Once harvested from the field, I dried litterbags for 4 days at 55°C. I removed and weighed the contents of the bags. Samples were ground in a Wiley mill, and then sub-sampled into a 0.2 g sample for ash determination (same ashing procedure as mentioned in the net primary production section) and a 0.2 g sample for %C and N determination (run on the LECO CHN-1000 analyzer). All litter decomposition rates were calculated on an ash-free basis. I calculated the carbon decomposition constant ( $k_c$ ,  $\text{yr}^{-1}$ ) for each year, site, and type of litter using the formula:

$$\ln(C_t/C_0) = -k_c t$$

where  $C_t$  and  $C_o$  are the carbon content of the litter at time  $t$  and time  $0$  (Olson 1963).

In order to compare the regional and interannual responses of decomposition to those of net primary production and soil respiration, I needed to express the decomposition of C on the same scale ( $\text{g C m}^{-2} \text{ yr}^{-1}$ ) as those measurements. To accomplish this, I assumed that all aboveground net primary production at the site for the year would eventually enter the litter pool and be decomposed at the rate we measured ( $k_c$  – which incorporates environmental constraints on decomposition occurring at a specific site). Therefore, I obtained an estimate of aboveground decomposition C loss ( $\text{g C m}^{-2} \text{ yr}^{-1}$ ) by multiplying the aboveground production values by  $k_c$ . I calculated this decomposition C loss from both the common and dominant litter  $k_c$  values at the SGS and KONZA.

### *Statistical Analyses*

The objective of this study was to determine patterns of net primary productivity (both above- and belowground), soil respiration, and litter decomposition across the Central Great Plains region and between years at individual sites. All response variables were statistically analyzed using a mixed model analysis of variance in SAS (version 8) statistical software program (SAS 1989). The fixed effects in the model were site (SGS, ARI, SVR, HAYS, and KONZA), location (between and under plants), and year (2000 and 2001). The random effects were plot nested within site and the year by plot

nested within site interaction (included only when location was incorporated into the model). This type of statistical approach incorporates a repeated measures design to handle the multiple years of data collected at the same sites. Preliminary statistical analysis indicated that location was not a significant main effect in the BNPP dataset; therefore, the distinction between locations was dropped from this dataset. A least significant difference mean separation test was utilized when significant main effects were found.

In addition to the analyses of variance described above, regressions were also used to evaluate the site-related regional relationships between the measured C inputs and outputs averaged across years and mean annual precipitation (MAP). The MAP of the sites was used in these regional regression models since previous work has shown MAP to be tightly correlated to long-term aboveground C input (Sala et al. 1988, Lauenroth and Sala 1992). A general linear model was used to assess statistically significant differences between the slopes of the regional C input (NPP) and output (soil respiration and decomposition) regression lines. All data were transformed as needed to fit the normality assumptions of the applied statistical test, and the level of significance of all tests was determined as a p-value < 0.05.

## **Results:**

### *Regional C Input and Output Relationships*

I found significant regional relationships for all the measured carbon input and output variables except BNPP-C (Figures 3.1 & 3.2). The carbon

inputs of ANPP-C and NPP-C and the carbon output variables SR-C,  $k_c$ , and the carbon lost via aboveground decomposition (Decomp. Loss) increased from western semi-arid shortgrass steppe to eastern sub-humid tallgrass prairie. Mean annual precipitation (MAP) explained 30-89% of the variability in these datasets (Figure 3.1 & 3.2). These significant regional relationships between the long-term MAP and the carbon input and output fluxes were also indicated in the analysis of variance (ANOVA) results. All measured variables (ANPP-C, BNPP-C, NPP-C, SR-C,  $k_c$  and Decomp. Loss of the common litterbags) had significant 'site' main effects, with the partial  $R^2$  of 'site' accounting for 70-99% of the variability in the dataset (Tables 3.2, 3.3, and 3.4). Comparison of the slopes of the regional regressions for the various carbon input and output fluxes (Figure 3.3) suggests that the loss of carbon via soil respiration is significantly more sensitive per mm of increase or decrease of mean annual rainfall than either of the carbon input variables found to have significant relationships across the regional MAP gradient (ANPP-C and NPP-C), or the carbon loss via aboveground decomposition (Decomp. Loss).

#### *Interannual Variability in C Inputs*

At all sites, total May-October precipitation was higher in 2001 than 2000 (Figure 3.4), although the actual magnitude of this difference in precipitation varied between sites and was highest at the HAYS and KONZA sites. Additionally, May-October total precipitation increased from shortgrass steppe to tallgrass prairie in both years, reflecting the mean annual precipitation trend.

The ANPP-C increased concomitantly with increasing May-October total precipitation across the sites in any given year; however, significant positive ANPP-C responses to increased May-October total precipitation in 2001 occurred at only three of the 5 sites (the shortgrass steppe sites (SGS, ARI) and one of the mixed grass prairie sites (SVR)) (Figure 3.4, hence the significant site\*year interaction term in the ANPP-C ANOVA, Table 3.3). A large proportion of the variance in the ANPP-C dataset was explained by 'site' ( $R^2=91\%$ ), while only 14% of the variance was explained by 'year' (Table 3.3).

Although there were significant 'site' differences in BNPP-C ( $p<0.0001$ ), the significant 'site\*year' interaction term precluded means separation tests. Our data indicate that BNPP-C does not vary systematically from semi-arid shortgrass steppe to sub-humid tallgrass prairie (Figure 3.1 & 3.4), although root:shoot production ratios do decrease across this transect (from an average of 3 to  $<1$ , data not shown). However, all sites except SGS exhibited a high degree of interannual plasticity in BNPP-C (Figure 3.4), increasing BNPP-C with increasing May-October total precipitation between the two years.

Combining these two datasets (ANPP-C and BNPP-C) together, the NPP-C data had significant 'site,' 'year,' and 'site\*year' interaction terms (Table 3.3). NPP-C was significantly higher in 2001 than 2000 at all sites except SGS, where no significant difference between the two years was found. Comparison of the positive increase in NPP-C to increases in May-October precipitation between the two years at each site (Figure 3.5) indicates that the regional interannual response does not appear to track the actual magnitude or

proportion of change in precipitation between the two years. For instance, both HAYS and KONZA experienced more than a 200 mm difference in May-October precipitation between the two years (representing more than 40% of the normal rainfall received during this period of the growing season), and yet, both sites had smaller differences in NPP-C between the two years than SVR (which experienced <50 mm difference in precipitation). At all sites where there was a significant difference in NPP-C between the two years, the majority of the NPP-C response was accounted for by BNPP-C (Figure 3.5).

#### *Interannual Variability in C Outputs*

May-October total soil respiration (SR-C) had significant 'site,' 'year,' 'site\*year,' and 'location' main effects (Table 3.3). In general, SR-C increased from western semi-arid shortgrass steppe to eastern sub-humid tallgrass prairie (Figure 3.6). The interspaces between plants usually had lower soil respiration rates than areas sampled directly under the crowns of plants. All sites except KONZA had higher SR-C in 2001 than 2000 (KONZA had no significant difference between the two years). This interannual trend corresponds with the increase in May-October total precipitation measured at the sites. Similar to the carbon input parameters, 'site' accounted for 96% of the variability in the SR-C data, but 'year' accounted for a much higher percent of the variability in the SR-C data ( $R^2 = 70\%$ ) than was found with the carbon input parameters (Table 3.2 & 3.3).

The carbon decomposition constant ( $k_c$ ) for the common litterbags increased from west-to-east across the transect tracking increases in precipitation and temperature (Figure 3.7, Table 3.4). The significant 'year' main effect suggests 2001 had higher rates of carbon decomposition than 2000, although this statistical result is primarily driven by increases in  $k_c$  between the two years at the SGS site (Figure 3.7, Table 3.4). These decomposition trends are paralleled and/or strengthened in the carbon lost via decomposition data (Figure 3.7). However, the dominant litter  $k_c$  was not significantly different between the SGS and KONZA sites (Table 3.4), suggesting that concurrent changes in litter quality (Table 3.1, (Murphy et al. In press) across the transect nullify the positive effect of increasing precipitation and temperature and keep the decomposition constants of the different grassland types similar (Figure 3.7). The dominant litter  $k_c$  is significantly higher in 2001 than 2000 at both sites (Figure 3.7, Table 3.4). When the aboveground decomposition carbon loss is calculated with the dominant litter  $k_c$ , statistically significant 'site,' 'year,' and the 'site\*year' interaction terms are found (Table 3.4). These data suggest that KONZA loses more carbon via aboveground decomposition than the SGS, and that only the SGS had significantly more aboveground decomposition carbon lost in 2001 than 2000 (Figure 3.7). However, these estimates of aboveground decomposition carbon loss are strongly driven by trends in ANPP-C across sites and between years.

The significant increases in SR-C and carbon lost via aboveground decomposition parallel increases in May-October precipitation between 2000

and 2001 (Figure 3.8). Similar to the NPP-C response, the interannual responses of these carbon output parameters across the region do not appear to track the actual magnitude or proportion of change in precipitation between the two years. In the case of the carbon output data, KONZA, the site with the largest interannual change in May-October total precipitation between 2000 and 2001 (an increase of >250 mm), had no significant differences measured in SR-C or decomposition C loss between the two years.

The loss of carbon via soil respiration is significantly more sensitive per mm of increased rainfall between years than either carbon input via NPP-C or carbon loss via aboveground decomposition (Figure 3.9). This result is similar to that of the comparison of the regional regression slopes (Figure 3.3) which demonstrated that SR-C was more responsive to increases or decreases in mean annual precipitation across the transect than the other carbon input and output variables. Both regionally and interannually SR-C, is the most responsive flux of C in these grassland ecosystems. Additionally, both inputs and outputs of C at ARI and SVR were more responsive to interannual variability in precipitation than the other sites. Carbon loss via aboveground decomposition on a per mm basis was low at all sites (Figure 3.9).

### **Discussion:**

*Do carbon input and output fluxes have similar regional trends?*

ANPP-C and NPP-C increased from western semi-arid shortgrass steppe to eastern tallgrass prairie; however, BNPP-C showed no regional



relationship with mean annual precipitation. While the ANPP regional relationship has been well documented (Sala et al. 1988, Lauenroth and Sala 1992), the lack of a regional trend in BNPP-C is somewhat surprising. Previous reports have suggested that proportional allocation to roots should increase in drier climates as the plants expend more energy and resources in acquiring water (Sims and Singh 1978, Milchunas and Lauenroth 2001); however, tallgrass prairie has been shown to be water and/or nitrogen-limited in most years (Blair 1997, Knapp et al. 2001), and the species existing there may benefit similarly from investing energy in root production. More years of data collection and additional efforts to minimize the artifacts associated with the root in-growth technique by using native root-free soil at each site, rather than the sandy loam soil I used at all sites would help elucidate the BNPP-C relationship across these different grassland communities. Surprisingly, although BNPP-C accounts for at least an equal amount of (if not 2-3x more) carbon input into these grassland ecosystems, I still found a significant relationship between total NPP-C and the mean annual precipitation gradient. Tallgrass prairie had almost twice the amount of NPP-C than the other grassland types included in the study, suggesting that the aboveground NPP trends are strong enough to overwhelm the lack of a belowground NPP trend.

Carbon output fluxes increased with increasing mean annual temperature and precipitation across the gradient. My growing season SR-C estimates, while crude in the sense that only one instantaneous measurement was made per month and these measurements were then scaled up to the

entire growing season, are comparable with more frequently made growing season SR-C totals from the tallgrass prairie (Bremer et al. 1998, Knapp et al. 1998b, Mielnick and Dugas 2000), and are similar to monthly instantaneous measurements made over the course of the growing season in the shortgrass steppe (Burke et al. 1999, LeCain et al. 2000, Mosier et al. 2002). My decomposition results concur with the general biogeochemical understanding that rates of microbial-mediated processes increase as temperature increases and water-limitations diminish.

Litter quality has a strong influence on regional trends in decomposition. Previous work has shown that increases in aboveground plant litter C:N and lignin:N ratios occur across this climatic gradient as a result of changes within species and alterations in species composition (Vinton and Burke 1997, Murphy et al. In press). The significant increase in dominant litter C:N ratios (Table 3.1) corresponded with no significant differences in the dominant litter decomposition constants between SGS and KONZA despite higher growing season precipitation at KONZA during both years of the study (Figure 3.7). Litter accumulation in the absence of fire and the resultant light attenuation, alteration in microclimate, and nitrogen cycling consequences are well-known characteristics of the tallgrass prairie (Knapp and Seastedt 1986, Knapp et al. 1998a). The mass loss of the dominant litterbags at KONZA after one growing season from this study compare well with previously reported rates of mass loss from this tallgrass prairie ecosystem (Seastedt 1988); however, mass loss from the *Bouteloua gracilis* common litterbags at KONZA was almost twice the

dominant litterbag rates. The fact that I used carbon decomposition constants from the common litterbags in my calculation of aboveground carbon decomposition loss most likely resulted in my estimates of this carbon flux being unrealistically high at the site-scale and artificially enhancing the slope of the regional regression. Even though I think these C loss estimates are inflated, it is interesting to note that the responsiveness of this parameter is still significantly lower than soil respiration C loss at the regional-scale (Figure 3.3) and is the least responsive C flux parameter measured interannually at specific sites (Figure 3.9).

Soil respiration rates in this study were more than 4x as sensitive to changes in mean annual precipitation across the regional gradient than the carbon input rates or the rates of aboveground C decomposition loss. However, interpretation of the soil respiration regional trend is difficult due to the fact that this measurement includes both autotrophic root respiration and soil microbial respiration resulting from soil organic matter and root exudate decomposition (Hanson et al. 2000). Root respiration contributions are thought to make up at least 30% of total soil respiration in grasslands (Herman 1977); however, differences between grassland species in rates of root respiration are essentially unquantified. Aboveground plant leaf and shoot respiration were not measured in this study (by definition, ANPP is the difference between gross photosynthesis and aboveground plant respiration), but it is probable that as water-limitation is somewhat alleviated across the precipitation gradient, plant species become less precipitation-use-efficient and lose more C via respiration.

Since the root respiratory C loss was captured in the soil respiration measurements but not in the net carbon input rates, it is not surprising that soil respiration rates appear more sensitive to changes in mean annual precipitation across the gradient than C inputs as they were measured here. The highly sensitive soil respiration response may be largely driven by autotrophic root respiration. Separating root and microbial contributions to soil respiration is a difficult task, but without that information, I am unable to determine whether the high regional sensitivity of soil respiration compared to the other measured carbon input and output rates is the result of increasing soil organic matter decomposition or simply due to alterations in root respiration and exudation.

*Are carbon input and output fluxes similarly sensitive to interannual variability in precipitation?*

Data from this study suggest that the SR-C output flux is the most sensitive flux of carbon to interannual variability in precipitation across these grasslands community types (Figure 3.9). The primary source of interannual variability in carbon inputs in these systems was attributed to belowground inputs, and even though my estimates of aboveground C decomposition loss are most likely too high (due the use of the common litterbag carbon decomposition constants and other assumptions made during the calculation), the magnitude and sensitivity of this C output flux was significantly lower than the other fluxes measured at all sites except SGS where the difference in the

aboveground C decomposition loss between the 2 years was equal to or greater than the NPP-C response.

SR-C output fluxes were higher in the year with greater precipitation inputs at almost all sites. This result is consistent with the previous soil respiration work that has indicated soil respiration rates are controlled primarily by temperature and water availability (Davidson et al. 1998, Mielnick and Dugas 2000). However, as in the previous section, interpretation of this interannual SR-C response is difficult as soil respiration is composed of both root and microbial components. It seems likely that both components of soil respiration would increase with increased water availability in these grassland ecosystems.

Work from the shortgrass steppe has shown a significant relationship between ANPP and interannual variability in precipitation (Lauenroth and Sala 1992), and my data do indicate that the 3 most western sites did experience significant increases in ANPP-C with increased precipitation in 2001; however, the more mesic, sub-humid grasslands did not. Regression relationships of annual precipitation and ANPP through time at the tallgrass prairie site have a lower  $R^2$  than those derived from similar data from the semi-arid shortgrass steppe (Knapp et al. 1998a), suggesting that the relative control of interannual variability in precipitation on aboveground production decreases in the more mesic grassland ecosystems. My data support this conclusion.

Little information exists on the BNPP response to interannual variability in precipitation. Hayes and Seastedt (1987) found that drought in the tallgrass prairie reduced root production, and my data support these findings. BNPP-C

was lower during the dry year 2000 at all sites except SGS. The large interannual BNPP-C response and its significant contribution to NPP-C in these grasslands argues that grassland ecosystem studies not incorporating belowground production are missing a significant and highly temporally variable input of carbon into these systems.

*Does the carbon flux sensitivity to interannual precipitation vary across grassland community types?*

The shortgrass steppe site, ARI, exhibited the most sensitive carbon cycling responses to interannual variability in precipitation (on a per mm increase of rainfall basis). Paruelo et al. (1999) found that regression models of ANPP and annual precipitation at different grassland community types across the Great Plains region suggested a maximum ANPP response to interannual variability in rainfall at the 475 mm mean annual precipitation zone. This mean annual precipitation (MAP) zone occurs between the ARI (450 mm MAP) and SVR (506 mm MAP) sites in this study; thus, my results concur with the previous work on this topic and suggest that both carbon inputs and outputs are more sensitive to interannual variability in precipitation at this MAP zone than at the semi-arid or sub-humid endpoints of the gradient. However, Paruelo et al. (1999) hypothesized that the maximum sensitivity zone coincides with mixed grass prairie vegetation, where vegetational constraints associated with the low relative growth rates of semi-arid shortgrass steppe vegetation and biogeochemical constraints associated with the relatively nitrogen limited

tallgrass prairie communities are both somewhat alleviated by the fact that these mixed grass communities consist of a mixture of shortgrass and tallgrass species. The most responsive site in this study, ARI, is classified as a shortgrass steppe community. Thus, while the mean annual precipitation zone encompassing the highest carbon flux sensitivity to interannual variability in precipitation agrees well with previous work, the type of plant community involved does not.

The regional trends in the interannual differences in carbon input and output fluxes at each site did not track the actual magnitude of difference in the amount of precipitation received between the two years (Figure 3.5 and 3.8). In fact, KONZA experienced the largest difference in precipitation between the two years and yet had no significant difference in ANPP-C, SR-C, and aboveground C decomposition loss. I believe this lack of carbon cycling response to a more than 250 mm difference in growing season precipitation is related to the fact that this site was burned in April of 2000, one month before the start of this study. My particular site at KONZA is located in a watershed that is burned once every 4 years. Fire plays a large role in the management of tallgrass prairie and has significant impacts on the ecosystem functioning of this grassland system (Blair et al. 1998, Collins and Steinbauer 1998, Knapp et al. 1998a, Rice et al. 1998). Results from the long-term studies at KONZA have shown that infrequent burning generally increases ANPP (Knapp et al. 1998a), belowground net primary production (Rice et al. 1998, Johnson and Matchett 2001), and soil respiration rates (Knapp et al. 1998b). Therefore, it is possible

that the lack of an interannual response in ANPP-C and SR-C reported here is the result of the spring fire in 2000 increasing both these input and output fluxes to higher levels than would have otherwise been achieved given the relatively dry conditions experienced that year. The difference in these fluxes between 2000 and 2001 may have been masked by the response to the fire. These results suggest that management practices, such as burning, and site history can be as or more important than interannual variability in precipitation in determining C fluxes in the tallgrass prairie.

#### *Potential Implications for Carbon Sequestration*

Grasslands experience relatively high levels of interannual variability in precipitation (Borchert 1950). This variability in precipitation coupled with the high relative growth rates of the dominant vegetation, most likely allows grasslands to exhibit higher levels of interannual variability in aboveground production than many other biome types (Knapp and Smith 2001). My data indicate that belowground production, a potentially more important source of carbon for carbon sequestration in these systems, is more responsive to interannual variability in precipitation than aboveground production. Thus, grassland ecosystems may have an even larger capacity for production responses to climate change than suggested by Knapp and Smith (2001). This high sensitivity of carbon inputs to alterations in precipitation means little in the discussion of potential carbon sequestration without a better understanding of the carbon output fluxes. While my data clearly demonstrate that the loss of



carbon via soil respiration is more sensitive to regional alterations in mean annual precipitation and interannual variability in precipitation than any of the other measured carbon fluxes, it is not possible to say how much of this soil respiration response is due to alterations in root respiration or microbial decomposition of root exudates and soil organic matter. A better understanding of the relative contribution and seasonality of the different soil respiration components across these grassland systems is necessary before predictions regarding the carbon sequestration potential of these native grassland systems can be made.

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**Table 3.1:** Characteristics of 5 sites located across a precipitation gradient in the Central Great Plains of the U.S. The sites are **SGS** – the Shortgrass Steppe Long-Term Ecological Research (LTER) site; **ARI** – the Arickaree Ranch, a shortgrass steppe site owned by The Nature Conservancy (TNC); **SVR** - the Smokey Valley Ranch, a mixed grass/shortgrass steppe site also owned by TNC; **HAYS** – the Hays Range Area, a mixed grass prairie owned by Ft. Hays State University; and **KONZA** – the Konza Prairie Natural Research Area tallgrass prairie LTER site. MAP = mean annual precipitation, MAT = mean annual temperature. All soil parameters are from the top 15 cm. Values are averages  $\pm$  standard deviations. Different letters indicate significant differences between means within rows ( $p$ -value  $\leq 0.05$ ). Statistics were the same on soil carbon and nitrogen pools expressed on  $\text{g m}^{-2}$  basis as the  $\text{mg g soil}^{-1}$  reported here. Plant species nomenclature follows that of the Great Plains Flora Association.

	<b>SGS</b> Shortgrass Steppe - LTER	<b>ARI</b> Arickaree - TNC	<b>SVR</b> Smokey Valley - TNC	<b>Hays</b> Ft. Hays State University	<b>Konza</b> Konza Prairie - LTER
<b>Climate &amp; Vegetation:</b>					
Latitude	40° 52'	39° 45'	39° 54'	38° 53'	39° 06'
Longitude	104° 41'	102° 30'	100° 58'	99° 23'	96° 32'
MAP (mm)	345	450	506	578	835
MAT (°C)	8.5	10.3	11.6	11.9	13
Vegetation Type	Shortgrass Steppe	Shortgrass Steppe	Mixed Grass	Mixed Grass	Tallgrass
Dominant Plant Species	<i>Bouteloua gracilis</i>	<i>Buchloe</i> <i>dactyloides</i>	<i>Bouteloua gracilis</i> / <i>Buchloe dactyloides</i>	<i>Schizachyrium</i> <i>scoparium</i>	<i>Andropogon</i> <i>gerardii</i>
Dominant Plant Litter C:N	29.15 $\pm$ 1.13 <sup>b</sup>	30.57 $\pm$ 0.30 <sup>b</sup>	33.83 $\pm$ 0.66 <sup>b</sup>	43.81 $\pm$ 5.21 <sup>a</sup>	45.75 $\pm$ 3.71 <sup>a</sup>
<b>Soils:</b>					
Textural Class	Clay loam	Sandy loam	Silt loam	Loam	Silty clay loam
% Sand	40 <sup>b</sup>	56 <sup>a</sup>	24 <sup>c</sup>	39 <sup>b</sup>	18 <sup>d</sup>
% Clay	30 <sup>b</sup>	18 <sup>d</sup>	25 <sup>c</sup>	24 <sup>c</sup>	38 <sup>a</sup>
Bulk Density ( $\text{g cm}^{-3}$ )	0.90 $\pm$ 0.10 <sup>b</sup>	1.17 $\pm$ 0.06 <sup>a</sup>	0.98 $\pm$ 0.09 <sup>b</sup>	1.05 $\pm$ 0.12 <sup>ab</sup>	1.01 $\pm$ 0.13 <sup>b</sup>

	<b>SGS</b> Shortgrass Steppe - LTER	<b>ARI</b> Arickaree - TNC	<b>SVR</b> Smokey Valley - TNC	<b>Hays</b> Ft. Hays State University	<b>Konza</b> Konza Prairie - LTER
pH <sup>§</sup>	7.6	7.4	7.3	7.4	6.9
Organic C (mg C g soil <sup>-1</sup> )	17.27 ± 1.72 <sup>c</sup>	8.74 ± 1.47 <sup>d</sup>	18.50 ± 1.40 <sup>c</sup>	34.31 ± 5.30 <sup>a</sup>	26.76 ± 3.76 <sup>b</sup>
Total N (mg N g soil <sup>-1</sup> )	1.95 ± 0.14 <sup>c</sup>	1.20 ± 0.11 <sup>d</sup>	1.89 ± 0.14 <sup>c</sup>	3.20 ± 0.67 <sup>a</sup>	2.70 ± 0.33 <sup>b</sup>
Soil C:N	8.87 ± 0.63 <sup>a</sup>	7.27 ± 0.98 <sup>a</sup>	9.78 ± 0.77 <sup>a</sup>	10.71 ± 0.61 <sup>a</sup>	9.92 ± 0.88 <sup>a</sup>

<sup>§</sup> - E.F. Kelly (unpublished data).



**Table 3.2:** ANOVA results of the main effects 'site' (SGS, ARI, SVR, HAYS, and KONZA) and 'year' (2000 and 2001) on the carbon input data (aboveground net primary production carbon – ANPP-C, belowground net primary production carbon – BNPP-C, and net primary production – NPP-C).

	ANPP-C				BNPP-C				NPP-C			
	df	F	p-value	Partial R <sup>2</sup>	df	F	p-value	Partial R <sup>2</sup>	df	F	p-value	Partial R <sup>2</sup>
<b>Site</b>	4	100.27	<0.0001	0.910	4	7.60	<0.0001	0.698	4	41.20	<0.0001	0.926
<b>Year</b>	1	61.42	<0.0001	0.140	1	132.80	<0.0001	0.578	1	158.67	<0.0001	0.633
<b>Site*Year</b>	4	16.54	<0.0001	0.153	4	10.18	<0.0001	0.176	4	8.90	<0.0001	0.141

**Table 3.3:** ANOVA of main effects 'site' (SGS, ARI, SVR, HAYS, and KONZA), 'year' (2000 and 2001), and 'location' (between or under plants) on May-October total soil respiration.

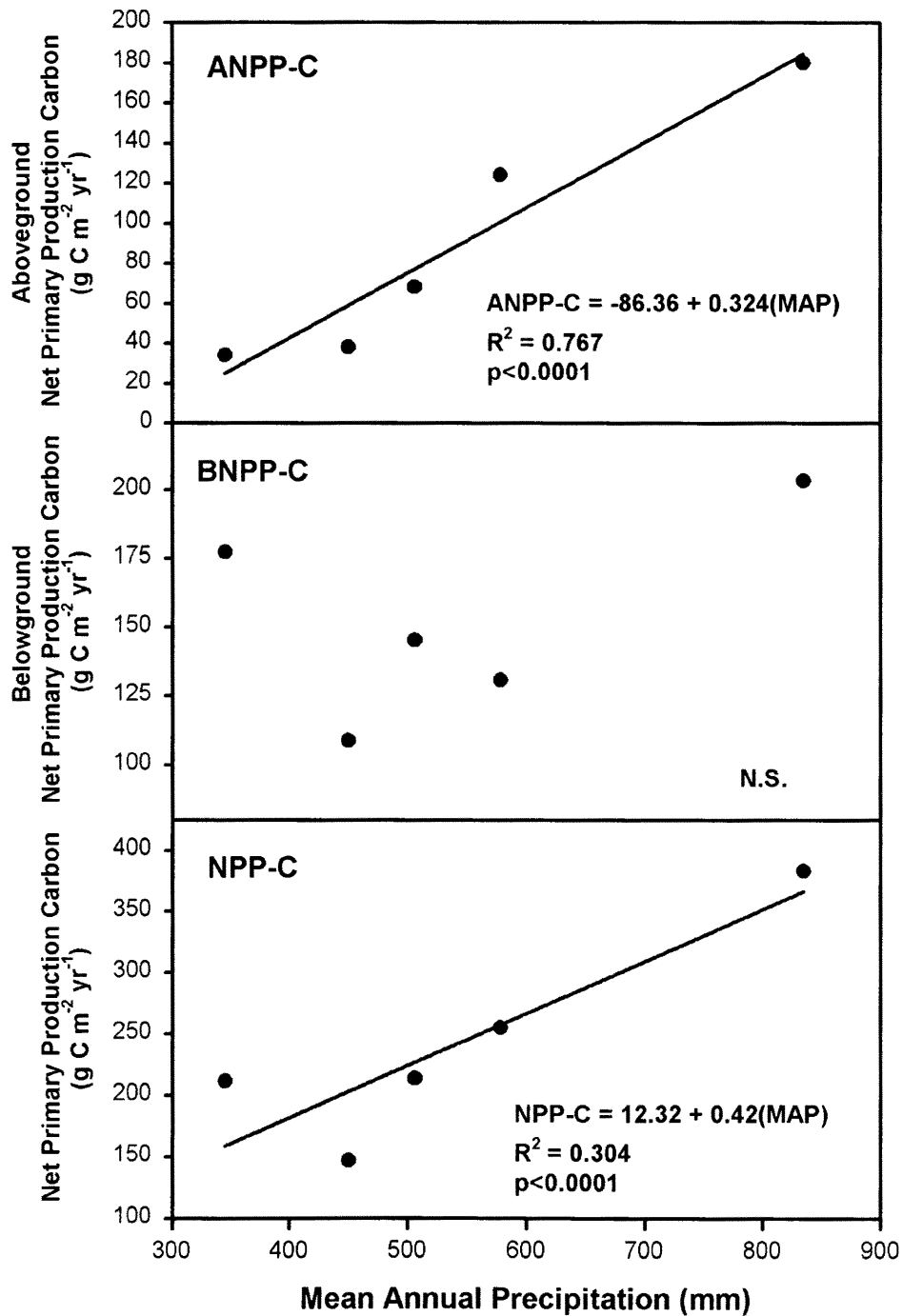
	<i>Soil Respiration</i>			
	df	F	p-value	Partial R <sup>2</sup>
<b>Site</b>	4	95.17	<0.0001	0.962
<b>Year</b>	1	185.79	<0.0001	0.702
<b>Site*Year</b>	4	16.00	<0.0001	0.242
<b>Location</b>	1	19.78	<0.0001	0.312
<b>Site*Location</b>	4	0.87	0.4885	0.055
<b>Year*Location</b>	1	0.15	0.6981	0.002
<b>Site*Year*Location</b>	4	2.40	0.0642	0.151

**Table 3.4:** ANOVA of the main effects 'site' and 'year' on the carbon decomposition constant ( $k_c$ ) and the carbon lost via decomposition on a  $\text{g C m}^{-2} \text{yr}^{-1}$  scale (for details on how this variable was calculated see the Litter Decomposition section of the Methods) on both the common litterbags (BOGR) and the dominant plant litterbags for KONZA and the SGS sites only.

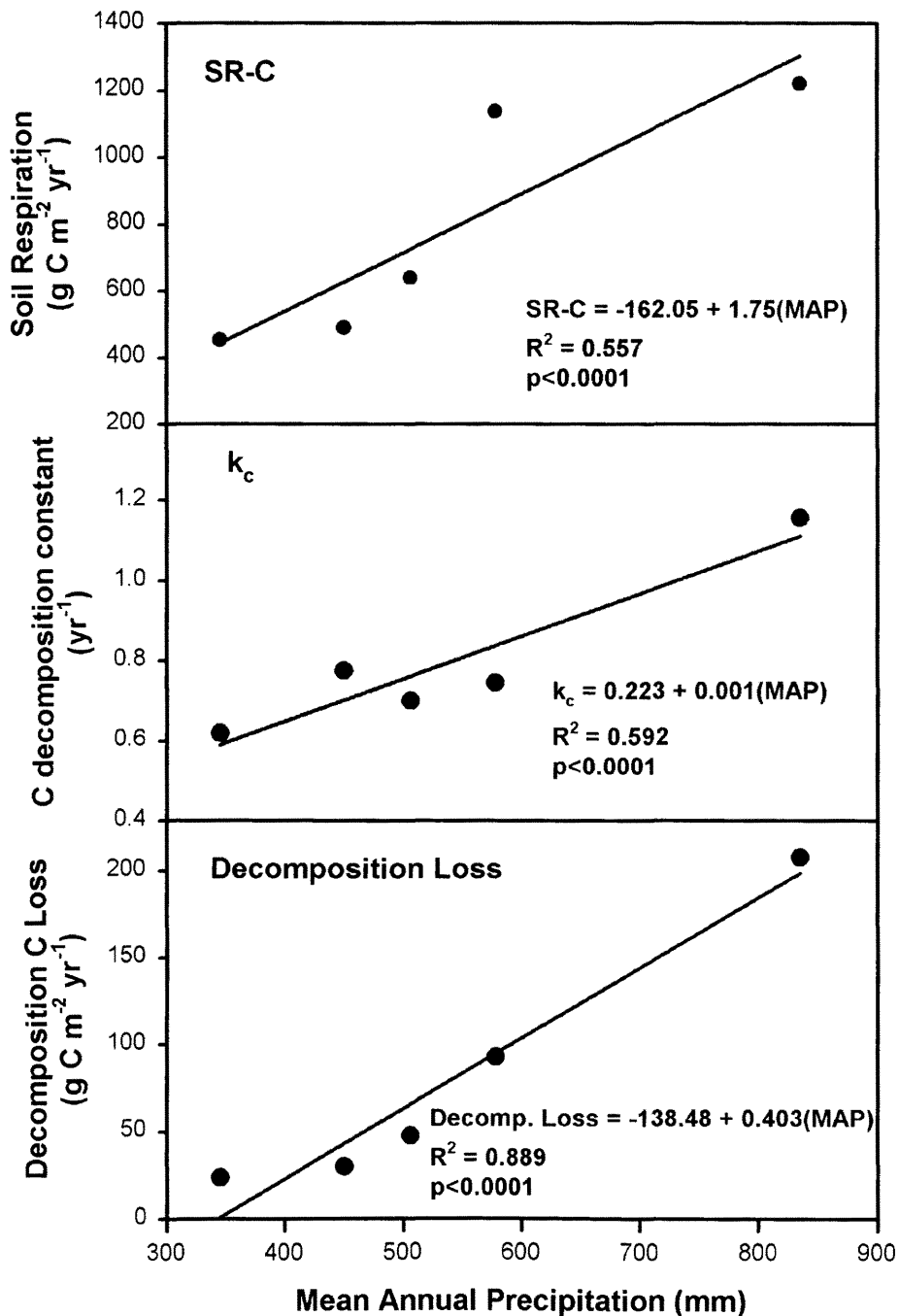
<b>BOGR</b>								
	<b><math>k_c</math></b>				<b>Decomposition Loss</b>			
	df	F	p-value	Partial $R^2$	df	F	p-value	Partial $R^2$
<i>Site</i>	4	46.90	<0.0001	0.883	4	738.43	<0.0001	0.992
<i>Year</i>	1	75.59	<0.0001	0.502	1	522.47	<0.0001	0.510
<i>Site*Year</i>	4	12.51	<0.0001	0.332	4	119.33	<0.0001	0.466

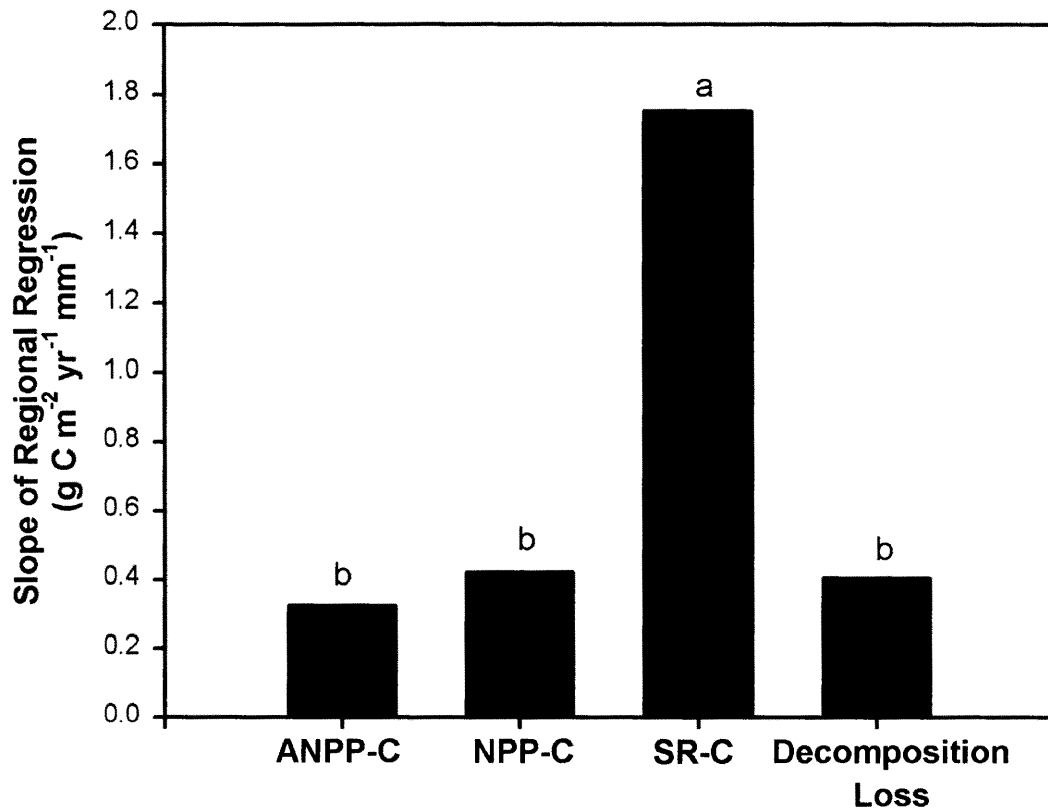
<b>Dominant</b>								
	<b><math>k_c</math></b>				<b>Decomposition Loss</b>			
	df	F	p-value	Partial $R^2$	df	F	p-value	Partial $R^2$
<i>Site</i>	1	3.48	0.0918	----	1	777.54	<0.0001	0.987
<i>Year</i>	1	44.52	<0.0001	0.763	1	198.09	<0.0001	0.627
<i>Site*Year</i>	1	3.83	0.0789	----	1	107.60	<0.0001	0.341



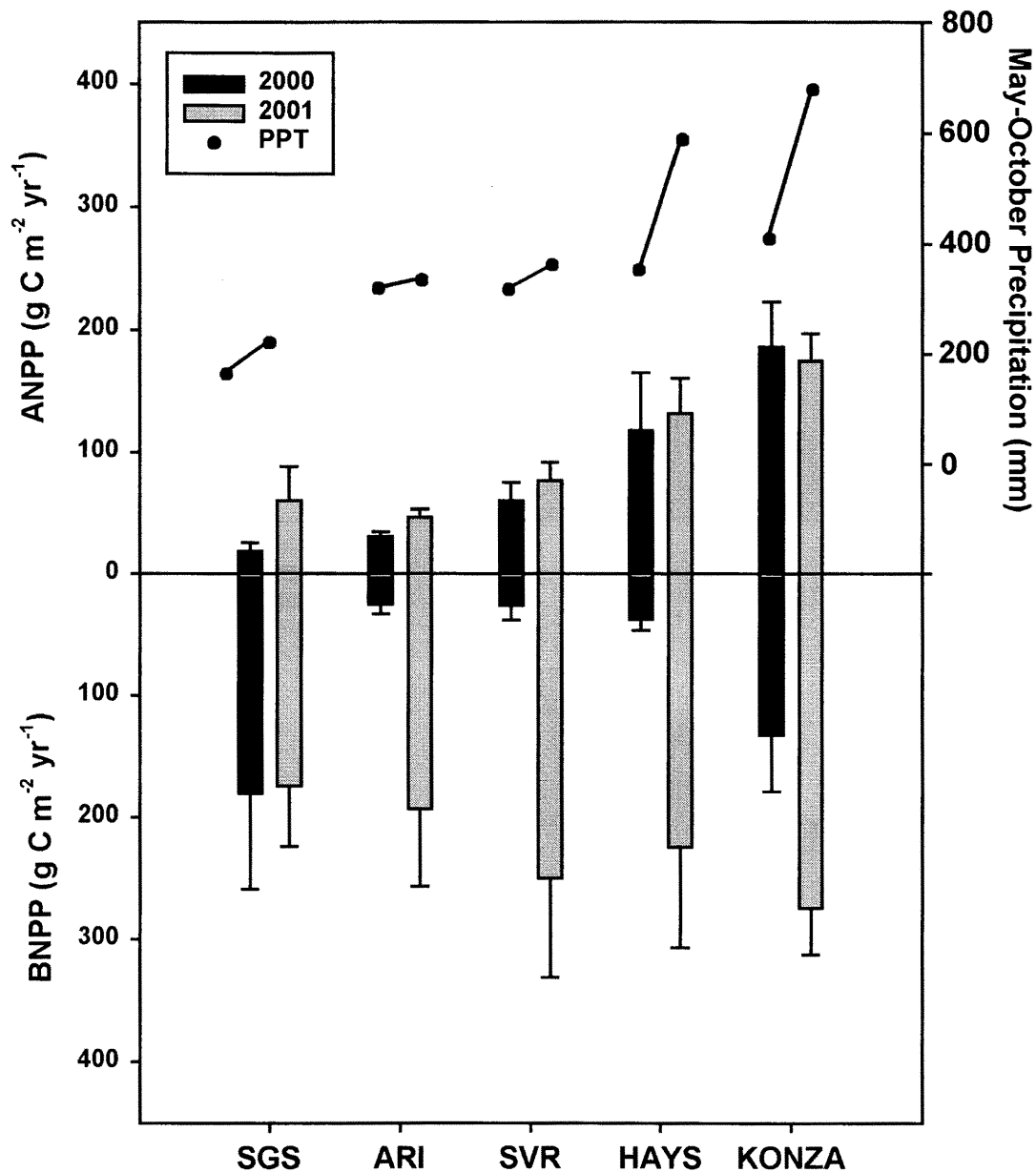
**Figure 3.1** - Regional regressions of aboveground net primary production carbon (ANPP-C), belowground net primary production carbon (BNPP-C), and the combined net primary production carbon (NPP-C) versus the mean annual precipitation (MAP, in mm) of the sites. The values presented here are the averages for the 2 years of the study (n=8-20 for each point). Regression line equations and statistics are presented when the model was significant (p<0.05, N.S. indicates no significant relationship was found).



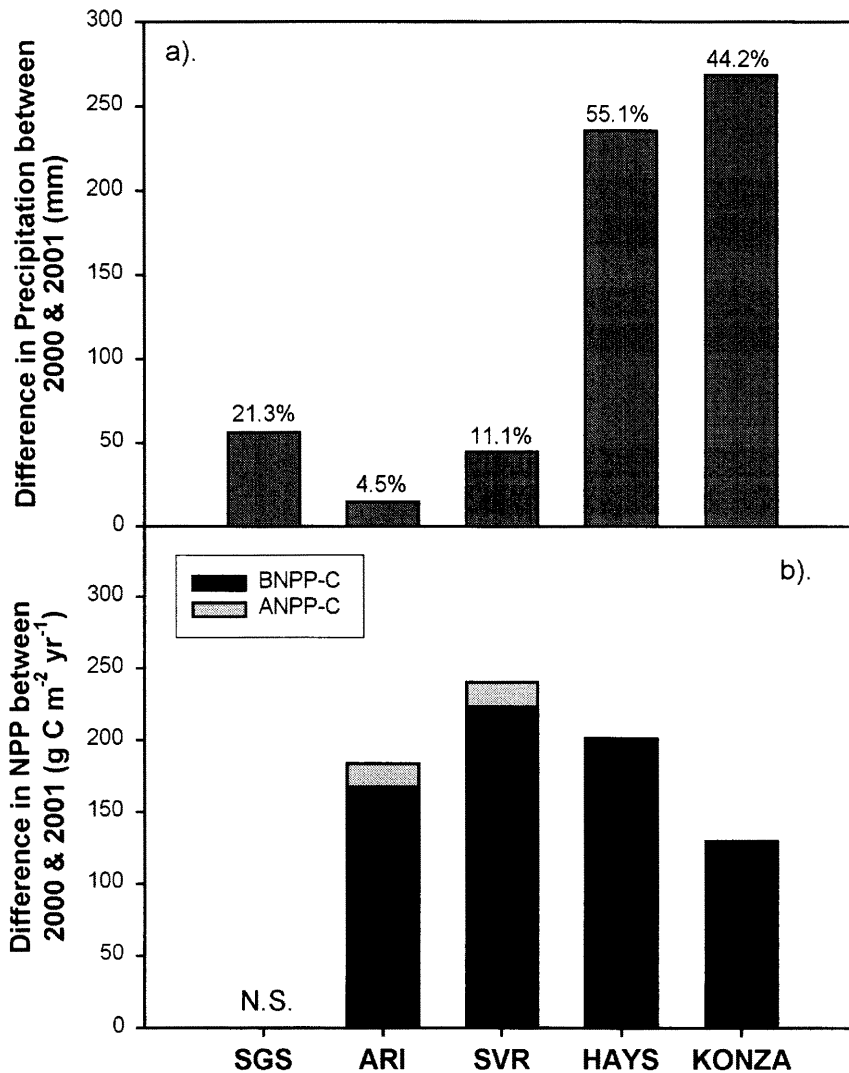
**Figure 3.2** - Regional regressions of carbon lost via soil respiration (SR-C), the carbon decomposition constant ( $k_c$ ), and the carbon lost via decomposition (Decomposition Loss - see the Litter section of the Methods for calculation details) versus the mean annual precipitation (MAP, in mm) of the sites. The values presented here are the averages for the 2 years of the study ( $n=6-8$  for each point). Regression line equations and statistics are presented when the model was significant ( $p<0.05$ ).



**Figure 3.3** - The slope of the significant C input and output ( $p < 0.05$ ) regional regressions. Significant differences ( $p < 0.0001$ ) between the slopes of the regional regression equations for the C input and output variables are indicated by different letters. The significant regional regression for the carbon decomposition constant ( $k_c$ ) could not be included on this graph as the units of  $k_c$  ( $\text{yr}^{-1}$ ) are not the same as the other measured parameters.

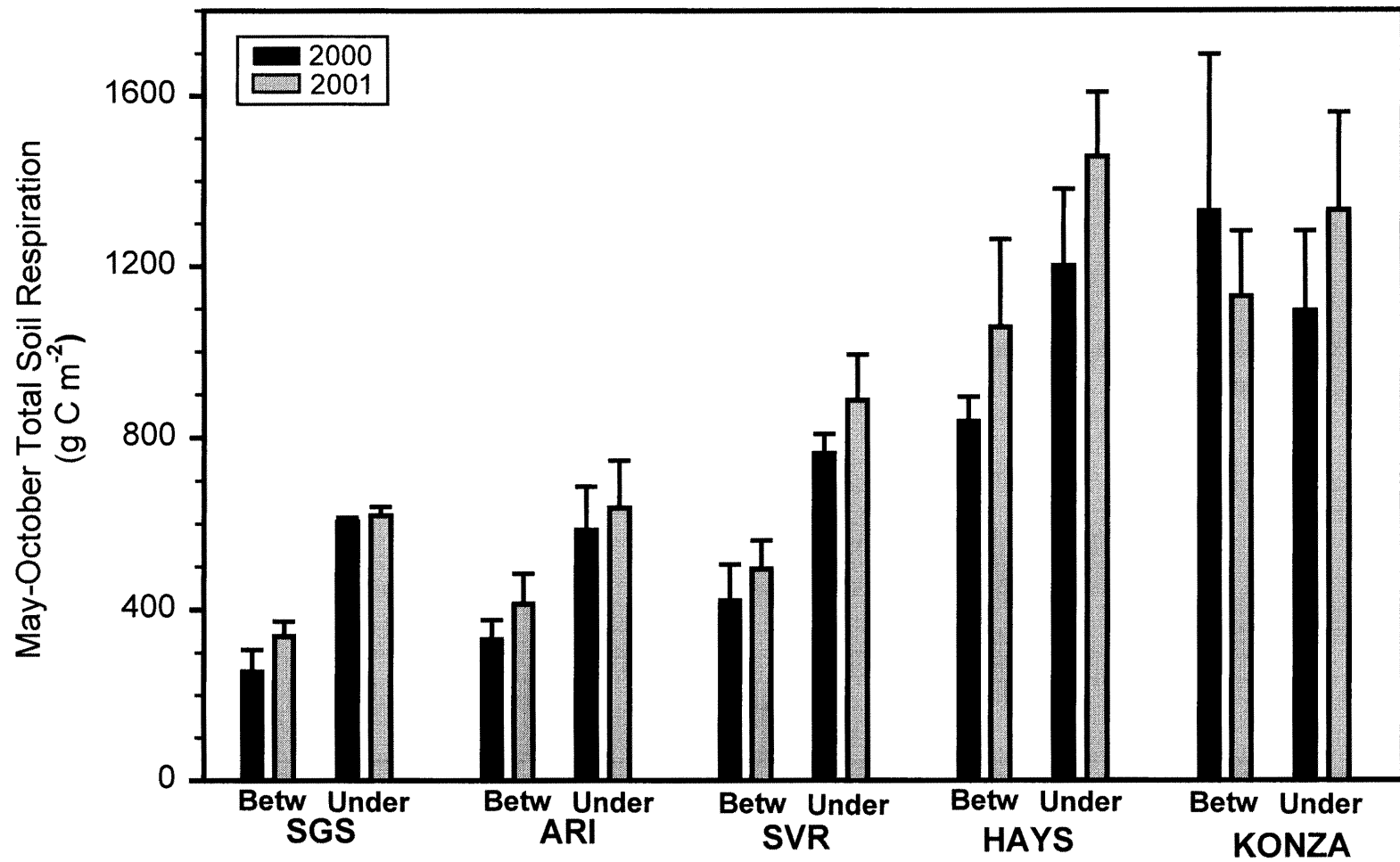


**Figure 3.4** - Carbon inputs (aboveground net primary production carbon - ANPP-C and belowground net primary production carbon - BNPP-C) at each of the sites (SGS, ARI, SVR, HAYS, and KONZA) for both 2000 and 2001. The scales are the same for both the ANPP-C and BNPP-C values. The top panel of the graph also includes the total amount of precipitation received during May-October for each year (2000 is the first dot and 2001 the second) at each of the sites.

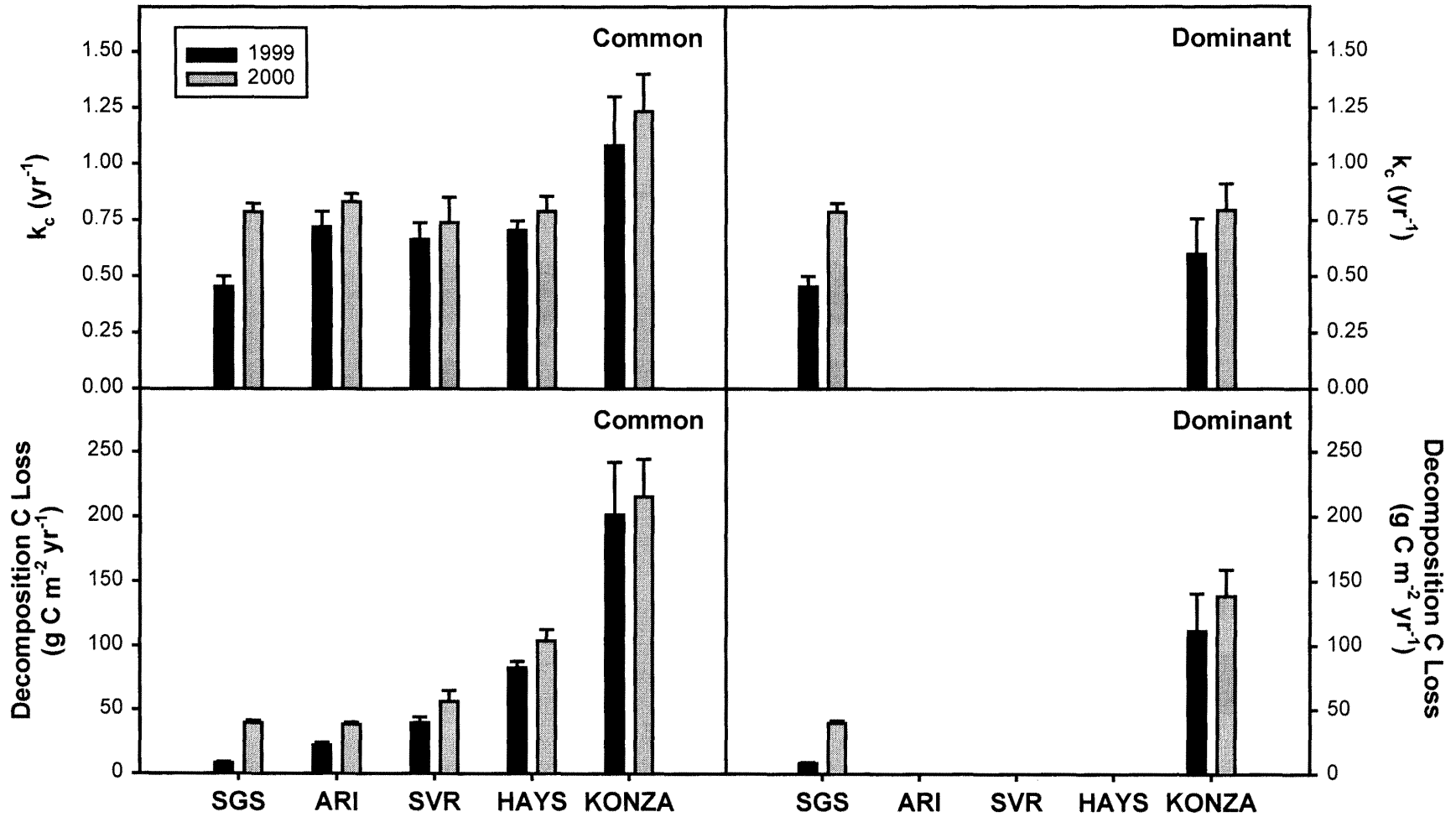


**Figure 3.5 -** (a) The difference in May-October total precipitation between 2001 and 2000 for each of the sites. The positive values indicate 2001 had more rainfall than 2000. The values on top of the bars for each site show the percentage of the long-term May-October total precipitation that is accounted for by the difference between the 2 years. (b) The difference between 2001 and 2000 NPP-C. Each NPP-C bar for every site is broken down into the above- and belowground components. For the SGS site, no significant difference in BNPP-C values were measured between the 2 years, and even though there was a measurable increase in ANPP-C in 2001, the total NPP-C was not significantly different between the 2 years (N.S.). At KONZA and HAYS, the entire difference in NPP-C between the two years was accounted for by the BNPP-C response. Significant differences ( $p < 0.05$ ) in NPP-C between the 2 years were observed at ARI, SVR, HAYS, and KONZA.

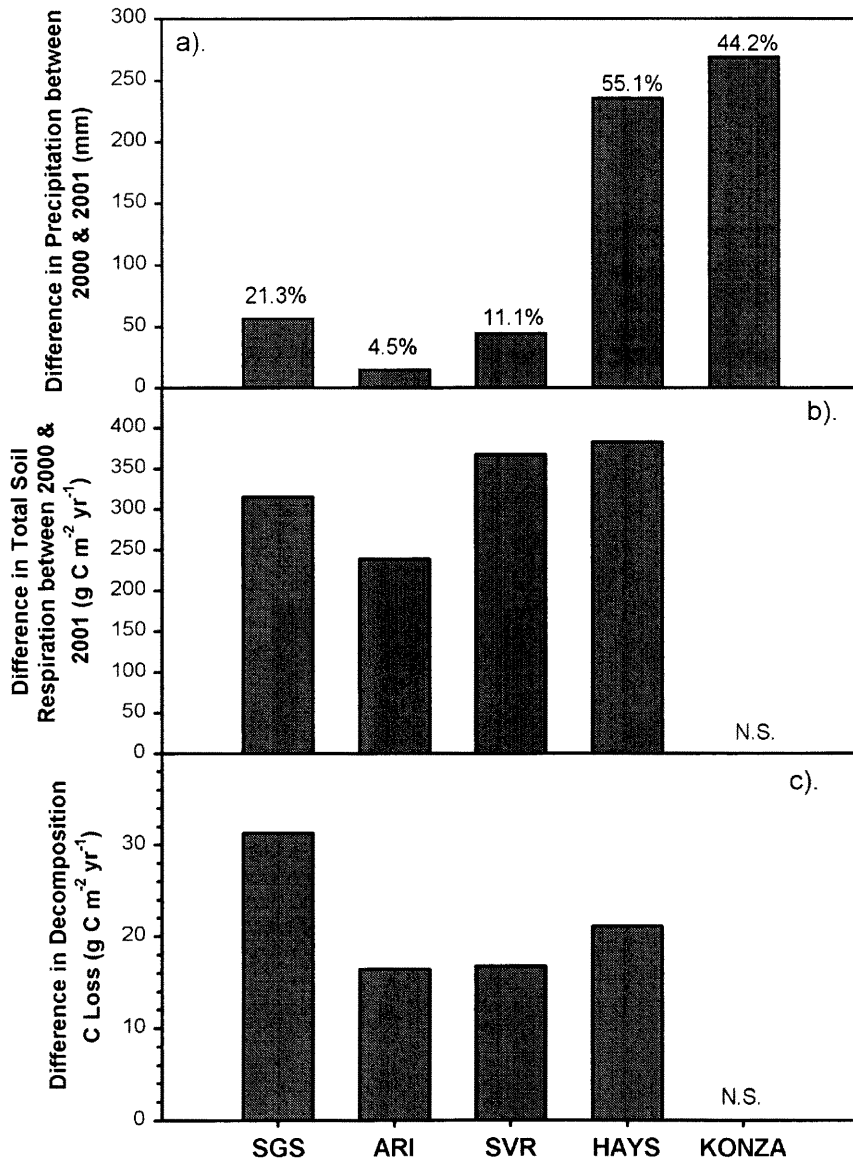




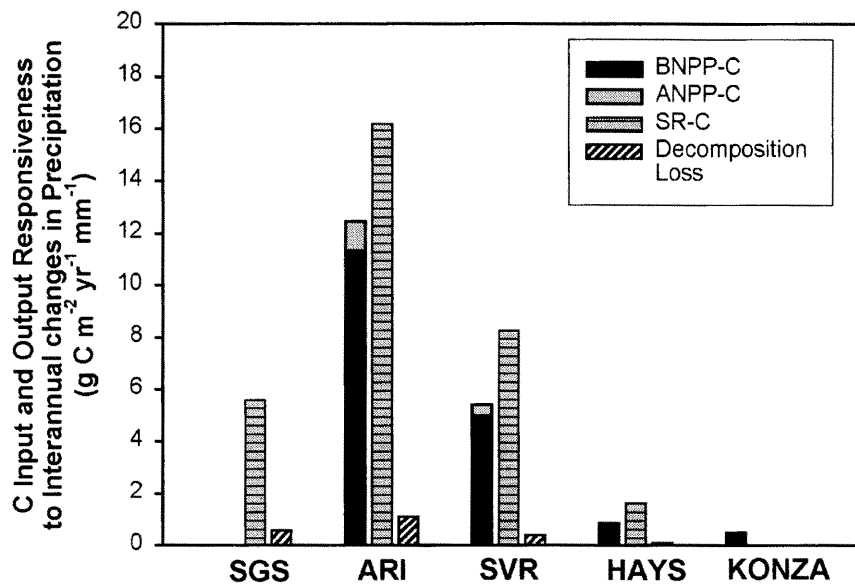
**Figure 3.6** - May-October total soil respiration for 2000 and 2001 and for both measured locations (in the interspaces between individual plants - Betw, and under crowns of plants - Under) at all sites (SGS, ARI, SVR, HAYS, and KONZA).



**Figure 3.7** - The carbon decomposition constant ( $k_c$ ) and the aboveground decomposition carbon loss for both the common litter (*Bouteloua gracilis* material) and the dominant litter types for both years of the study (2000 & 2001). At the SGS, the common and dominant litter types are the same, but at KONZA, the dominant litter is composed of *Andropogon gerardii* material. There were no dominant litterbags put out at the intermediate sites (ARI, SVR, and HAYS). Refer to the Litter section of the Methods for the calculation of the aboveground decomposition carbon loss measurement.



**Figure 3.8** - (a) The difference in May-October total precipitation between 2001 and 2000 for each of the sites. The positive values indicate 2001 had more rainfall than 2000. The values on top of the bars for each site show the percentage of the long-term May-October total precipitation that is accounted for by the difference between the 2 years. (b) The difference between 2001 and 2000 in the May-October total soil respiration. All sites, except KONZA which had no significant difference between the 2 years (N.S.), had higher rates of soil respiration in 2001 than 2000. (c) Differences between 2001 and 2000 in the aboveground decomposition carbon lost calculated using the common litter  $k_c$  values. All sites had significantly more carbon lost via aboveground decomposition in 2001 than 2000 except for KONZA (N.S.).



**Figure 3.9** - The interannual response of net primary production carbon (broken into the aboveground, ANPP-C and belowground, BNPP-C components), soil respiration (SR-C), and aboveground decomposition carbon loss (Decomposition Loss) calculated on a "per mm of rainfall" basis. Only response variables with significant interannual differences at individual sites are shown.

## CHAPTER IV: MICROBIAL COMMUNITY COMPOSITION ACROSS THE CENTRAL GREAT PLAINS OF NORTH AMERICA: LANDSCAPE VERSUS REGIONAL VARIABILITY

### Introduction:

Mean annual precipitation and temperature increase from west to east across the Central Great Plains of North America. Associated with these climatic gradients, plant community type changes across this region from western semi-arid shortgrass steppe to mixed grass and finally eastern sub-humid tallgrass prairie (Sims et al. 1978, Epstein et al. 1996, Lauenroth et al. 1999, Lane et al. 2000). Shifts in the dominant plant species associated with these different community types influence production allocation patterns (root:shoot ratios) (Sims and Singh 1978, Sims et al. 1978, Lane et al. 2000, McCulley 2002) and litter quantity and quality (Murphy et al. In press). These trends in plant production and litter chemistry may be important factors in governing the pool size and activity of soil microbiota (Hobbie 1992, Zak et al. 1994, Aerts 1997). In fact, previous work across this region has shown that total soil carbon and nitrogen, net primary production, soil microbial biomass, litter decomposition, and rates of soil respiration increase from shortgrass steppe to tallgrass prairie (Sala et al. 1988, Burke et al. 1989, Zak et al. 1994, Vinton and Burke 1997, Lauenroth et al. 1999, McCulley 2002). Similarly, rates of *in situ* net nitrogen mineralization are predicted to increase from west to east

across this region (Burke et al. 1997), although field estimates have not verified this prediction (McCulley 2002). Thus, across the Central Great Plains, increases in substrate availability and generally more favorable climatic conditions appear to result in larger microbial biomass pools and higher microbial activity.

In addition to these regional trends in substrate availability, microbial biomass, and biogeochemistry, landscape-level variability in these same parameters exists across topographic gradients at individual site locations. For example, work from the shortgrass steppe and tallgrass prairie has shown that lowlands have greater aboveground biomass than uplands (Schimel et al. 1985, Schimel et al. 1991, Briggs and Knapp 1995, Blair 1997, Turner et al. 1997). Additionally, soil organic carbon and total nitrogen, potential carbon and nitrogen mineralization rates, and soil respiration rates are generally higher in lowlands than uplands in shortgrass steppe (Schimel et al. 1985, Burke et al. 1999, Hook and Burke 2000). Reports of topographic trends in *in situ* net nitrogen mineralization measurements are inconsistent in the shortgrass steppe (lowlands higher than uplands in Schimel *et al.* 1985, but no differences in Hook and Burke 2000), but results from the tallgrass prairie indicate that uplands have higher *in situ* net nitrogen mineralization rates than lowlands (Turner et al. 1997). While these landscape-level patterns in substrate availability and nutrient cycling are perhaps more subtle than the regional patterns, these studies do suggest biogeochemical variability at the landscape-level does exist in plant communities across the Central Great Plains.

Thus, there is evidence that microbially-mediated biogeochemical processes vary across landscapes from uplands to lowlands and regionally from shortgrass steppe to tallgrass prairie. This observed landscape and regional variability could be the result of improved microclimatic conditions supporting higher microbial activity for longer periods of time during the growing season, increased substrate availability allowing larger microbial biomass pools, and/or shifts in the microbial community composition in response to the alterations in microclimate and substrate availability as one moves from uplands to lowlands or from shortgrass steppe to tallgrass prairie. While there is some support for improved climatic conditions and increased substrate availability across topographic gradients and regionally, little is known about the regional or topographic variability present in the microbial community composition of these grassland communities.

As mentioned previously, plant community composition changes across the region. Shortgrass steppe, mixed grass prairie, and tallgrass prairie have distinct floristic assemblages with varying proportions of C<sub>3</sub> and C<sub>4</sub> grasses, shrubs, and forbs (Coupland 1992, Kucera 1992, Lauenroth and Milchunas 1992, Epstein et al. 1997). In addition to these regional plant community composition changes, landscape-level variability in plant community composition also occurs in these grassland communities; differences in the relative abundance of C<sub>3</sub> and C<sub>4</sub> grasses and forbs between upland and lowland topographic positions are commonly reported (Barnes et al. 1983, Milchunas et al. 1989, Hartnett and Fay 1998). Previous work has shown that

plant species can impact both soil microbial biomass and community composition (Groffman et al. 1996, Grayston et al. 1998). Thus, I hypothesized that due to plant community composition and substrate availability changes associated with the landscape and regional microclimatic gradients, I would find differences in the microbial communities existing across these gradients.

In addition to the spatial variability in microbial biomass and community composition associated with these regional and landscape gradients, both microclimatic conditions and substrate availability exhibit a high degree of temporal variability across seasons. Grassland communities of the Central Great Plains are characterized as receiving the majority of their annual rainfall during the May through September growing season. However, dry periods are common, with a relatively high frequency of drought (Lauenroth and Burke 1995). Aboveground plant production (i.e., substrate availability) is significantly related to precipitation trends across the region and through time at specific sites (Sala et al. 1988, Lauenroth and Sala 1992, Knapp et al. 1998); however, while belowground production, a potentially large microbial substrate in grasslands, does appear to respond to interannual variability in precipitation, the relationship is not as responsive as aboveground production and is not well quantified across the region (Hayes and Seastedt 1987, Milchunas and Lauenroth 2001). Peaks in dominant plant species photosynthetic rates generally occur in May-June in most grassland types of this region (Knapp 1985, Monson et al. 1986), and while little data exist on the seasonal variability of root chemistry and quality of root exudates, it seems reasonable to



hypothesize that some type of seasonal variation in this potentially important labile carbon source does exist (Klein 1977, Myers et al. 2001). Thus, microbial biomass and community composition might be expected to reflect these seasonal trends in plant activity and microclimatic conditions.

My objective for this study was to determine whether microbial community composition and biomass differed regionally across three grassland community types (shortgrass steppe, mixed grass and tallgrass prairie), topographically across uplands and lowlands at specific sites, and temporally through the growing season. I was also interested in quantifying whether spatial variability in microbial community composition across the regional scale was larger or smaller than the local spatial variability displayed across landscape-level topographic units, or the temporal variability at individual sites throughout the season.

## **Methods:**

### *Sampling Design*

To accomplish these objectives, I established 5 grassland sites spanning a 800 kilometer transect from eastern Colorado to eastern Kansas (Table 4.1). All 5 sites were sampled in October 2000 and June 2001 to determine whether microbial community composition and biomass differed regionally across these grassland community types and between seasons at individual sites. To address my objectives associated with landscape-level topographic variability, 4 uplands and lowlands at 3 of the 5 sites were also sampled in October 2000.

## Study Area

All sites are native grassland managed with moderate levels of cattle grazing and have not been previously cultivated, as evidenced by detailed site histories and well-developed soil profiles that lack a plow layer. The two westernmost sites, the Shortgrass Steppe Long-term Ecological Research site (SGS) and a Nature Conservancy owned ranch near the Arickaree River (ARI), are typical shortgrass steppe plant communities, dominated by *Buchloe dactyloides* and *Bouteloua gracilis* (Association 1986, Lauenroth and Milchunas 1992). Smokey Valley Ranch (SVR), also owned by The Nature Conservancy, has a mixture of both shortgrass steppe and mixed grass prairie vegetation. In central Kansas, the range area owned and managed by Ft. Hays State University (HAYS) is dominated by plants typically found in southern mixed grass prairie, *Schizachyrium scoparium* and *Bouteloua curtipendula* (Coupland 1992). The Konza Prairie Biological Station (KONZA), is owned by The Nature Conservancy, managed by Kansas State University, and is also a Long-term Ecological Research site. KONZA is a tallgrass prairie with dominant plant species being *Andropogon gerardii* and *Sorghastrum nutans* (Silletti and Knapp 2000). KONZA is the only site where typical grassland management includes fire. Our plots at this site are located in a watershed that burns once every four years. The plots were burned in April 1996 and 2000. Both mean annual temperature and precipitation increase from west to east across this transect (Table 4.1). I selected these sites while attempting to minimize textural

differences across the gradient. Selected soil characteristics and taxonomy, as well as a summary of biogeochemical parameters measured as part of a concurrent study (McCulley 2002) for all five sites are presented in Table 4.1. At each site, 4 permanent plots (4m x 4m) on a level upland were established in 1999. These permanent plots at all five sites represent the “regional dataset” discussed in this paper. The landscape-level sampling occurred at SGS, HAYS, and KONZA, each representing one of the grassland types encountered across this transect (shortgrass steppe, mixed grass and tallgrass prairie). The 4 upland and lowland samples from these sites comprise the “landscape dataset” discussed herein.

### *Field Sampling*

I collected soil samples to address the questions about regional trends with a soil corer (0-10 cm depth, internal diameter = 4.8 cm) at 1, 3, and 5 m along 5 m transects run from one randomly determined corner of 3 of the permanent plots at each of the 5 sites in October 2000 (n=9 per site). Previous work from the semi-arid shortgrass steppe has shown that presence and absence of plants can have significant effects on measured biogeochemical parameters (Hook et al. 1991), and that this effect varies across the regional gradient (Burke et al. 1999). However, the objective of this paper was to detect microbial community variability associated with plant community type across the region and at differing landscape positions; therefore, I sampled soils randomly (both under and between plants) in an attempt to capture the maximum

variability representative of the plant community type. One of the 5 m transects sampled in October 2000 was re-sampled in June 2001 (n=3 per site) at each of the 5 sites to determine seasonal differences in microbial community composition. June 2001 soil samples were taken randomly from within 50 cm of the October 2000 sample.

On the same day I sampled the permanent plots for the regional dataset in October 2000, 4 uplands and 4 lowlands were sampled at the SGS, HAYS, and KONZA sites. At all uplands and lowlands, one soil sample (0-10 cm) was collected.

I removed all soil samples from the soil corer with the smallest amount of disturbance possible, stored them in plastic bags, and put them immediately on dry ice. Samples were kept on dry ice for less than 24 hours before being transferred to a  $-70^{\circ}\text{C}$  freezer, where they were stored for up to 6 months before I performed microbial community composition analysis. While previous work has shown that storage of samples can affect microbial community composition, storage effects were small compared to differences associated with soil type and extraction method (Schutter and Dick 2000). All of my samples were stored and extracted in a similar manner. Therefore, differences between sites and landscape positions are reflective of actual microbial community composition differences and not simply storage artifacts.

### *Microbial Community Composition*

I assessed microbial community composition using phospholipid fatty acid (PLFA) analysis (Vestal and White 1989). PLFA analysis allows quantification of the viable microbial biomass and the functional group composition at the time of sampling based on the total amount of phospholipids extracted and the chemical make-up of the constituent fatty acids (Vestal and White 1989). Frozen samples were allowed to thaw for 15-30 minutes. Each sample/core was homogenized, and then ~20 g of field moist soil was extracted in a single phase, phosphate buffered  $\text{CHCl}_3\text{-CH}_3\text{OH}$  solution to remove PLFAs (Bligh and Dyer 1959). I separated PLFAs by silicic acid chromatography and derivitized the PLFAs (cleave the fatty acids from the phospholipid glycerol backbone and replace the glycerol bonds with methyl groups) in an alkaline system to form fatty acid methyl esters (FAMEs) (White et al. 1979). I separated and quantified FAMEs on a gas chromatograph linked to a mass spectrometer. Individual FAME spectra were identified using an existing FAME library (D.C. White, 1999, unpublished data).

FAMEs are described by standard nomenclature (IUPAC-IUB 1977) ( $\text{A:B}\omega\text{C}$ ), where "A" indicates the total number of carbon atoms, "B" the number of unsaturated bonds, and " $\omega\text{C}$ " indicates the number of carbon atoms between the aliphatic end of the molecule and the first unsaturated bond. *Cis* and *trans* geometric isomers are indicated by the suffixes "c" and "t", respectively. Other notations are "Me" for methyls, "cy" for cyclopropyl groups, and the prefixes "i" and "a" for *iso* and *anteiso* methyl branching, respectively.

### *Soil Characteristics*

After soil samples were processed for microbial community composition, a 10 g sub-sample was dried at 110°C for three days to determine gravimetric water content. I air-dried and sieved (2 mm) the remaining soil. From this sieved soil, a sub-sample was ground in a ball mill, acid-washed to remove inorganic C, and analyzed on a LECO CHN-1000 analyzer (St. Joseph, MI) for organic C and total N. Additional sub-samples were used for texture analysis using the hydrometer method (Gee and Bauder 1986). HAYS soils contained significant quantities of calcium carbonate; therefore, soil organic C was determined via the loss-on-ignition method (Nelson and Sommers 1996).

### *Statistical Analyses*

I utilized two statistical approaches to determine whether the microbial community composition and biomass data differed regionally across the grassland types, topographically across uplands and lowlands at specific sites, and temporally through the growing season. Analysis of variance was used to determine whether significant differences in the total quantity of PLFA extracted from the soil samples (and the soil covariate parameters) existed across the regional gradient of sites (CPER, ARI, SVR, HAYS, and KONZA), across the topographic landscape-level gradients (location being either upland or lowland), and between the two sampling periods over the course of the growing season (October 2000 and June 2001). I transformed data as needed to fit the

normality assumptions, and I used least significant difference means separation tests to interpret the significant main effects of 'site,' 'location,' and 'season.'

For the microbial community composition data, I first ran a principal component analysis (PCA) on the mole fraction of 19 FAMEs that were consistently found in all the samples and represent a wide array of microbial functional groups. To maximize the power of the PCA, I incorporated both the landscape and regional datasets into one analysis. I log transformed the mole fraction of each FAME used in the PCA. Once I acquired the first and second principal component axes/weights for each sample, I then ran an ANOVA similar to that used for the total PLFA data to determine whether the principal component axes scores for the samples were significantly different across the regional 'site' gradient, landscape-level topographic positions, and the season of sampling. To further evaluate the change in microbial community composition, the mole fractions of the 19 FAMEs used in the PCA were separated into their representative functional groups (gram positive bacteria, gram negative bacteria, non-specific bacteria, actinomycetes, fungi, and protozoa) and summed so that each sample had one relative abundance value for each functional group. I then ran these functional group data through the afore-mentioned ANOVA to assess the 'site', 'location' and 'season' main effects. The gram-negative and actinomycetes groups were log transformed to achieve normal distributions, and least significant difference means separation tests were used to evaluate significant main effects.

I also quantified the FAME richness, diversity, and evenness patterns for each of the microbial community composition samples. FAME richness is simply the total number of FAMEs identified per sample, and FAME diversity was calculated as the Shannon index,  $H' = - \sum(P_i \times \log P_i)$ , where  $P_i$  is the proportional amount of each FAME in a sample. I calculated FAME evenness as  $E = H'/\ln(\text{Richness})$ . The same analysis of variance approach used on the total microbial biomass data was utilized on these calculated FAME indices to evaluate the significance of the main effects of 'site,' 'location,' and 'season.' All of these indices were normally distributed.

In an attempt to address whether the spatial variability in microbial community composition and biomass across the region was larger or smaller than the local spatial variability across landscape-level topographic units or the temporal variability at individual sites throughout the season, I calculated the proportion of variance accounted for by the main effects of the regional 'site,' the landscape-level 'location,' and the temporal 'season' from the ANOVAs with the microbial functional group and total PLFA data. This provides a quantitative estimate of each of these main effect sources of variability within their respective analysis of variance models that can then be compared.

Additionally, stepwise regressions were performed to assess the relationship of total PLFA, the weights of the first and second principal component axes for the microbial community composition data, the FAME richness, diversity and evenness indices, and the relative abundances of the functional groups to the soil characteristics measured (%C and N, %sand and



clay, and gravimetric water content). All ANOVAs, the PCA, and regressions were performed using SAS, version 8 (SAS 1989).

## **Results:**

I found significant regional, topographic, and seasonal influences on both the soil characteristics and microbial data. In the following sections, I discuss these spatial and temporal influences on each type of data collected: soil characteristics, microbial biomass and community composition (including FAME richness, diversity, and evenness indices), and microbial functional groups.

### *Soil Characteristics & Gravimetric Moisture*

Soil %C, %N and C:N ratio increased from western semi-arid shortgrass steppe to eastern sub-humid mixed and tallgrass prairie (Table 4.1). Averaged across the June and October sampling dates, gravimetric soil moisture ranged from a low of 19% at ARI to a high of 40% at KONZA. Gravimetric soil moisture was significantly different across sites ( $p < 0.0001$ ) but not significantly different between sampling dates ( $p=0.29$ ); however, a significant site\*season interaction term ( $p=0.0005$ ) precluded means separation tests. The coarsest and finest textured soils occurred at ARI and KONZA, respectively. Bulk density mirrored these textural trends, with highest bulk density corresponding to coarsest soils, and soil pH decreased from west to east across the region (Table 4.1).

In the landscape dataset, all soil variables had a significant site main effect ( $p < 0.05$ ). Percent C, %N, and the C:N ratio all had significant site\*

landscape position interaction terms ( $p < 0.01$ ), precluding means separation tests, but trends across the region for these parameters are similar to those found in the regional dataset (Table 4.2). %Clay and gravimetric soil moisture increased significantly from shortgrass steppe to mixed and tallgrass prairie ( $p=0.011$  and  $p<0.0001$ , respectively). %Sand was significantly different at all 3 sites (KONZA<HAYS<SGS,  $p<0.0001$ ). Only %sand and gravimetric soil moisture were significantly different between upland and lowland landscape positions. Uplands had higher sand content and lower soil moisture values than lowlands ( $p<0.005$ ). These results were somewhat surprising given that previous studies have reported generally higher %C and N in lowland soils (Schimel et al. 1985, Yonker et al. 1988, Hook and Burke 2000). Both SGS and HAYS data concurred with these previous reports, but KONZA did not (Table 4.2). It is possible that the surficial upland soils at KONZA have higher concentrations of C and N than would otherwise occur if plant rooting volume was not constrained by limestone bedrock.

### *Microbial Biomass*

Total PLFA is an estimate of viable microbial biomass at the time of sampling (White et al. 1996). In general, total PLFA increased from western shortgrass steppe to eastern tallgrass prairie (Figure 4.1 and 4.2). Means separation tests on the regional dataset indicated increasing total PLFA with ARI < SVR < CPER, KZ, HAYS ( $p<0.0001$ ). Somewhat different results for the means separation tests on the main effect site were found for total PLFA in the

landscape dataset (CPER, HAYS < KZ, Figure 4.2,  $p < 0.0001$ ). Total PLFA was significantly higher in June 2001 than in October 2000 (Figure 4.1,  $p = 0.003$ ), although this significant season main effect was primarily driven by the large difference between October and June at the HAYS site. There were no significant differences between uplands and lowlands at any of the 3 sites sampled for the landscape dataset (Figure 4.2).

Regressions of total PLFA and the measured soil characteristics indicated that 66% of the variability in the regional total PLFA data was accounted for by %C and clay content (Table 4.3). However, in the landscape dataset, variability in total PLFA was primarily related to gravimetric soil moisture ( $R^2 = 63\%$ , Table 4.3).

#### *Microbial Community Composition*

The PCA on the combined datasets identified three significant axes explaining a combined total of 58% of the variance in the FAME multivariate data (Figure 4.3). To simplify presentation and interpretation of the data, only the first and second principal component axes are discussed here. Principal component axis 1 (PC 1) accounted for 24.5% of the variability and was significantly related to site for both the regional ( $p < 0.0001$ ) and the landscape dataset ( $p = 0.015$ ) but was not related to season or landscape location, suggesting that the sites differed significantly in their microbial community composition. Means separation tests of PC 1 weights were different for the two datasets (regional data set – ARI, KZ > SVR, CPER > HAYS, landscape data

set: – CPER > KZ, HAYS). Regressions of the principal component weights with the soil characteristics indicated that PC 1 was significantly related to %C and gravimetric soil moisture for the regional data set, but only %C for the landscape data set (Table 4.3). FAMEs indicative of fungi were negatively weighted on PC 1, while actinomycetal and bacterial groups had positive weights (Table 4.4).

Principal component axis 2 (PC 2) explained 18.9% of the variability in the FAME data, with negative weights on FAMEs indicative of eubacterial anaerobes, gram-negative, gram-positive, and non-specific bacteria. FAMEs with positive weights on PC 2 included gram-negative and gram-positive bacteria, as well as fungi (Table 4.4). Similar to PC 1, PC 2 was significantly related to site in both the regional and landscape datasets ( $p < 0.0001$ , for both), and means separation tests on PC 2 weights for both datasets indicated shortgrass steppe > mixed grass > tallgrass prairie. While no relationship between PC 2 and season was found ( $p = 0.719$ ), landscape location was significant ( $p = 0.004$ ), with uplands having higher PC 2 weights than lowlands particularly at HAYS (Figure 4.3b). Regression relationships for both datasets indicated PC 2 is related to % sand and gravimetric soil moisture (Table 4.3).

These PCA results indicate microbial community composition differed across the grassland types represented in this study. However, the PCA detected no significant shift in microbial community composition associated with changing seasons (June 2001 versus October 2000) at any of the sites, but

there were small differences between upland and lowland microbial communities, particularly at the mixed grass prairie site, HAYS.

The fact that PC 1 and PC 2 weights were significantly related to the main effect 'site' and were found via regressions to be related to the soil parameters %C, % sand, and gravimetric water content suggested that at least a portion of the categorical 'site' effect might have been accounted for by %C, % sand and gravimetric water content. To test whether the categorical 'site' effect was entirely accounted for by the significant soil parameters identified by the regression models, the soil parameters were added to the PC 1 and PC 2 ANOVA analyses with the main effect 'site' in the model statement. Addition of the soil parameters into the ANOVA analyses would cause the main effect 'site' to become insignificant only if the soil parameters completely accounted for the same variability encompassed in the categorical 'site' effect. In almost all cases, the significant soil parameters failed to completely capture the "site" main effect present in the principal component axes (only with the landscape dataset does % C account for all the 'site' variability in PC 1) suggesting that other variables not measured in this study contribute to the 'site' differences found in the PCA.

FAME richness and diversity were significantly different across sites in both the regional and landscape datasets (Figure 4.5); however, no significant differences between season or across landscape locations were found for either parameter. The significant 'site\*season' interaction term for the FAME diversity data precluded means separation tests for the site differences in the regional

dataset, but the trends were similar to those of the landscape dataset that showed significantly less FAME diversity at KONZA and HAYS than at SGS. In general, both FAME richness and diversity decreased from western semi-arid shortgrass steppe to eastern sub-humid tallgrass prairie. Regression analyses with these indices and the soil characteristics data indicated that FAME diversity was negatively related to soil %C and FAME richness increased with increasing sand content (Figure 4.6). No significant 'site,' 'season,' or 'location' main effects were found in the FAME evenness data.

### *Microbial Functional Groups*

As noted above, statistical analyses on FAMEs associated with different microbial groups indicate that there are significant differences among the sites in microbial community composition. I separated the mole fraction of the 19 FAMEs into their representative functional groups (gram positive bacteria, gram negative bacteria, non-specific bacteria, actinomycetes, fungi, and protozoa) and added the mole fractions of all FAMEs associated with a functional group together. This resulted in each sample having one relative abundance value for each microbial functional group. I conducted analysis of variance on the relative abundances of the different microbial functional groups. In the regional dataset, the relative abundance all microbial functional groups were significantly related to site except protozoa (Table 4.5). The shortgrass steppe site ARI generally had higher relative abundances of gram-positive bacteria, non-specific bacteria, and actinomycetal functional groups than the other sites

(Figure 4.4). KONZA had higher relative abundances of gram-negative bacteria, particularly of eubacterial anaerobes as indicated by large quantities of FAME cy19:0 (data not shown), and lower relative abundances of fungi than the other sites (Figure 4.4). Relative abundances of gram-negative bacteria, non-specific bacteria, and actinomycetal functional groups were also significantly different across sites in the landscape dataset (Table 4.5), and means separation tests on these groups yielded similar results to those of the regional dataset.

The relative abundance of non-specific bacteria, actinomycetal, and fungi functional groups were significantly different between seasons (Table 4.5). Bacteria and actinomycetes had higher relative abundances in October 2000 than in June 2001, but lower relative abundances were observed in October for fungi. This significant effect of season was primarily driven by changes at the shortgrass steppe sites (CPER and ARI). The marginal significance ( $p=0.043$  and  $0.047$  for bacteria and fungi, respectively, Table 4.5) and the non-uniform response of the 3 microbial functional groups to changes in season may account for the PCA indicating no seasonal shift in the microbial community composition.

Non-specific bacteria were the only microbial functional group to vary across upland and lowland locations ( $p=0.012$ , Table 4.5). Lowlands had greater relative abundances of these non-specific bacterial markers than uplands. This result suggests that the FAMEs associated with this functional

group (i14:0, 14:0, i17:0, and 18:0) are responsible for the separation of uplands and lowlands along PC 2 in the PCA.

Soil characteristic data were able to explain 31-60% of the variability in the microbial functional groups of the regional dataset (Table 4.3), except for protozoa for which the regression model found no significant soil variables. Fewer significant models were found in the landscape dataset. Only gram-negative bacteria, actinomycetes, and protozoa had significant relationships with any of the measured soil parameters (Table 4.3).

### **Discussion:**

Microbial biomass increased from shortgrass steppe to tallgrass prairie and was accompanied by alterations in the microbial community composition, such that the different grassland communities appear to have different associated microbial communities. Soil parameters were significantly correlated to both the microbial biomass and community composition data, but they do not account for all of the observed regional, 'site'-related variability in the data. In the following discussion, I elaborate on the regional, landscape-level topographic, and temporal trends in the microbial biomass and community composition data, as well as compare these different sources of variability in the microbial community composition data.



## *Microbial Biomass*

Microbial biomass and activity are largely governed by soil moisture and temperature and the quantity and quality of available substrates. The three grassland community types I studied exist across a 500 mm mean annual precipitation and a 4°C mean annual temperature gradient, with net primary production and rates of biogeochemical processes increasing concomitantly from western semi-arid shortgrass steppe to eastern sub-humid tallgrass prairie (Table 4.1). Along this gradient of decreasing climatic limitations and increasing substrate availability, I found larger viable microbial biomass pools in the tallgrass prairie than the shortgrass steppe. This result is consistent with microbial biomass estimates from chloroform fumigation-incubations on both shortgrass steppe and tallgrass prairie soils (Zak et al. 1994). Unfortunately, comparison of the specific values derived from the chloroform fumigation-incubation and phospholipid fatty acid techniques cannot accurately be made, although comparative equations to relate PLFA to both chloroform fumigation-extraction and substrate induced respiration measurements of microbial biomass do exist (Bailey et al. *In press*). My microbial biomass values for the tallgrass prairie site, KONZA, are within the range of those reported from restored tallgrass prairie in Illinois (KONZA: 80 to 150 nmol PLFA g soil<sup>-1</sup>; Illinois: 79 to 123 nmol PLFA g soil<sup>-1</sup>; (Bailey et al. *In press*). Zak et al. (1994) report an almost ten fold difference in microbial biomass carbon between the shortgrass steppe and tallgrass prairie sites, but my findings indicate at most only a four-fold difference in total viable microbial biomass. These differing

magnitudes of regional trends in microbial biomass are probably the result of the two methodologies estimating different microbially-associated parameters. The chloroform fumigation-incubation technique most likely incorporates measures of microbial biomass (viable or non-viable at the time of sampling) as well as some labile soil organic carbon pools (Jenkinson and Powlson 1976), while phospholipid fatty acid analysis estimates only viable microbial biomass, as phospholipids are found primarily in intact cell membranes and degrade quickly upon cell lysis (Vestal and White 1989).

In a study using the phospholipid fatty acid procedure, similar trends were found across a regional climatic gradient extending from arid to semi-arid Judean desert ecosystems (Steinberger et al. 1999). Microbial biomass increased with increasing precipitation, and the specific values of total PLFA reported from those arid and semi-arid ecosystems are within the same range as those reported in this study. Similarly, my values correspond well with total PLFA reported from temperate agricultural grasslands in the UK (Bardgett et al. 1999) and are within the range of those reported for various forest types in Lower Michigan (Myers et al. 2001).

While total PLFA generally increased from shortgrass steppe to tallgrass prairie in both the regional and landscape datasets, means separation tests on the regional dataset indicated one of the shortgrass steppe sites, SGS, had total PLFA values similar to those measured at the mixed grass or tallgrass prairie sites. I believe this result may be related to soil texture. The regional SGS site was intentionally located on a fine-textured soil type in order to reduce

textural differences across the regional dataset. However, the SGS landscape sites as well as the second regional shortgrass steppe site, ARI, were both located on coarser, sandy loam soils and had lower total PLFA and % C values. By modifying soil microclimatic conditions and soil organic carbon content, soil texture is known to play a role in determining microbial biomass and activity as well as impacting microbial community composition (Schimel et al. 1994, Zak et al. 1994, Scott et al. 1996, Schutter and Dick 2000). This study was not designed specifically to address the role of soil texture on viable microbial biomass; however, the differences in total PLFA measured between the SGS regional and landscape sites (which were sampled on the same day in October) and % clay being a significant parameter in the regional total PLFA regression model (Table 4.3) support the idea that increased soil clay content leads to more stabilization of soil C and higher microbial biomass (Schimel et al. 1994).

Although previous studies have found increased substrate availability and improved microclimatic conditions in lowlands compared to uplands, and although I measured higher gravimetric soil moisture contents and lower percentages of sand in lowlands than uplands at all sites in the landscape dataset, I did not find increased viable microbial biomass in lowlands compared to uplands (Figure 4.2). Similarly, Zak et al. (1994) found no differences in microbial biomass across catenary sequences in either the shortgrass steppe or tallgrass prairie. These results may be due to the fact that both studies sampled uplands and lowlands at only one point in time (October in this study; July or August for Zak et al. (1994)). By October, plant activity is largely

reduced, and depending on water limitations during the Zak et al. (1994) study, the plants could have been largely dormant at the sites. Landscape-level variability in substrate availability via root exudation, perhaps a more important control on the total viable microbial biomass pool, may be accentuated early during the growing season at these sites (Klein 1977) and might then promote differentiation in total PLFA between uplands and lowlands. Temporally, I did find increased total PLFA during the peak growing season (Figure 4.1). This result combined with the knowledge that there was no significant difference in gravimetric soil moisture content between the two sampling periods suggests that plant activity may be partially controlling the viable microbial biomass pool.

#### *Microbial Community Composition & Functional Groups*

The three grassland community types sampled in this study have floristically distinct species assemblages (Coupland 1992, Kucera 1992, Lauenroth and Milchunas 1992). Plant species composition has been shown to influence the size and composition of the microbial pool, most likely via the quantity and types of root exudates made available to the soil microbial community (Klein 1977, Groffman et al. 1996). Many studies have found distinct microbial communities associated with different management practices, soil type, and/or plant species dominance (Bossio et al. 1998, Grayston et al. 1998, Donnison et al. 2000, Calderon et al. 2001, Schutter et al. 2001). (Johnson et al. 1992, Bossio et al. 1998, Grayston et al. 1998, Steinberger et al. 1999, Donnison et al. 2000, Saetre and Baath 2000, Calderon et al. 2001,

Grayston et al. 2001, Myers et al. 2001, Schutter et al. 2001, Wilkinson et al. 2002) Given these previous results and the known differences in plant species assemblages across our sites, I predicted and found differences in microbial community composition across the different grassland types (Figure 4.3a & 4.3b).

All the microbial functional groups identified, except protozoa, varied significantly across sites. The main trends in the microbial functional group dataset were that the tallgrass prairie site had more gram-negative bacteria, particularly eubacterial anaerobes, and less fungi than the other grassland communities. The tallgrass prairie site in this study had higher percentages of clay and gravimetric soil moisture than all of the other sites; thus, it is not surprising this site had the highest relative abundance of anaerobic bacterial markers, as both factors would result in an increase in anaerobic conditions within the soil. However, based on the idea that fungi become more abundant as the recalcitrance of soil organic matter and litter increase (Alexander 1977, Grayston et al. 2001) and that the percent of lignin in the aboveground biomass of certain species increases from shortgrass steppe to tallgrass prairie (Murphy et al. In press), I hypothesized that the relative abundance of fungi would increase from west to east across the regional gradient, but this is not what I found. Previous work has shown that fungal biomass comprises a large portion of the total microbial biomass in tallgrass prairie soils (Miller et al. 1995, Rice et al. 1998), and one comparative study suggested that fungal biomass was 8-9 times greater in tallgrass prairie than shortgrass steppe (Paul et al. 1979).

However, my results indicate that fungal biomass does not increase across the gradient and that fungi make up a smaller proportion of the microbial community in tallgrass prairie than shortgrass steppe or mixed grass prairie. These conflicting results could be due to the different techniques employed, and the reasons for the lower relative abundance of fungi that I estimated at the tallgrass prairie site remain unclear.

Seasonal variability in microbial community composition is also commonly reported (Bardgett et al. 1999, Saetre and Baath 2000, Grayston et al. 2001). I was surprised at the lack of seasonal microbial community variability exhibited in our data given the large differences in plant activity (and probably quantity and quality of root exudation) and daily temperature between the peak and the end of the growing season in this region. I did find marginally significant seasonal shifts in some of the microbial functional groups measured (Table 4.5), but the results of the PCA suggest these shifts were not large enough to result in distinct differences in microbial community composition between the two sampling periods. It is possible that the reduced sampling size ( $n=3$ ) at the second time period (June) limited our ability to detect seasonal changes.

I observed increased total viable biomass in June relative to October, but no change in the microbial community composition between the two time periods. This result suggests that seasonal trends in microbial-mediated biogeochemical processes in this region, such as soil organic matter decomposition and nitrogen mineralization, may be due to a larger viable

microbial biomass pool rather than a change microbial community composition. Conversely, I measured no difference in viable microbial biomass between upland and lowland topographic positions, but the PCA did identify microbial community composition differences between uplands and lowlands, primarily due to higher non-specific bacterial markers in lowlands. These results suggest that landscape-level variability in microbial community composition may be larger than temporal variability in these grassland ecosystems; however, my sampling frequency and extent were limited. More extensive seasonal and landscape-level sampling is needed.

I was surprised by the trends of decreasing FAME richness and diversity from west to east across the regional gradient since mean annual precipitation and temperature, net primary productivity, and plant species diversity increases in this same direction (Burke et al. 1991, Gross et al. 2000, McCulley 2002). My FAME richness numbers are within the range reported for vegetable farm soil but my FAME diversity values are much lower than those reported in the vegetable farm study (Schutter et al. 2001). Interpretation of the data are difficult as there are no other published reports of these values from native grasslands.

#### *Comparison of Regional, Topographic, and Seasonal Variability*

I found strong evidence that microbial biomass and microbial community composition varies across the regional gradient I sampled in the Central Great Plains. However, I found seasonal effects only in the total viable

microbial biomass data, and landscape-level topographic variability in the microbial community composition data but not the biomass dataset. The proportion of variance explained in the ANOVA by the site, season, and location main effects (the  $R^2$  for each main effect) for both the total viable microbial biomass and microbial community composition data (PC axes 1 and 2 weights) indicate that the main effect 'site' accounted for more than 10x of the variation explained by either the 'season' or 'location' main effects. These results suggest that regional variability in microbial biomass and community composition is much larger than either seasonal or landscape-level topographic variability. Most likely this is due to the variability in the controlling factors of microbial biomass and community composition, such as climate, soil organic matter, and plant species composition, being larger across the Central Great Plains than the topographic or seasonal variability in these parameters at any particular site.

### **Conclusions:**

Microbial biomass and community composition varied regionally, with different microbial communities associated with each of the 3 grassland types sampled. While microbial biomass was greater in June than October and community composition was slightly different between upland and lowland topographic positions, less variability in both parameters was explained by the landscape-level topographic and seasonal effects than the regional site main effect. Soil parameters, such as texture, soil organic C pools and gravimetric



soil moisture, were related to the regional site differences in microbial biomass and community composition; however, these soil parameters were unable to account for all the variability associated with the 'site' main effect. This suggests a potentially large influence of the plant community and/or the chemical composition of the available microbial substrate pools in determining the type of microbial community present. The total phospholipid values reported in this study compare well with those from restored tallgrass prairie and with the general trends in microbial biomass previously reported for the endpoints of the transect using a different technique. However, some interesting microbial community discrepancies exist, namely, the low relative abundances of fungi at the tallgrass prairie site and the trends of decreasing FAME diversity and richness from shortgrass steppe to tallgrass prairie. Additional phospholipid analyses and regional microbial community work would assist in clarifying the nature of these types of discrepancies.

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**Table 4.1:** Regional Site Characteristics (MAP = mean annual precipitation, MAT = mean annual temperature, NPP = net primary production, LTER = Long-term Ecological Research site, TNC = The Nature Conservancy). All soil parameters are from the top 10 cm. Values are averages  $\pm$  standard deviations. Different letters indicate significant differences between means within rows ( $p$ -value  $\leq 0.05$ ).

	<b>SGS</b> Shortgrass Steppe - LTER	<b>ARI</b> Arikaree River - TNC	<b>SVR</b> Smokey River - TNC	<b>Hays</b> Ft. Hays State University	<b>Konza</b> Konza Prairie - LTER
<b>Climate &amp; Vegetation:</b>					
Latitude	40° 52'	39° 47'	39° 47'	39° 47'	39° 47'
Longitude	104° 41'	104° 26'	102° 54'	99° 49'	96° 43'
MAP (mm)	345	450	506	578	835
MAT (°C)	8.5	10.3	11.6	11.9	13
Vegetation Type	shortgrass steppe	shortgrass steppe	mixed grass	mixed grass	tallgrass
<b>Biogeochemistry:</b>					
NPP (g m <sup>-2</sup> ) *	433 $\pm$ 121	343 $\pm$ 189	460 $\pm$ 220	504 $\pm$ 164	746 $\pm$ 123
Litter Decomposition (k, yr <sup>-1</sup> ) *	0.5 $\pm$ 0.19	0.55 $\pm$ 0.19	0.63 $\pm$ 0.06	0.66 $\pm$ 0.08	0.89 $\pm$ 0.16
Soil Respiration * (g C m <sup>-2</sup> season <sup>-1</sup> )	492 $\pm$ 254	328 $\pm$ 124	529 $\pm$ 239	918 $\pm$ 230	904 $\pm$ 214
<b>Soils:</b>					
Textural Class	Clay loam	Sandy loam	Silt loam	Loam	Silty clay loam
% Sand	40 <sup>b</sup>	56 <sup>a</sup>	24 <sup>c</sup>	39 <sup>b</sup>	18 <sup>d</sup>
% Clay	30 <sup>b</sup>	18 <sup>d</sup>	25 <sup>c</sup>	24 <sup>c</sup>	38 <sup>a</sup>
Bulk Density (g cm <sup>-3</sup> ) *	0.90 $\pm$ 0.10	1.17 $\pm$ 0.06	0.98 $\pm$ 0.09	1.05 $\pm$ 0.12	1.01 $\pm$ 0.13
pH <sup>s</sup>	7.6	7.4	7.3	7.4	6.9

	<b>SGS</b> Shortgrass Steppe - LTER	<b>ARI</b> Arikaree River - TNC	<b>SVR</b> Smokey River - TNC	<b>Hays</b> Ft. Hays State University	<b>Konza</b> Konza Prairie - LTER
% C	2.13 ± 0.29 <sup>c</sup>	1.21 ± 0.35 <sup>d</sup>	2.04 ± 0.09 <sup>c</sup>	3.36 ± 0.11 <sup>a</sup>	2.71 ± 0.22 <sup>b</sup>
% N	0.22 ± 0.03 <sup>c</sup>	0.12 ± 0.02 <sup>d</sup>	0.20 ± 0.01 <sup>c</sup>	0.32 ± 0.02 <sup>a</sup>	0.25 ± 0.02 <sup>b</sup>
C:N	9.58 ± 0.32 <sup>b</sup>	9.88 ± 1.26 <sup>b</sup>	10.12 ± 0.31 <sup>ab</sup>	10.67 ± 0.59 <sup>ab</sup>	11.01 ± 0.47 <sup>a</sup>

\* - see McCulley (2002) for details.

§ - E.F. Kelly (unpublished data).

**Table 4.2:** Landscape soil characteristics (0-10 cm) from uplands and lowlands at three of the regional sites (SGS – shortgrass steppe, Hays – mixed grass prairie, Konza – tallgrass prairie).

	SGS		Hays		Konza	
	Upland	Lowland	Upland	Lowland	Upland	Lowland
Textural Class	Sandy Loam	Sandy Clay Loam	Loam	Silt Loam	Clay Loam	Silt Loam
% Sand	69	59	36	24	24	22
% Clay	19	22	26	27	33	24
% C	0.90 ± 0.19	1.40 ± 0.68	3.67 ± 0.39	4.73 ± 0.60	4.25 ± 0.22	3.52 ± 0.36
% N	0.09 ± 0.02	0.13 ± 0.06	0.34 ± 0.05	0.45 ± 0.06	0.37 ± 0.02	0.33 ± 0.04
C:N	9.77 ± 0.46	10.4 ± 0.31	10.7 ± 0.52	10.4 ± 0.23	11.4 ± 0.16	10.6 ± 0.38
Gravimetric H <sub>2</sub> O (%)	17.8 ± 0.62	21.5 ± 4.43	31.2 ± 4.44	33.3 ± 1.25	39.4 ± 1.13	47.4 ± 4.06

**Table 4.3:** Regression relationships (model p-values <0.001) of the total PLFA abundance (TotPLFA=log transform of total pmol of PLFA), the principal component first and second axes (PC1&2), and the microbial functional groups (GramPos=Gram positive bacteria, L\_Gneg = log transform of gram negative bacteria, Bact =non-specific bacteria, L\_actino=log transform actinomycetes) for both the regional and landscape datasets with the covariate soil parameters (sq\_H2O=square root transform of gravimetric soil moisture). N.S. indicates no significant relationship with covariates.

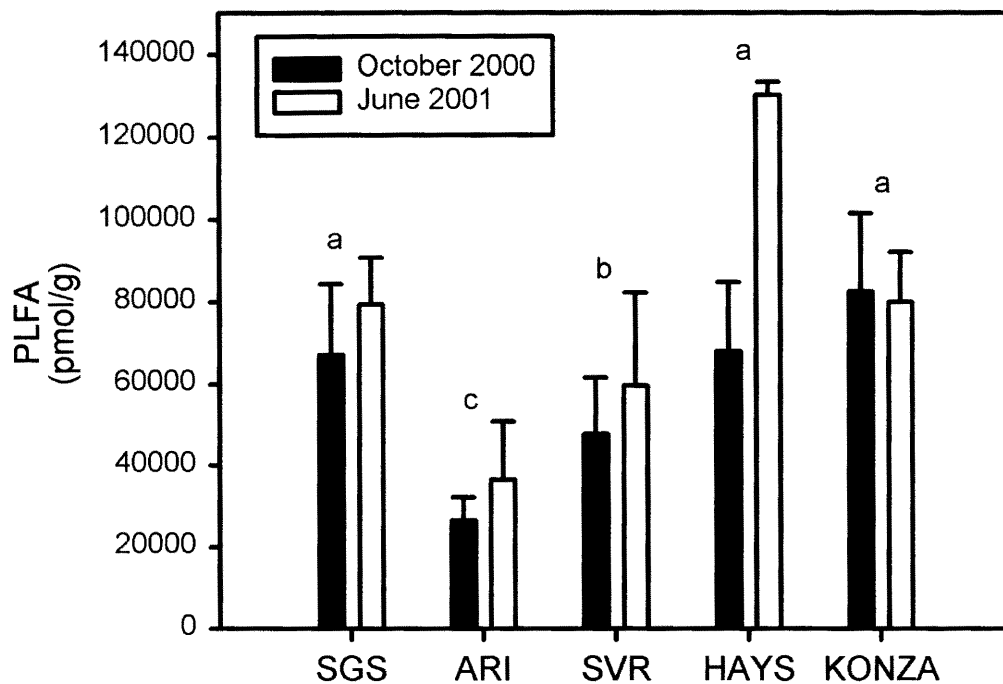
<b>Regression Equation</b>	<b>R<sup>2</sup></b>
<b>Regional Data</b>	
TotPLFA = 9.36 + 0.36(%C) + 0.03(%Clay)	0.66
PC1 = 1.37 – 2.16(%C) + 0.72(sq_H2O)	0.55
PC2 = 4.87 + 0.04(%Sand) – 1.22(sq_H2O)	0.58
GramPos = 11.33 + 0.09(%Clay)	0.31
L_Gneg = 2.23 + 0.01(%Clay) + 0.12(sq_H2O)	0.60
Bact = 12.96 – 22.8(%N) + 0.15(%Clay)	0.52
L_actino = 1.05 – 0.17(%C)	0.42
Fungi = 55.79 + 2.83(%C) – 5.62(sq_H2O)	0.37
Protozoa	N.S.
<b>Landscape Data</b>	
TotPLFA = 8.54 + 0.45(sq_H2O)	0.63
PC1 = 2.86 – 0.97(%C)	0.41
PC2 = 9.35 – 1.77(sq_H2O)	0.81
GramPos	N.S.
L_Gneg = 3.34 - 0.005(%Sand)	0.54
Bact	N.S.
L_actino = 0.92 – 1.08(%N)	0.46
Fungi	N.S.
Protozoa = 0.48 + 0.09(%C)	0.19

**Table 4.4:** Microbial PLFAs receiving large positive (> 0.2) or negative weights (< -0.2) on the first and second principal component axes.

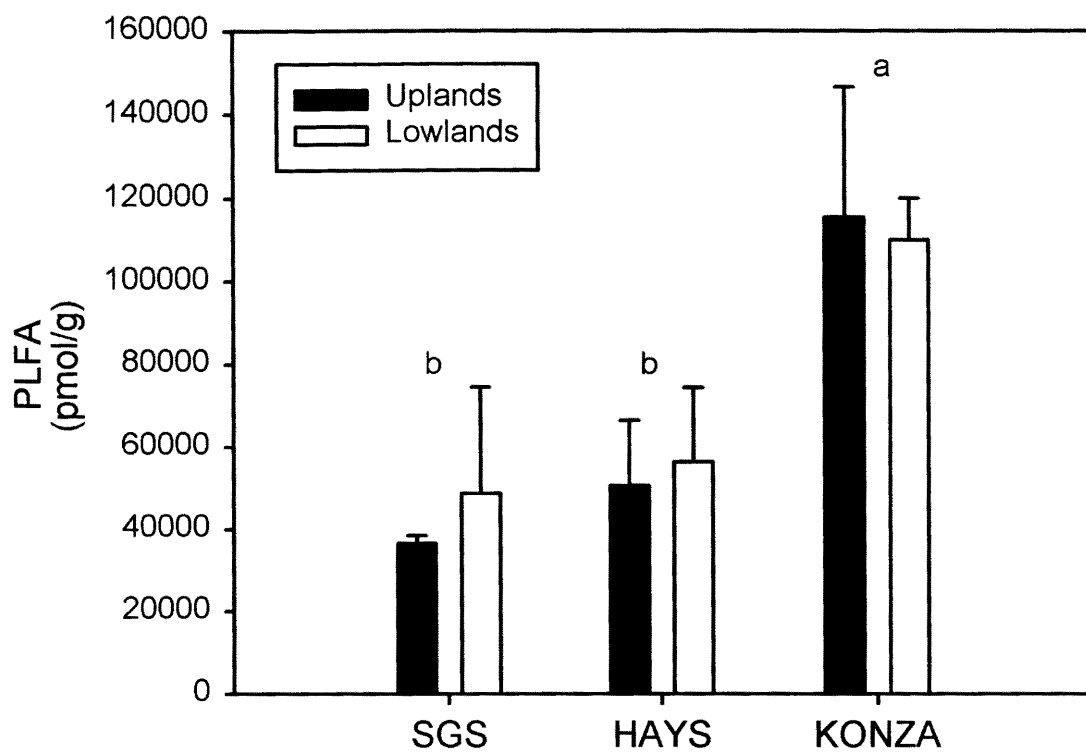
<i>FAME</i>	<i>Weights</i>	<i>Specificity as a Marker</i>
		PC Axis 1
18:1w9t	-0.369	Fungi
18:2w6,9	-0.106	Fungi
10Me18:0	0.408	Actinomycetes
a17:0	0.390	Gram-positive bacteria
i17:0	0.300	Gram-positive bacteria
i16:0	0.298	Gram-positive bacteria
17:0	0.296	Non-specific bacteria
11Me16:0	0.279	Gram-positive bacteria
i14:0	0.273	Non-specific bacteria
cy17:0	0.228	Eubacterial anaerobes, gram-negative bacteria
		PC Axis 2
cy19:0	-0.490	Eubacterial anaerobes, gram-negative bacteria
14:0	-0.379	Non-specific bacteria
18:1w7c	-0.265	Gram-negative bacteria
i17:0	-0.223	Gram-positive bacteria
16:1w7c	0.372	Gram-negative bacteria
18:2w6,9	0.323	Fungi
18:1w9c	0.273	Fungi
11Me16:0	0.258	Gram-positive bacteria

**Table 4.5:** ANOVA results (p-values) for the effects of site, location, season, and their interactions on the microbial functional groups (GramPos = gram positive bacteria, GramNeg = gram negative bacteria, Bacteria = non-specific bacteria, Actino. = actinomycetes, N.S. indicates p-value > 0.05).

	GramPos	GramNeg	Bacteria	Actino.	Fungi	Protozoa
<b><i>Regional Analysis</i></b>						
Site	0.0101	<0.0001	<0.0001	0.0002	<0.0001	N.S.
Season	N.S.	N.S.	0.0428	0.0171	0.0473	N.S.
Site*Season	N.S.	0.0091	N.S.	N.S.	N.S.	0.0198
<b><i>Landscape Analysis</i></b>						
Site	N.S.	0.0002	0.0004	0.0077	N.S.	N.S.
Location	N.S.	N.S.	0.0119	N.S.	N.S.	N.S.
Site*Location	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.



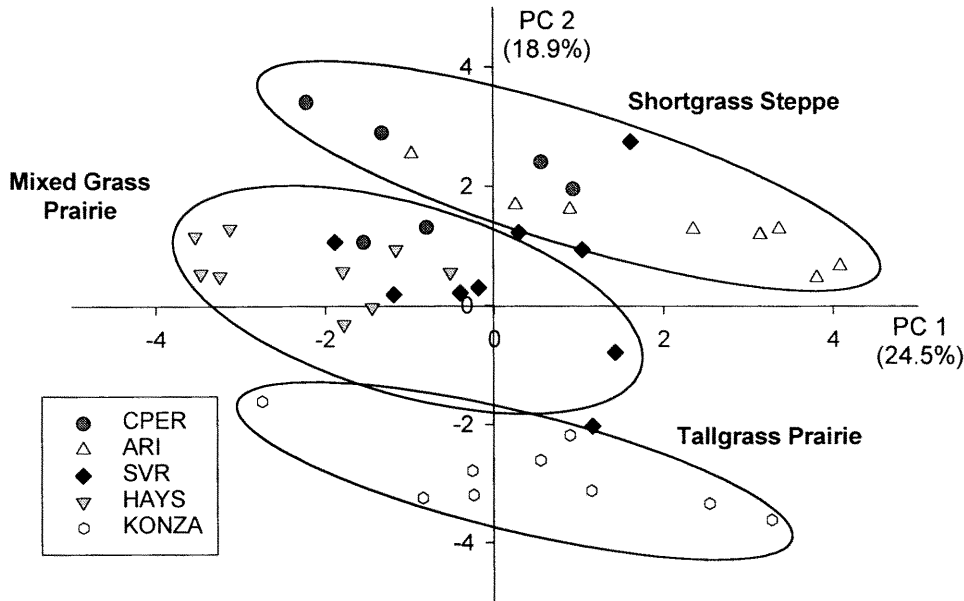
**Figure 4.1** - Total PLFA (mean  $\pm$  1 S.D.) for the 5 sites across the regional transect for both the October 2000 and June 2001 sampling time periods. Repeated measures ANOVA indicated the main effects 'site' and 'season' were significant ( $p < 0.0001$  and  $p = 0.003$ , respectively), but the interaction term was not significant. Results from means separation tests indicated June had larger total PLFA than October. The significant differences between sites are represented by different letters on the graph.



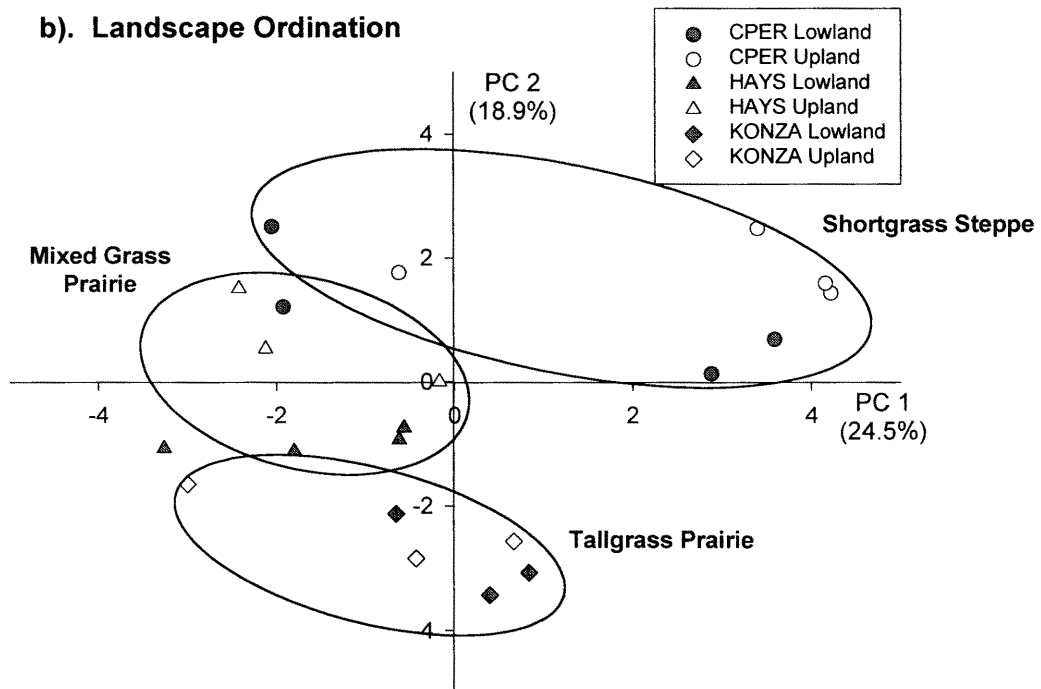
**Figure 4.2** - Total PLFA (mean  $\pm$  1 S.D.) of uplands and lowlands (location) for the landscape dataset. ANOVA results indicated significant differences existed between sites (indicated by different letters between sites), but there were no significant differences between locations for any of the sites measured.



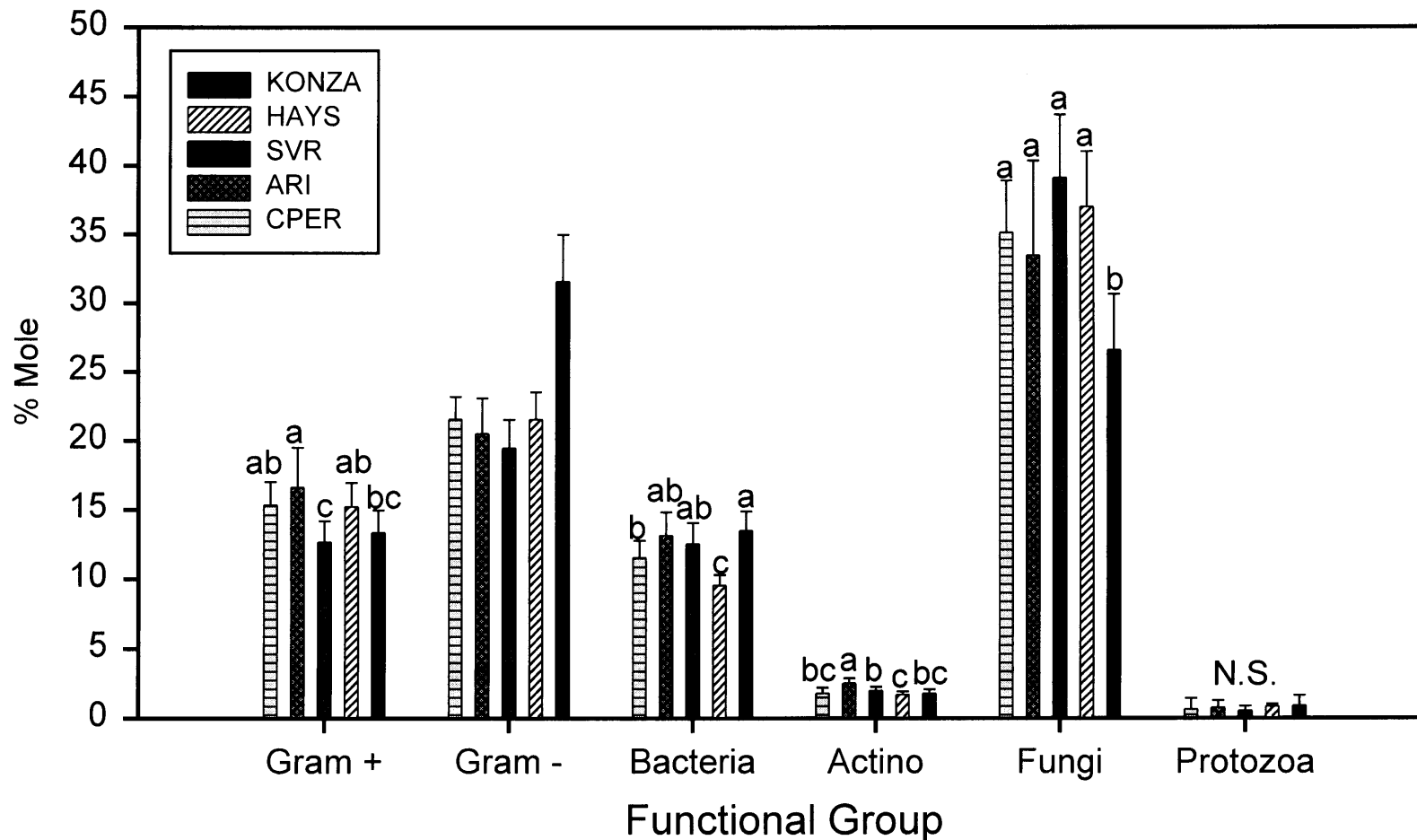
**a). Regional/Seasonal Ordination**



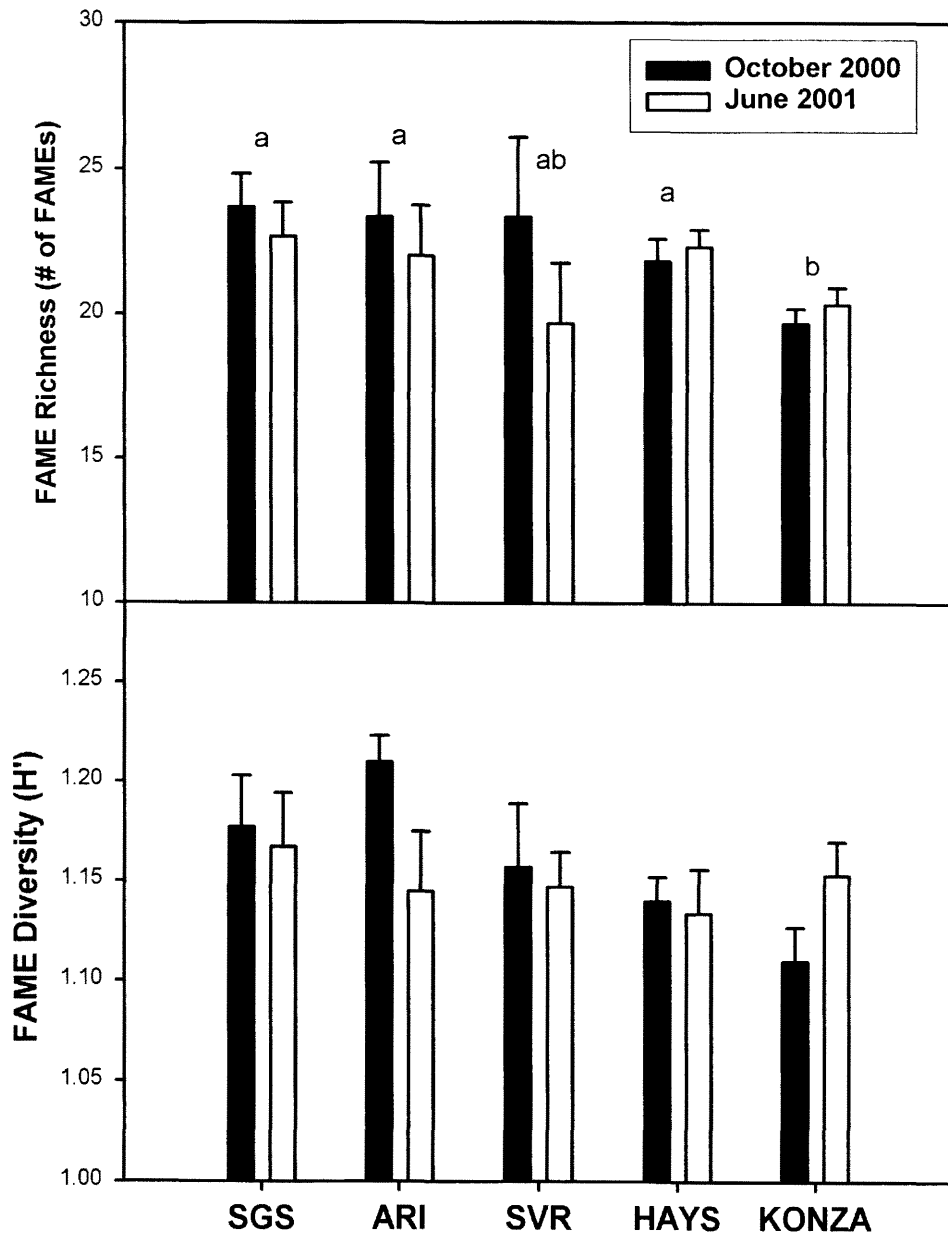
**b). Landscape Ordination**



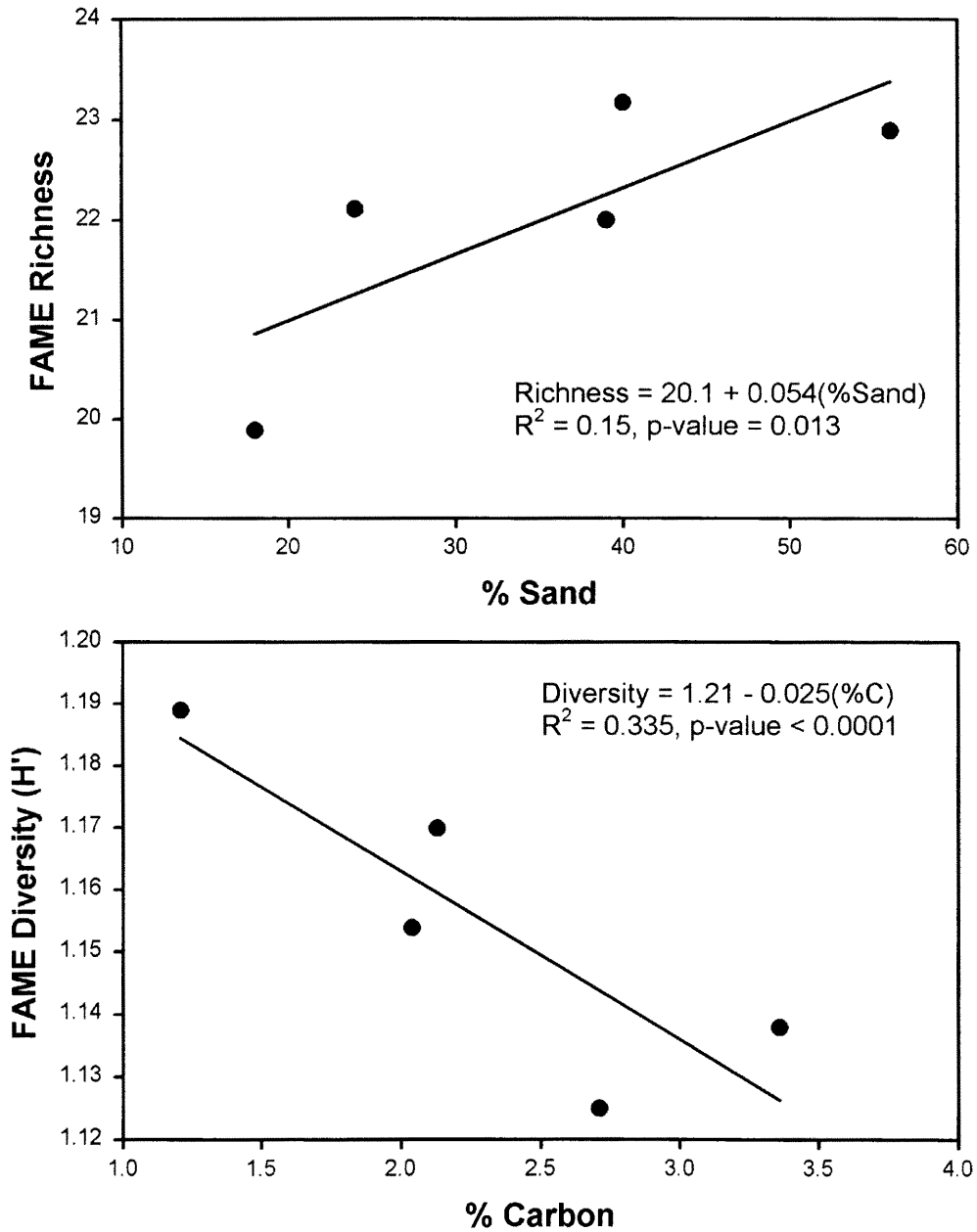
**Figure 4.3 – a)** Ordination of the regional dataset (principal component axes 1 and 2 – PC 1 and PC 2). Repeated measures ANOVA indicated neither PC 1 nor PC 2 were significantly related to the main effect “season,” so no distinction between the October 2000 and June 2001 time periods are presented here. Microbial community composition was distinct among grassland community types, with shortgrass steppe sites (CPER and ARI) located in the first and second quadrants, the tallgrass prairie site (KONZA) located in the third and fourth quadrants, and the mixed grass prairie sites (SVR and HAYS) occurring intermediately in the principal component space between the other two community types. **b)** Ordination of the landscape dataset (with the same PC 1 and PC 2 as in Figure 3a since both datasets were analyzed together in one PCA). Distinct microbial communities were associated with the different grassland community types and occur in the same general principal component space as in Figure 3a. Uplands and lowlands also exhibit distinct microbial communities, with uplands generally higher on the PC 2 axis than lowlands for each site.



**Figure 4.4** - Mean  $\pm$  1 S.D. of the % mole fraction of the different microbial functional groups represented by the 19 FAMES included in the PCA. Data are from the regional dataset and are averaged across sampling dates. Different letters represent significant differences between site means within a functional group type ( $p < 0.05$ ). N.S. indicates no significant difference between sites. A significant site\*season interaction term precluded means separation tests being performed on the gram-negative functional group.



**Figure 4.5** - The FAME richness and diversity index (Shannon's - H') for all 5 sites across the region in October 2000 and June 2001. For FAME richness, only 'site' was significant ( $p < 0.01$  - different letters indicate significant difference between sites). The FAME diversity data had significant 'site' ( $p < 0.001$ ) differences and a significant 'site\*season' interaction term ( $p < 0.001$ ), precluding means separation tests. However, in general, FAME diversity decreased from SGS to KONZA.



**Figure 4.6** - The regression relationships for the FAME richness and diversity data from the regional dataset (averaged across season) and the soil characteristic data.

## CHAPTER V: SUMMARY AND CONCLUSIONS

The primary objective of my dissertation was to quantify regional, seasonal and interannual trends in nitrogen (N) and carbon (C) cycling of grasslands in the U.S. Great Plains. A secondary objective was to describe the microbial community composition of the grassland types represented in the study and attempt to determine what spatial (local landscape or regional) and/or temporal (seasonal) scale accounted for the largest proportion of the variance in the microbial community composition data. To address these objectives, I analyzed biogeochemical pools and processes across a transect of 5 sites that span a precipitation gradient in the Great Plains over a 3 year period.

The results of my research show that on a regional scale C cycling and microbial biomass increase in parallel with increases in mean annual precipitation and temperature, aboveground primary production, and soil organic matter pools. However, contrary to simulation model predictions (Burke et al. 1997), *in situ* net N mineralization rates show no regional trend, perhaps due to the variability in N immobilization potential across the sites. Similarly, I did not observe strong seasonal or interannual *in situ* net N mineralization trends. However, carbon cycling parameters (above- and belowground net primary production, litter decomposition rates, and soil respiration) exhibit large interannual differences that appear primarily related to the differences in

growing season precipitation between the two years of data collection. In almost all cases, C cycling rates are higher in the wet year (2001) than the dry (2000). However, variability in other factors (i.e., temperature) between the two years may also influence these parameters. Carbon loss via soil respiration is more sensitive to changes in precipitation across the regional gradient and between years at specific sites than either C inputs (via net primary production) or C loss via aboveground decomposition. Similar to results reported by Paruelo et al. (1999), the grassland community occurring at a mean annual precipitation of ca. 450 mm appears to be the most sensitive in terms of C cycling to alterations in interannual precipitation. Finally, microbial biomass is greater during the growing season (June) than in the fall (October), and microbial community composition is different for the 3 grassland types represented in the study. Regional-scale differences between sites account for more of the variability in microbial community composition data than either the landscape- or seasonal-scale parameters.

In conclusion, regional and interannual variability in precipitation are important in determining both C inputs and outputs in these grassland ecosystems of the Central Great Plains. Carbon fluxes are quite responsive to interannual variability in precipitation, and the degree of responsiveness is not uniform across the grassland types present in the region. I did not find evidence to support regional, seasonal, or interannual trends exist in net N mineralization rates, but studies quantifying gross N mineralization and N immobilization may help elucidate patterns in this important biogeochemical

process. Regional differences in microbial biomass and community composition occur across the gradient, suggesting that the increases in C cycling across the region are the result of both increasing microbial biomass and alterations in the types of microbes present.

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## **APPENDIX I**

**Table A.1:** Soil pedon description for the Shortgrass Steppe LTER site located near Nunn, Colorado.

Location CPER Date 5/16/01 Described by Kelly/McCulley

Vegetation Shortgrass Geomorphic Surface Flood Plain/Terrace Parent Material(s) Loess/Alluvium/ Shale

Depth (cm)	Horizon	Color Moist/Dry	Structure	Gravel %	Consistency			Texture	pH	Clay films	Boundaries	Notes
					Wet	Moist	Dry					
0-10	A	10YR 3/2 10YR 5/2	1 vf gr	0	ss po	vfr	so	SiL CL	7.6	n	a s	-e 3 vff
10-32	ABt	10YR 3/2 10YR 4/2	1 m sbk	0	ss ps	fr	so	CL	7.7	l	cs	e 3 vff
32-47	Bt	10YR 5/3 10YR 6/3	2 m sbk	0	ss ps	fr	ss	CL	7.8	n	cs	es 2 vf
47-75	Btk	10YR 5/2 10YR 7/2	2mf sbk	0	ss ps	fr	ss	CL	7.9	n	cs	es 2 vf 1 c
75-100	BCK	10YR 5/2 10YR 7/2	1m sbk	0	ss ps	fr	so	L	8.2	n		ey 1 vff 1 c

**Table A.2:** Soil pedon description for the Arickaree River site owned by The Nature Conservancy located near Idalia, Colorado.

Location Arickaree Date 5-20-01 Described by Kelly/McCulley

Vegetation Shortgrass Geomorphic Surface Terrace Parent Material(s) Loess/ss/limestone

Depth (cm)	Horizon	Color Moist/Dry	Structure	Gravel %	Consistency			Texture	pH	Clay films	Boundaries		Notes
					Wet	Moist	Dry						
0-8	A	10YR 3/2 10YR 4/3	1 f gr	0	Ss po	lo	so	L	7.4		A	S	1vf 2f eo
8-33	BAt	10YR 3/3 10YR 5/3	1 f sbk	0	Ss Ps	vfr	so	L+	7.6		C	S	1vf 1f e
33-64	Bt	10YR 4/4 10YR 6/4	2 f pr m sbk	0	S Ps	fr	sh	CL	7.8		C	S	2vf es
64-85	Btk	75YR 5/4	3 m abk	0	S Ps	fr	h	CL	8.0		C	W	3vf es
85-105	BCK	75YR 25/4 10YR 6.4	M m sbk l	0	Ss Po	vfr	so	L-Cl	8.1				3vf ex

**Table A.3:** Soil pedon description for the Smokey Valley River site owned by The Nature Conservancy located near Oakley, Kansas.

Location Smokey Valley Date 5-19-01 Described by Kelly/McCulley

Vegetation shortgrass/mixed grass Geomorphic Surface Terrace Parent Material(s) loess-ss

Depth (cm)	Horizon	Color Moist/Dry	Structure	Gravel %	Consistency			Texture	pH	Clay films	Boundaries		Notes
					Wet	Moist	Dry						
0-14	A	10YR 3/2	1 f gr 2 m sbk	0	ss ps	pfr	so	L-Sil	7.3		a	s	eo
14-46	ABt	10YR 3/2	2 m pr sbk	0	ss ps	vfr fr	so sh	CL	7.3		c	s	eo
46-70	Btk	10YR 5/2	2 m pr sbk	0	s p	vfr	sh	CL	7.9		c	s	e
70-97	BK	10YR 5/2	2 m sbk	0	ss ps	fr	sh	CL	7.9		c	w	es
97-110+	BCK	10YR 6/3	m	0				CL-	7.9				ev

**Table A.4:** Soil pedon description for the Hays site owned by Fort Hays State University located in Hays, Kansas.

Location Hays, KS Date 5-19-01 Described by Kelly/McCulley

Vegetation mixed grass Geomorphic Surface \_\_\_\_\_ Terrace \_\_\_\_\_ Parent Material(s) loess-Sh-limestone-ss

Depth (cm)	Horizon	Color Moist/Dry	Structure	Gravel %	Consistency			Texture	pH	Clay films	Boundaries	Notes
					Wet	Moist	Dry					
0-90	A	10YR 3/2	1 gr	25	Ss ps	lo	so	L	7.4		C s	3 vf f 2 f e
10-26	AB	10YR 4/2 10YR 3/2	1 f sbk	50	Ss ps	vfr	sh	SCL	7.8		C s	3f 3f es
26-45	BtK	10YR 7/4	2 f sbk	75	S po	fr	sh	CL	7.8		C w	2vf 2f es
45-58	BtK2	10YR 7/6	M 1 f sbk	75	S po	fr	sh	CL	7.8		A s	Es 1vf
58+	R		M						8.3			Ev 1vf

**Table A.5:** Soil pedon description for the Konza LTER site owned by located near Manhattan, Kansas.

Location Konza Date 5-17-01 Described by Kelly/McCulley

Vegetation Andropogon Geomorphic Surface Terrace Parent Material(s) Loess-Shale/Limestone

Depth (cm)	Horizon	Color Moist/Dry	Structure	Gravel %	Consistency			Texture	pH	Clay films	Boundaries		Notes
					Wet	Moist	Dry						
0-13	A	10YR 3/1	2 f gr	0	s ps	vfr	sh	SiCL	6.9		c	s	3vf 2f eo
13-30	AB	10YR 3/2	2 m sbk	0	s p	fr	h	C	6.6		c	s	2vf 1f eo
30-55	Bt	10YR 4/2	3 m sbk	0	s p	fr fl	h	C	7.0		c	s	1vf  eo
55-86	Btk	10YR 4/3 7.5YR 6/4	2 m sbk	0	s p	fr	h	SCL-C	7.7		c	w	1vf  e
86-100	Btkg	10YR 5/3 7.5YR 6/4	m	0	ss p	fr	so	CL	7.8				MnO <sub>2</sub> 1vf - Fe-oxides es CaSO <sub>4</sub>