DISSERTATION

MICROBIAL AND BIOGEOCHEMICAL RESPONSES TO CHANGING PRECIPITATION PATTERNS IN GRASSLAND ECOSYSTEMS

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ABSTRACT

MICROBIAL AND BIOGEOCHEMICAL RESPONSES TO CHANGING PRECIPITATION PATTERNS IN GRASSLAND ECOSYSTEMS

Global circulation models predict that precipitation patterns in grasslands will both intensify and be characterized by more severe drought in the future. In these systems, the availability of water strongly controls ecosystem function, so changes in precipitation are likely to significantly alter biological communities and biogeochemical dynamics. Since these biogeochemical changes could feed back on climate drivers by influencing regional to global scale energy and water balance, predicted changes in grassland precipitation call for a better understanding of relationships between water availability and grassland biogeochemical dynamics.

My dissertation aimed to address how changing rainfall patterns affect biogeochemical cycling and soil microbial communities in grasslands. I first tested the generality of controls over soil organic matter storage in temperate grasslands by studying existing spatial gradients in soil carbon and nitrogen, as they relate to the spatial variation in average precipitation and temperature, and soil texture. I found that statistical models developed in US grasslands overestimated soil organic carbon and underestimated soil organic nitrogen in Chinese grasslands. However, when I incorporated nitrogen deposition and historical land use using a simulation model, it resulted in more accurate model estimates for this region. This work suggests that nitrogen deposition and historical land use legacies may need to be considered to accurately describe biogeochemical dynamics in Chinese grasslands and better predict the vulnerability of global carbon stocks to loss.

Responses of ecosystems to changes through time are often somewhat different than relationships gleaned from large-scale spatial gradients. At the local scale, I found that an 11-year drought can significantly alter biogeochemical and ecosystem dynamics in the highly drought-resistant shortgrass steppe. Here, soil inorganic nitrogen availability increased up to 4-fold in plots receiving 25% of summer precipitation. This accumulation of nitrogen under drought may explain the higher plant tissue nitrogen

and N_2O flux observed under recovery. A more "open" nitrogen cycle that I observed following severe drought could affect the impact of drought on grassland ecosystems, as well as the timescale of recovery.

Soil microbial community composition was also altered by this 11-year drought manipulation in the shortgrass steppe, and these differences persisted even after communities were subject to the same moisture conditions for 36 hours in the lab. In this lab experiment, I also identified specific microbial groups that grew under a certain moisture levels, presenting evidence of moisture niche partitioning in microbial communities. However, this niche differentiation wasn't realized in the field; communities that grew under dry conditions in the lab were not similar to those that emerged under long-term drought plots. Overall, this work suggests that contrary to previous assumptions, microbial communities display legacies from long-term field treatments, and that although soil moisture has the potential to drive microbial community composition through niche partitioning, this factor may not always be the primary driver of long-term community composition.

Microbial communities were also sensitive to altered precipitation timing in the tallgrass prairie. In addition, communities that were subject to intensified precipitation patterns in the field respired less than control soils after laboratory rewetting events, but respiration rates of the different field treatments converged after 100 days under the same conditions. Surprisingly, species composition of these communities was more sensitive to drying and rewetting pulses in the lab than those from the control. Together, these results show that microbial communities display legacies to altered precipitation timing, in addition to drought, but community composition is not necessarily tightly linked to respiration.

Overall, my dissertation work suggests that grasslands will be sensitive to extreme shifts in precipitation, and that biogeochemical and microbial responses could influence how grasslands are altered under future precipitation regimes. However, my work also shows that precipitation is not the only factor controlling biogeochemical and microbial community dynamics in grasslands, even under rainfall manipulations and across precipitation gradients. Therefore, the response of grasslands to other environmental factors – that shift with precipitation changes or are predicted to change independently – should not be overlooked.

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Chapter 1: Introduction

Water has been identified as the main controller of ecosystem function in semi-arid ecosystems (Noy-Meir 1973). These ecosystems cover over a fifth of global land area (Leith 1978), store more than a third of the world's soil carbon (Anderson 1991, Scurlock and Hall 1998, White et al. 2000), and provide much of the land used for pastoral and crop farming worldwide (FAO 2005). Climate models predict that there will be future changes in both the timing and amount of precipitation in semi-arid grasslands (Karl et al. 1995, Easterling 1999, IPCC 2007, Jentsch et al. 2007). As grasslands are sensitive to changes in water availability, shifts in rainfall are likely to significantly alter these ecosystems and the services they provide. In addition, other global changes, such as shifts in land use (Chuluun and Ojima 2002) and increased nitrogen deposition (Galloway et al. 2004), will affect grassland dynamics, and may interact with precipitation changes in novel ways (Miller et al. 2004). Overall, impending environmental change has given new context to the study of water-ecosystem relationships in semi-arid systems, and call for a greater understanding of how precipitation and other predicted changes will affect various components of grassland ecosystems.

Because grassland organisms are frequently water limited, precipitation has proven to be a good predictor of many semi-arid processes. Extensive work has shown that plant growth in grasslands is often limited by water availability, resulting in a strong positive relationship between annual net primary production (ANPP) and annual precipitation (Sala et al. 1988, Lauenroth and Sala 1992). However, relationships between precipitation and belowground processes are less clear. Although decomposition can be linearly related to precipitation in arid and semi-arid environments (Steinberger and Whitford 1988, Jacobson and Jacobson 1998, Epstein et al. 2002), previously established relationships between precipitation and decomposition may shift under new rainfall regimes (Yahdjian et al. 2006). The relationship between net nitrogen mineralization and precipitation is even less clear. Net N mineralization is not sensitive to changes in precipitation across regions (Barrett et al. 2002, McCulley et al. 2009), and changes in net N mineralization after water exclusions and additions are not consistent (Yahdjian et al. 2006). Relationships between precipitation and biogeochemical processes may be further complicated by

these processes' sensitivity to not only the amount but also the timing of precipitation events (Sala et al. 1992, Austin et al. 2004, Collins et al. 2008). A better understanding of interactions between water and belowground processes is needed, especially because shifts in biogeochemical dynamics have the potential to feed back on atmospheric climate drivers and alter the trajectory of climate and other global changes (Finzi et al. 2011).

Many biogeochemical transformations in the soil are controlled by soil microorganisms. Despite the important role that microorganisms play in ecosystems, methodological challenges and immense microbial biodiversity has impaired our ability to address fundamental questions in microbial ecology. Recent evidence suggests that, contrary to traditional assumptions (Baas-Becking 1934), microorganisms can constrain the biogeochemical functions they mediate (Allison and Martiny 2008, Strickland et al. 2009), display biogeographical patterns (Fierer and Jackson 2006, Green et al. 2008), and adapt to local conditions (Waldrop and Firestone 2006, Wallenstein and Hall 2012). These new observations have motivated an increased interest in the response of microbial communities to environmental factors, and in the role of microorganisms in biogeochemical feedbacks to climate drivers (Bardgett et al. 2008, Singh et al. 2010).

Microorganisms are highly sensitive to changes in soil moisture (Harris 1981, Schimel et al. 2007), and moisture sensitivity can be highly variable among microbial groups (Van Gestel et al. 1993). Thus, predicted changes in precipitation are likely to affect soil microbial community composition and function in grasslands in addition to aboveground communities and biogeochemical processes. For example, fungi may be more tolerant to drought than bacteria. Shifts in the abundance of these organisms, and the stoichiometry of microbial biomass, could affect larger-scale biogeochemical cycling (Bapiri et al. 2010, Hawkes et al. 2010, Yuste et al. 2010). In addition, drying and rewetting events that will become more frequent with increased precipitation variability can alter the functional potential of microbial communities, even up to 6 weeks after moisture pulses end (Fierer et al. 2003). Although these studies suggest that microbial communities will be sensitive to shifts in rainfall patterns (Williams and Rice 2007, Fierer et al. 2009), grassland microbial communities can also be highly resistant to changes in

moisture regime (Cruz-Martinez et al. 2009). Further microbial biogeography and enzyme activity are not always well-correlated to precipitation (Gonzalez-Polo and Austin 2009, Lauber and Fierer 2009). Thus, more research is needed to determine how microorganisms will respond to future precipitation changes and how these changes will in turn influence biogeochemical dynamics.

The objective of my dissertation is to improve our understanding of grassland responses to future climate change by describing microbial and biogeochemical dynamics under different rainfall regimes. I address the following specific questions:

1. Are the relationships between soil carbon, soil nitrogen, and environmental factors the same across two similar environmental gradients in temperate grasslands of the US Great Plains and Inner Mongolia, China?
2. How are carbon and nitrogen linkages altered by long-term drought in the shortgrass steppe, and how does this affect drought recovery?
3. Does moisture niche partitioning drive shifts in microbial community composition under long-term drought in the shortgrass steppe?
4. Does a history of more extreme rainfall events in the tallgrass prairie alter the response of microbial communities to drying and rewetting?

In the following chapters, I address each of these questions in temperate grasslands using regional gradients, long-term field rainfall manipulations, and coupled field-lab studies. In doing so, I describe the microbial and biogeochemical responses to precipitation in several grassland types, and investigate the mechanisms likely to control these patterns to improve our overall understanding of grassland-precipitation dynamics.

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Chapter 2: Controls on soil organic carbon and nitrogen in Inner Mongolia, China: a cross-continental comparison of temperate grasslands[1]

Introduction

A central challenge in global biogeochemical modeling is developing a generalizable structure that accurately captures variation among ecosystems. Capturing variation in controls on carbon cycling is especially important as it is coupled to and often drives other biogeochemical cycles. Soil organic carbon (SOC) storage is determined by the long-term net balance of photosynthesis and total respiration in terrestrial ecosystems. Therefore, in all systems, factors that influence these processes such as climate, topography, soil texture, and land use management, exert strong control over SOC and soil organic nitrogen (SON) dynamics. However, the relative importance of these parameters, and their relationships to soil organic matter, may vary depending on many different ecosystem properties. In an attempt overcome this variation, most global ecosystem models assume that in climatically similar regions, such as grasslands, relationships between SOC and its environmental controls are the same, despite regions evolving independently. The extrapolation of these relationships in ecosystem models allows us to predict ecosystem dynamics in the future and over large regions for which we have little data, and improve our understanding of these systems. However, to do this we must be certain whether controls that have been established in one temperate grassland can indeed be generalized to other climatically similar regions.

Grassland ecosystems play a significant role in the global carbon cycle, covering nearly one fifth of global land area (Leith 1978) and storing between 200 and 300 Pg of soil carbon (Anderson 1991, Eswaran et al. 1993, Scurlock and Hall 1998). Climate and soil texture are considered major controls of total soil carbon and the relative proportions of carbon pools in grasslands (Miller et al. 2004, Wang et al. 2005, Plante et al. 2006); SOC, SON, and C:N generally increase with increasing precipitation and clay content and decreasing temperature (Burke et al. 1989, Paruelo et al. 1998). However, even within

[1] Evans, S. E., I. C. Burke, and W. K. Lauenroth. 2011. Controls on soil organic carbon and nitrogen in Inner Mongolia, China: A cross-continental comparison of temperate grasslands. *Global Biogeochemical Cycles* **25**.

grasslands, the relative importance of each of these factors may shift under altered climate, plant species composition, nutrient input, or land use management (Miller et al. 2004), due to imperfect ecological convergence and recent global change. Considering these potential influences, it is important to continue to test the generality of grassland models developed in one region for their application for all regions of similar climate.

In this study, I aim to test the generality of the relationships of SOC and SON with environmental factors in temperate grasslands by 1) identifying the most important drivers of soil SOC, SON, and organic matter fractions across a major environmental gradient in China, and 2) assessing the extent to which predictive relationships from North American grasslands are accurate for Inner Mongolia. I do this by examining the relationships among SOC and SON data collected in Inner Mongolia with environmental controls, and also by testing the ability of other grassland models to accurately predict observed values. I use a grassland regression model developed in the Great Plains (Burke et al. 1989) to test its applicability to Chinese grasslands, and the more highly parameterized Century model (Parton et al. 1987) to investigate which parameters are most important for simulating predictions comparable to Chinese grassland data.

Much of China's temperate grassland lies in the northern province of Inner Mongolia. This arid and semi-arid region is predicted to see some of the strongest and earliest effects of climate change (OIES 1991, IPCC 2007). In addition, increasing population in Inner Mongolia has led to increased nitrogen (N) deposition (Lu and Tian 2007) and intensification of land use, which, in addition to altering ecosystem carbon dynamics, has altered soil fertility and threatened personal livelihoods (Chuluun and Ojima 2002, Jiang et al. 2006). Therefore, in addition to possible differences due to spatially-independent evolutionary paths, changes unique to this grassland region could alter fundamental relationships developed in grassland SOC models. In particular, other studies have found that historical land use may alter the relationship between SOC and its environmental controls in this region (Wang et al. 2005, Zhou et al. 2007). Further, Chuluun and Ojima (2002) suggest that, although both are currently changing, land use may be more important than climate parameters in predicting SOC values in the future.

I hypothesize that land use management and nitrogen deposition may be more important controlling factors of SOC and SON in Inner Mongolia than in other temperate grasslands, and that this interaction could alter carbon turnover and fractional pools in the short-term, and in the long-term, challenge the predictive relationships previously proposed for SOC in temperate grasslands. Land management in Inner Mongolia has a longer history compared to other grassland regions, and has recently intensified (Xiong et al. 2008). Carbon balance in this area is also sensitive to additional N inputs (Zeng et al. 2010), and has been used to explain observations of higher plant production in this region for a given climate (Xiao et al. 1996). Therefore, SOC and SON in Inner Mongolia may be even more affected by N deposition (Lu and Tian 2007) occurring as a result of increased population density in this region (Jiang et al. 2006).

Methods

Experimental Approach and Sites

To assess the generality of controls over SOC and SON in semiarid temperate grasslands in this study, I first collected new data from a precipitation gradient in Inner Mongolia, China and analyzed it for significant trends and predictor variables. I then compared this data to output from a model developed from data in the US Great Plains (Burke et al. 1989) and the Century model (Parton et al. 1987). These transects in the Great Plains and Inner Mongolia span mid-latitude, semi-arid temperate grasslands, and were identified by the Global Change and Terrestrial Ecology (GCTE) International Geosphere-Biosphere Program (IGBP) as key gradients that incorporate trends over large spatial scales with regional and global implications (Koch et al. 1995) (Fig. 2.1). I used both a correlative, regional model (Burke et al. 1989) and a simulation model that has been widely validated, the Century model (Parton et al. 1987), to test the generality of the control variables. In contrast to other modeling approaches that focus on parameter optimization and testing of mechanisms, by testing the generality of a simple model and then a highly parameterized model, I could better explain discrepancies that arise between predicted values and values

that were measured across the Inner Mongolia transect, and improve understanding of how ecosystem dynamics may differ between the two regions.

The Northeast China Transect (NECT) is located between 112° and 130° E and 42° and 46°N in Inner Mongolia, China. I selected 12 sites on the western 1000 km of the transect. This area spans three types of grasslands: meadow steppe, typical steppe, and desert steppe (Table 2.1, Fig. 2.2). Mean annual precipitation (MAP) at the sites ranged from 170 to 450 mm, mean annual temperature (MAT) from 0.78 to 5.6°C, and altitude from 478 to 1550 m (Fig. 2.2). Land use history information was acquired from a variety of sources, and although it was collected for every site, uncertainty about land use history varied among sites. Most sites were previously established as research sites (Sites 3, 4, 5, 6, 7, 8, 9, 10, 11) and therefore I could accurately and confidently describe the number of years these site had been fenced, or the current grazing intensity (quantified by percent biomass removed per year), and when possible, the land use before the site became a research site. Other sites were private farms (site 2), or had been recently abandoned (site 1) and land use was estimated based on information from the land managers. All information, when possible, was verified with other studies that have previously used this gradient to examine climate and land use effects on environmental factors (Zhang et al. 1997, Ni and Zhang 2000, Wang et al. 2005). I also used accounts from land managers and several accounts from the literature to obtain information on longer history more general to the region. Much of the land in this region experienced drastic land intensification as a result of population increases and settlement of local farmers in the 1950's (Sneath 1998, Jiang et al. 2006, Xiong et al. 2008), and this was confirmed by many site managers and farmers. In sum, I collected the best possible information about land use but given the very long settlement history of the region, there is substantial uncertainty.

Sampling

I collected soils in 12 sites across the Northeast China Transect in July of 2008. Within each site, I established two (or in site 2, three) 100 m transects in two areas at least 500 m apart. I estimated soil texture in the field (and later quantified texture in the lab), aiming to maximize variation in soil texture

between these transects within a site. I randomly located and collected three 5 x 20 cm cores along each transect, separating soil into a 0-10 cm depth and 10-20 cm depth. Soils were returned to the Chinese Academy of Sciences Institute of Botany laboratory in Beijing within one week. They were dried at 60°C and sieved to remove the soil fraction > 2 mm. In all regression analyses, I averaged independent variables over the three cores along each transect, but did not average between transects within a site as soil textures, which were quantified more exactly in the lab, provided additional variation I did not want to ignore. Therefore, I had 12 sites total, but 25 points in the regression analysis because all sites had two transects and site 2 had three transects (Table 2.1).

Particulate organic matter (POM) fractionations

I used size and density fractionations to estimate coarse and fine particulate organic matter pools (Cambardella and Elliott (1992), modified by (Kelly et al. 1996)). These fractions are also called POM 500 and POM 53 fractions, respectively, referring to the particle size in μm. I shook 30 g soil samples in 0.5 mol L^{-1} sodium hexametaphosphate solution for 18 h and separated the coarse and fine fraction using 0.5 mm and 53 μm sieves, respectively. Carbonates were present in some typical and desert steppe soil samples, as I observed effervescence when soils came in contact with 1M HCl. In these samples, carbonates were removed using an acid pretreatment method (Nelson and Sommers 1982) after fractionation, so removal treatment would not interfere with particle dispersion. I measured C and N on dried, ground soils using a LECO CHN-1000 analyzer. I calculated the mineral associated organic matter (MAOM, Cambardella and Elliott [1992]) fraction by subtracting the two POM fractions from the total C. The presence of a significant fraction of labile carbon in the total C would cause an overestimation of MAOM as determined by subtraction, but respiration measurements and previous work in grasslands characterizing highly labile SOC pools (e.g. (Kelly et al. 1996, Gill et al. 1999)) show that this pool is very small relative to other fractions. Therefore, in this analysis, I felt justified labeling the fraction remaining after subtracting coarse and fine POM from total C and N as MAOM.

Statistical analysis of the response of C and N fractions to environmental factors

To describe the major controls over SOC and SON dynamics in Inner Mongolia and their relationship to environmental factors, I first determined the linear relationship of total C and N in soil organic matter and its fractions to *each* independent variable using Pearson's correlation coefficients in proc corr, SAS 9.2 (SAS Institute, Cary, NC). Independent variables included mean annual precipitation (MAP, in cm), mean annual temperature (MAP, °C), silt (%), clay (%), and land use variables that included an estimate of the percent biomass removed per year due to grazing, and the number of years (if any) the area had been fenced. Coarse (500) and fine (53) POM values were better predicted when combined into one POM pool, representing a carbon fraction more labile than MAOM. I added an additional type of dependent variable by calculating the relative proportion of C or N in the POM or MAOM pool as a percentage of total C.

I used a multiple linear regression approach to identify and evaluate the contributions of the strongest predictive variables for all dependent variables. To identify the best predictive models for each variable, I used an all possible subsets regression analysis (SAS proc reg) to select the 5 models that best fit the data, then a likelihood approach to determine the best predictive model. This approach ranks competing models relative to one another, instead of assuming a true model. Specifically, I used the corrected Akaike information criterion (AICc) to rank models because it includes a correction term for potential bias produced by sample size (Hurvich and Tsai 1989). AICc judges a model by how closely the fitted values tend to be to the "true values," but also penalizes the model with each added parameter (Burnham and Anderson 2002). With the best model, I estimated the parameter for each independent variable, tested it for significance ($p<0.01$), and calculated the standardized coefficient to allow comparison among independent variables that have different units by placing them on the same scale. When independent variables are correlated in multiple linear regression models, estimations of regression coefficients are not accurate. Therefore, I tested all variables for the occurrence of collinearity, and

accounted for this by testing collinear variables individually for significance in case the presence of both caused both to be insignificant parameters.

Comparison of US and Inner Mongolia using regression and simulation modeling

i. Statistical comparison of predicted and observed values for all models

My goal was to assess the generality of the Great Plains model, first by using a simple regression model, then by varying parameters in the Century model, and comparing the resulting predictions from both to observed values in Inner Mongolia. To statistically evaluate how these values compared, I performed linear regressions between observed values (y) and predicted values (x) for each of the models (regression and Century models), as suggested by Piniero et al. (2008). I calculated the r-squared of this relationship and tested the null hypotheses that the estimated slope (β1)=1 and intercept (β0)=0.

To evaluate overall goodness-of-fit, I calculated the root mean squared deviation (RMSD) as

$$\text{RMSD} = \sqrt{\frac{1}{n-1}} \sum_{i=1}^{n} (pre_{\text{i}} - obs_{\text{i}})^2 \quad (1)$$

where pre_{i} represents the predicted values, obs_{i} the observed values, and *n* the number of observations. This value represents the mean deviation of the predicted values from the observed. Like the sum of squares, RMSD evaluates the overall goodness-of-fit of the model to the data, but has the advantage of calculating values in the same units as the model variables it describes.

To further partition model error I also calculated Theil's partial inequality coefficients (Theil 1958, Smith and Rose 1995, Paruelo et al. 1998), which separate error into three parts: U_{bias}, which compares the differences in means of observed and predicted values; $U_{\beta=1}$, which quantifies the proportional difference of the slope of the predicted versus observed regression from a 1:1 line; and U_e, which describes the variance that is unexplained after a model is fit to the predicted and observed values. This analysis allowed us to evaluate whether the model residuals are systematic in some way that have functional significance, or the result of unexplainable variability. I calculated these errors terms as follows:

$$U_{bias} = \left[n\left(\overline{obs} - \overline{pre}\right)^2\right]/\text{SSPE} \quad (2)$$

$$U_{\beta=1} = \ [(\beta - 1)^2 \sum_n (pre_i - \overline{pre})^2]/\text{SSPE} \quad (3)$$

$$U_e = \ \sum_n (est_i - obs_i)^2/\text{SSPE} \quad (4)$$

Where *obs* and *pre* represent the observed and predicted value (i subscript) or mean (bar), β represents the slope of the regression between observed and predicted, est_i are the values estimated from a linear model developed from the relationship between observed and predicted values, and *n* is the number of observations. SSPE is the squared sum of the predicted error, calculated as:

$$SSPE = \ \sum_n (obs_i - pre_i)^2 \quad (5)$$

I calculated these error terms, in addition to the slope and intercept, to describe how the observed data compared to each prediction using the models described below.

ii. Predictive ability of Great Plains regression model

To compare relationships in Inner Mongolia with a previous model developed in the US Great Plains (Burke et al. 1989) for both rangeland and cultivated land, I entered climate and texture data obtained and collected from Inner Mongolia, used the Great Plains model to predict SOC and SON, and regressed the model output against the observed data. Although site information indicated that none of the sites were ever cultivated (only grazed), I compared predictions from the model developed in cultivated sites to the data I collected as an exploratory exercise to see if simulated carbon losses due to cultivation would better predict data values, and to provide insight into a somewhat uncertain historical past of these soils.

iii. Incorporating additional parameters and predictive power using the Century model

I used the Century model (v4.5) to ask whether additional parameters acting within a dynamic model could predict soil C and N values observed in this region better than the simple regression model. Because I knew that this region may have undergone significant land use intensification in the 1950's

(Sneath 1998, Xiong et al. 2008), and that recent estimates report N deposition levels higher than that in the Great Plains (Lu and Tian 2007), I focused on N deposition and land use as possible influences of simulated SOC and SON. Century is a model that simulates biogeochemical fluxes on a monthly time step (Parton et al. 1987). It was originally designed in the US Great Plains but has been used extensively all over the world (Parton et al. 1993). In the model, soil fluxes are controlled by temperature, water, and soil texture, in addition to lignin/N and C/N ratios. Land use history is implemented in Century by designating certain land use types in repeating blocks for specific periods of time. Climate input data for simulations were obtained from Zhou Guangsheng (*pers comm.*) from weather stations nearest to the experimental sites. Maximum and minimum monthly temperature and precipitation were averaged over the 50-year climate record; monthly values were stochastically generated by Century based on these means. Soil texture parameters were obtained from texture analyses on soil samples. I tested both observed bulk density values and values calculated using soil texture and SOC (Rawls 1983) to see if this affected the model output, but differences were negligible.

iv. Sensitivity of SOC, SON, and ANPP to elevated nitrogen deposition

I was first interested in how elevated N deposition, simulated by Century, affected SOC, SON and aboveground net primary production (ANPP) in Inner Mongolia. I examined the sensitivity of these parameters to N deposition 1) at equilibrium (light grazing for 5000 years), and also 2) when including probable land use histories of the sites and region, in order to better compare simulated values to observed values.

N deposition has increased in this area in the last 60 years, as population levels have increased (Jiang et al. 2006). Current estimates in western Inner Mongolia are within the range of 0.32-1.15 g N m^{-2} yr^{-1} (Lu and Tian 2007). The default values for Century, stemming from estimates in the Great Plains, are about 0.3 g N m^{-2} yr^{-1}. I tested three N deposition values at equilibrium: 0.05, 0.9, and 1.5 g N m^{-2} yr^{-1}. I treated these parameters as fixed (not as a function of precipitation) in order to simplify the sensitivity analysis, and did not include any additional land use changes after the time when the model reached

equilibrium. By doing this, I was first able to see how SOC, SON, and ANPP responded to changes in N deposition in this area *at equilibrium.*

However, studies reporting land use changes in the last 60 years in this region suggest the assumption that these sites are currently at SOC and SON equilibrium is not valid, and that these values would not be comparable to observed data. To simulate SOC and SON values that were more comparable to the values I observed in Inner Mongolia, I did a second analysis of N deposition, which included a 60-year period of intensive grazing (Xiong et al. 2008), and the current known land use type. Under these conditions I simulated two N deposition levels: 1) parameter values for N deposition used for Great Plains Century simulations and 2) 0.9 g N $m^{-2}yr^{-1}$. Century4.5 simulations for the Great Plains model N deposition as a function of precipitation, using two parameters, epnfa(1) and epnfa(2) as slope and intercept. Parameterization for the Great Plains (epnfa(1)=0.21 and epnfa(2)=0.0028) result in an average N deposition of 0.3 g N m^{-2} yr^{-1} over all Inner Mongolia sites (such that N deposition = 0.21+ precip*0.0028), as sites have an average mean annual precipitation of about 35 cm.

v. Sensitivity of SOC and SON to inclusion of periods of intensive land use

Given that this region experienced significant land use intensification in the 1950's (Sneath 1998, Xiong et al. 2008), and that current land use practices on each site varied, I was also interested in whether the inclusion of specific periods of changes in land use would result in SOC and SON predictions closer to observed values than predictions by the Great Plains regression model. Century simulates land use changes over time by separating land use into periods within which specified events repeat. I separated the history of these sites into 3 periods: equilibrium, which consisted of light grazing and lasted 5000 years; 60 years of intensive grazing, as a result of population growth, settlement, and land use intensification in the area beginning in the 1950's (Sneath 1998, Jiang et al. 2006, Xiong et al. 2008); and current (20 years or less) land use based on knowledge obtained from each site (described in Table 2.1). Although I could not confirm that all sites experienced increases in grazing intensities in the 1950's, studies suggest that this trend occurred in the region as a whole, and I was interested to know whether this

was an important factor. Grazing effects were determined based on relationships described in Ojima et al. (1990) and Holland et al. (1992), in which the relationship of grazing to biomass production changes as grazing intensity increases. This approach has been used to simulate grazing variation in other studies in this region (Wang et al. 2007).

I wanted to investigate how the inclusion of these periods in the model, individually and in combination, affected SOC and SON output for sites in Inner Mongolia. Therefore, I tested three different "histories": 1) a 5000 year equilibrium period, and a period of current known land use 2) a 5000 year equilibrium period, and 60 year period of more intense grazing and 3) a 5000 year equilibrium period, a 60 year period of more intense grazing, and a period of current known land use. I used a scaled N deposition of 0.9 g N m^{-2} yr^{-1} for each of these runs.

vi. Comparison of model predictions of ANPP to observed ANPP in Inner Mongolia

Because most ecosystem carbon enters the system through photosynthesis, ANPP represents a major control over organic matter storage. In this way, simulated ANPP values can provide additional insight into variation in SOC and SON under different modeling scenarios. Adjustments in N deposition and land use affect ANPP, and I was interested in whether simulated ANPP fit with observed ANPP in Inner Mongolia under the same scenarios that simulated SOC and SON fit with observed SOC and SON in Inner Mongolia. Because I did not measure ANPP in Inner Mongolia in 2008, I used ANPP values described in the literature across the Northeast China Transect (Yu et al. 2004, Zhou et al. 2006, Hu et al. 2007). I compared these "observed" values to ANPP simulated by Century under parameters that produced the best fit to SOC and SON (elevated N deposition and inclusion of intensive land use). I also compared observed values to ANPP predicted for these sites by two regression models relating ANPP to mean annual precipitation: one developed in the Great Plains (Sala et al. 1988), and one developed along the Northeast China Transect (Zhou et al. 2002).

Results

Response of C and N fractions to environmental factors

Total C and N increased as precipitation and fine-textured soil increased, and decreased as mean annual temperature (MAT) increased (Table 2.2). Total C contained in the intermediate POM fraction increased with MAT and decreased with percent silt and clay, whereas percent C in MAOM fraction, representing passive C associated with silt and clay particles, had the opposite response to these factors. Total C:N significantly correlated with total SOC, but surprisingly, decreased as total SOC increased. Both 'Biomass Removed per Year' and 'Time Fenced' were tested against all response variables in linear regressions, but alone did not significantly explain any of the variation (and therefore are not listed in Table 2.2).

Multiple regression models for observed independent variables

Best-fitting models revealed that with climate, texture, and interactions terms alone, I could explain 76% of the variability in total SOC and 71% of total SON in this region (Table 2.3). Land use terms ('Biomass Removed per Year' and 'Time Fenced') were not significant explanatory variables for total SOC when included in the model, but contributed significantly to the POM-C, POM-N and MAOM-C models. Longer periods of time that sites were fenced resulted in increased MAOM passive C, but decreased POM-C.

Generality of Great Plains regression model

To determine the generality of the SOC and SON models developed in the US Great Plains compared to other temperate grassland regions, I compared the observed carbon values in Inner Mongolia to values predicted by a regression model for the US Great Plains (Burke et al. 1989) (Fig. 2.3). The model from the Great Plains explained a significant proportion of the variation in the China soils (r^2=0.58, $p<0.0001$, Table 2.4). However, on average, observed values for China were 30% lower than predicted values from the U.S. Great Plains model, and regressions between predicted and observed values included

an intercept term significantly different than 1 for both SOC and SON (Table 2.4). Predictions of soil N by the U.S. model also explained a large proportion of the variability (r^2=0.85, p<0.0001), but in contrast, *under*estimated total N values along the Northeast China Transect (intercept significantly greater than 1, Table 2.4). When error was partitioned, more error was found in U_{bias} and U_e terms, and $U_{\beta=1}$ error was low.

Burke et al. (1989) also developed a model predicting C and N in cultivated sites, and I measured how predictions from this model fit the data as a general investigation of how the incorporation of land use might affect the goodness of fit (Fig. 2.3). The cultivated model did not produce a higher r^2 value (SOC: r^2=0.39, SON: r^2 =0.77), but predicted lower C and higher N than for range soils (closer to observed values), a lower bias term, and a lower RMSD.

Century simulations

i. Sensitivity of SOC, SON and ANPP to elevated nitrogen deposition

Century simulations revealed that adjusting N deposition and land use history parameters produced simulated SOC and SON closer to observed values. At *equilibrium* (no land use scenarios included), SOC, SON, and ANPP were sensitive to changes in N deposition, but showed a greater response to deposition changes from 0.05 to 0.9 g N m^{-2} yr^{-1} than from 0.9 to 1.5 g N m^{-2} yr^{-1} (Fig. 2.4). Comparisons among simulations of the three N deposition levels at equilibrium and observed values suggested that SOC and SON simulated at an N deposition level of 0.9 g N m^{-2} yr^{-1} produced the best fit with observed data (smallest RMSD, Table 2.4). When decomposing model error, U_e and U_{bias} error terms were highest, suggesting that the predicted values have a consistent relationship to observed values at different N deposition levels at equilibrium ($U_{\beta=1}$ was low), but simulations at equilibrium left a large amount of variability unexplained (high U_e, Table 2.4).

I also tested Great Plains and elevated N deposition levels, while including land use conditions according to our knowledge for the region and each site (60-year period of intensive grazing and current known land use for each site) to better compare model output with observed data. After including these

periods, SOC and SON under elevated N deposition (0.9 g N m^{-2} yr^{-1}) was significantly related to the observed values (SOC: r^2=0.53, p<0.0001; SON: r^2=0.67 p=0.002) and had a lower RMSD than values under N deposition parameters used for the Great Plains (SOC: r^2=0.221 p=0.78; SON: r^2=0.043 p=0.062) (Fig. 2.5, Table 2.4). The lack of fit in the Great Plains model was primarily related to unexplained variance (U_e), and bias (U_{bias}). Although overall error was low, any remaining error in the elevated N deposition model was most attributed to unexplained variance (U_e) for SOC, and lack of consistency ($U_{\beta=1}$) for SON.

ii. Sensitivity of SOC and SON to changes in land use history

SOC and SON were closest to observed values under land use scenario 3, as determined by the lowest RMSD when compared with the data (Table 2.4). This scenario included a 5000 year period of equilibrium, a 60-year period of intensive grazing and the current known land use for each site (e.g. fenced 7 years, heavily grazed 3 years, etc. depending on the site). The inclusion of only the current land use period (scenario 1) produced the worst fit with the observed values, including a slope significantly lower than 1 and an intercept significantly higher than 0 (Fig. 2.6, Table 2.4). Inclusion of the 60-year period (scenario 2) produced predictions with a statistically significant relationship to observed values. The unexplained variance (U_e) made the largest contribution to the lack of fit for the competing models, and remained the largest source of error even in the best-fitting model. Thus, the superior fit of the best model was the result of reduced error in model consistency ($U_{\beta=1}$) and bias (U_{bias}).

iii. Simulated ANPP for Inner Mongolia compared to values observed in the literature

I examined the ANPP output from Century under the model scenario that produced SOC and SON closest to observed values (0.9 g N m^{-2} yr^{-1} N deposition and intensive and current land use periods, Table 2.4). Simulated ANPP by this Century model *were comparable* to those estimated in the literature for these sites (r^2=0.72 p<0.001) (Fig. 2.7, Table 2.4). Values predicted by the Great Plains ANPP regression model (Sala et al. 1988) were also significantly related to estimated ANPP from the literature

(r^2=0.54 p<0.01), but values from the regression model were generally *lower* than observed ANPP (intercept= 32.6, p<0.05). Much of the observed lack of fit was associated with mean differences (U_{bias}) and lack of consistency ($U_{\beta=1}$). A regression model developed in this area by Zhou et al. (2002b) provided the best fit to observed values (r^2=0.76 p<0.001) and the lowest RMSD of the three models.

Discussion

I found that although SOC and SON in Inner Mongolia are controlled by the same climate and texture variables used to predict SOC and SON in the Great Plains, in this region, values of SOC were lower, SON higher, and ANPP higher than those predicted by regression models developed in the Great Plains. The incorporation of both elevated N deposition and an intensive land use history in Century was necessary to obtain simulated values of SOC, SON, and ANPP near the values observed across the Northeast China transect.

Response of SOC to land use and texture in Inner Mongolia

I used two approaches to compare soil organic matter response to climate and land use in grasslands across continents: (1) I determined the relationships – and importance of the relationships – of climate, texture, and land use to total SOC and SON and fractions, and compared them to relationships described for other grasslands in previous studies and (2) I compared the values predicted by previous grassland regression and simulation models to those observed in Inner Mongolia.

My results show that climate and texture variables exert dominant controls on all fractions of soil organic matter in Inner Mongolian grasslands. SOC increased with increasing precipitation and decreased with increasing temperature. Other studies have found similar trends in temperate semiarid regions (Burke et al. 1989, Alvarez and Lavado 1998, Paruelo et al. 1998), and across this transect (Zhou et al. 2002). This trend can be explained by production responding more than decomposition to increased precipitation across the spatial gradient in water-limited areas, and decomposition rates responding more strongly than production to higher temperatures across the gradient (Epstein et al. 2002, Guo et al. 2006). However, the

total SOC explanatory power (R^2=0.76, Table 2.3) with only these variables was surprising; I sampled across a land use gradient, and studies quantifying the losses due to degradation in this area suggest that land use is a strong determinant of SOC – and perhaps an even stronger control than climate – of SOC in this area (Chuluun and Ojima 2002b). For this reason, I originally hypothesized the land use in this area would alter the predictive power that climate variables have over SOC and SON values. One possible reason for the lack of a significant role of land use parameters in the multiple regression is that variables describing land use were only describing present or recent (decadal scale) practices, and could not describe longer-term effects that would have a larger impact on current SOC and SON levels. However, this as an explanation alone would have resulted in a much lower R^2 value than I produced with climate and texture data. By comparing these results with data and models developed in other continents, I could gain more insight into the importance of historical land use on SOC values in this area.

Relationships of SOC to environmental variables

Overall, other models relating environmental variables to observed SOC and SON found similar relationships (positive or negative, see Table 2.2) as I did among variables. For instance, Burke et al. (1989) found that similar factors that I included in the model I developed should be included in a predictive model in the Great Plains, but found a change in a standardized MAT unit caused the largest change in SOC. In this study, MAP was the most important factor, as Guo et al. (2006) found for areas receiving less than 1000 mm MAP in the United States. In US forests, Homann et al. (1995) found climate and texture variables explained a large proportion of the variation in SOC, but MAT had a stronger, and positive, effect on SOC. Percival et al. (2000) sampled sites in New Zealand and found that soil chemical characteristics, rather than climate or soil texture, explained much more of the variation in SOC in grasslands there.

In contrast to the most dominant controls over total C, POM-C and MAOM-C fractions were most strongly related to temperature, texture, and land use (Table 2.3). Percent MAOM-C decreased with MAT, suggesting that the proportion of soil carbon that is recalcitrant decreases with increasing

temperature. Previous studies across climatic gradients have suggested that SOC recalcitrance *increases* with increasing temperature (and decreasing total SOC) (Trumbore et al. 1996), but several studies have challenged this idea and its implications for possible decomposition feedbacks to predicted temperature increases (Giardina and Ryan 2000). Percent POM-C and MAOM-C were not significantly correlated to land use variables in simple regressions (Table 2.2), but these variables were significant predictors in multiple regressions (Table 2.3). Percent POM-C declined with increased grazing intensity, possibly because this fraction is more reduced by decreases in plant inputs than the total C pool (Kelly et al. 1996). Current grazing intensity was not a significant predictor of MAOM-C, but MAOM-C was higher in sites that were fenced. Studies examining recovery after cessation from cultivation (Burke et al. 1995) and grazing (Steffens et al. 2011) have similarly detected changes in C fractions and not total C in decadal recovery, but it is surprising that the return of C in this study occurs in the pool with the slowest turnover time. Studies examining recovery after cultivation, and a few after grazing, have found that recovery of this pool is not detectable on a decadal timescale (Robles and Burke 1998, Burke et al. 1999). However, several recent studies on grazing have reported an increase in the passive pool with grazing exclosures, with no change in the intermediate (POM) pool (Altesor et al. 2006, Pineiro et al. 2009).

Comparison of model predictions to observed values in Inner Mongolia

i. Regression model

Soil C and N in the US Great Plains were also best predicted by precipitation, temperature, and soil texture, and had the same relationships (positive or negative) as response variables in the model I developed did to each predictor variable (Burke et al. 1989). The model produced a good fit to observed values, capturing a large amount of variability in the Inner Mongolia dataset, which was surprising given the simplicity of this model and considering what my remaining analyses reveal (i.e. a strong sensitivity of soil organic matter to unknown past land use and N deposition). The main discrepancy was not consistency (slope deviating from 1), but that the US Great Plains model overestimated C values and underestimated N values observed in Inner Mongolia. Paruelo et al. (1998) used this Great Plains model

to assess the SOC drivers and predictability of another GCTE temperate grassland transect in Argentina, and found SOC observations at this site fit well with the Great Plains model (r^2=0.63), and, unlike data collected in Inner Mongolia, fell evenly above and below the model predictions (y-intercept of best-fitting line did not differ significantly from 0). These model-data comparisons suggest that the relationship of SOC and SON to climate variables predicted by the Great Plains model is *consistent* (slope near 1), but that the Great Plains regression models produce a poorer fit to observations because of bias (differences of means, intercept greater or less than 0) and additional variance in the data that could not be explained by the model (Fig. 2.3, Table 2.4).

In Inner Mongolia, observed carbon values that were *closest* to predicted values for rangeland models were from those sites along the Inner Mongolia transect that had been fenced for 12 and 20 years. In addition, the US model developed from cultivated sites in the US produced less bias than the rangeland model. Inner Mongolia sites were *not* cultivated, but the improved model-data fit suggested the gaps between observed and predicted values from the rangeland model may be due to reduced carbon storage as a result of long-term intensive land use in the Inner Mongolia region that could not be quantified by current land use metrics. Although this is impossible to know due to lack of precise knowledge of historical land use at these sites, several empirical and modeling studies have demonstrated the extent of the effect of historical land use on current SOC observations and processes. Sandor et al. (1986) found SOC was still 40% lower in areas that had been intensively cultivated but abandoned 1000 years ago in New Mexico. Although grazing may have varying short-term effects on SOC, studies suggest that when it does greatly reduce SOC, either due to its high intensity or the absence of evolutionary adaptation to grazing, effects can be similar to that of cultivated areas. Pineiro et al. (2006) used coupled grazed and fenced sites and found that long-term grazing reduces SOC by 15-30%, with largest reductions occurring in the slow and passive pools. Century simulations suggest that 400 years of low intensity grazing would produce a 10% reduction in SOC (Alvarez 2001). Few long-term studies testing the effect of long-term grazing and subsequent exclosures exist due to the absence of a reliable control, but observed reductions in slow and passive carbon pools suggest that recovery would be similar to recovery from these effects

due to cultivation (i.e. on the order of centuries), and be longest in areas receiving the least precipitation (Paustian et al. 1997).

ii. Century model

Using the US regression model (Burke et al. 1989), I found observed SOC in Inner Mongolia was lower than predicted by the US model, and observed SON was higher than predicted by the model (Fig. 2.3, Table 2.4). Therefore, I used the Century model to ask what other factors – that I did not measure – might explain these results, and what changes were needed to simulate values closer to observed data. SOC and SON were both sensitive to changes in N deposition, especially changes from very low (0.05 g N m^{-2} yr^{-1}) to values of deposition that might be occurring today (0.9 g N m^{-2} yr^{-1}) (Fig. 2.4, Table 2.4). SOC and SON were not as sensitive to changes in N deposition from 0.9 g N m^{-2} yr^{-1} to 1.5 g N m^{-2} yr^{-1}, but sites in the meadow steppe (wetter end of the gradient) were more responsive than sites in the drier end, suggesting that the diminished response could be related to water limitation.

SOC and SON were, on average, closest to observed values when deposition was 0.9 g N m^{-2} yr^{-1}, after incorporating the land use history that is likely for this region (addressed below). This value for N deposition is approximately 0.4-0.5 g N m^{-2} yr^{-1} higher than parameterized simulations in the Great Plains. When I used the same parameters used in the Great Plains, in addition to all land use scenarios, I found carbon was underestimated by an average of 1.45 kg C m^{-2} yr^{-1} and N was underestimated by 0.18 kg N m^{-2} yr^{-1} (Fig. 2.5). These results are in line with my findings from the US regression model, which did not include N deposition and underestimated N (and arguably underestimated C as well, when no land use was included) compared to observed values. I was able to explain this discrepancy by increasing N input, and increasing ANPP, using the Century model. This suggests that N deposition, or some N input, is important in elevating ANPP in Inner Mongolia and producing SOC and SON values that are higher than expected based on Great Plains data.

SOC and SON were also sensitive to changes in land use history. The inclusion of 60 years of intensive grazing before the current land use period had a stronger effect on SOC and SON than the

inclusion of the current known period of grazing (1-20 years) (Table 2.4). These current land use periods varied depending on site, but included fencing treatments or various grazing intensities for different (known) lengths of time. The simulations, however, suggest that the potential degradation and reduction in SOC and SON caused by the period of land intensification as a result of settlement and increased population was more important in determining SOC levels. However, SOC and SON responses to changes in simulated land use history were not consistent among site (Fig. 2.6). This may be because differences in site climate and soil type cause sites to respond differently to intensive land use in this area, but it is more likely due to inaccuracy of land use history before the current period (i.e. some sites may have experienced more or less intensive land use prior to the current land use). This is supported by the fact that unexplained error (U_e) still contributed to any remaining lack of fit in the model with the best-fitting land use scenarios (Table 2.4).

I did not measure ANPP at Inner Mongolia sites in the summer of 2008, but previous studies recorded ANPP at these and other sites along the Northeast China Transect (Yu et al. 2004, Hu et al. 2007), and Zhou et al. (2002) developed a regression model to predict ANPP in this region using annual precipitation. Perhaps not surprisingly, ANPP for the Northeast China Transect sites fit well with those predicted by this model developed in Inner Mongolia (Zhou et al. 2002) (Fig. 2.7, Table 2.4). However, values reported for the desert steppe, typical steppe, and meadow steppe in Inner Mongolia in the literature were slightly *higher* than values predicted by the widely-used ANPP regression model presented by (Sala et al. 1988), developed in the Great Plains. Similarly, ANPP values in Inner Mongolia were also higher than ANPP simulated by Century when N deposition parameters from the Great Plains were used (data not shown). This follows results from early Century model validations (Parton et al. 1993), which reported that peak live biomass was underestimated by Century for Asian sites, in contrast to the other 9 sites (Gilmanov et al. 1997). Previous studies have suggested that this discrepancy may be due to a higher prevalence of C3 plants in this area compared with regions in North American with a similar climate (Tieszen et al. 1999). However, a hypothesis that fits with the rest of the data from this study is that this higher ANPP is related to higher nitrogen inputs in China. Century simulations *did* produce ANPP values

within the range reported by the literature under *elevated* N deposition (0.9 g N m^{-2} yr^{-1}). This is also the Century scenario that, with the inclusion of intensive land use, estimated SOC and SON values nearest to those observed (Table 2.2). This suggests that in Inner Mongolia a higher ANPP (C input), enabled by increased N deposition, is necessary to produce the greater equilibrium-stage SOC and SON values, which fit observed values when losses due to intensive land use are accounted for as well.

Conclusions

The results of this study challenge the generality of relationships between environmental factors and C and N pools in temperate grasslands. SOC and SON data I collected in Inner Mongolia were strongly related to texture and climate, as they are in other similar regions of the world, and data had consistent relationships with values predicted from Great Plains models across this range of sites. However, these models showed strong bias (overestimation of C and underestimation of N) in predicting SOC and SON values in Inner Mongolia. We tested two possible factors that may have influenced this. Elevated N deposition levels did simulate accurate predictions for biogeochemical pools in Inner Mongolia, so it is possible there is unaccounted for nitrogen input in this region, or differences in fundamental nitrogen cycling properties, such as nitrogen use efficiency, compared to other grasslands. In addition, including historical overgrazing produced simulated values closer to those observed in this area. The relationships of environmental controls with SOC and SON in grasslands are perhaps not as generalizable as many widely-used models, and modelers, assume. The possible divergence of these relationships in Inner Mongolia from those used in models for grassland ecosystems could affect our ability to predict regional ecosystem dynamics, and also add uncertainty to global predictions of carbon flux.

Tables

Table 2.1. Description of Sites Sampled across the Northeast China Transect

Site	Grassland type	MAP (mm)	MAT (°C)	% Clay range	% Silt Range	Land use	# Sub-plots	Mean C (kg m^{-2})
1	Meadow steppe	380	4.77	14-18	31-41	Not grazed	2	0.851
2	Meadow steppe	350	5.08	2-4	5-9	50% grazed	3	2.010
3	Typical steppe	331	3.16	12-17	24-27	Fenced 20 years	2	1.595
4	Typical steppe	331	2.02	10-16	29-37	80% grazed	2	1.899
5	Typical steppe	331	2.02	11-13	20-32	Fenced 9 years	2	2.009
6	Typical steppe	300	0.78	9-23	24-31	Fenced 7 years	2	2.582
7	Typical steppe	300	0.78	8-12	17-24	70% grazed	2	1.853
8	Typical steppe	277	2.67	15-19	33-40	Fenced 10 years	2	2.336
9	Typical steppe	277	2.67	12-15	34-39	70% grazed	2	2.936
10	Desert steppe	178	2.1	8-12	12-29	Fenced 1 year	2	0.611
11	Desert steppe	171	2.1	8-11	16-21	60% grazed	2	0.771
12	Desert steppe	160	2.41	9-13	15-21	60% grazed	2	1.167

Table 2.2. Correlations between Parameters (top) and Dependent Variables Measured in Inner Mongolia[a]

	MAP	MAT	%Silt	%Clay	TotC[b]
Total C	+	-	+	+	
POM-C	+	+	+		
%POM-C		+	-	-	
MAOM-C	+	-	+	+	
%MAOM-C		-	+	+	
Total N	+	-	+	+	+
POM-N		+	+	+	+
%POM-N		+	+	+	+
MAOM-N		-	-		
%MAOM-N		-	-	-	-
C:N					-
C:N POM		-	-	-	-
C:N MAOM				+	

[a] All relationships reported were significantly related in a Pearson Correlation ($p<0.05$). "Percent biomass removed per year" and "Time Fenced" were not reported because there were no significant relationships (positive or negative) with dependent variables.
[b]Total Carbon is listed as an independent variable to show with which dependent variables it correlated.

Table 2.3. Best Predictive Models[a] for SOC, SON and C and N Fractions Measured in Inner Mongolia.

Variable	Coefficient	Standardized Coefficient	p-value
Total Carbon: R^2=0.75, Adj R^2=0.75			
MAT^2	0.163	0.6825	0.0322
MAT	-1.085	-0.7529	0.0137
MAP^2	-0.016	-3.2491	<.0001
MAP	0.858	3.3207	<.0001
%Clay	0.164	0.3291	0.0005
%Silt	0.089	0.4396	<.0001
Intercept	-9.725		<.0001
% POM-C: R^2=0.37, Adj R^2=0.34			
MAT	0.092	0.4333	<.0001
%Silt	-0.008	-0.2655	0.0116
%BiomassRemvd/yr	-0.003	-0.4510	0.0048
Years fenced	-0.021	-0.5211	0.0012
Intercept	0.571	0	<.0001
% MAOM-C: R^2=0.475 Adj R^2=0.442			
MAP*MAT	-0.003	-0.5370	<.0001
%Silt	0.013	0.4248	<.0001
%BiomassRemvd/yr	0.003	0.4527	0.0021
Years fenced	0.023	0.5209	0.0005
Intercept	0.234	0	0.0155

Variable	Coefficient	Standardized Coefficient	p-value
Total Nitrogen: R^2=0.71 Adj R^2=0.68			
MAT^2	0.026	1.147	0.0110
MAP*MAT	-0.006	-1.839	0.0004
MAP	0.007	0.269	0.0531
MAP*%silt	0.001	0.965	<.0001
%Biom Remvd/yr	0.002	0.335	0.0043
Years fenced	0.011	0.448	0.0007
Intercept	0.031	0	0.6064
% POM-N: R^2= 0.851 Adj R^2= 0.839			
MAT^2	-0.333	-2.443	<.0001
MAP^2	-0.028	-11.048	<.0001
MAP	1.322	9.834	<.0001
MAP*MAT	0.077	3.834	<.0001
Years fenced	-0.051	-0.355	<.0001
Intercept	-14.977	0	<.0001
% MAOM-N: R^2=0.474 Adj R^2=0.453			
MAP*MAT	0.846	1.459	<.0001
MAT*%silt	-0.577	-1.254	<.0001
Intercept	1.608	0	0.0768

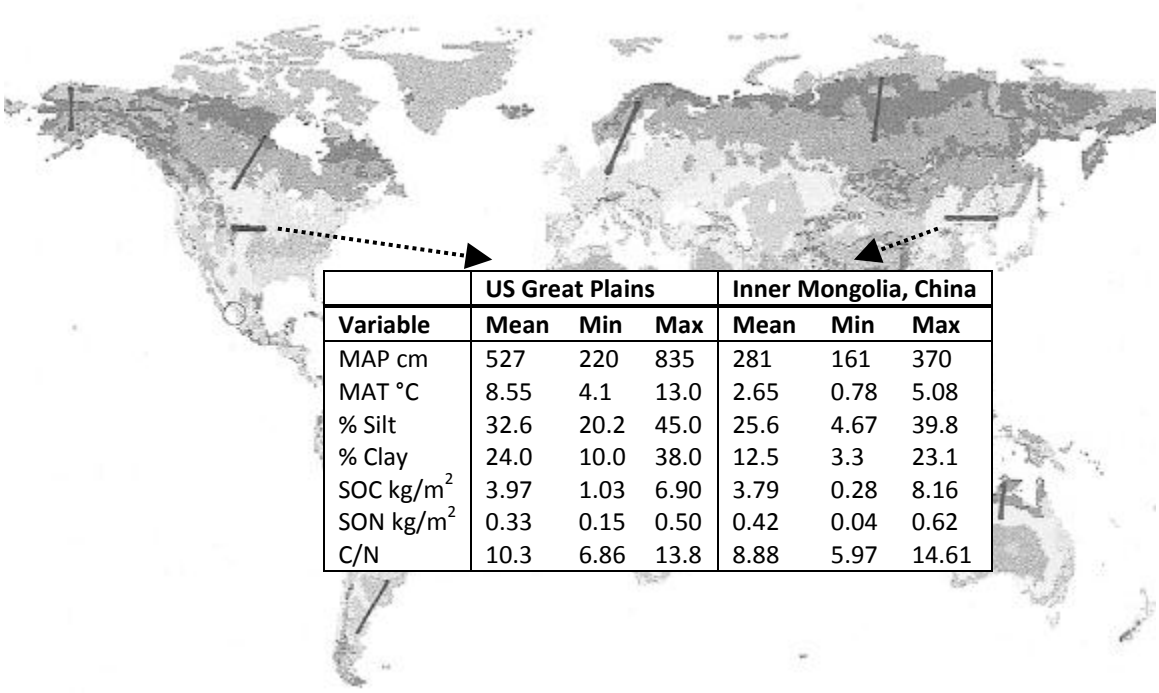
[a]Multiple linear regression models were determined using all possible subset selection of 6 independent variables: mean annual temperature (MAT in °C), mean annual precipitation (MAP in cm), silt and clay (%), and two metrics of land use: number of years fenced, and percent biomass removed per year due to grazing.

Table 2.4. Summary of Regression[a] and Goodness of Fit Statistics for Model Simulations Compared to Observed Data.

Model Information		r^2		Slope		Intercept		RMSD[b]		U_{bias}[c]		$U_{\beta=1}$[d]		U_e[e]	
		SOC	**SON**	**SOC**	**SON**	**SOC**	**SON**	**SOC**	**SON**	**SOC**	**SON**	**SOC**	**SON**	**SOC**	**SON**
CENTURY: Sensitivity to elevated N-deposition at equilibrium															
N deposition	Land Use														
0.05	Equilibrium	**0.17**	0.077	-1.524	0.447	**4.332**	0.381	3.428	1.34	0.850	0.068	0.012	1.4E-03	0.998	0.073
0.9	Equilibrium	**0.849**	**0.472**	1.09	**2.51**	-0.253	**-0.791**	0.579	1.2	8.2E-03	2.9E-03	0.037	2.8E-03	0.320	0.012
1.5	Equilibrium	**0.78**	**0.227**	**0.684**	0.738	0.637	-0.087	1.153	1.18	0.465	0.052	0.267	4.3E-04	0.419	0.057
CENTURY: Sensitivity to inclusion of land use periods															
N deposition	Land Use														
0.9	Current land use only	0.134	0.231	**0.211**	**0.216**	**2.434**	**0.297**	2.579	1.21	0.388	0.003	0.053	1.1E-03	0.934	0.022
0.9	60-year intensive grazing	**0.456**	0.232	0.536	0.482	1.103	0.192	1.873	1.18	0.534	0.010	0.145	0.013	0.774	0.035
0.9	60-year grazing & current land use	**0.531**	**0.672**	0.993	0.725	0.024	0.257	1.43	0.24	4.9E-05	0.074	3.7E-05	0.004	0.036	0.059
CENTURY: Elevated vs. Great Plains N-deposition with best-fitting land use scenarios															
N deposition	Land Use														
0.9	60-year grazing & current land use	**0.531**	**0.672**	0.993	0.725	0.024	0.257	1.43	0.24	4.9E-05	0.074	3.7E-05	0.004	0.036	0.059
0.3[f]	60-year grazing & current land use	0.221	0.043	0.888	**0.648**	**1.788**	0.095	2.006	0.19	0.653	0.192	2.0E-03	0.025	1.002	0.535
Great Plains Regression															
Citation	Developed in														
Burke et al. [1989]	US Great Plains rangeland sites	**0.577**	**0.853**	1.045	1.094	**-2.243**	**0.064**	2.267	0.351	0.795	0.076	0.001	0.001	0.892	0.083
Burke et al. [1989]	US Great Plains cultivated sites	**0.389**	**0.772**	1.124	1.087	-2.029	0.054	1.878	0.346	0.568	0.060	0.003	0.001	0.868	0.067
ANPP predictions from Century and regressions models compared to ANPP in Inner Mongolia															
Citation	Developed in	**ANPP**[h]		**ANPP**		**ANPP**		**ANPP**		**ANPP**		**ANPP**		**ANPP**	
Sala et al. [1988]	US Great Plains for ANPP	**0.538**		0.887		**32.569**		33.646		0.359		0.517		0.340	
CENTURY[g]	Best-fitting SOC and SON model	**0.717**		1.168		-2.937		31.862		0.295		0.288		0.659	
Zhou et al. [2002]	Inner Mongolia grasslands for ANPP	**0.763**		0.923		18.346		27.705		0.014		0.208		0.097	

[a] Regressions represent observed (y) versus predicted (x). Bold indicates regression model (r-squared) is significant, slope is significantly different than 1 and intercept significantly different than 0 ($p<0.01$).
[b] Root Mean Squared Deviation, represents overall goodness-of-fit of model (see Equation 1)
[c] Error term that compares the differences in means of observed and predicted values (see Equation 2)
[d] Error term which quantifies the proportional difference of the slope of the predicted vs. observed regression from a 1:1 line (see Equation 3)
[e] Error term that describes the variance unexplained by a model fit to the observed values (see Equation 4)
[f] Value represents average nitrogen deposition simulated for the Great Plains; nitrogen deposition varied with precipitation.
[g] This model was developed in this paper using Century and included an elevated N deposition level (0.9) and a period of 60-year grazing and current land use
[h] Regression of Century model simulations of ANPP (y) compared to observed ANPP from literature (x)

Figures



	US Great Plains			Inner Mongolia, China		
Variable	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
MAP cm	527	220	835	281	161	370
MAT °C	8.55	4.1	13.0	2.65	0.78	5.08
% Silt	32.6	20.2	45.0	25.6	4.67	39.8
% Clay	24.0	10.0	38.0	12.5	3.3	23.1
SOC kg/m^2	3.97	1.03	6.90	3.79	0.28	8.16
SON kg/m^2	0.33	0.15	0.50	0.42	0.04	0.62
C/N	10.3	6.86	13.8	8.88	5.97	14.61

Figure 2.1. International Geosphere-Biosphere Program (IGBP) Global Change and Terrestrial Ecosystems (GCTE) global terrestrial transects, modified from Koch et al. (1995). Lines represent transects of climatic gradients. Data from Inner Mongolia, China were collected in this study and compared with data from other studies to assess generality of environmental controls over soil organic matter.

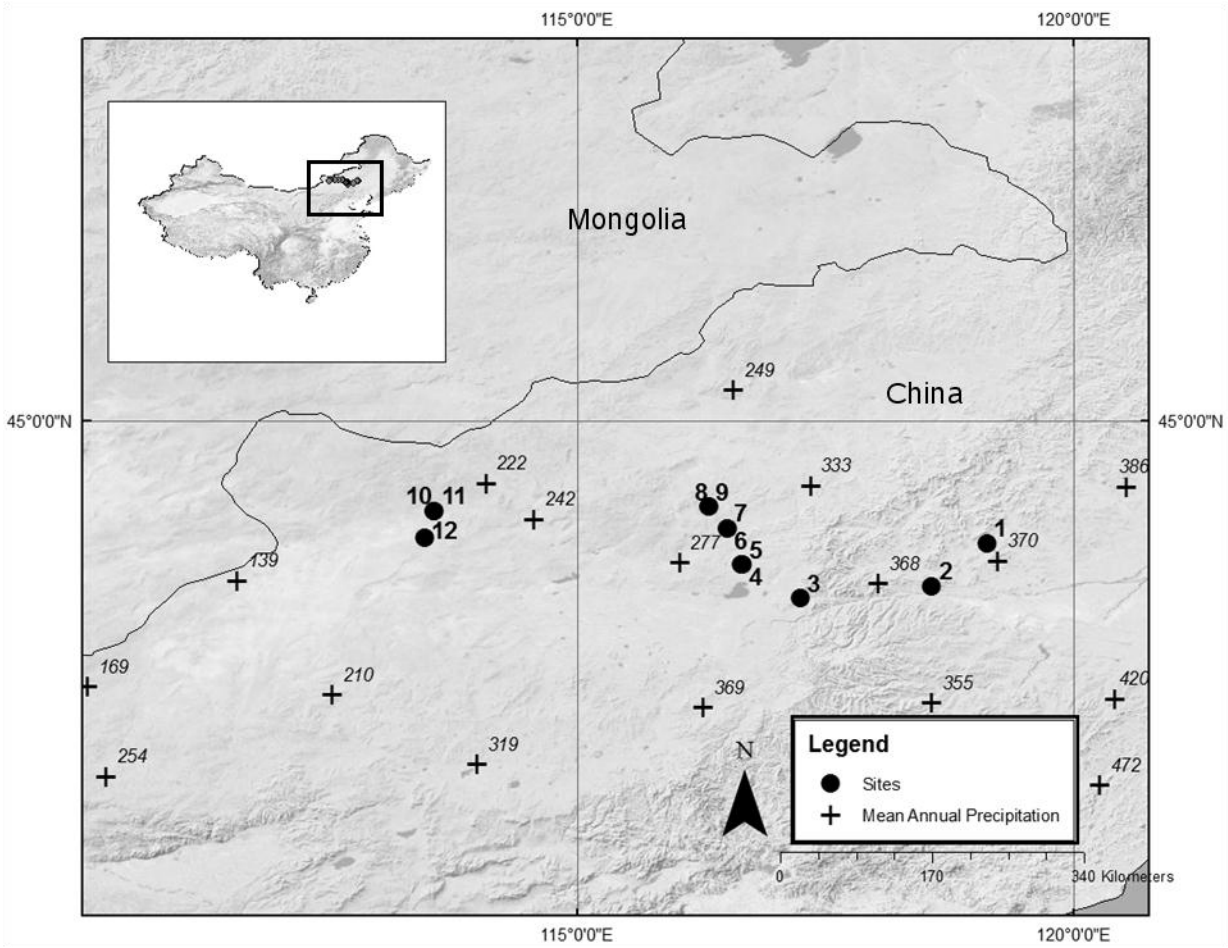


Figure 2.2. Map of study region in the Inner Mongolia province of China (inset), displaying study sites (●) and average mean annual precipitation (+, italic font) for the area.

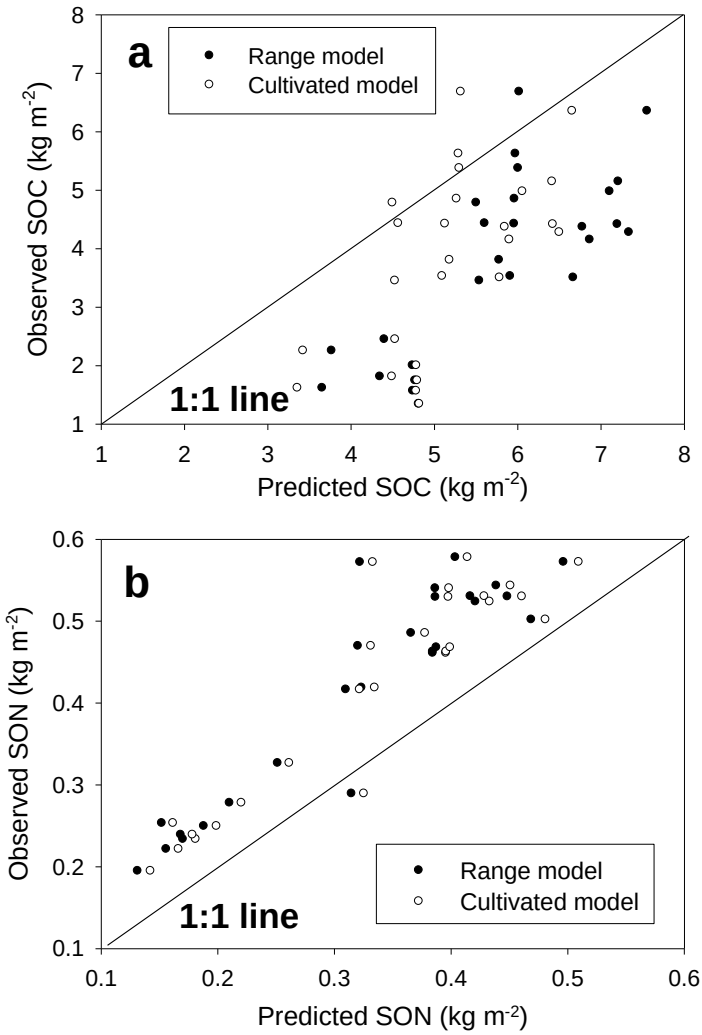


Figure 2.3. Predicted values from the Great Plains regression model [Burke et al., 1989] compared to observed values from Inner Mongolia for SOC (a) and SON (b). I simulated values for Inner Mongolia using both the model developed for US rangeland (●) and cultivated land (○).

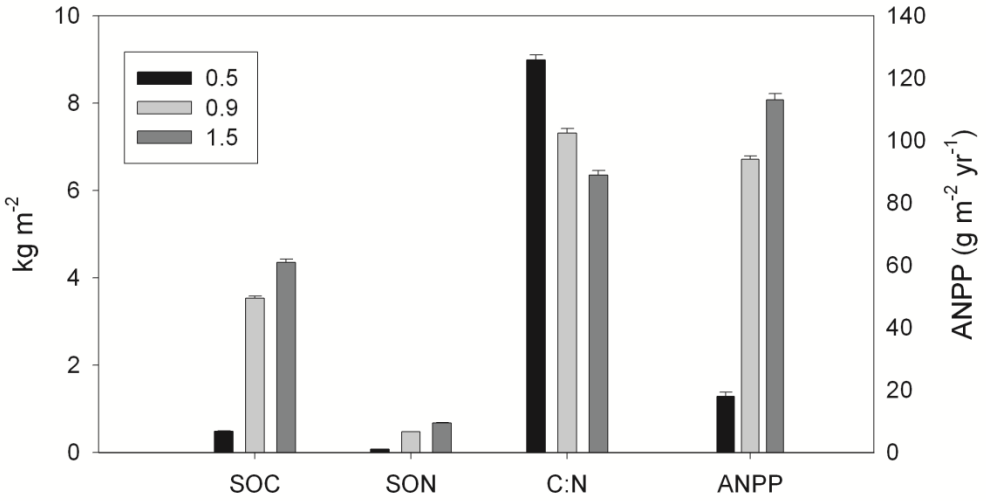


Figure 2.4. Average SOC (kg C m^{-2}), SON (kg N m^{-2}), C:N, and ANPP (g m^{-2} yr^{-1}, second y-axis) across all sites simulated by Century at 3 different nitrogen deposition levels (g N m^{-2} yr^{-1}) for Inner Mongolia sites. Output was recorded after 5000 years of equilibrium conditions at each site, with no current land use periods.

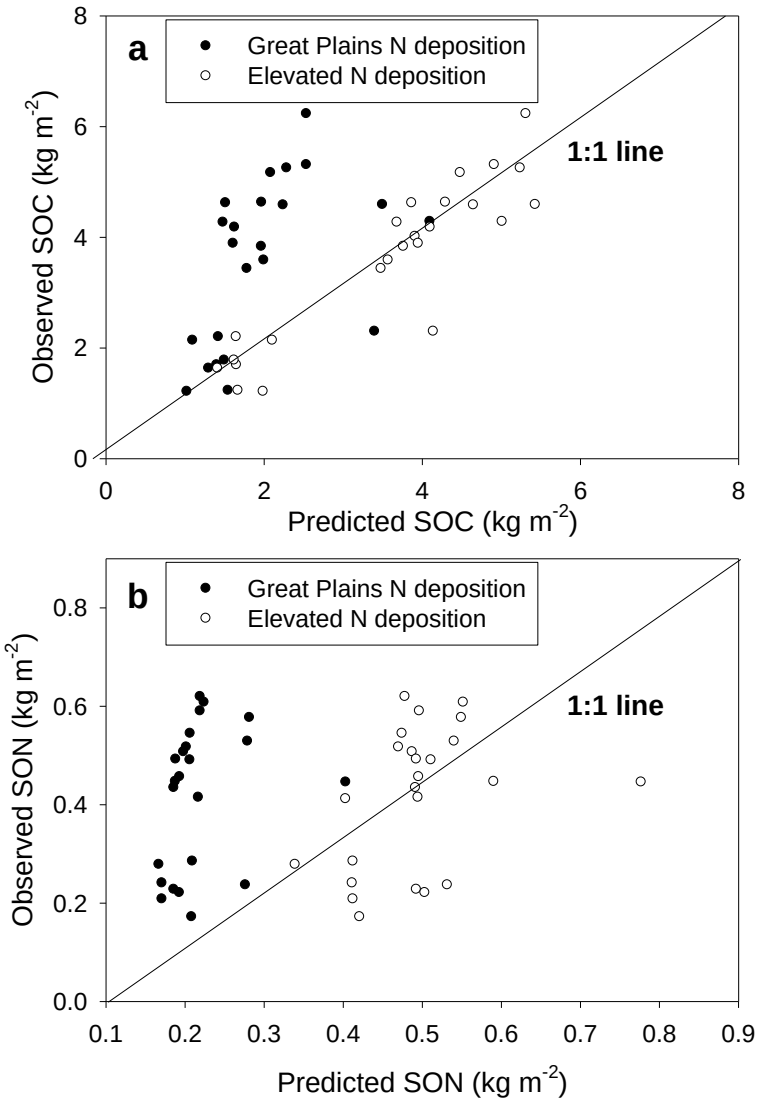


Figure 2.5. SOC (a) and SON (b) predicted by Century using nitrogen deposition as parameterized in the Great Plains (●), which varies with precipitation but results in an average of 0.3 g N m^{-2} yr^{-1}, and fixed at 0.9 g N m^{-2} yr^{-1} (○), compared to observed values in Inner Mongolia at all sites. All runs included a 60-year intensive grazing period followed by a unique current land use period depending on the site. This combination proved to be the land use scenario that best predicted observed values (see Table 2.4).

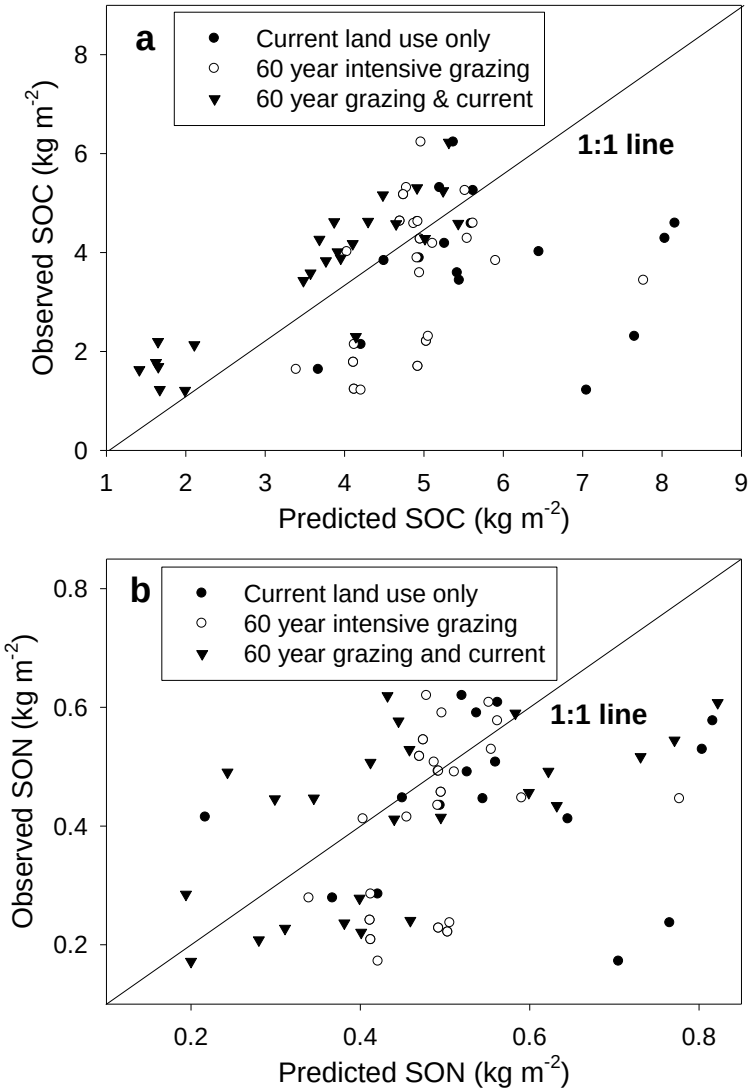


Figure 2.6. SOC (a) and SON (b) predicted by Century when I varied the inclusion of certain land use periods, compared to observed values in Inner Mongolia at all sites. Current land use refers to the information I acquired at sites from the current manager, and is a 1 to 20 year history depending on the site. 60-year intensive grazing is a period *before* the "current" land use that was implemented in all sites to represent the increase in grazing intensity as population density increased in Inner Mongolia around the 1950's. All runs used nitrogen deposition of 0.9 g N m^{-2} yr^{-1}.

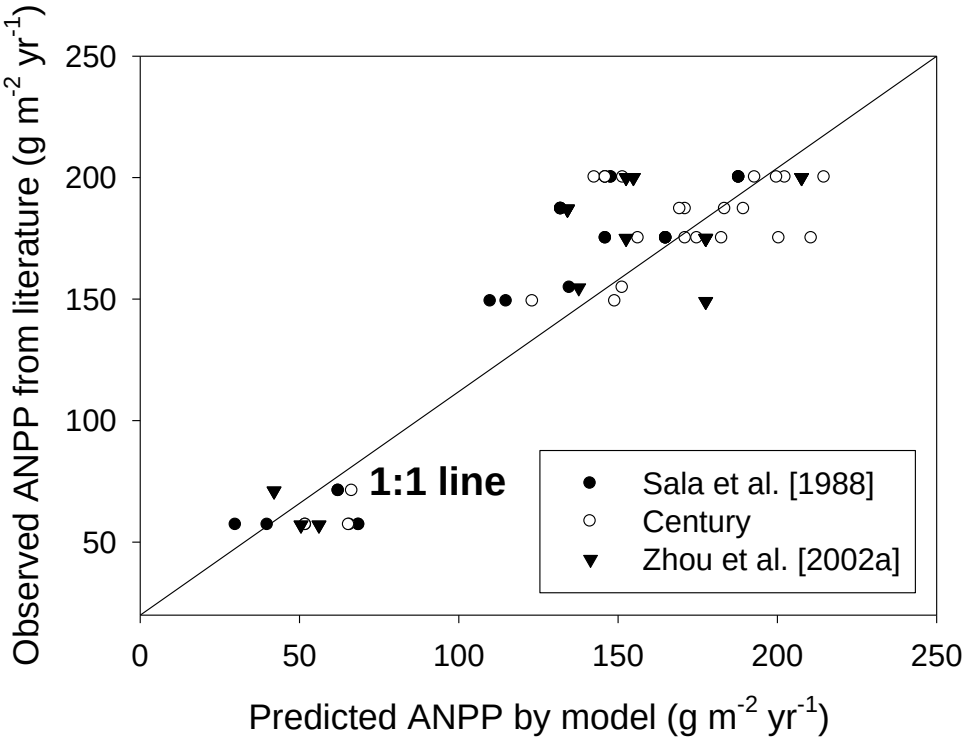


Figure 2.7. Simulated ANPP (g m^{-2} yr^{-1}) from Sala et al., (1988) (ANPP=-34+0.6*MAP), Zhou et al., (2002a) (ANPP=-84.8+0.7905*MAP) and as simulated by Century (all land use periods, N-deposition of 0.9 g N m^{-2} yr^{-1}) versus ANPP estimates from the literature across the Northeast China Transect in Inner Mongolia grasslands.

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Chapter 3: Carbon and nitrogen decoupling under an 11-year drought in the shortgrass steppe: implications for increased nutrient loss and prolonged ecosystem recovery[2]

Introduction

Precipitation is the major control on ecosystem processes in semiarid ecosystems (Noy-Meir 1973), where it is also highly variable within and among years. Because organisms in these ecosystems have adapted to variable rainfall and frequent water limitation, ecosystem processes, including processes that couple carbon (C) and nitrogen (N) cycles such as the accumulation of biomass, remain relatively stable under historical ranges of precipitation variability. However, future climate regimes in semiarid systems are expected to be characterized by more frequent summer droughts, increases in temperature (IPCC 2007, CCSP 2008) and possibly, decadal-scale "megadroughts" with no known recent analogues (Cook and Seager 2010, Woodhouse et al. 2010). So although semiarid systems are highly resistant to drought, processes such as biomass accumulation and decomposition will likely be altered, and perhaps in different ways, by these extreme events. Major changes due to disturbance, and different responses among ecosystem processes, can cause C and N to "decouple" (Asner et al. 1997). This decoupling causes asynchrony in N supply and demand that can increase nutrient loss and create new biogeochemical feedbacks. Such changes in fundamental ecosystem properties can intensify the impact of disturbances such as drought, and result in legacies that impact ecosystem processes beyond the timescale of the disturbance.

Because N that is linked to C in biomass is less vulnerable to loss (Vitousek and Reiners 1975), C and N decoupling as a result of disruptions of biomass accumulation can alter N retention. In semiarid systems, the majority of N flux occurs through internal cycling; rates of "open" fluxes, such as leaching and gaseous emissions, are low (Burke et al. 2008), or limited to brief precipitation pulses (Austin et al.

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2004). Although these low loss rates suggest that seasonal supply and demand of N are highly synchronized in drier ecosystems (Risser and Parton 1982), N loss rates may increase relative to internal fluxes as precipitation decreases (Austin and Sala 2002, McCulley et al. 2009). In addition, N has been seen to accumulate in soil as inorganic N during dry periods (Jackson et al. 1988, Whitford et al. 1995, Reynolds et al. 1999, Augustine and McNaughton 2004) and under short-term drought manipulations (Yahdjian et al. 2006). These studies suggest that water limitation in semiarid systems may cause N availability to be asynchronous with plant and microbial N demand just as short-term fluctuations in precipitation can lead to periods of greater N loss and limitation (Austin 2004).

Long-term changes in N-retention as a result of precipitation changes may cause organisms to be more frequently N-limited, and alter plant-N-soil interactions. After water, N is most likely to limit productivity in semiarid systems (Burke et al. 1997, Hooper and Johnson 1999), and N and water availability are highly interdependent (Harpole et al. 2007, Bai et al. 2008). Because of this N-limitation, N that has accumulated in dry years can result in higher-than-expected plant productivity in years following drought (Briggs and Knapp 1995). In addition, new plant-soil-N feedbacks following drought could cause increased variability in production (Haddad et al. 2002). Alternatively, plant, root, and tiller mortality, and reduced meristem density that occur as a result of drought can also generate "structural vegetative constraints" (Lauenroth and Sala 1992) that reduce the capacity of plants to respond to both ambient moisture conditions and any increases in N availability that occurred under drought (Benson et al. 2004). In addition, C and N decoupling could occur in the short-term, but changes in plant species composition and N use efficiency (NUE) that occur as long-term drought persists could cause a "re-coupling" of C or N in a way that more closely resembles a drier system. In sum, although previous studies have documented possible ecosystem impacts of C and N decoupling under moisture limitation, the nature and timescale of this decoupling under long-term drought and recovery is unclear.

Although there has been much previous work on interactions among rainfall, N, and C cycling in grasslands (Austin et al. 2004, Burke et al. 2008, Yahdjian and Sala 2008), most findings are based either on the monitoring of natural variability across space and time (Augustine and McNaughton 2004,

McCulley et al. 2009), and therefore not ideally suited to investigate responses to novel events, or on short-term rainfall manipulations (1-4 years) (Yahdjian et al. 2006), which may not sufficiently test the limits of drought-resistance in semiarid systems. The shortgrass steppe, a semiarid ecosystem on the drier Western edge of the US Great Plains, is extremely resistant to drought and other disturbances (Milchunas et al. 1988, Burke et al. 2008, Peters et al. 2008), but is likely to experience droughts of novel lengths and severities in the future (IPCC 2007). A large body of research describing interactions among climate, vegetation, and biogeochemistry in the shortgrass steppe ecosystem provides an excellent context for interpreting N responses to drought (Sala et al. 1992, Vinton and Burke 1995, Lauenroth et al. 2008a). In this study, I use an 11-year rainfall manipulation in the shortgrass steppe to ask how a drought of unprecedented duration and severity affects N-conservation, and how this affects ecosystem recovery. To address this, I measure C and N dynamics in the 10^{th} and 11^{th} year of a long-term drought experiment where plots received 25% and 50% of growing season rainfall, then in the first and second year of recovery, when plots received ambient rainfall.

I hypothesized that drought decreases N conservation in the shortgrass steppe because C-N decoupling induced by high moisture limitation results in increased rainfall use efficiency and decreased nitrogen use efficiency. I predicted that 1) after 11 years of drought, plant production decreases and inorganic N accumulates in the soil, leaving it more vulnerable to loss through gaseous flux; and 2) under recovery, structural vegetative constraints result in lags in plant production such that accumulated N is assimilated by plants less efficiently.

Methods

Study site and rainfall manipulations

I conducted this study in the semiarid shortgrass steppe at the Central Plains Experimental Range (CPER) Long Term Ecological Research Site (Lauenroth et al. 2008a), 60 km northeast of Fort Collins, Colorado (40° 49’ N latitude, 104° 46’ W longitude). Mean annual temperature is 8.2 °C, and mean annual precipitation is 341 mm (65-year average), with 83% of precipitation occurring between April and

September (Sala et al. 1992). Soils are frequently dry but experience brief wet periods, and as a result, soil water content is highly variable (Lauenroth and Bradford 2006). Precipitation patterns are dominated by small events (< 5 mm), but differences in the size of large events (> 30 mm) accounts for most of the variability in interannual rainfall (Lauenroth and Sala 1992).

Dominant vegetation in the shortgrass steppe includes short-stature C_4 grasses *Bouteloua gracilis* (blue grama) and *Bouteloua dactyloides* (buffalograss). Other common shrubs are *Opuntia polyacantha* (plains pricklypear cactus), *Artemisia frigida* (prairie sagewort), *Eriogonum effusum* (spreading buckwheat), *Chrysothamnus nauseosus* (rubber rabbitbrush), and *Gutierrezia sarothrae* (broom snakeweed) (Lauenroth 2008). Soil types at this site are Renohill and Ascalon fine sandy loams (Aridic Argiustoll and Ustic Haplargid) (Natural Resource Conservation Service 2008).

In spring of 1998, two blocks with similar vegetation were identified near the headquarters of the SGS LTER field site, and divided into four 3.5 m by 1.7 m plots (Fig. 3.1). Blocks were selected to represent slightly different topographies (slight slope and toeslope) and soil textures, although differences in soil texture between blocks were not significant when measured in 2008. During the growing season (average dates 26 April – 7 October), two rainout shelters automatically covered plots with a sliding roof when rainfall was detected by an electronic rainfall sensor (AeroChem Metrics, Bushnell, FL). Each week, a proportion (100%, 25% or 50%) of ambient rainfall measured by a rain gauge was added back to the plots to simulate drought. The 100% treatment was used to test the shelter effect and specifically, the effect of changes in rainfall timing caused by the re-additions. The control plot was never covered by shelters and received ambient precipitation. To evaluate treatment effectiveness, I monitored soil moisture each year of the study. In summer 2008, I measured soil moisture approximately every two weeks using a handheld 10 cm TDR Probe (Campbell Scientific, Logan, UT). From 2009-2011, I inserted one 10 cm TE probe (Decagon Devices, Pullman, WA) in each plot that measured hourly soil temperature and volumetric soil moisture.

These 11-year drought manipulations gave us the unique opportunity to monitor long-term drought of unprecedented severity and length. However, the large investment required to set up and

maintain rainfall manipulations often results in smaller plots sizes that make careful sampling essential to preserve the long-term integrity of the plots. In an effort to use the most accurate but least destructive method for measuring plant and biogeochemical dynamics, methods sometimes differed interannually, but properties and processes were always measured consistently across plots within years.

Overall, to test how drought affected N conservation in these plots, I monitored C and N pools that are good indicators of biogeochemical decoupling and that are important for ecosystem function. I measured plant production and N content, soil inorganic N dynamics, and soil trace gas flux in the 10th and 11th year of drought (2008 and 2009), and under recovery (2010 and 2011), when shelters were removed. In 2009 I also subjected plots to three moisture pulses by adding an equal amount of water to each plot, investigating dynamics while controlling for soil moisture differences and simulating recovery.

Plant production and N content

I measured parameters that described aboveground and belowground plant production each year between the 1st and 15th of August, which is within range of expected peak biomass in the shortgrass steppe (Lauenroth et al. 2008b). In 2008, I estimated aboveground net primary productivity (ANPP) using a First Growth digital canopy camera (Decagon Devices 2004), establishing a calibration between measured ANPP and percent cover as determined by the camera using a greenness index. Because the relationship between greenness and ANPP was significant ($p < 0.05$) but weak ($R^2 = 0.45$), and this method has since been found to be less accurate than other non-destructive measures of production in the shortgrass steppe (Byrne et al. 2011), I estimated 2009-2011 ANPP using a point-intercept method (Jonasson 1988, Frank and McNaughton 1990) modified by Byrne et al. (2011). The frequency of graminoid, shrub, and forb contact with intercept points on a 62 x 80 cm grid was a good predictor of harvested ANPP values in calibrated plots (2009: $R^2 = 0.84$, 2010: $R^2 = 0.79$, 2011: $R^2 = 0.87$, all p-values < 0.05).

To assess root dynamics, I measured belowground net primary production (BNPP) in 2008, and standing root biomass in 2010 and 2011. I estimated BNPP using a root in-growth core technique (Vogt

and Persson 1991, Lauenroth 2000), quantifying new root production that grew into a 5 x 15 cm core lined with 1 mm mesh (McCulley et al. 2009). Under drought recovery (2010 and 2011), I destructively sampled eight (2010) and five (2011) 5 x 15 cm randomly sampled cores from each plot to measure standing root biomass. In both methods, cores were dried for 1 week at 50 ºC, and roots were separated from cores by sieving and hand-picking roots for a standard amount of time, then weighed.

I measured the C and N content of above and belowground plant biomass sampled in August 2010 and August 2011. Since I did not harvest ANPP, I collected aboveground biomass for tissue analysis by collecting leaves from grasses, shrubs and forbs nearest to the soil core sampled. I dried leaves and roots from biomass cores at 50 ºC for 1 week, ground them on a Wiley mill, and analyzed tissues for total C and N content on a LECO CHN-1000 analyzer (St. Joseph, MI). Plant tissue N (%) served as an indicator of N use efficiency (NUE). I also estimated the N-yield of different plant growth forms by multiplying plant tissue N by production, using plant species cover data (described in Evans et al. [2011]) to partition total ANPP among growth forms. I calculated rainfall use efficiency (RUE) as the plant production per cm rainfall plots received in the previous year (e.g. September 2010 to August 2011 for 2011 RUE).

Soil N dynamics

To address the extent of C and N decoupling, and how this affects N-conservation under drought and recovery, I measured both net N mineralization and the soil inorganic N that is vulnerable to loss at any given time. I used ion-exchange membrane probes or "Plant Root Simulators" (Western Ag Innovations, Inc., Saskatoon, Canada) as an index of net N-mineralization and to quantify plant- and microbial-available N (Dodd et al. 2000, Hook and Burke 2000). In 2008 (during drought) and 2010 (first year of drought recovery), I buried three pairs (an anion and cation probe) of 10 cm membranes for two 2-month periods. Probes were analyzed for NH_4-N and NO_3-N on an Alpkem Analyzer (Pulse Instruments Ltd., Saskatoon, SK) at Western Ag. I report inorganic N accumulation per day to account for small

variation (± 2 days) in the time the probes remained in the soil, and per cm rainfall to control for differences in rainfall in the different sampling periods.

In addition to measuring available N, I quantified N that is potentially available at any one time by using 2 M KCl extractions at multiple time points. Although the number of cores sampled per plot differed each year (2008, 2 cores, 2009, 3 cores, 2010, 8 cores, 2011, 5 cores), core sampling and lab analyses were identical. I extracted ammonium (NH_4^+) and nitrate (NO_3^-) from 15 x 5 cm cores using 2 M potassium chloride (KCl) and Whatman (#40) filter paper, and analyzed extracts on a Perstorp Analytical Alpkem autoanalyzer (Wilsonville, OR).

Trace gas flux

I installed three flux chambers in each plot (24 chambers total) in the 2008 and 2009 growing season. Trace gas measurements were carried out following Livingston and Hutchinson (1995) with modifications similar to von Fischer et al. (2009). In brief, I measured the concentration of gas in the headspace of 20-cm PVC base rings every 5 minutes for 20 minutes and analyzed gas subsamples using a Shimadzu GC14B gas chromatograph with N_2 carrier gas.

In 2008, I measured CO_2 and N_2O every 2 weeks throughout the growing season, but N_2O flux was too small to be detected. In 2009, I also tested for the vulnerability of C and N to gaseous loss under equal-moisture conditions (simulating recovery) by subjecting manipulated plots to equal pulses of moisture (100% of ambient rainfall from previous week) and measuring CO_2 and N_2O flux. To ensure plots still received the correct overall reductions of growing season rainfall (i.e. 25%, 50%) after these three pulses (June 12, June 30, July 14), I adjusted the following week's water additions to account for additional rainfall received during the experimental moisture pulse.

Statistical analysis

I analyzed differences in these C and N cycling parameters among treatments to evaluate how drought affects N conservation. I used a randomized block model to analyze C and N responses under

drought and recovery, estimating responses using the restricted maximum likelihood (REML) method. For variables that were measured at a single time point (e.g. BNPP), the randomized block mixed model included treatment as a fixed factor, and block a as random effect. For variables measured at several time points throughout the experiment (e.g. soil cores analyzed for inorganic N, PRS probes, ANPP, etc.), but in different locations within a plot, I accounted for correlations among measurements on the same plot over time by treating time and its interactions as an additional fixed effects, and adding a block by treatment by time interaction as an additional random effect.

For most sampling times, I collected more than one sample in each plot (e.g. 5 cores or 3 quadrats per plot). These "pseudoreplicates" served to capture within-plot variation and strengthen my assessment of treatment effects, but did not add as much statistical power as independent replication. However, when samples were taken in the same location within plots over time, such as trace gas measurements on bases that remained in the same location in the plot throughout the year (2008 and 2009), I tested whether including a random effect of sample (e.g. trace gas base 1, 2, 3) nested within the block by treatment interaction was needed to account for additional variation produced by the sampling location.

In all repeated measures models, I also considered whether to include an autoregressive correlation between plots over time, but did not include this additional parameter when it resulted in higher or nearly equal Akaike Information Criterion constant (AICc) values compared to the model without it. When significant treatment by time interactions occurred in any model ($p < 0.05$), I compared treatments within each year or time point.

Results

Effectiveness of treatments

Treatment plots receiving 25% and 50% of ambient rainfall resulted in significantly lower soil moisture in 2008 and 2009 drought years, according to hourly soil moisture data (averages presented in Fig. 3.2). Plots that received 100% of rainfall (the same amount as control plots, but re-added weekly) displayed higher variation in soil moisture, and often lower soil moisture than control plots. The three

water additions in 2009 increased soil moisture in all treatments, and there were no significant differences in soil moisture among treatments ($p > 0.05$) within each water addition.

Plant production under drought and recovery

Long-term drought significantly reduced ANPP in 2008 and 2009, the 10^{th} and 11^{th} year of the rainfall manipulation (Fig. 3.3a). ANPP decreased with drought severity in both years, but 25% and 50% treatments were only significantly different from each other in 2009 ($p = 0.03$). Decreases in ANPP were not proportional to rainfall reductions, as treatments receiving 25% and 50% of rainfall resulted in an average drought-year ANPP of 50% and 65% of control ANPP, respectively.

Root in-growth cores revealed that BNPP in the 25% drought plots was 16% lower than the BNPP in control ($p = 0.007$), but there was no significant difference between BNPP in 50% and control plots (Fig. 3.3b). In 2010 and 2011, after drought had ended, root biomass in 25% and 50% plots was significantly lower than root biomass in control plots ($p < 0.01$). Reductions in ANPP also persisted two years after drought, but only in 25% drought plots. In rainout shelter treatments that received 100% of rainfall, ANPP and root biomass were often lower and sometimes significantly lower than the control treatments, following patterns of lower soil moisture in 100% treatments compared to control (Fig. 3.1, 3.2).

Under drought, RUE (plant production per cm rainfall) was higher in drought plots than the control in 2008 ($p < 0.05$), but not in 2009 (Fig. 3.4). RUE was lower in drought plots in 2010 and 2011 ($p < 0.05$), when all plots received the equal rainfall (Fig. 3.4).

Nitrogen dynamics under drought and recovery

Nitrogen content (%) of plant tissue varied by vegetation type, and in general, drought plots had higher N-content in plants and roots than control and 100% treatments in both recovery years (Fig. 3.5a,b inset). Due to differences in production among plots, when I calculated ANPP-N, or the overall aboveground N yield (g N / m^2), there were fewer trends among treatments (Fig. 3.5). However, 25%

drought plots did have significantly lower total ANPP-N and root biomass-N than control plots, largely mediated by overall greater production in control plots, and shifts in plant species composition (Evans et al. 2011) (Fig. 3.5).

There were no significant differences in plant available N (as measured by resin probes) among treatments under drought in 2008 and 2009 (Fig. 3.6). However, when differences in rainfall among treatments were accounted for, plant available N was higher in drought plots (per cm rainfall received, Fig. 3.6 inset). In 2010, the first year of recovery, drought plots had significantly higher available N than control plots in the June-July measurement period, and this trend, although not significant, continued in the August-September period. Total soil inorganic N (as measured by KCl extracts) was consistently higher under drought, and up to 5 times that of inorganic N in control plots (Fig. 3.7). Soil N continued to be higher in both sampling dates in 2010 and in 2011.

Trace gas flux under drought and moisture pulses

CO_2 flux in 25% and 50% drought plots was an average of 39% and 28% lower than control treatments across all measurement dates in 2008, respectively (Fig. 3.8). Soil moisture differed among treatments at the time of sampling, and variation in soil moisture explained 42% of the variance in CO_2 flux. The three water additions in 2009 resulted in average volumetric soil moistures of 22.0%, 19.6%, and 14.5% (data not shown), and were not significantly different among treatments within a sampling date. After water additions, N_2O flux in the 25% drought treatment was nearly double N_2O in 100% plots on June 12 ($p = 0.006$), and remained significantly higher than N_2O in 100% plots on June 30 ($p = 0.01$), but there were no significant differences among treatments on July 14 ($p = 0.21$) (Fig. 3.9b). In contrast to N_2O, CO_2 flux in drought plots was consistently lower than 100% plots after 2009 water additions (Fig. 3.9a).

Discussion

I found that long-term drought causes significant reductions in plant biomass and CO_2 flux, and an accumulation of inorganic N in the shortgrass steppe. This decoupling of C and N under long-term drought increased the openness of the N cycle *upon recovery*, decreasing both NUE and RUE when rainfall returned to normal levels, in accordance with my predictions. The accumulation of N in soil and plant tissue and reductions in plant biomass persisted two years after manipulations were removed, suggesting that biogeochemical feedbacks associated with these changes could alter fundamental ecosystem properties of the shortgrass steppe ecosystem, and increase the recovery-time from future decade-long droughts.

Effectiveness of treatments and implications for altered rainfall timing

Drought treatments effectively reduced rainfall, but I also found variables responded differently to control (ambient rainfall) and 100% treatments that received weekly re-additions. Although rarely statistically significant, these differences could have been caused by slight chemical differences in the water used to irrigate plots (well water vs. rainfall) or the altered timing of water inputs to manipulated plots. The timing of precipitation plays a large role in ecosystem dynamics in the shortgrass steppe (Sala and Lauenroth 1982), and changes in rainfall event size and timing can significantly alter ecosystem C cycling in grasslands (Knapp et al. 2002, Heisler-White et al. 2009). The majority of rainfall events in the shortgrass steppe are small (< 5 mm), and water inputs in the form of larger events in manipulated plots could have reached deeper soil layers where water is less vulnerable to evaporative loss (Sala et al. 1981).

Heisler-White et al. (2008) suggest that this mechanism (increases in water availability caused by deeper infiltration) may explain increases in production they observed when total rainfall was distributed in fewer, larger events in the shortgrass steppe. I was not testing the effect of altered rainfall timing on ecosystem dynamics in this study, and data do not suggest there is a statistically significant effect. However, in contrast to these previous findings, I found a trend of *decreased* production in the 100% plots, and on average, lower soil moisture (in the top 10 cm) compared to control (Fig. 3.1). I also observed a slight increase in N availability and larger extractable N pools in 100% treatments compared

to control treatments, and others studies suggest that size of precipitation events affects N availability (Yahdjian and Sala 2010). Overall, these observations suggest that 1) more work is needed to examine both the ecosystem and biogeochemical consequences of altered rainfall timing and 2) if anything, the rainout design exacerbated drought effects, rather than negating them.

Therefore in the context of this study, the shelter effect does prevent us from making direct conclusions (and predictions) about specific amounts of rainfall reductions (i.e. predicted responses to future droughts 25% and 50% of ambient rainfall), but I can still be confident that shelters induced long-term drought conditions. In addition, although results from these long-term manipulations provide inference unobtainable from monitoring or short-term studies, low field replication and the resulting restriction of sampling to less destructive techniques does limit my scope and ability to make conclusions about specific mechanisms. In this study, I targeted important C and N pools and found that drought can cause significant C-N decoupling and decreases in N conservation under recovery, confirming my hypothesis. These results can direct future research toward describing full ecosystem N budgets under drought and recovery, and toward more precise estimations of fluxes and fates of N and H_2O throughout the ecosystem.

Does long-term drought alter C and N coupling?

Overall, C fluxes (plant production, CO_2 flux) were lower in drought plots, but N fluxes (plant N uptake, N_2O flux) were higher. The large accumulation of inorganic N under drought provided further evidence of C and N decoupling. Although inorganic N pools at any single time point are not useful in interpreting rates of processes (Schlesinger 1997), I consistently found higher inorganic N in soil subject to drought (Fig. 3.7), as other studies have (Garcia-mendez et al. 1991, Davidson et al. 1993, Whitford et al. 1995, Reynolds et al. 1999, Yahdjian et al. 2006).

This C and N decoupling may be caused by different ecosystem compartments or processes having different sensitivities to drought, or by abiotic mechanisms. Belowground and especially aboveground production were significantly reduced by long-term drought (Fig. 3.3), but microbial

biomass was not significantly different among treatments (data not shown, but see Table 4.3). Microorganisms may be capable of decomposing plant litter at moisture levels not high enough to stimulate plant production (Sala and Lauenroth 1982, Ogle and Reynolds 2004). This difference in moisture sensitivity may affect ecosystem flux in pulse-driven arid ecosystems (Huxman et al. 2004) and could explain the accumulation of N under drought in this study (continued microbial mineralization but decreased plant uptake). In addition, N-mineralization and decomposition may have different sensitivities to moisture (Yahdjian and Sala 2008). I found that net N-mineralization (as indexed by N accumulated on exchange membranes) was not significantly different among drought plots, that inorganic N accumulated under drought, and CO_2 flux was significantly lower under drought. Although this does not provide a direct comparison of the sensitivity of C versus N-mineralization to drought, it does support other studies that have suggested that N-mineralization may be less sensitive to moisture than decomposition across wet and dry seasons (Hook and Burke 2000), over precipitation gradients (Barrett and Burke 2002), and under other rainfall manipulations (Yahdjian et al. 2006).

Finally, the physical properties of the products of these processes, rather than differences in process sensitivity to moisture, may contribute to N accumulation and C-N decoupling under drought (Yahdjian et al. 2006). Specifically, nitrate, which dominates inorganic N pools (relative to ammonium) in the shortgrass steppe, is highly mobile under high water content. Reduced soil water content may alter nitrate mobility, slowing the movement of nitrate to deeper soil layers and limiting diffusion of nitrate to plants and microbes.

How does drought affect N conservation in semiarid grasslands?

Two metrics in this study provided an indicator of openness of the N cycle: percent N in biomass, which has been used as a proxy for NUE (Vitousek 1982), and N_2O release, which, when controlling for moisture differences, I used as an indicator of *potential* gaseous N loss. I hypothesized that drought would increase the openness of the N cycle in the shortgrass steppe, as other studies have found that N may be less conserved in drier sites (Austin and Vitousek 1998, McCulley et al. 2009) and after disturbance

(Vitousek and Reiners 1975). Overall, I found evidence that this occurs or has the potential to occur, but only in the years following the drought, when water availability increases and plants and microbes are more active. Although I did observe an accumulation of inorganic N under drought, which could certainly be vulnerable to loss, the N exchange probe data suggests that the diffusion limitation induced by drought might make N inaccessible to plants and microbes. Because of this, *under drought*, although I did not construct a full N-budget, my results suggest N_2O flux was not a significant pathway of N loss, and plant NUE might not have been as strongly reduced due to the overriding moisture limitation.

However, supporting my hypothesis, I found that this C-N decoupling that occurs under drought is likely to result in a more open N cycle *when moisture returns*, and that this increased openness could extend for at least 2 years after drought has ended. When adding equal moisture pulses to drought plots that simulated recovery, I found greater N_2O flux in plots that had been experiencing drought for 11 years (Fig. 3.9b). Greater N_2O flux under drought was likely mediated by greater inorganic N in these plots (Fig. 3.7), and this correlated has been reported in N-addition experiments (Mosier et al. 1996). In contrast to N_2O, CO_2 flux remained lower in drought plots under drought (Fig. 3.8), and when soil moisture was constant across plots (Fig. 3.9a). This is likely because reduced production under drought resulted in both decreased root respiration and lower availability of labile C to microbes. I did not measure N_2O flux in the years following drought, but as soil inorganic N continued to be higher under drought plots, it is likely that when these plots received equal rainfall, this N was vulnerable to loss, which could result in N-limitation when plants recover.

Greater plant tissue N following drought provides further evidence supporting my hypothesis that drought results in decreased conservation of N (though under recovery). This decrease in NUE may also have been due to the higher availability of N in the soil, and has implications for changes in plant-soil interactions as well as forage quality and land use in the shortgrass steppe. High-N litter produced from inefficient plant N use can lead to increased net N mineralization and increased available N that is then advantageous for N inefficient plants (Vitousek 1982, Vinton and Burke 1995). This positive feedback could be mediated by changes in plant community composition. “Weedy” species have increased relative

to grasses under these manipulations (Evans et al. 2011), and under N additions, these changes can persist long after N additions have ceased (Lauenroth et al. 1978, Milchunas and Lauenroth 1995). However, although foliar plant N increased the year after drought, reduced ANPP and altered species composition in drought plots resulted in no significant difference in N-yield (g biomass N / m^2, Fig. 3.5), suggesting that a reduction in forage quantity under drought is not necessarily compensated for by increased forage quality.

Ecosystem lags and implications for long-term drought recovery

My results show that severe drought can induce significant lags in production such as those observed after dry years (Lauenroth and Sala 1992), and that these lags may be the result of structural vegetative constraints. I also observed lags in soil N dynamics: soil inorganic N remained higher in drought plots for the two years I monitored after drought, and higher soil water content may have made this N more accessible to plants and microbes. However, it is unclear what the long-term fate of this soil inorganic N will be. The greater nutrient supply after drought did not increase production, as it can in mesic grasslands (Briggs and Knapp 1995, Haddad et al. 2002). Instead, biomass continued to be constrained, and the increased nutrient supply caused an increase in plant tissue N, indicating less efficient use of N by plants, in line with my predictions. These changes in plant and soil N could persist in the long-term through plant-soil-N feedbacks, and can increase inter-annual variability in production (Haddad et al. 2002). On the other hand, the data suggest that greater soil inorganic pools also make N more vulnerable to loss, and increases in N loss could leave vegetation N-limited once plants can fully respond to rainfall (Yahdjian et al. 2006).

Although trends 2 years following drought are not enough to determine how plant-N interactions will affect long-term recovery, I did see that the weedy plant species that increased under drought, and that have increased under N additions (Lauenroth et al. 1978, Evans et al. 2011), accounted for post-drought increases in ANPP in 50% plots (unpublished data). This may indicate that over time, more soil N will be taken up by these N-inefficient plants compared to loss through gaseous flux, which represents

a small component of the N budget in semiarid systems. In either case, when C and N dynamics converge ("re-couple") through plant uptake and loss of inorganic N, the system may reach a steady state that is characterized by new biogeochemical properties.

Conclusions

I found that an 11-year rainfall manipulation in the highly drought-resistant shortgrass steppe significantly altered C and N dynamics, and affected ecosystem function up to 2 years into recovery. Soil N accumulated under drought and reductions in plant biomass persisted after drought, resulting in less efficient plant N assimilation and higher gaseous N loss from drought plots when water was available. These results suggest that long-term drought decreases N conservation in the shortgrass steppe when moisture returns. The de-coupling of C and N, and subsequent increases in N loss and initiation of new plant-soil-N feedbacks, are likely to alter the course and timescale of shortgrass steppe recovery from future decade-long droughts, and possibly analogous disturbances in other ecosystems, and therefore alter our ability to predict responses of semiarid ecosystems to novel precipitation regimes.

Figures

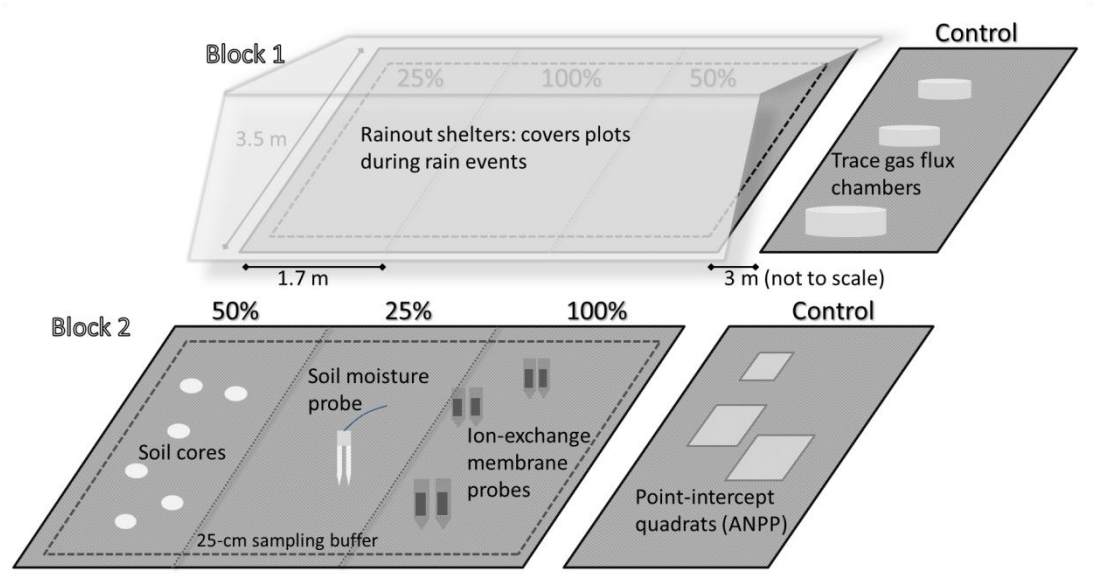

Figure 3.1. Growing season precipitation in 25% (white), 50% (light grey), and 100% and control (darker grey) treatments, and non-growing-season precipitation (black, the same for each treatment) and during the 11-year drought experiment and 2-year recovery, when shelteres were removed. Each full bar shows annual precipitation. Lines represent long-term (1959–2009) mean annual precipitation (341 mm, solid line) and growing season precipitation (241 mm, dashed line).

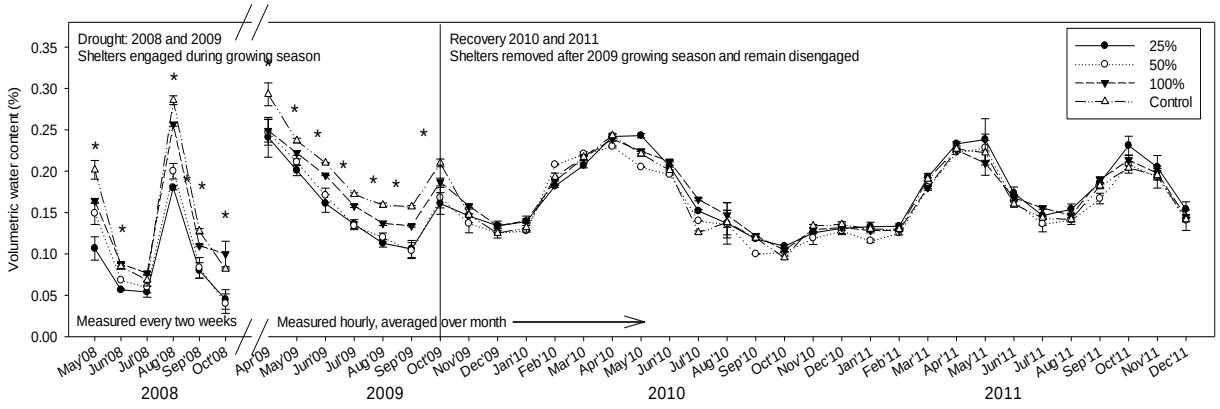

Figure 3.2. Soil moisture dynamics under rainfall manipulations during 2008 and 2009 growing season,and after manipulations were removed after the growing season of 2009 and remained disengaged in 2010 and 2011. Soil moisture was measured every 2 weeks in the summer of 2008, and hourly from spring of 2009 through 2011. Error bars are standard errors of means (N=2). The * indicates a significant difference among treatments ($p < 0.05$). Differences between 25% and 50% treatments, and between Control and 100% were significant two times, both when shelters were engaged.

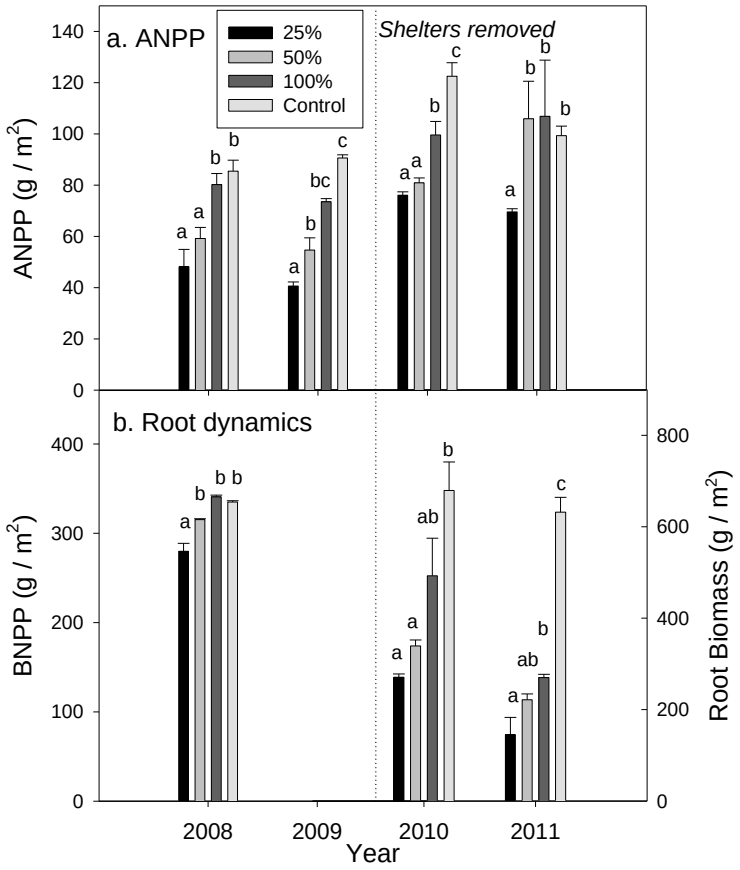

Figure 3.3. Aboveground primary productivity (a) and root dynamics (b) in drought manipulations receiving 20%, 50% and 100% of ambient precipitation on the shortgrass steppe for a period of 10 and 11 years (2008 and 2009), then released from drought treatments (indicated by dashed line, 2010 and 2011). Root dynamics (b) were described by estimating belowground net primary production (BNPP) in 2008 using an in-growth core, and by harvesting root biomass in 2010 and 2011 (b, second axis). Bars show mean and standard error and different letters indicate significant differences between treatments ($p < 0.05$).

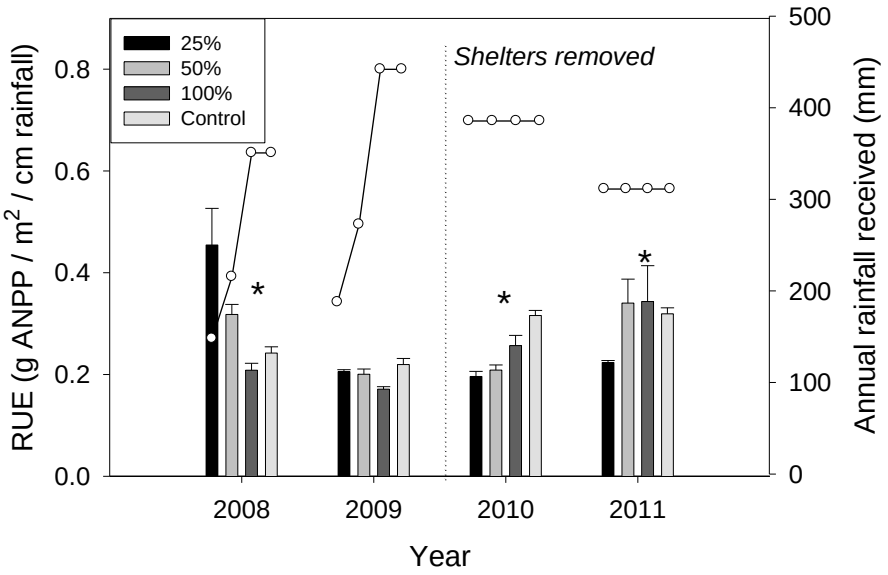

Figure 3.4. Mean rainfall use efficiency (RUE, bars) under drought manipulations in 2008 and 2009, and recovery from drought in 2010 and 2011 (after dashed line) in the shortgrass steppe. RUE was as calculated by dividing ANPP for each treatment by annual rainfall each treatment received in the previous year, shown by open dots.

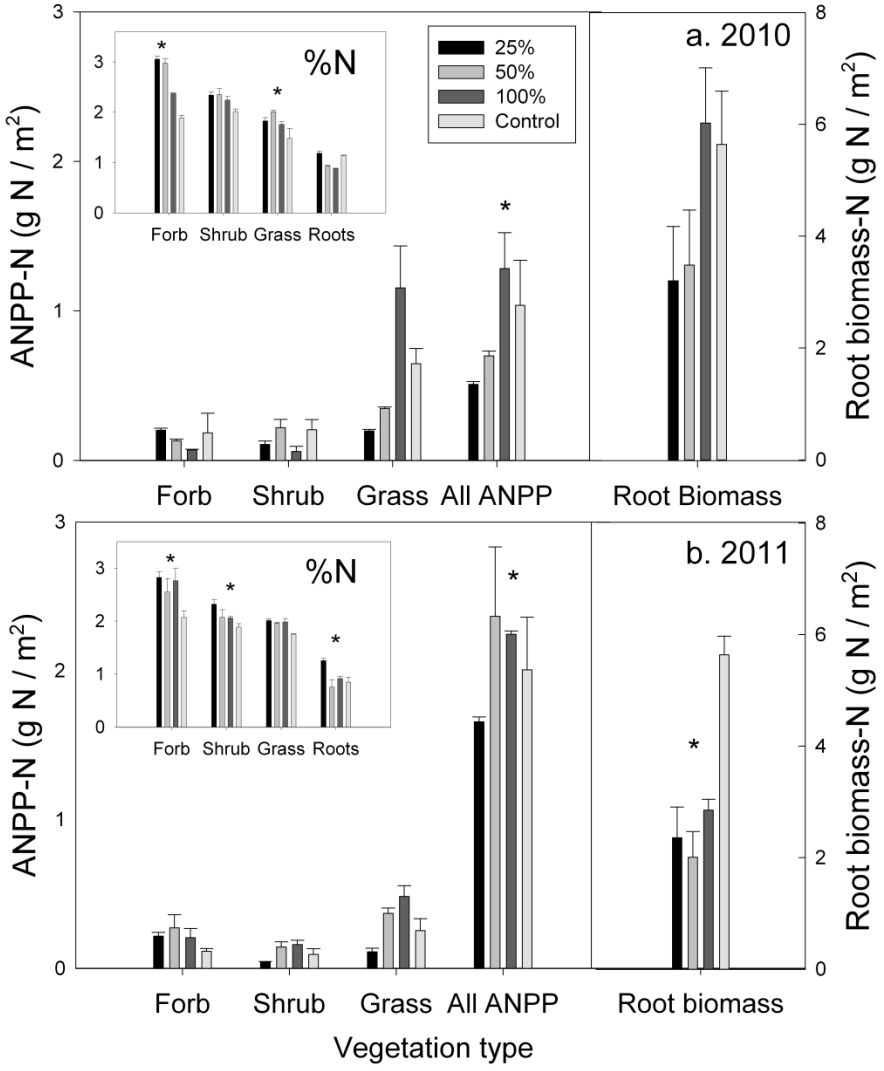

Figure 3.5. Aboveground and belowground N-yield of vegetation in the first (2010, a) and second year (2011, b) of recovery after an 11-year drought in the shortgrass steppe. N-yield was determined using the percent N in vegetation (inset) and the belowground biomass and aboveground production for each growth form. The * indicates significant difference among treatments ($p < 0.05$).

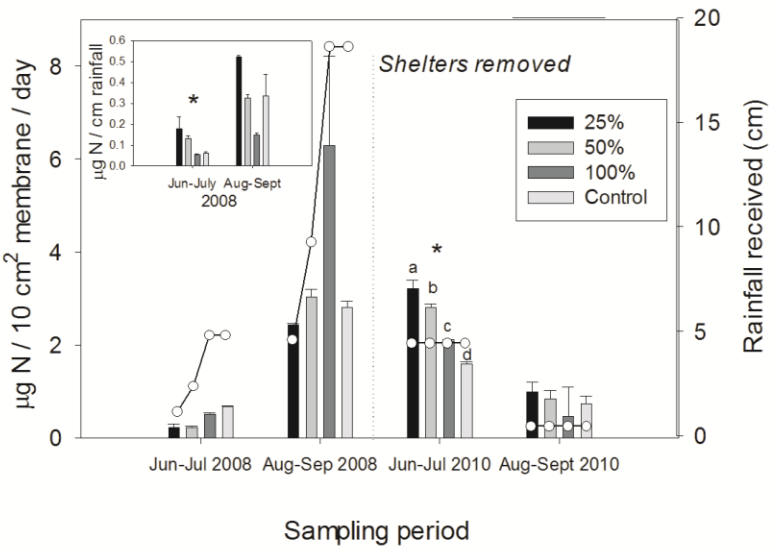

Figure 3.6. Total inorganic N (NO_3^--N + NH_4^+-N) captured on ion-exchange membrane probes in the 10th year of a long-term drought manipulation in the shortgrass steppe (2008), when rainfall the plots received varied (shown by open dots) and the first year of recovery (2010), when all plots received the same amount of rainfall. Bars show ug-N accumulated per 10 cm probe per day in each 2-month period of burial, while inset shows ug-N per cm rainfall during 2008, when treatments received unequal rainfall. Where significant differences among treatments occurred, significant pairwise differences ($p < 0.05$) are indicated by letters. Ratios of nitrate to ammonium were consistent across treatments within any given time point, so are not shown separately.

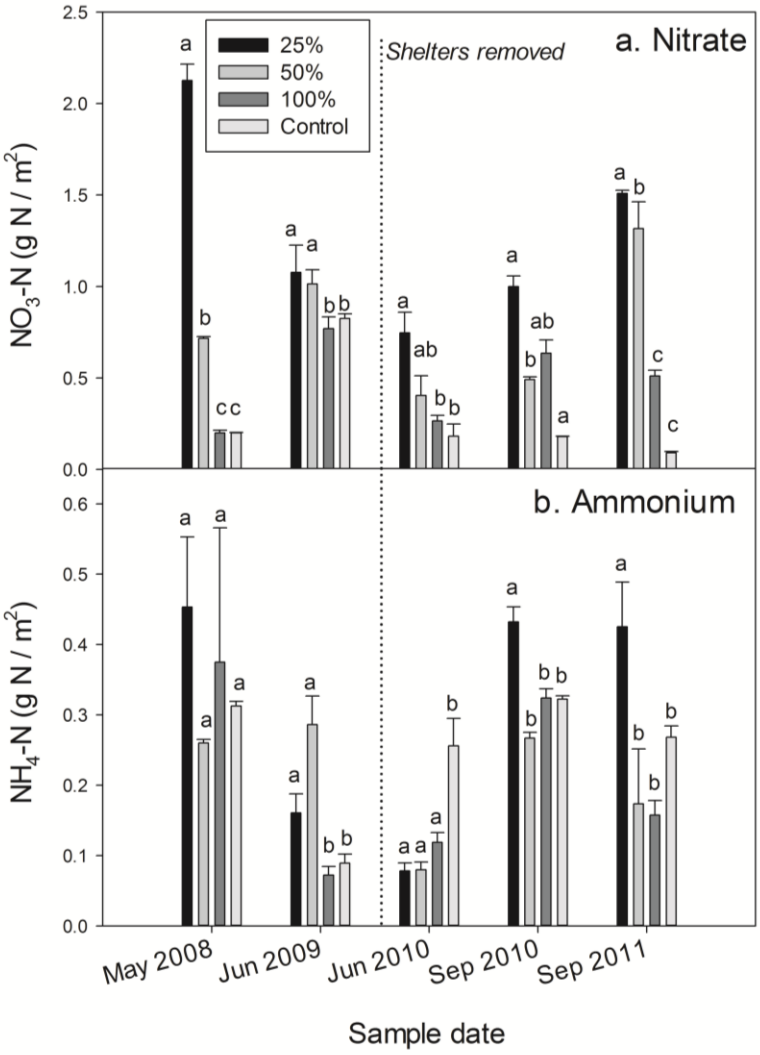

Figure 3.7. NO_3^--N (a) and NH_4^+-N (b) extracted with 2M KCl in the 10th and 11th year of a drought manipulation (2008 and 2009), and after release from the manipulation (indicated by dashed line, 2010 and 2011). Bars show means (N=2) and standard error and different letters indicate significant differences between treatments ($p < 0.05$).

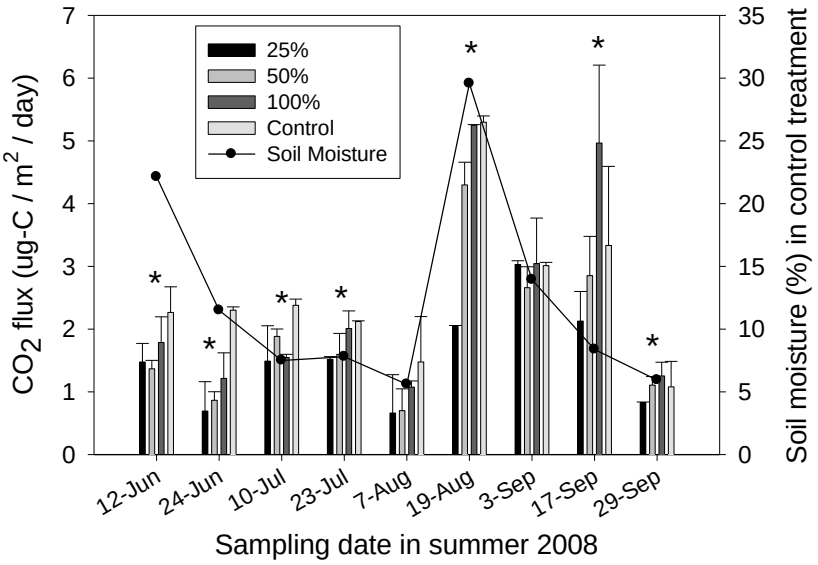

Figure 3.8. Mean soil CO_2 flux (bars) in the growing season of 2008, the 10th year of a long-term drought manipulation in the shortgrass steppe. Soil moisture is shown for control plots (symbols and line) to display the variation in soil moisture at the time of sampling; drought treatments had reduced soil moistures. The * indicates a significant difference ($p < 0.05$) among treatments.

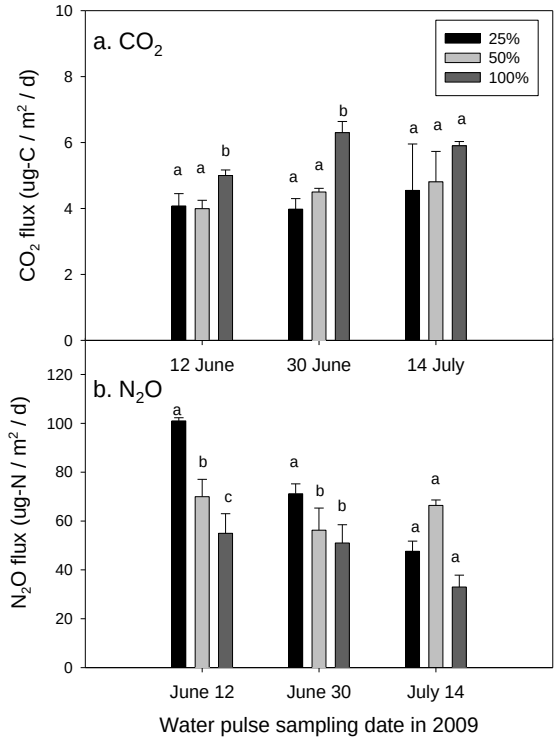

Figure 3.9. Mean and standard error of soil CO_2 (a) and N_2O (b) flux after three experimental water pulses in the summer of 2009. Soil moisture was not significantly different among treatments within a moisture pulse date. Different letters indicate significant differences among treatments.

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Chapter 4: Does moisture niche partitioning drive shifts in microbial community composition under long-term drought in the shortgrass steppe?

Introduction

Identifying the most important environmental factors that structure biological communities has been a persistent goal in ecology (Clements 1916, Gleason 1926, Tilman 1996, Hooper et al. 2005), and is essential for predicting species distributions under future environmental conditions and for preserving global biodiversity (Sala 2000). Although efforts to determine controls on species composition have only recently targeted microbial communities, an increase of in-depth microbial sequencing studies has already increased awareness of previously unrecognized biogeographical patterns in microorganisms (Fierer and Jackson 2006, Green et al. 2008). This work has identified several factors that are strongly correlated to microbial community structure and diversity, such as pH (Lauber et al. 2009), carbon quality (De Deyn et al. 2011) and quantity (Fierer and Jackson 2006), and soil water content (Bossio and Scow 1998, Frey et al. 1999, Lauber et al. 2009). These biogeographical patterns provide a strong foundation on which to test the relative importance of different drivers of microbial community composition across ecosystems, and to compare to factors that drive communities of larger organisms.

Precipitation is a major driver of global biome distribution, primary productivity (Chapin et al. 2002) and microbial activity (Parton et al. 1987), and is an especially important driver of ecosystem function and vegetation dynamics in grassland ecosystems (Noy-Meir 1973, Sala et al. 1988, Lauenroth and Sala 1992). Several studies have shown that microbial communities can also be sensitive to precipitation changes in these water-limited systems (Castro 2010, Drenovsky 2004, Williams and Rice 2007). These shifts in microbial community structure in response to changes in moisture regime suggests that variation in moisture sensitivity among microbial groups, which has been observed (Drenovsky 2004), is driving community composition as soil moisture varies in space and time. However, other studies also suggest that grassland microbial communities can be highly *resistant* to changes in rainfall

(Griffiths et al. 2003, Landesman and Dighton 2010), even over many years (Cruz-Martinez et al. 2009), making it unclear what factors control microbial community sensitivity to moisture, and whether the persistence of certain taxonomic groups under specific moisture conditions (i.e. moisture niche partitioning) is actually, and always, driving the changes observed.

What can resolve this discrepancy and provide insight into when and how moisture drives microbial community composition? Precipitation patterns in grasslands are expected to shift in the future (IPCC 2007, Jentsch et al. 2007), and identifying the role of moisture in microbial community dynamics will be essential for predicting long-term microbial and biogeochemical responses to future climates. One way to understand drivers and better predict patterns of species composition is to not only investigate microbial groups that are present in a specific environment, but also identify groups that actively respond to certain environmental conditions, or in other words, occupy a defined niche. Most studies investigating drivers of microbial community composition characterize total microbial DNA, which captures active microbial groups as well as a potentially large portion of communities that is inactive or dormant (Cole 1999). Total microbial community composition reflects which microbial groups have competitively prevailed in that environment in the long-term, and provide a measure of community potential, but they do not necessarily show which groups are thriving and favored under certain conditions (as well as contributing functionally). By describing microbial groups active at certain moisture levels, I could assess the extent to which microorganisms vary in their response to different moisture levels, and whether this partitioning of moisture niches across groups is an underlying driver of long-term shifts in microbial community composition under different precipitation regimes.

In this study, I asked 1) whether long-term drought alters microbial community composition, activity, and respiration in response to moisture and 2) whether variation in moisture sensitivities among microbial groups (moisture niche partitioning) could explain the distribution of microbial groups I observed under long-term drought. Based on previous studies that identify moisture as a primary driver of microbial community structure (Bossio and Scow 1998, Frey et al. 1999, Drenovsky et al. 2004, Lauber et

al. 2009, Brodie et al. 2010, Castro et al. 2010), I hypothesized that declines in microbial sensitivity to reduced moisture availability under drought would result in long-term shifts in microbial community structure in the shortgrass steppe. If this hypothesis were correct, I would expect a) antecedent drought to alter microbial function and activity in response to moisture; b) distinct communities to be active under different moisture levels (moisture niche partitioning); and c) this moisture niche partitioning to provide a good indicator of the shifts in long-term communities under distinct moisture regimes in the field.

Methods

Study site and field rainfall manipulation

The study site is located on the semiarid shortgrass steppe at the Central Plains Experimental Range (CPER), 60 km north-east of Fort Collins, Colorado (40° 49' N latitude, 104° 46' W longitude). The CPER is administered by the USDA Agriculture Research Service and is also a National Science Foundation Long Term Ecological Research site (Lauenroth et al. 2008a). Mean annual temperature is 8.2 °C and mean annual precipitation is 341 mm (65-year average), with with 83% of precipitation occurring between April and September (Sala et al. 1992). Soils are frequently dry but experience brief wet periods, and as a result, soil water content is highly variable (Lauenroth and Bradford 2006). Precipitation patterns are dominated by small events (< 5 mm), but differences in the size of large events (> 30 mm) accounts for most of the variability in interannual rainfall (Lauenroth and Sala 1992). The site is dominated by typical upland vegetation, including the short-stature C_4 grasses blue grama (*Bouteloua gracilis*) and buffalograss (*Bouteloua dactyloides*), and plains pricklypear cactus (*Opuntia polyacantha*). Soil types at this site are Renohill and Ascalon fine sandy loam (Aridic Arguistoll and Ustic Haplargid) (Natural Resource Conservation Service 2008).

Rainfall manipulations are described in Evans et al. (2011). In brief, in 1998, two blocks were divided into four 3-m^2 treatment plots: a control plot receiving ambient rainfall and three manipulated plots that were automatically covered by rainout shelters during rain events in the growing season

(average dates 26 April – 7 October). Each week, water was re-added to these plots as a proportion of the ambient rainfall received that week (25% or 50% treatments) or including all rainfall in one re-watering (100% treatment). Thus, I investigated two drought severity levels (25% and 50% of growing season rainfall), one treatment that also received weekly re-additions, but at 100% of the week's ambient rainfall, and a untreated control treatment receiving ambient rainfall.

Soil characterization

Four soil cores from each plot were collected in May of 2009 (11^{th} year of drought). After soils were sieved, I measured water holding capacity, initial soil moisture, and several soil properties for later correlation with community and functional response. Microbial biomass carbon (MBC) and nitrogen (MBN) were determined by chloroform fumigation extractions (Vance et al. 1987). I placed a 6 g soil subsample into an acid-washed 50 mL tube and fumigated with chloroform for five days, while another 6 g subsample that was not fumigated acted as a control. Dissolved C and nitrogen (N) were extracted from both subsamples by shaking soil in 10 ml of 0.5 M K_2SO_4 for two hours then filtering through #40 Whatman filter papers. Extractions were analyzed on a Shimadzu TOC analyzer. Microbial biomass was determined by subtracting C and N in fumigated samples from non-fumigated control, (Vance et al. 1987) and no correction factors were applied. Extractable C and N values were obtained from the non-fumigated control samples. I measured percent organic carbon in soils by running ground subsamples on a LECO CHN-1000 analyzer, and pH using a 1:1 mixture of soil and deionized H_2O and a pH meter (Sparks 1996). The remainder of soil samples were stored at -10°C until initiation of the lab experiment in May of 2010.

Incubation of soils from drought treatments at a range of soil moistures

Before incubating soils, I subsampled soil cores described above to characterize microbial communities in the non-incubated, long-term field treatments (4 cores x 8 plots = 32 samples). With the

remaining soil, I set up lab incubations that subjected soils from each field treatment to 5 moisture levels to examine a) function (respiration rate) and b) the species composition (active and total) of microbial communities (Fig. 4.1). I selected incubation moisture levels based on the range of soil moistures I observed using hourly volumetric soil moisture measurements in the field, with the lower limit set by the solubility of bromodeoxyuridine (BrDU) in water, which was used to isolate the active community (see below). I converted gravimetric moisture data to water potentials using a calibration developed for this soil, and added water that resulted in incubation water potentials of -0.001, -0.01, -0.1, -0.5, and -1.5 MPa (Fig. 4.3). In these soils, this range of water potentials corresponded to gravimetric water contents between 6% and 25%.

To monitor the soil respiration of drought treatments under this range of moisture levels, I incubated 14-16 g of soil in 1 quart mason jars stored at 25 °C. I measured soil respiration rates by analyzing the accumulation of CO_2 in the headspace of the jars with a LiCor Infrared Gas Analyzer (IRGA) every four hours for the first 48 hours of the incubation, then weekly for 6 months.

To describe how microbial communities, both active and total, responded to contemporary moisture conditions, and to test for niche partitioning, I incubated 5-g soil samples in sterile jars with BrDU. BrDU is an analogue of thymidine, and therefore can be used to analyze the proliferation of living cells (Urbach et al. 1999). I added BrDU to samples by dissolving a consistent amount (300 ng/g soil) in water I added to each incubation. I incubated the vials at 25 °C for 36 hours, while growing microorganisms incorporated BrDU molecule into replicating DNA (Borneman 1999). This amount of time (24 - 48 hours) is consistent with other studies using BrDU to isolate actively growing microbial communities in soil (Artursson et al. 2005, Allison et al. 2008, Hanson et al. 2008) and allows time for replication and BrDU-incorporation to occur, but not enough for significant turnover of the microbial biomass.

I extracted DNA from incubated soils using MoBio PowerSoil Kit (MoBio Laboratories, Solano, CA) and performed an antibody immunocapture to separate labeled DNA (containing the BrDU

molecule) from inactive DNA (Allison et al. 2008, McMahon et al. 2009, Hirsch et al. 2010). To obtain enough BrDU-DNA for pyrosequencing preparation, I pooled separately-incubated and extracted DNA from field cores (4 from each plot) for a total of 40 BrdU-DNA samples and 40 total (active + inactive) samples (5 water-levels x 2 blocks x 4 treatments, see Fig. 4.1).

Bacterial community pyrosequencing

I analyzed the community structure of total and active communities (from moisture incubations) and field treatment communities (not subject to moisture incubations) using a pyrosequencing-based analysis of the 16S rRNA gene as described in Fierer et al. (2008) to maximize both sequencing (phylogenetic) depth and the number of communities profiled (sample breadth). I amplified the 27 to 338 portion of the 16S rRNA gene using error-correcting bar-coded primers (Hamady et al. 2008). The forward primer contained a Roche 454 'A' pyrosequencing adapter, connected with a TC linker, and reverse primer contained a 12-bp bar-coded sequence, Roche 454 'B' sequencing adapter, and a TC linker. Polymerase chain reactions (PCR) were conducted with 0.5 μL (10 μM) of each forward and reverse primer, 3 μL template DNA, and 22.5 μL Platinum PCR SuperMix (Invitrogen, Carlsbad, CA), similar to Fierer et al. (2008). I amplified samples in triplicate, pooled and cleaned reactions using a PCR Cleanup Kit (MoBio Laboratories, Carlsbad, CA), then sequenced them on a Roche FLX 454 pyrosequencing machine at the Environmental Genomics Core Facility at the University of South Carolina.

I followed previously-described protocols to analyze pyrosequencing data (Fierer et al. 2008, Hamady et al. 2008, Lauber et al. 2009) using QIIME (Quantitative Insights Into Microbial Ecology) (Caporaso et al. 2010b). I first removed sequences < 200 bp and with a quality score < 25. I identified bacterial operational taxonomic units (OTU's) as those organisms whose 16S rRNA gene sequences were 97% similar, and used the most abundant sequence per OTU as the representative sequence for that OTU. I aligned sequences using PyNAST (Caporaso et al. 2010a) and assigned taxonomies to these

representative OTU phylotypes using the RDP Classifier (Wang et al. 2007a). I performed basic filtering on all datasets that excluded all OTU's that were only present in one sample.

Generation of species distributions from moisture response and comparison to communities from long-term drought treatments

To assess the extent to which microbial responses to different moisture levels explained shifts in community composition under long-term drought treatments, I examined the overlap between communities active under certain lab moisture conditions and communities under long-term field manipulations. I first did this qualitatively by comparing responses of individual phyla to lab moistures and field treatments. If the group abundances shifted similarly from wet to dry lab incubations as from control to drought plots in the field, this would suggest that differences in soil moisture among drought treatments – and differential soil moisture sensitivity among phyla – was driving changes in phyla distribution under drought. However, I also wanted to evaluate the extent to which this overlap occurred if I considered how actual field soil moisture conditions varied among drought treatments. To do this, I generated moisture frequency distributions for each field treatment (25%, 50%, 100%, control, Table 4.1) based on hourly soil moisture data collected in 2008, during drought treatment. I then used the abundance of species active at different moisture levels (in control soils) to weight the abundances of certain species, generating "niche-extrapolated" communities one might expect to emerge in long-term drought treatments if specific moisture conditions favored certain microbial groups (i.e. if moisture response were driving changes in species abundance). I compared community composition from the communities I generated with those I observed in the field (to examine the extent of overlap), as well as original active communities (collapsed over lab moisture levels, but not weighted) using multivariate distance metrics described below. Although this analysis can be considered a simplified version of species distribution models (although these predict across space, not time) and niche models recently emerging in plant

community ecology (Iverson and Prasad 1998, Austin 2002), for simplicity's sake I simply refer to them as niche-extrapolated communities in this paper.

Data analysis

I aimed to describe how microbial communities respond to both long-term drought treatments and lab moisture incubations. Under this design, I therefore treated field drought treatment (4 levels), lab moisture (5 levels), and a treatment by moisture interaction as fixed factors both in multivariate analyses of differences in whole communities, and univariate analyses of differences in single variables (e.g. respiration, single phyla) among treatments and moisture levels.

I examined variation in community composition among samples (beta-diversity) using the Unifrac distance metric (Lozupone and Knight 2005). Unifrac calculates the fraction of branch length unique to a sample or environment compared to overall branch length, computing similarity distances by using only presence or absence of phylotypes ("unweighted Unifrac"), and also when including abundances of phylotypes ("weighted Unifrac"). The use of this distance metric allowed us to consider the phylogenetic relationship of groups when determining the similarity of one community to another. Using these Unifrac distances, I created ordinations using non-metric multidimensional scaling (NMDS). Then, using PerMANOVA analyses (Anderson 2001) in Primer v6, I tested for significant differences between communities in different treatments, across moisture levels, and between communities I observed in long-term treatments and niche-extrapolated communities. PerMANOVA is a permutation-based multivariate analysis that can accommodate many sampling designs, and allowed me to include "block" as a random effect and examine a moisture by treatment interaction. This test calculates a pseudo *F*-statistic (and p-value) by comparing the total variance explained by sample identities (i.e. treatment, moisture) to that explained by random permutations of sample identities.

I also tested for correlations between long-term field community composition and environmental variables (DOC, pH, etc.) using Monte Carlo permutations (9999) and NMS vector fitting (in R), and for

relationships between plant community composition and bacterial community composition by correlating plant and microbial species distance matrices using a Mantel test (in R).

To describe the relative influence of treatment and moisture on respiration rate (univariate data), I used a mixed model in SAS (proc mixed) that included treatment and moisture as fixed effects, and block as a random effect. I calculated partial r^2, indicating the relative explanatory power contributed by each factor by dividing sum of squares of a factor by the total model's corrected sum of squares. I also analyzed the correlation between respiration rate (at 36 hours and 6 months) and each environmental variable (transformed when not meeting normality assumptions) using linear regressions (SAS proc reg).

Results

Community composition under long-term drought treatments

Field community composition was significantly different across 11-year rainfall reduction treatments and control treatments (p=0.02, Fig. 4.2). Differences were not significant either across drought treatments (moderate vs. severe), nor across types of "control" (unmodified plots vs. plots covered by the shelter but receiving 100% of moisture re-added weekly). Several individual phyla also showed significant responses to drought: Actinobacteria were less abundant under drought than control (p=0.001), while abundance of Bacteroidetes was significantly higher in the 25% treatment than in all other treatments (Fig. 4.3). The environmental variables that most strongly correlated to field community composition were DOC, DOC/N, and pH (Table 4.2).

Active and total community composition under moisture incubations

By isolating BrDU-DNA from total DNA, I examined community composition of both the active and the total (active + nonactive) bacterial community from each drought treatment under 5 moisture levels (ψ -1.5, -0.5, -0.1, -0.01, -0.001 MPa, see Fig. 4.1). Overall, active communities showed higher variation than total community composition (Fig. 4.4a), and were more driven by lab moisture level than

field experimental treatment, although both factors were significant over several taxonomic levels (Fig. 4.5). In addition, differences that emerged among moisture levels were primarily driven by changes in the relative abundance of active groups (as opposed to simply the presence or absence), as moisture was a weaker driver of betadiversity using unweighted Unifrac (data not shown).

Total community composition was only marginally affected by moisture conditions, but primarily determined by the soil's field treatment (25%, 50%, etc.) (Fig. 4.4a,b, Fig. 4.5). This effect was also consistent across the breadth of taxonomic levels, but for total communities, factors had a stronger effect at the phylum level, while active communities were more responsive at the species level. The distribution of phyla in total communities subject to moisture incubations (not shown) was very similar to that of the distribution of phyla in non-incubated communities from field treatments (Fig. 4.3).

Functional response of different drought treatments to moisture

I examined the relative influence of field treatment and lab moisture level on respiration rate. In both short-term (36 hour) and long-term (6 month) incubations, soil moisture was a stronger determinant of respiration rate than field treatment; that is, the current conditions for the incubation were a more important control than the 11-year field treatments (Fig. 4.6). In short-term incubations, however, field treatment was also a significant driver of respiration rate, explaining 21% (partial r^2) of the variation out of 86% of total explanatory power. Specifically, in the short-term, respiration rates of soils from control treatments were higher than 25% rainfall reduction at -0.001 MPa ($p = 0.08$) and control soils were higher than 50% and 25% at -0.1 MPa ($p = 0.04$). Variation in short-term respiration rates among field treatments was also not significantly correlated to variation in environmental factors among field treatments ($p > 0.1$ in regressions with DOC, DON, microbial biomass C and N, SOC, and pH in Table 4.3, regression results not shown).

Separation of microbial groups across moisture niches

By examining active communities from the control field treatment at different moisture levels, I could identify the whole communities and individual phyla that characterized different moisture niches, ignoring effects of field drought treatment. As described above for all field treatments, moisture strongly influenced bacterial community composition, including in the control treatment (Fig. 4.4b). Moisture also significantly altered the distribution of several phyla (Proteobacteria, Bacteroidetes, Actinobacteria) in the control treatment, providing an indicator of phyla separation across moisture niches (Fig. 4.7).

Overlap between moisture niche separation and community shifts under drought

I evaluated the overlap between bacterial response to lab moisture and community shifts under long-term drought plots by directly comparing change in phyla, and at the species level, by generating communities one would expect to see under rainfall manipulations in the field (niche-extrapolated communities). In general, response to moisture in the lab (i.e. phyla more abundant under wet conditions, etc.) was not a good predictor of changes in abundance under long-term drought treatments. For example, Actinobacteria, relative to other groups, were significantly more abundant in dry moisture incubations, and although this -1.5 MPa water potential did occur more frequently in the drought plots, Actinobacteria were actually lower in drought plots than control plots in the field. Similarly, the abundance of Proteobacteria (specifically, alphaproteobacteria) was directly related to increasingly wet water potentials, but was not significantly different among drought and control plots in the field (Fig. 4.3, 4.7).

Niche-extrapolated communities, generated by weighting the relative abundance of species active under certain moisture with soil moisture frequency distributions in the field (see Table 4.1), were slightly more similar to communities from long-term drought plots than communities that had not been weighted (Table 4.4). However, the community composition was still quite distinct from long-term drought plots, and much more similar to the composition of original active communities that were not weighted based on field moistures.

Discussion

In this study, I asked 1) whether long-term drought alters microbial community composition, activity, and respiration in response to moisture and 2) whether variation in moisture sensitivities among microbial groups (moisture niche partitioning) could explain the distribution of microbial groups I observed under long-term drought. Overall, I observed significant shifts in bacterial community composition under 11-year drought treatments in the shortgrass steppe, and differences in the short-term respiration rate and active community composition as a result of these changes in the field. I also documented bacterial moisture niche partitioning by describing communities that actively grew under a range of moisture levels. However, I did not find evidence that these differences in microbial sensitivity to moisture (niche partitioning) was directly driving long-term shifts in community composition, as abundances of species in field treatments was significantly different from those expected based on bacterial moisture response and field moisture conditions.

Does long-term drought alter microbial community growth and function in response to moisture?

As I had hypothesized, microbial communities were significantly different under drought plots compared to control. Other studies have also shown that microbial community composition is affected by shifts in moisture regime (Drenovsky et al. 2004, Williams and Rice 2007, Castro et al. 2010), but many also show microbial communities are resistant to changes in moisture (Cruz-Martinez et al. 2009, Landesman and Dighton 2010), or that precipitation is a weaker driver of microbial communities (Lauber et al. 2009) than one might expect based on its importance in driving plant community composition and ecosystem function (Fan 1993, Epstein et al. 1997, Epstein et al. 2002). Although drought resulted in community changes, the severity of drought (25% vs. 50% of ambient growing season precipitation) did not significantly affect composition. This lack of difference could indicate that there is a threshold of bacterial sensitivity to moisture, especially since moisture stress may act through different mechanisms as moisture levels decrease (Stark and Firestone 1995, Chowdhury et al. 2011). It could also indicate that

differences in the extremity of disturbances might not explain discrepancies microbial sensitivity among previous experiments (Landesman and Dighton 2010). However, soil moisture regimes in the 25% and 50% treatments was also more similar than one might expect based on the proportional differences in rainfall (see Chapter 3 and Evans and Burke in revision), and it is likely this also influenced the degree community composition differed among these treatments.

Soil moisture patterns also differed slightly among 100% and control plots (Evans and Burke in revision), perhaps because weekly re-additions resulted in changes in the timing of rainfall in 100% plots. This difference could explain the marginal difference in community composition I observed between 100% and control treatments. Notably, I also saw a shift in pH under 100% treatments, and lower plant cover (Evans and Burke in revision). Overall, although methodological caveats prevent us from extrapolating results from this study to larger areas, and from making predictions for specific future rainfall shifts (i.e. a 50% reduction in ambient precipitation), my monitoring does show that drought treatments effectively reduced soil moisture, and that this significant difference resulted in altered microbial community composition in the shortgrass steppe.

The composition of communities that were active under different moistures was affected by both contemporary moisture conditions and by field moisture treatment (Fig. 4.4, 4.5). The fact that communities did not completely (and immediately) converge, even when subject to the same moisture conditions in the lab, suggests that the change in community composition under drought may have affected community potential (i.e. the microbial 'seed bank') and influenced which species are active under certain conditions, at least in the short-term (Fig. 4.4b). Other studies have also shown that historical conditions (or "life history envelope" (Waldrop and Firestone 2006) can affect the response of communities to moisture conditions (Fierer et al. 2003, Evans and Wallenstein 2012), and other environmental changes (Tobor-Kaplon et al. 2006, Ayres et al. 2009), challenging previous assumptions that bacterial communities respond uniformly and immediately to their contemporary environment (Schimel and Gulledge 1998, Schimel 2001). These results call for further investigation into how a

microbial community's climate envelope determines its response to contemporary conditions, and how potential constraints from antecedent conditions affect biogeochemical cycling (Prosser et al. 2007).

Total bacterial community composition was more strongly affected by long-term field treatment than contemporary moisture level, in contrast to the relative influence of factors structuring active communities (Fig. 4.4, 4.5). Williams (2007) also found total community structure was more related to long-term irrigation treatments in the tallgrass prairie than it was to contemporary moisture conditions, which had a greater influence microbial physiology and activity. In this study, total communities were quite distinct from active communities (Fig. 4.4a), both in composition and in the significance of factors influencing composition across taxonomic levels (Fig. 4.5). Active communities were more significantly affected by moisture and treatment at the species level, while total communities were significantly different across moistures and treatments at coarser taxonomic scales. Other studies have reported active microbial community composition can differ significantly from that of the total community (Griffiths et al. 2003), just as plant seed banks can differ from existing plant communities (Coffin and Lauenroth 1989). In this study, some species that I observed in the active community were not even detected in the total community, showing that species active under certain conditions can be so rare when the inactive (or slow-growing) portion of the community is included that these species can appear absent in the total community at this depth of sampling. These observations highlight the importance of considering both active and total microbial community composition, as even species that are the most responsive to environmental conditions – and that may be the highest contributors to function – may not be detected by commonly used total DNA profiles.

Long-term drought treatment altered the functional response of microbial communities to moisture in the short-term, but respiration rates were only affected by contemporary moisture level in the long-term (Fig. 4.7). Differences in soil respiration due to drought treatment – in the short-term – could be due to shifts in microbial community composition (Wallenstein and Hall 2012), or to shifts in other environmental factors, like carbon availability. However, although environmental factors did change

under drought (see Table 4.3), differences in short-term respiration rate were not significantly correlated to variation in this factors ($p > 0.1$ for all correlations, data not shown). Thus, this change in moisture response may have been due to shifts in the functional potential of the microbial community, which I did observe in short-term incubations (Fig. 4.3).

Although respiration data suggest the observed shift in community potential and activity may not affect respiration-moisture relationships in the long-term, observed shifts in short-term response may be equally important for semiarid carbon budgets. The immediate pulse of CO_2 that occurs after frequent rewetting events can constitute a large proportion of total carbon flux in semiarid and arid ecosystems (Huxman et al. 2004, Munson et al. 2010), and accurately predicting the magnitude of this pulse has been challenging (Yuste et al. 2005, Borken and Matzner 2009, Lawrence et al. 2009). My results suggest that some of the uncertainty in predictions of this CO_2 pulse could be explained by shifts in microbial community potential under antecedent conditions. Interestingly, I did not observe a significant interaction between moisture and treatment, suggesting that a history of drought may have reduced overall potential of the microbial community (i.e. respiration was lower than control at each moisture level), but did not necessarily result in communities that can better take advantage of dry conditions.

Can bacterial moisture niche partitioning explain shifts in community composition under long-term drought?

I hypothesized that the shifts in microbial community composition I observed under drought would be directly driven by variation in moisture sensitivity among microbial species. To test this hypothesis, I first examined whether different microbial groups were active under different moisture conditions, and then compared microbial groups active in relatively drier conditions to those abundant under long-term drought treatments.

Although many studies have documented variation in microbial sensitivity to moisture (Harris 1981a, Avrahami and Bohannan 2007), few have presented a comprehensive assessment of those

microbial groups that grow in response to a range of moisture levels, or moisture niche partitioning in soil bacteria. My findings are similar to some observation in previous studies, but not all. Actinobacteria and Firmicutes have been shown to benefit from dry conditions (Griffin 1969, Drenovsky et al. 2004). Actinobacteria were indeed most active at driest and intermediate moisture conditions, but Firmicutes were significantly more abundant at an intermediate moisture (-0.1 MPa). Proteobacteria have also been shown to have higher abundances under wet conditions (Castro et al. 2010), as I observed, and as a gram-negative bacteria, may be more sensitive to drought (Harris 1981a, Nesci et al. 2004).

If moisture were the primary driver of microbial community structure in the field, one would expect microbial distribution among moisture niches to serve as a good predictor of changes in total community composition under long-term drought. Although adjusting relative species abundance based on microbial moisture niche and field moisture conditions *did* improve my ability to predict community composition in the long-term field plots (Table 4.4), these niche-extrapolated and long-term field communities were still significantly different. This was likely because a large degree of the dissimilarity of community composition among field communities and those active under lab incubations was due to the presence or absence of certain microbial species, and not simply changes in abundance, which is the only thing I could manipulate with weighting analyses. However, my inability to detect many of the species active under dry conditions in total plots provides further evidence that other drivers are causing these species to be too rare to be detected.

Comparisons of community shifts in the lab and field at the phyla level also did not support my hypothesis that moisture drives long-term community shifts under drought. Phyla that increased in response to drought were not the same phyla that were more active at low moisture levels in the lab (Fig. 4.3, 4.7). Specifically, Actinobacteria, which were more active at drier moistures, were *lower* in drought treatments than control (Fig. 4.3, 4.7), and Proteobacteria (largely alphaproteobacteria), which were more abundant under high moistures, were not significantly different among field treatments. Bacteroidetes was one phyla that did increased in abundance under drier (specifically, -0.5 MPa) conditions and groups in

this phyla were also more abundant under drought conditions. This distribution of Bacteroidetes across moisture conditions is one thing that made niche-extrapolated communities more similar to field communities (Table 4.4), but still only had small effects. My inability to generate communities closer to those under long-term drought also may have been influenced by differences in the taxonomic level at which moisture and treatment effects were expressed in active versus communities under long-term treatments (Fig. 4.5).

Together, these results suggest that there may be other factors that affect microbial community composition, even when only rainfall is manipulated, and challenges assumptions that differences in microbial sensitivity has driven previously observed shifts under precipitation manipulations. In this study, microbial communities in the field were affected by DOC/N, and although less strongly, pH (Table 4.2). These variables were also correlated to drought treatment (Table 4.3), but can also drive microbial community composition independent of moisture (Bossio and Scow 1998, Rousk et al. 2010), or more strongly than soil water content (Lauber et al. 2009) and may have altered the direct effect of changes in moisture on microbial communities in these long-term treatments. Williams (2007) also suggests that the differences in microbial community composition he observed under long-term irrigation plots were due to aggregated effects of the rainfall manipulation on the ecosystem, like shifts in rhizodeposition. These trends could suggest that the extent other factors shift under drought may determine whether or not microbial communities are sensitive to changes in moisture, but other studies have also recorded community resistance with changes in environmental factors, and vice versa (Cruz-Martinez et al. 2009, Evans and Wallenstein 2012). Overall, my results call for further studies that separate direct and indirect drivers of microbial community composition.

Conclusions

In summary, I observed differences in bacterial communities under drought but did not find evidence that these differences were driven by changes in moisture regime in the shortgrass steppe. I

documented moisture niche partitioning in bacteria by showing that unique communities are active under different moisture conditions. This suggests that long-term shifts in soil moisture regime have the potential to drive the relative abundance of bacterial groups, which could affect microbial community dynamics under new precipitation regimes. However, I did not find evidence that the shifts in microbial community composition I observed under long-term drought were driven by variation in bacterial moisture sensitivity. If longer and more severe droughts in the shortgrass steppe are accompanied by changes in soil and plant properties, as previous results from this manipulation suggest (Evans et al. 2011, Evans and Burke, in revision, Chapter 3), these factors could also be important drivers of microbial community composition under future climate scenarios.

Many recent studies describe the sensitivity or resistance of microbial community composition to global changes. Additional observations of shifts in microbial communities with environmental fluctuations are certainly needed to make larger generalizations about microbial community dynamics (see Allison and Martiny (2008), but coupling these observations with novel approaches could allow these results to more effectively contribute to much-needed predictive frameworks and ecological theory in microorganisms (Prosser et al. 2007) by determining the factors that actually drive these changes. In this study, I used a novel approach in which I examined active microbial communities, comparing them first to total communities (to describe how shifts in microbial potential affect contemporary activity and function) and then to communities subject to long-term field treatments (to evaluate drivers of community composition). I extrapolated community composition based on moisture niche separation and also used phylogenetic information to examine controls on community composition. Although largely absent from microbial ecology, both complex species distribution models (Iverson and Prasad 1998, Austin 2002), and inference based on phylogenetic conservation of responses, traits, or niches (Webb et al. 2002, Ackerly 2003, Silvertown et al. 2006, Lennon *in review*) are being used to gain insight into controls on species assembly in other organisms. Overall, a myriad of hypothesis-driven approaches will be needed to

determine the most important factors structuring microbial communities, and to establish theory that facilitates predictions of species distributions under future climates.

Tables

Table 4.1. Frequency distribution of soil moisture in each drought treatment over one year (2009) with rainout shelters engaged during the growing season. These weights were used to generate a community expected under long-term soil moisture conditions of each treatment.

Ψ (MPa)	Frequency (weights)			
	25%	50%	100%	Control
-0.001	0.293	0.202	0.275	0.175
-0.01	0.078	0.058	0.243	0.197
-0.1	0.296	0.222	0.071	0.149
-0.5	0.083	0.201	0.325	0.361
-1.5	0.006	0.010	0.076	0.064

Table 4.2. Nonmetric Multidimensional Scaling correlations (r^2) between field community composition (non-incubated) and environmental variables

Community Distance metric	DOC	DON	DOC:N	%OC	pH	Plant sp. composition[α]
Unifrac based on presence-absence	0.57	0.284	0.823**	0.37	0.41*	0.19
Unifrac based including abundance	0.68*	0.33	0.616*	0.65	0.27	0.18

* indicates $p < 0.1$ and **indicates $p < 0.05$
[α]Mantel test correlation statistic (r)

Table 4.3. Mean (and standard error) of environmental variables across precipitation treatments measured in the last year of drought and at the time of sampling for soil microbial communities

	25%		50%		100%		Control	
DOC	123.75	*(13.3)*	156.77	*(31.1)*	168.3	*(31.6)*	77.3	*(7.5)*
DON	27.60	*(1.1)*	34.92	*(6.2)*	33.9	*(0.3)*	20.0	*(0.9)*
DOCN	4.51	*(0.3)*	4.55	*(0.1)*	5.1	*(0.8)*	4.0	*(0.6)*
MBC	137.61	*(8.5)*	165.59	*(29.0)*	187.4	*(32.9)*	148.4	*(8.2)*
MBN	21.12	*(7.9)*	34.77	*(12.4)*	21.38	*(3.6)*	26.81	*(5.9)*
MBCN	10.42	*(4.0)*	27.87	*(17.0)*	19.71	*(10.1)*	6.92	*(1.6)*
SOC	1.42	*(0.07)*	1.39	*(0.04)*	1.46	*(0.01)*	1.37	*(0.02)*
pH	7.61	*(0.35)*	7.70	*(0.07)*	7.72	*(0.48)*	6.23	*(0.18)*

Table 4.4. PerMANOVA distances between microbial communities simulated based on niche partitioning and field moisture conditions, and total communities present in long-term rainfall manipulations

Pairwise comparison between communities			Distance*
Communities from drought treatment		Active communities (post- and pre- extrapolation)	
25%, field treatment	-	Niche-extrapolated 25% community[α]	0.584
	-	Active 25% community[†]	0.651
50% field treatment	-	Niche-extrapolated 25% community	0.512
	-	Active 25% community	0.566
100% field treatment	-	Niche-extrapolated 25% community	0.730
	-	Active 25% community	0.745
Control field treatment	-	Niche-extrapolated 25% community	0.813
	-	Active 25% community	0.845

*Distance (larger indicates more dissimilar) between communities based on Unifrac distances based on abundance
[α] Community composition after weighting species abundance based on species moisture niche partitioning from control treatments and field moisture distributions from each rainfall treatment
[†] Sum of active community composition across moistures without niche weighting

Figures

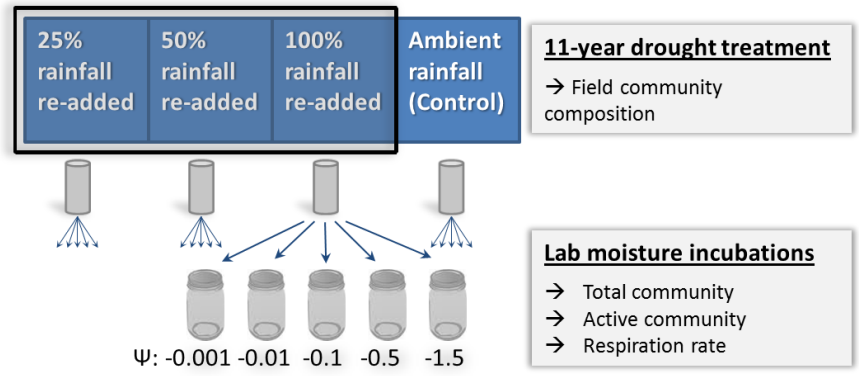

Figure 4.1. Experimental design

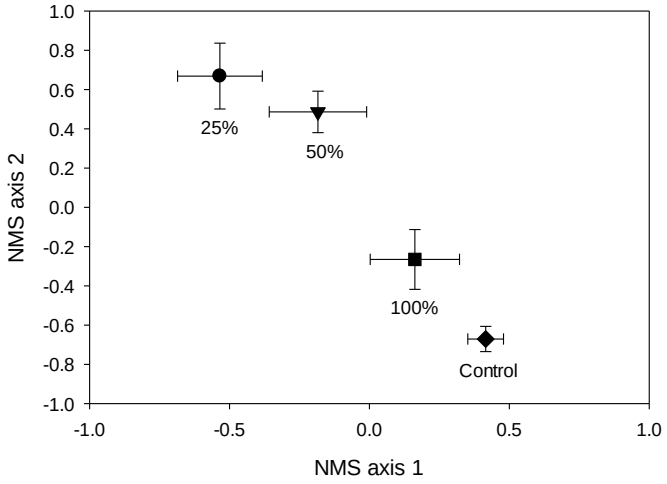

Figure 4.2. Nonmetric multiple dimension scaling (NMS) ordination of community similarity among long-term drought treatments in the field. Error bars represent the standard error of mean coordinates (N=8)

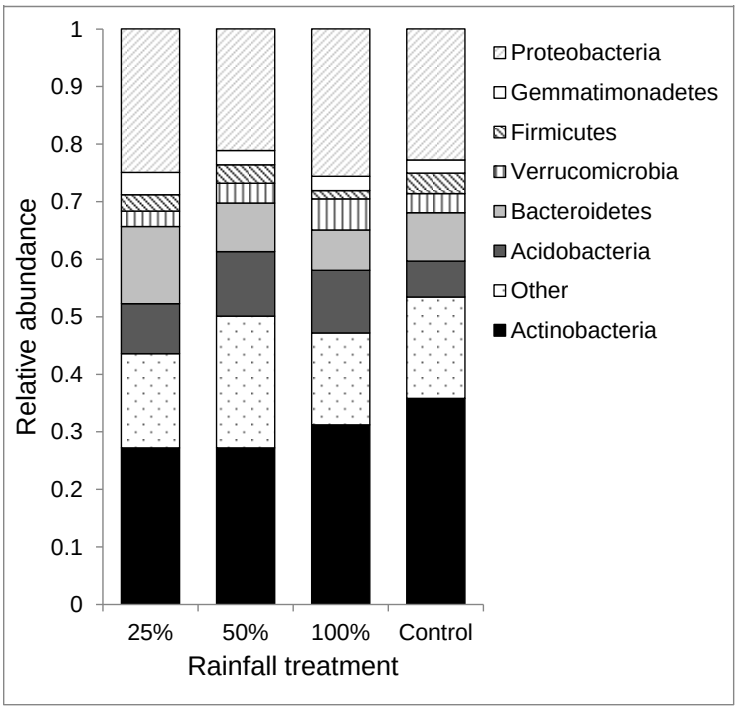

Figure 4.3. Relative abundance of dominant bacterial Phyla in (non-incubated) Field soils.

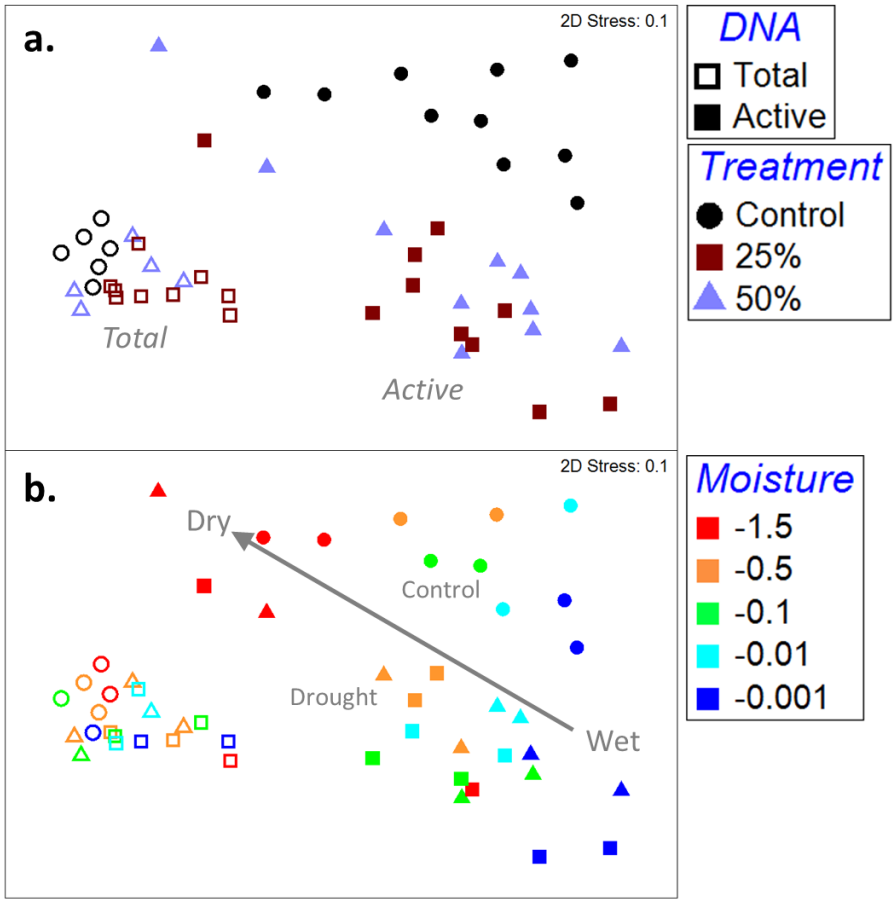

Figure 4.4. Active and total community composition from different drought treatments (indicated in A) subject to different moisture conditions in the lab (indicated in B), as analyzed by Nonmetric multidimensional scaling using weighted unifrac distances. A and B are identical except for labels and 100% treatments were excluded for clarity, but not significantly different from Control treatments.

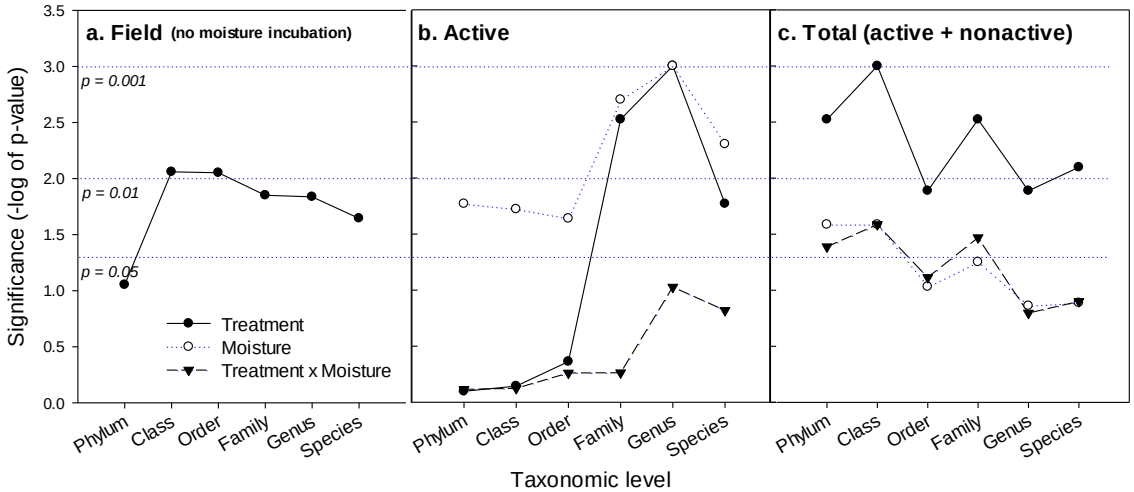

Figure 4.5. Significance (as determined by log of p-value) of factors influencing community composition across taxonomic level in (non-incubated) drought treatments (a), and Active (b) and Total (c) communities from drought treatments subject to moisture incubations

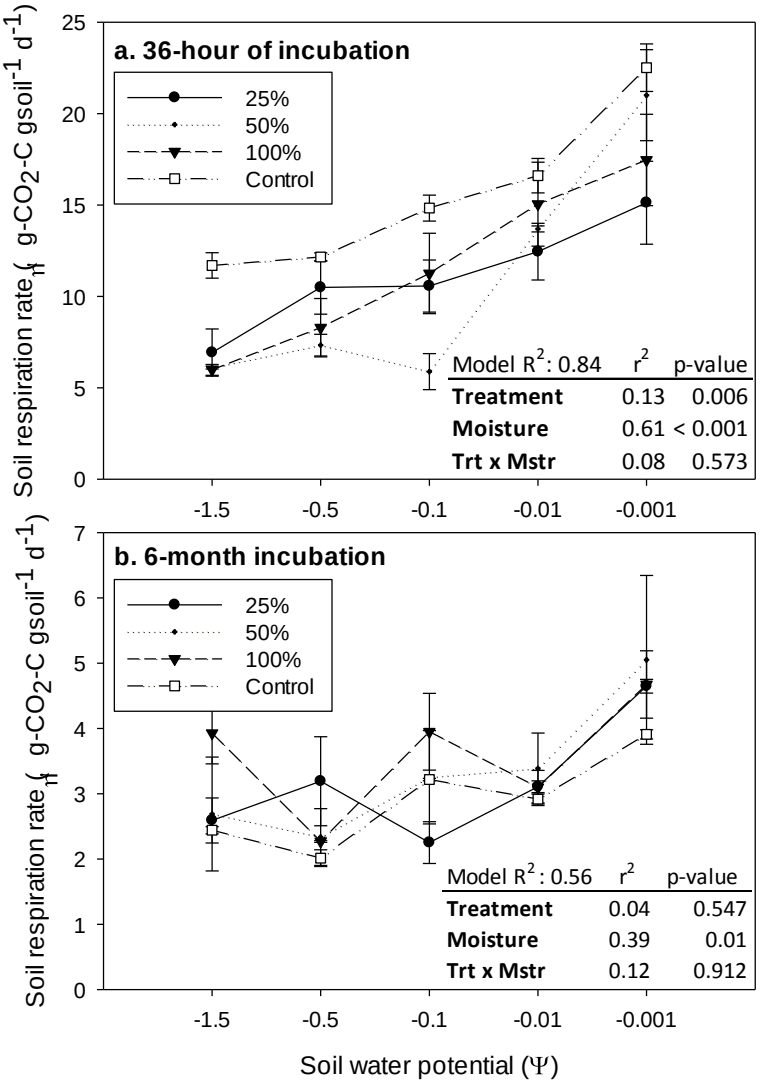

Figure 4.6. Respiration rate of soils from rainfall manipulations in the shortgrass steppe incubated at 5 water potentials in the lab over 36 hours (a) and 6 months (b).

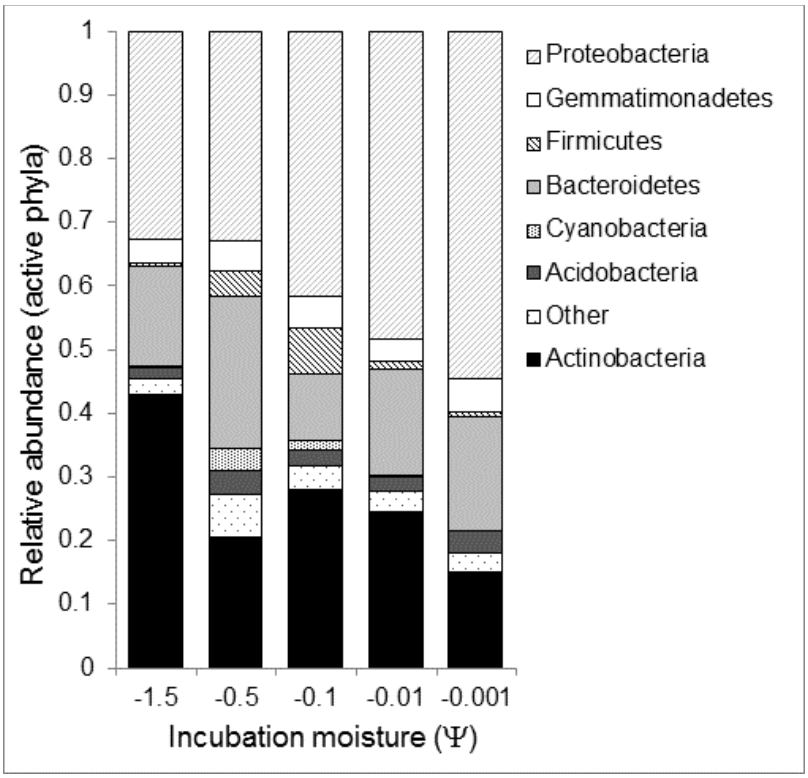

Figure 4.7. Relative abundance of dominant Phyla from Control plots active under moisture levels in lab incubations

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Chapter 5: Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter?[3]

Introduction

While soil moisture is an eminent control on the rates of biogeochemical processes in all terrestrial ecosystems, responses to moisture pulses driven by dynamic precipitation patterns are especially complex and difficult to predict (Collins et al. 2008). These drying-rewetting events can result in large pulses of soil CO_2 efflux that can strongly impact net ecosystem carbon (C) balance (Birch 1958, Austin et al. 2004, Parton et al. 2012) and in increased nitrogen (N) leaching (Miller et al. 2005, Gordon et al. 2008). Earth system climate models predict an impending intensification of the hydrologic cycle that will result in longer dry periods and more intense rainfall events (Huntington 2006). Under these conditions, the role of moisture pulses in regulating ecosystem function may become increasingly important, and changes in rainfall timing may alter the relationships between mean annual precipitation and rates of ecosystem processes (Knapp et al. 2002).

Since soil microorganisms are key drivers of biogeochemical cycling, the way they respond to changes in rainfall timing could be an important factor for predicting changes in ecosystem processes. Sudden changes in moisture are stressful to microbes, as they must expend energy to regulate osmotic pressure to their microenvironment. To achieve osmotic regulation as soils dry, many microbes synthesize solutes such as polyols and amino acids (Csonka 1989). As soil water potential increases rapidly after precipitation events, microbes must release solutes before osmotic pressure bursts cells (Wood et al. 2001). Fungi and bacteria have a wide range of tolerances to moisture stress, and have adopted many different strategies to cope with this stress (Van Gestel et al. 1993, Schimel et al. 1999). For example, fungi may be more drought tolerant than bacteria (with the exception of actinomycetes) because their hyphae can transfer moisture from water-filled micropores (Harris 1981b, de Boer et al. 2005), whereas

[3] Evans, S. and M. D. Wallenstein. 2012. Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter? *Biogeochemistry* **109:**101-116.

bacteria require water films for motility and substrate diffusion (Stark and Firestone 1995). These physiological adaptations to moisture pulses require a large investment of resources, and are likely to reduce population fitness in environments where they are less important to survival (Schimel et al. 2007). Therefore, as precipitation regimes intensify, frequent and extreme drying-rewetting events may select for microbial taxa that are more tolerant to desiccation stress, and these changes may result in a community that responds differently to moisture stress. On the other hand, the frequency of large magnitude drying-rewetting events may not drive changes in community composition or function: selection for stress tolerant taxa may occur with even a single drying-rewetting event and may persist over a period of years.

A ubiquitous underlying assumption about microbial communities is that fast turnover and widespread dispersion precludes any influence of antecedent conditions on contemporary structure and function (Allison and Martiny 2008). However, there is a growing body of evidence suggesting that, like plant communities, historical conditions influence responses of microbial communities to their environment (Van Gestel et al. 1993, Fierer et al. 2003, Waldrop and Firestone 2006, Stres et al.). Although temporal lags in process rates could simply be mediated by the persistent changes of the drivers of microbial function, such as substrate quality or quantity, soil texture, or even moisture (through ecosystem water storage), these findings suggest that altered biotic potential through persistent changes in microbial community composition could be an additional mechanism fostering inertia (Lauenroth and Sala 1992). Indeed, microbial communities previously exposed to disturbances such as precipitation stress (Fierer et al. 2003), freeze-thaw cycles (Schimel et al. 2007, Stres et al. 2010), or redox fluctuations (DeAngelis et al. 2010) have proven more resistant to these stresses than those that have not. In this way, whole microbial communities may "adapt" to a particular environment, and resultant shifts in community-level traits may alter relationships between environmental factors and function. Further, the timescale on which these legacies persist could determine their contribution to biogeochemical feedbacks and will influence our ability to predict ecosystem responses to novel climate regimes (Allison and Martiny 2008).

In this study, I isolate the effects of a single environmental change — intensified rainfall patterns — and test whether decade-long exposure to these conditions alters microbial responses to drying-

rewetting events that are more commonly experienced under the intensified precipitation regime. The Rainfall Manipulation Plot Study (RaMPS) in the U.S. tallgrass prairie is ideal experiment on which to test this. In these manipulations, the timing and quantity of precipitation events was experimentally altered for 10 years to simulate a more extreme rainfall regime (fewer, larger rainfall events separated by longer dry periods). Harper et al. (2005) reported that the experimentally increased duration of drought and intensity of rainfall events at this site led to a reduction in mean annual soil respiration. Soil moisture explained less than half of the variation in respiration rates, and although decreased plant C inputs was hypothesized to influence reduced respiration (Fay et al. 2002), the authors suggest that changes in whole-community microbial responses, brought on by the stress of the precipitation manipulation, may also be affecting respiration rates. It is unknown how the long-term modifications to the timing and magnitude of discrete rainfall events have altered microbial community composition and function in this experiment, whether community-level adaptations to climate persist in microbial communities, and whether microbial adaptation to precipitation regimes can affect soil respiration. With a coupled field-lab experiment, I examine whether precipitation history alters functional response to drying-rewetting through persistent changes in environmental drivers or through community-level microbial adaptation either to precipitation changes or other environmental variables altered by precipitation.

I hypothesize that a history of rainfall intensification will cause changes in microbial respiration in response to drying-rewetting due to persistent changes in microbial community composition. As species sensitive to drying-rewetting would have died or decreased in abundance, and tolerant species would remain, I predict that soils from manipulations that altered rainfall timing will change less in response to drying-rewetting pulses in the lab, but that functional and compositional differences among field treatments will subside after soils are subjected to the same conditions for the duration of the 4-pulse incubation (115 days).

Methods

In order to test how different precipitation histories affect the response of soil microbial communities to drying-rewetting pulses, I subjected soils from an existing field rainfall manipulation in the tallgrass prairie to drying-rewetting lab incubations. By monitoring both the function and composition of the community throughout the lab incubation (Fig. 5.1), I could could examine the sensitivity of the microbial community to drying-rewetting pulses, and determine whether how this response was influenced by antecedent precipitation patterns and other soil factors.

Field site and sampling

I sampled soils from the Rainfall Manipulation Plot Study (RaMPS) at Konza Prairie Biological station in northeast Kansas (Fay et al. 2000). Twelve 7.6 m x 7.6 m plots were established in 1997 on annually burned native tallgrass prairie. In six "Delayed" rainfall treatment plots, rainfall timing was altered such that the dry periods were 50% longer than ambient conditions. Irrigation systems then re-applied all ambient rainfall that occurred in that period, creating larger, but less frequent, rainfall events in Delayed plots (Fay et al. 2000). Two cores were taken from each RaMPS plot in late December 2007, and homogenized to pass a 2 mm sieve. Soils from 0-10 cm depths were sent to Colorado State University and stored at -10 °C until lab analysis.

Lab incubation

In early 2009, I set up a lab incubation that exposed soils from both field treatments to four drying and rewetting events that mimicked the conditions experienced for 10 years under the Delayed treatment in the field (Fig. 5.1). Pseudoreplicate cores from each plot were combined, and soils were thawed and allowed to thermally equilibrate over five days at 25 °C. Initial soil moisture and water holding capacity (WHC) were determined on a small subsample of soil from each field plot. Incubations were run in duplicate; approximately 5 g soil was placed in sterile 50 mL tubes with septa in the lids to facilitate gas measurements. After temperature equilibration, I brought all soils to 45% gravimetric soil moisture using sterile distilled H_2O, and allowed them to incubate at this moisture with the caps on for 3

days. I then placed all tubes subject to drying-rewetting pulses in a fume hood with their lids off to air-dry for three days. I chose to wet up soils to 45% soil moisture (by weight) and allow twenty days between moisture pulses because these were average values obtained from 1998-2002 field data under the Delayed rainfall treatments at Konza Biological Station. Control ("continuously wet") treatments were not dried out and kept at this soil moisture for the duration of the experiment, and served as a comparison to dried and rewet samples to account for successional changes in microbial and soil properties over the course of the experiment. I subjected dried-rewet soils to a total of four drying-rewetting periods, destructively harvesting samples from initial soils (Fresh, field-moist), after the initial wetting up period (field-moist soils brought to 45% soil moisture), after the first rewetting pulse (Pulse 1), and after the last rewetting pulse (Pulse 4) (Fig. 5.1). With the exception of the Fresh soils, all samples were harvested on the third day of incubation after the 45% soil moisture pulse, in order to facilitate comparisons among each time point and to the continuously wet control.

Respiration readings

I measured soil respiration rates by analyzing the accumulation of CO_2 in the headspace of the 50 mL tubes with a LiCor Infrared Gas Analyzer (IRGA). Readings were taken during the three days after a moisture pulse, and approximately weekly throughout the experiment on the continuously wet control.

Microbial biomass

Microbial biomass was determined by chloroform fumigation extractions (Vance et al. 1987). I placed a 4 g soil subsample into an acid-washed 50 mL tube and fumigated with chloroform for five days, while another 4 g subsample that was not fumigated acted as a control. Dissolved C and N were extracted from both subsamples by shaking 4 g soil subsamples in 10 ml of 0.5 M K_2SO_4 for two hours then filtering through #40 Whatman filter papers. Extractions were analyzed on a Shimadzu TOC analyzer. Microbial biomass was determined by subtracting C and N in fumigated samples from non-fumigated

control, and no correction factors were applied. Extractable C and N values were obtained from the non-fumigated control samples.

Quantitative PCR

I extracted soil DNA from each sample using the Power Soil DNA Isolation Kit (MoBio, Carlsbad, CA) according to the instructions of the manufacturer. I performed quantitative polymerase chain reactions (QPCR) in triplicate using 96-well plates on an iCycler iQ thermal cycler (BioRad). Reactions consisted of 12.5 μL of Absolute QPCR SYBR Green mix (ABgene), 2.5 uL of 5 ng/μL bovine serum albumin (BSA), 0.25 μL of a 10 μM mixture of each primer (final volume 0.1 μM), 5 μL of template DNA, and PCR-grade H_2O to a final volume of 25 μL. For 16S rRNA bacterial genes, I used EUB338 (Lane 1991) and Eub518 (Muyzer et al. 1993) at an annealing temperature of 55 °C; for fungal rRNA genes I used ITS1f (Gardes and Bruns 1993) and 5.8s (Vilgalys and Hester 1990) at an annealing temperature of 53 °C. Other conditions included: 95 °C for 2 minutes, followed by 40 cycles of 95 °C for 15 s, annealing temperature for 30 s, and 72 °C for 30 s. I diluted DNA to 1ng/μl for bacterial assays and 5 ng/μL for fungal assays, and adjusted to report copies per ng DNA.

I generated melting curves for each run to verify product specificity by increasing the temperature from 55 °C to 95 °C. Standards were run in triplicate in each assay, and standard curves were developed using a serial dilution of genomic DNA extracted from pure cultures. For all quantitative PCR assays there was a linear relationship between the log of the standard copy number and the calculated threshold cycle across the standard concentration range ($R^2 > 0.95$ in all cases).

Pyrosequencing of bacterial communities

I analyzed the bacterial community structure of Fresh, Pulse 1, and Pulse 4 soils (see Fig. 5.1) using a pyrosequencing-based analysis of the 16S rRNA gene in total soil DNA as described in Fierer et al. (2008). I amplified the 27 to 338 portion of the 16S rRNA gene using error-correcting bar-coded primers (Hamady et al. 2008). The forward primer contained a Roche 454 ‘A’ pyrosequencing adapter,

connected with a TC linker, and each reverse primer contained a unique 12-bp bar-coded sequence, Roche 454 'B' sequencing adapter, and a TC linker. PCR reactions were conducted with 0.5 μL (10 μM) of each forward and reverse primer, 3 μL template DNA, and 22.5 μL Platinum PCR SuperMix (Invitrogen, Carlsbad, CA), similar to Fierer et al. (2008). I amplified samples in triplicate, and pooled and cleaned them using a PCR Cleanup Kit (MoBio Laboratories, Carlsbad, CA), then sequenced them on a Roche FLX 454 pyrosequencing machine at the Environmental Genomics Core Facility at the University of South Carolina. Of 36 samples intended for pyrosequencing, 5 samples did not successfully amplify and therefore were not included in the 31 pooled barcoded samples submitted for sequencing.

I followed previously-described protocols to analyze pyrosequencing data (Fierer et al. 2008, Hamady et al. 2008, Lauber et al. 2009) using QIIME (Caporaso et al. 2010b). I first removed sequences < 200bp and with a quality score < 25. I identified OTU's as 97% similarity and used the most abundant sequence per OTU as representative of that OTU. I aligned sequences using PyNAST (Caporaso et al. 2010a) and assigned taxonomies to sequences representative of each phylotype using the RDP Classifier (Wang et al. 2007a).

Data analysis

I aimed to test how microbial communities from two different rainfall manipulations responded to a series of moisture pulses in the laboratory. The experimental design consisted of 3 factors: field treatment (2 levels, Delayed and Ambient, fixed), time point in the lab incubation (4 levels, fixed), and a treatment by time point interaction, with 6 field replicates. To analyze univariate data, I first log-transformed data for certain variables (Microbial biomass C and N, Extractable C and N, fungal:bacterial ratio) to adjust for unequal variances. I then used a repeated measures model (SAS, proc mixed) to account for the correlation among plots over time throughout the lab incubation, with plots nested within treatment. When significant differences occurred in an ANOVA, I compared treatments separately within a time point and compared time points within treatments. I also used this model to compare changes in individual taxonomic groups in my community analyses.

To quantify how field treatments differed in variability in response to moisture pulses in the lab (Table 5.1), I calculated the proportional change in response variable (Y) from one moisture pulse to the next ($[Y_{t+1} - Y_t]/Y_t$) for each sample. I also calculated the proportional change between pulse 4 and the continuously wet control ($[Y_{Pulse4} - Y_{Wet}]/Y_{Wet}$), which were measured at the same time point (the conclusion of the experiment), to describe the integrated effect of drying-rewetting compared to a continuously wet incubation, and the coeffiecient of variation (standard deviation divided by absolute value of the mean) to describe the samples' total variability throughout the lab incubation. I then compared Ambient and Delayed groups within the same univariate model as above.

To describe beta diversity and still account for differences in the number of sequences per sample, I constructed rarefaction curves that describe how the number of unique phylotypes (< 97% sequence similarity) increased as sequences in a sample increased. I determined similarity of overall community composition among samples using Unifrac (Lozupone and Knight 2005). Unifrac calculates the fraction of branch length unique to a sample or environment compared to overall branch length, computing similarity distances using only presence or absence of a phylotype (unweighted), and including abundance of phylotype (weighted). The use of this distance metric allowed us to consider the phylogenetic relationship of groups when determining the similarity of one community to another.

After removing outliers, I created ordinations with Unifrac distances using Non-metric multidimensional scaling (NMDS) with the remaining 27 samples (N=3-6 in each group), and tested for significance of differences between communities in different treatments and across time points using PerMANOVA (Anderson 2001) in Primer v6. PerMANOVA is a permutation-based multivariate analysis that can accommodate more complex and unbalanced sampling designs. This test calculates a pseudo *F*-statistic by comparing the total variance explained by sample identities (i.e. Time, Treatment) to that explained by random permutations of sample identities. As with univariate data from the same design, I tested the effect of Time (fixed), Treatment (fixed), Time*Treatment, and nested plots within treatments (random) on community similarity, and examined significance of pairwise comparisons within both Time and Treatment compared to 9,999 permutations. To examine more specific species responses, I also

performed a Similarity Percentage (SIMPER) analysis (Clarke and Gorley 2006) to identify the relative contribution of each species to the differences in groups I observed using PerMANOVA.

Results

To determine whether a long term treatment of altered rainfall timing affected microbial community response to drying-rewetting, I measured variables that describe both the functional response and changes in the composition of microbial communities. I was interested in whether differences caused by rainfall manipulation persisted in the lab, whether this persistence could be explained by environmental variables or microbial community composition, and if a history of this stress caused variables to fluctuate less in response to moisture pulses.

Respiration

Respiration rates were highest in both Ambient and Delayed soils at the beginning of the lab incubation, and respiration pulses were smaller with each subsequent moisture pulse (Fig. 5.2). Soils from Ambient field treatments showed significantly higher respiration rates at the initial Wetting up period and after the second drying-rewetting pulse. Dry-rewet soils showed higher respiration pulses than continuously wet soil at the beginning of the experiment, but both the difference between field treatments (Ambient and Delayed) and the difference between pulsed and continuously wet soils was small at the end of the 115-day incubation (Pulse 4).

Microbial Biomass

Long-term treatment (Ambient or Delayed) also affected microbial biomass, but there were no significant differences by the end of the incubation. Microbial biomass C and N were significantly higher under Delayed rainfall timing manipulations at the time of sampling compared to soils from Ambient plots (Fig. 5.3), and responded differently to drying and rewetting in the lab. Microbial C increased after the first pulse in Ambient soils but was reduced by Pulse 4. Microbial N increased in Delayed soils after

the first pulse but decreased in Ambient soils and in subsequent moisture pulses. Microbial C in Delayed soils was relatively unchanged by moisture pulses, but Microbial N in Delayed was more variable than Ambient across time points (Fig. 5.3, Table 5.1).

Extractable organic carbon (EOC) and nitrogen (EN)

There was a large increase of EOC (but not EN) during the first moisture pulse, especially in Ambient soils, and a later (Pulse 4) increase of N in soils from both field manipulations (Fig. 5.4). There was not a significant difference between EOC or EN in soils that had undergone drying rewetting pulses and those that were continuously wet, or significant differences between field treatments within any one time point. However, soils that experienced drying-rewetting in the field did have less variation in EOC in response to lab pulses (Fig. 5.4, Table 5.1).

Fungal: bacterial ratio

Soils that experienced Delayed rainfall timing had higher fungal: bacterial ratios in Fresh soils and after Pulse 1 (Fig. 5.5). Fungal: bacterial ratio increased in both field treatments as pulses progressed in the lab, and pulsed soils had a higher ratio than soils kept in continuously wet conditions. Ambient soils changed more over the course of the lab incubation (Fig. 5.5, Table 5.1) and there were no significant differences in field treatments at the end of the incubation in soils that experienced drying-rewetting or between field treatments in the continuously wet incubation.

Bacterial community

Pyrosequencing resulted in 99,048 sequences and 14,207 unique phylotypes (1 phylotype=97% similarity). Sequences per sample ranged from 41 to 7,485 with an average of 3,302. Four samples were removed from the community similarity and diversity analysis because they were outliers in the NMDS analysis, and also had less than 250 sequences per sample. Rarefaction curves continued to increase with additional sequences even up to 7000 sequences, and diversity did not significantly differ between

Ambient and Delayed soils in Fresh soils or after Pulse 1, but was higher in Ambient after Pulse 4 (Fig. 5.6).

Bacteria dominated soil communities compared to Archaea, but this proportion was not affected by field or lab manipulations. The most abundant Phyla in all groups were Actinobacteria (23%), Proteobacteria (23%), Verrucomicrobia (14%), and Acidobacteria (11%) (Fig. 5.7), and there were trends of higher variability across time points in Delayed soils compared to Ambient. According to my SIMPER analysis, a species from Verrucomicrobia (in the Xiphinematobacteriaceae family) most strongly contributed to differences among groups, which was more abundant in both Delayed soils compared to Ambient and in soils at Pulse 4 compared to Fresh (Table 5.2). Other notable groups that contributed to differences among treatments were Acidobacteriaceae (increased by Pulse 4), and Alphaproteobacteria (Rhizobales more abundant in Delayed but decreased in response to lab drying-rewetting).

When communities were analyzed for similarity based on Unifrac distances, there was significant variation within groups (Fig. 5.8), but lab treatment explained more similarity among samples than field treatment (Ambient or Delayed) (Table 5.3). PerMANOVA pairwise comparisons (among time points within treatments and between treatments within time points) revealed that no communities were significantly different using Unweighted Unifrac differences. When taking relative abundance into account (Weighted Unifrac), treatments were significantly different at Pulse 4 ($p < 0.05$) and in Fresh soils ($p < 0.1$). Soils from Delayed treatments changed from Pulse 1 to Pulse 4, showing greater differences as the lab incubation progressed, but Ambient soils did not significantly change over time (Table 5.1, 5.3).

Discussion

Did long-term rainfall manipulations conditions influence microbial response to drying rewetting?

While there is little doubt that soil microbial activity responds quickly to changes in environmental conditions, the role of environmental history in driving contemporary rates of microbially-mediated processes is largely unknown. Previous studies have documented differences in microbial

function induced by historical legacies in climate (Fierer and Schimel 2002, Fierer et al. 2003), litter quality (Ayres et al. 2009, Keiser et al. 2010), or disturbance regime (Tobor-Kaplon et al. 2006), but the mechanisms driving these legacies is often unclear.

My study provides evidence that, while soil moisture at any instant is the dominant driver of microbial function, the long-term treatment of changed soil moisture regime also affects the response of soil microbes to drying and rewetting events. For example, I observed a lower respiration rate following initial soil rewetting in Delayed soils compared to Ambient soils (Fig. 5.2). This could be explained by persistent changes in other drivers like microbial biomass or substrate availability, but these pools did not explain a reduction in respiration in Delayed soils at the beginning of the experiment (Fig. 5.3, 5.4). The different long-term precipitation regime induced by these rainfall timing manipulations may have altered the aggregate community-level traits (*sensu* Wallenstein and Hall (2011)) that control soil respiration including carbon use efficiency, soil moisture sensitivity and stress tolerance. These changes are most likely driven by changes in the relative abundance and activity of taxa that differ in physiology (Wallenstein and Hall 2012), which could occur at any phylogenetic level, depending on the degree to which these traits are conserved across evolutionary history. In my study, differences in community-level responses to the initial experimental rewetting could be attributed to the higher fungal:bacterial ratio in soils from the Delayed treatment (Fig. 5.5). In this manner, historical precipitation regimes can act as a distal control on contemporary rates of microbial processes (e.g. respiration) by modifying the traits of microbial communities that act as transducers between contemporary abiotic drivers (e.g. soil moisture, substrate availability) and microbial function (as Wallenstein et al. (2006) proposed for denitrification.

Do long-term treatment effects persist when soils are subject to the same conditions?

The relative importance of environmental history on contemporary process rates depends, in part, on the degree to which historical effects persist following environmental change. In this study, the ecological importance of historical precipitation regime depends on whether the differences in moisture pulse response between Delayed and Ambient soils that I observed during the initial pulse persisted when

the soils were subjected to the same moisture pulse regime. I predicted that soil microbial communities adapted to extreme rainfall patterns (i.e. drying-rewetting events of greater magnitude) would change less in response to drying-rewetting pulses than those that experienced ambient rainfall, and that Ambient soils would become more similar to Delayed through time as they adapted to moisture pulses. Consistent with this hypothesis, I found that respiration, biomass-C and extractable-C changed less in Delayed soils than Ambient soils throughout the 115-day laboratory experiment (Table 5.1), and that the effect of precipitation history declined throughout the experiment such that initial differences among soils from different field treatments were negligible by the end of the lab experiment.

Other studies suggest that the effects of drying - rewetting events may cause changes in C-mineralization long after the moisture pulse (Schimel et al. 1999, Fierer and Schimel 2002). Fierer and Schimel (2002) showed that differences in function persisted 6 weeks after drying-rewetting, with little convergence once subjected to the same conditions. The incubation in this study extended longer than this, and although I examined how control (Ambient) and stressed (Delayed) soils responded to a stress (instead of how they recover), I did observe similar respiration rates, suggesting that the effects of a decade of an altered precipitation regime on respiration may not persist beyond a single growing season in this particular prairie ecosystem. The persistence of historical legacies observed by Fierer and Schimel (2002) was at least partially explained by differences in substrate availability, which did not differ at the end of the experiment. Thus, the persistence of long term treatment effects may depend on the mechanism through which these legacies are generated.

Mechanisms antecedent conditions influence contemporary response

There are two mechanisms by which long-term rainfall manipulation treatments may have affected contemporary microbial function in this experiment. First, the experimental intensification of precipitation regime induced by RaMPS could have caused changes in plant and soil properties that persisted after soils were removed from the field and placed under identical conditions in the laboratory (Fig. 5.8a,b, Table 5.3). In this study, the laboratory experiment isolated the effects of drying-rewetting

by subjecting two soils that differed only in historical moisture regime (i.e. no other previous ecosystem differences that would result in soil texture or chemical differences) to moisture pulses in a controlled lab environment in the absence of plants and other environmental drivers. Therefore, any changes that occurred reflect direct responses to shifts in precipitation, or indirect responses such as shifts in plant growth or chemistry affecting the quantity and quality of C inputs to soils. Although Fay et al. (2002) found decreased aboveground net primary production under Delayed rainfall in the field, I did not observe a difference in soluble (labile) C or N in initial soil measurements from each treatment. Therefore, I do not believe the persistence of differences in respiration between soils with different histories were primarily due to differences in substrate. Increased drying-rewetting can alter other abiotic factors such as soil physical structure (Adu and Oades 1978) that may also persist, although it is unlikely these changes significantly affected respiration rates because soils were initially identical and many of these variables change on much longer timescales (Jenny 1941).

The second mechanism by which environmental history can affect contemporary microbial function is through changes in the composition and aggregate physiology of microbial communities. Altered precipitation patterns could induce community-level adaptation to the stress associated with drought and intensified rain events. This biotic selection could be driven directly by osmotic stress, or indirectly through abiotic factors that shifted under altered precipitation timing. Changes in community structure, such as the differences in fungal:bacterial ratio that I observed in this study, are likely to alter the aggregrate function of microbial communities (Wallenstein and Hall 2012). Although I did not explicitly test fungal versus bacterial tolerance to drying or rewetting, increases in fungal:bacterial ratios do suggest that fungi and bacteria have differing sensitivities to drying-rewetting, as other studies have also suggested (Bapiri et al. 2010, Hawkes et al. 2010, Yuste et al. 2010). Ratios converged by Pulse 4, and Delayed soils changed less in response to drying-rewetting (Fig. 5.5, Table 5.1), suggesting biotic community adaptation to drying-rewetting stress could be captured at this broad level, and possibly explaining the persistence of observed differences in respiration rate.

Historical legacies in bacterial community composition

While my data show that the fungal:bacterial ratio increased as a direct result of increased drying-rewetting, a more detailed investigation of bacterial community composition revealed only subtle differences in community structure between field treatments, but increasingly dissimilar communities when subjected to identical conditions in the lab (Fig. 5.7, 5.8). Bacterial community data from pyrosequencing do not support a biotic mechanism for the historical legacies I observed in function, although precipitation history clearly influenced bacterial community composition throughout the timescale of the incubation, and this lack of initial dissimilarity does not preclude this mechanism's expression on different timescales and through other microbially-mediated functions.

I suggest two reasons why a 10-year rainfall timing manipulation may not have resulted in more distinct bacterial communities. First, it is possible that most taxa in the tallgrass prairie soils are pre-adapted to some degree of moisture fluctuation, and the increased magnitude induced by these manipulations did not induce further selection. Other studies have observed no significant change in bacterial community composition under rainfall manipulations (Cruz-Martinez et al. 2009, Landesman and Dighton 2010). The differences I observed, either from field or lab treatments, emerged due to changes in relative abundance of particular taxa, rather than the presence or absence of certain taxa (as quantified by Unweighted Unifrac distances, Fig. 5.8b, Table 5.3). Delayed soils might have been better adapted to drying-rewetting. However, since the magnitude of the pulses that occurred in this precipitation regime also occurred in the natural historical climate, although less frequently, Ambient soils may have also contained the microbial taxa that allowed the extant community to adapt to laboratory moisture pulses quickly.

Second, the lack of detectable effects of the RaMPS experiment on plant community structure and function may have buffered soil microbial communities from direct drying-rewetting selection pressures. Plant community properties, which remained relatively unchanged under this rainfall manipulation, have been shown to stabilize microbial dynamics; for example, plant diversity has been shown to diminish changes in microbial biomass and denitrification rates across seasons (McGill et al.

2010). In the absence of plant-mediated environmental buffering, exposure to direct drying-rewetting in the lab may have may have induced stronger selection on community composition. Consistent with this hypothesis, subtle differences observed in field soil communities under the RaMPS appeared to drive divergent trajectories for community composition in the lab. For example, a greater abundance of a Verrucomicrobia species in Delayed soils most strongly contributed to whole-community dissimilarity of Delayed and Ambient soils at Pulse 4 (Table 5.2). This increase in abundance with each subsequent lab pulse could have emerged from this species' slightly greater abundance in Delayed plots in the field which enabled them to capitalize on preferred conditions once plant-mediated buffers were removed.

Individual responses of certain species to drying-rewetting pulses, when examined across time, varied significantly (see Sparklines in Table 5.2). It is possible that the divergence in community composition I observed, and general variability within samples, may relate to the nature of drying-rewetting as a disturbance. Unlike the Verrocomicrobia example discussed above, an Acidobacteria species that was more abundant under altered rainfall timing in the field changed very little in the lab, perhaps reflecting an alternative strategy of shifting resource allocation from growth to structural stability, instead of capitalizing on short-lived optimal conditions. A climate shift toward more extreme conditions (intensified rainfall) may more strongly induce diverse life strategies compared to a unidirectional shift (drought), which may result in more specialization (Wallenstein and Hall 2012). Other studies have suggested similar delineation of life strategies as a framework for predicting responses of microbial communities to disturbance (Van Gestel et al. 1993, Fierer et al. 2007).

Methodological idiosyncrasies could also have influenced measured trends in community composition and the absence of a link between community composition and function. First, tolerance to drying and rewetting may not have been expressed on the phylogenetic level I chose (97% similarity for OTU's) because it requires complex mechanisms involving multiple genes. Keiser et al. (2010) examined the effect of historical substrate exposure on function and community composition on this phylogenetic level and also found community composition, which converged under similar conditions, did not follow a similar trajectory as function, as decadal supply of litter type from the treatments continued to affect

decomposition rate after 100 days. Second, I only sequenced bacterial communities, and fungi could display unique and strong responses to moisture stress. Efforts to determine the phylogenetic level at which microbial stress tolerance is expressed will be important for the development of predictive frameworks. In addition, assessing overall microbial community composition (as opposed to only the active members) may mask discreet changes in species assemblage that are better linked to function (or stress tolerance) (McMahon et al. 2011). A final methodological concern is whether communities were affected by long-term storage at -10 °C. Although physical effects on soils from the same site were likely similar, certain microbial communities could be more sensitive to cold-stress than others, and this could alter microbial community composition and responses to moisture upon rewetting (Lee et al. 2007, Gonzalez-Quinones et al. 2009). However, as I found no significant difference (yet communities were also not statistically the same), it is unlikely cold storage either affected soils differently or selected for species in a systematic way.

Implications of historical legacies for predicting ecosystem responses to novel climates

A current challenge for ecologists is to establish whether existing relationships between abiotic factors and community and ecosystem properties can be extrapolated over time to predict ecosystem-atmosphere feedbacks and the direction and rate of global change. My results suggest, as other studies have, that long term treatment conditions do play a role in determining the functional and composition response of microbial communities to environmental factors (Gulledge and Schimel 1998, Lundquist et al. 1999, Fierer and Schimel 2002, Fierer et al. 2003). In this study, differences in respiration rates – that could not be explained by substrate availability or microbial biomass – persisted when soils were incubated under the same conditions, but for less than 115 days. The increase in frequency of stressful conditions that already occur within an ecosystem's historical range of variability might cause lags in function, perhaps mediated by changes in community composition (in this case fungal:bacterial ratio), but these lags will be short. Decadal-scale conditions may more strongly influence contemporary functional response when disturbances are further outside an environment's historical range of variability (Veblen et

al. 1999), crossing potential thresholds, or when acting through indirect drivers like changes in plant properties. In contrast to short functional lags, effects of historical precipitation continued to cause differences in bacterial community composition through the end of the experiment. This suggests that biologically-mediated legacies at least have the potential to cause longer functional lags, perhaps in functions controlled by narrow phylogenetic groups (McGuire et al. , Schimel 1995). Thus, legacies of environmental conditions may affect microbially-mediated processes on different timescales, and vary in magnitude for different functions. More detailed descriptions of the temporal dynamics of microbial responses could improve predictions for how microbially-mediated processes will respond to global changes (Treseder et al. 2012).

These results call for further work to 1. isolate direct and indirect mechanisms of historical conditions on responses of microbial communities through coupled field-lab studies (see Docherty et al. 2012, Brown et al. 2012) 2. determine the phylogenetic level at which adaptations to stress, and functional linkages, are expressed, and 3. identify factors controlling the timescale on which historical legacies affect contemporary microbial responses. Under novel climate regimes, historical legacies may impair our ability to predict ecosystem responses with current predictive relationships. Some studies have begun to investigate whether carbon dynamics under moisture pulses can be better predicted using explicit microbial mechanisms (Lawrence et al. 2009, Li et al. 2010). Results from this study suggest that microbial adaptation to climate conditions may influence this response as well, and further research is needed to quantify how microbial legacies to climate could affect predicted changes in carbon flux at the ecosystem scale (Todd-Brown et al. 2012).

Tables

Table 5.1. Summary of resistance of microbial communities from different long-term rainfall manipulation (Ambient and Delayed rainfall timing) subject to multiple drying-rewetting pulses in the lab

Parameter	**Field rainfall manipulation**	**Proportional change between two time points in lab manipulation**						
		Fresh to Pulse 1	Pulse 1 to 2	Pulse 2 to 3	Pulse 3 to 4	Pulse 1 to 4[a]	CV[b]	Pulse 4 - Wet Control[c]
Respiration	Ambient	**-0.439**[a]	**-0.106**	-0.516	1.019	6.31	0.702	-0.003
	Delayed	**-0.209**	**-0.405**	-0.404	1.254	4.08	0.710	-0.100
Microbial	Ambient	**1.071**				-0.363	**0.410**	0.164
Biomass C	Delayed	**0.147**				-0.222	**0.268**	0.410
Microbial	Ambient	-0.223				-0.561	0.416	0.037
Biomass N	Delayed	0.182				-0.650	0.668	-0.332
Extractable C	Ambient	**8.975**				-0.564	**0.769**	0.098
	Delayed	**4.014**				-0.663	**0.619**	-0.071
Extractable N	Ambient	-0.029				1.479	0.553	0.148
	Delayed	0.185				1.358	0.562	0.217
Fungal:	Ambient	**2.068**				**1.101**	**0.784**	-0.342
Bacterial	Delayed	**0.991**				**0.290**	**0.428**	-0.351
Community	Ambient	0.2098				0.2333		
Dissimilarity[c]	Delayed	0.2181				0.2990		

Bold indicates a significant difference ($p < 0.1$) between the proportional change (or CV) in Ambient and that in Delayed

[a] All variables other than respiration were measured only at Fresh, Pulse 1 and Pulse 4 time points, so proportional change could not be calculated among each time point.

[b] Coefficient of Variation of all time points measured (excluding wet control)

[c] $Y_{Pulse\ 4} - Y_{Wet\ Control} / Y_{Wet\ Control}$

[d] Average Weighted Unifrac distance in ordination space between two communities of two groups. I could not test for significance of degree of change

Table 5.2. Summary of response of most abundant unique phylotypes (greater than 0.85% abundance averaged across all groups) to field treatments and moisture pulses. Difference between Delayed and Ambient represents averages across all time points (D-A), as average differences between Pulse 4 and Fresh (P4-Fr) are averaged across both treatments. Contribution to community difference was determined by SIMPER analysis in Primerv6, which determines the contribution of each species to driving the dissimilarity of communities in a group. Sparklines ("Response to Pulses") represent the relative abundance of that species in Ambient (black line) and Delayed (blue line) treatments in Fresh, Pulse 1, and Pulse 4 lab treatments.

Organism taxonomy	Response to pulses	Avg abundance (%)	Avg diff: Del - Amb	% Contrib to Del/Amb difference	Avg diff: P4-Fr	% Contrib to P4/Fr difference
Bacteria Verrucomicrobia Verrucomicrobiae Verrucomicrobiales Xiphinematobacteriaceae Xiphinematobacteriaceae_genera_incertae_sedis		11.526	4.879	5.92	3.184	4.28
Bacteria Proteobacteria Alphaproteobacteria Rhizobiales		2.812	0.712	1.3	-0.823	1.66
Bacteria Proteobacteria Alphaproteobacteria Rhizobiales Bradyrhizobiaceae Bradyrhizobium		2.388	0.016	1.02	-0.804	1.05
Bacteria Acidobacteria Acidobacteria Acidobacteriales AcidobacteriaceaeGp4		2.100	-0.275	0.97	1.754	0.94
Bacteria Actinobacteria Actinobacteria Actinobacteridae Actinomycetales		1.382	-0.658	0.94	-0.810	0.86
Bacteria Actinobacteria Actinobacteria Rubrobacteridae Rubrobacterales Rubrobacterineae		1.338	0.121	0.83	-0.420	0.83
Bacteria Proteobacteria Betaproteobacteria Burkholderiales		1.312	0.189	0.77	-0.557	0.75
Bacteria Acidobacteria Acidobacteria Acidobacteriales AcidobacteriaceaeGp16		1.234	-0.315	0.71	-0.378	0.74
Bacteria Actinobacteria Actinobacteria Rubrobacteridae Rubrobacterales Rubrobacterineae Rubrobacteraceae Solirubrobacter		1.035	-0.090	0.69	-0.565	0.72
Bacteria Verrucomicrobia Verrucomicrobiae Verrucomicrobiales Xiphinematobacteriaceae Xiphinematobacteriaceae_genera_incertae_sedis		1.006	0.081	0.65	1.006	0.59
Bacteria Actinobacteria Actinobacteria		0.954	-0.100	0.63	-0.159	0.58
Bacteria Actinobacteria Actinobacteria Actinobacteridae Actinomycetales Corynebacterineae Mycobacteriaceae Mycobacterium		0.941	-0.272	0.61	0.186	0.58
Bacteria Acidobacteria Acidobacteria Acidobacteriales Acidobacteriaceae Gp6		0.886	-0.210	0.59	0.054	0.53
Bacteria Gemmatimonadetes Gemmatimonadetes Gemmatimonadales Gemmatimonadaceae Gemmatimonas		0.878	-0.138	0.56	0.381	0.53
Bacteria Actinobacteria Actinobacteria Actinobacteridae Actinomycetales		0.860	-0.543	0.55	0.082	0.51

Table 5.3. PerMANOVA results for Main effects and Pairwise comparisons within the Trt*Time interaction (field treatments within each time point and time points within each field treatment)

Distance metric	Test	Factor	Pairwise comparison	Mean distance[a]	P-value[b]
Weighted Unifrac	Main effects	Trt			0.278
		Time			**0.001**
		Trt*Time			**0.085**
	Pairwise within Time	Fresh	Ambient-Delayed	0.1766	**0.0771**
		Pulse 1	Ambient Delayed	0.2294	0.2109
		Pulse 4	Ambient-Delayed	0.2244	**0.0486**
	Pairwise within Trt	Ambient	Fresh - Pulse 1	0.2098	0.2486
		Ambient	Pulse 1 - Pulse 4	0.2333	0.2121
		Ambient	Fresh – Pulse 4	0.2412	0.1200
		Delayed	Fresh - Pulse 1	0.2181	0.2043
		Delayed	Pulse 1 - Pulse 4	0.2990	**0.0464**
		Delayed	Fresh- Pulse 4	0.2338	**0.0383**
Unweighted Unifrac	Main effects	Trt			0.6606
		Time			**0.0440**
		Trt*Time			**0.0665**
	Pairwise within Time	Fresh	Ambient-Delayed	0.6239	0.1639
		Pulse 1	Ambient Delayed	0.6565	0.4874
		Pulse 4	Ambient-Delayed	0.6505	0.4428
	Pairwise within Trt	Ambient	Fresh - Pulse 1	0.6397	0.3869
		Ambient	Pulse 1 - Pulse 4	0.6510	0.3502
		Ambient	Fresh – Pulse 4	0.6485	0.2068
		Delayed	Fresh - Pulse 1	0.6654	0.2094
		Delayed	Pulse 1 - Pulse 4	0.6720	0.3635
		Delayed	Fresh – Pulse 4	0.6592	0.2491

[a]Pairwise mean distances were derived from different distance metrics (Weighted and Unweighted Unifrac) and therefore are only comparable within that distance matrix.
[b]Bold indicates $p < 0.1$

Figures

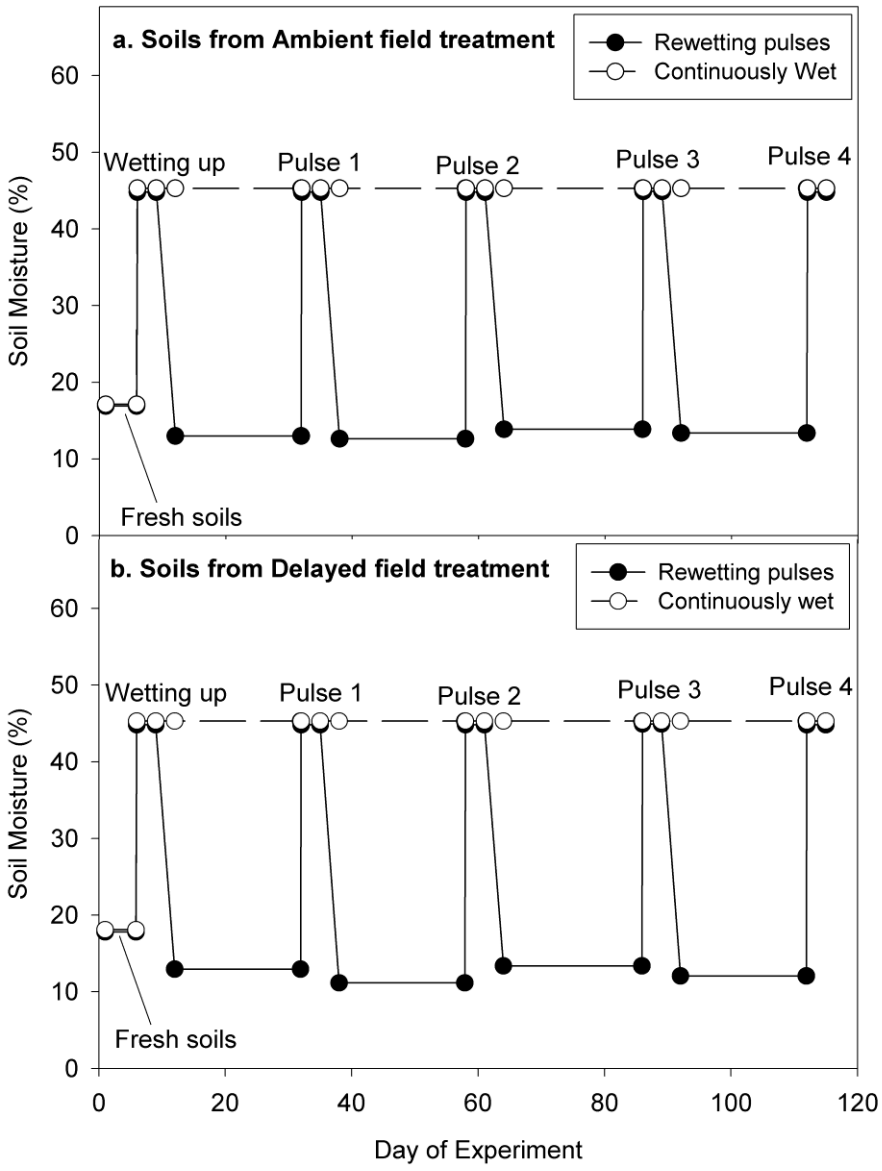

Figure 5.1. Average soil moisture in lab incubation treatments throughout the experiment and time points of sample. Soils from Ambient (a) and Delayed (b) field manipulations were equivalently subject to either drying-rewetting pulses (filled circles, solid line) or kept continuously wet (open circles, dashed line). Error bars represent standard error of mean soils moisture at that time point, but often smaller than symbol

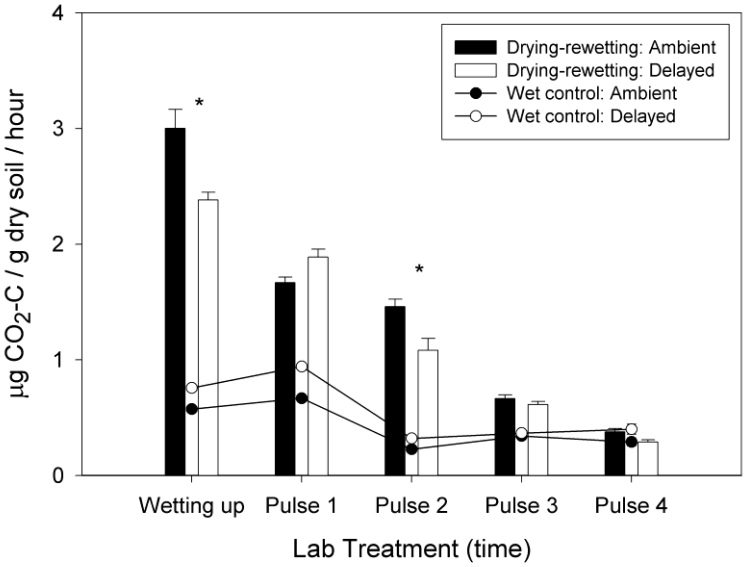

Figure 5.2. Average respiration rate for soils from Ambient (filled) and Delayed (open) field plots when subject to drying rewetting pulses (bars) and continuously wet incubation (symbols). Rates for drying-rewetting incubations were calculated for the first 48 h after receiving each moisture pulse. * indicates a significant difference ($p<0.05$) between Ambient and Delayed treatments within that time point. Error bars are standard errors for means (N=6).

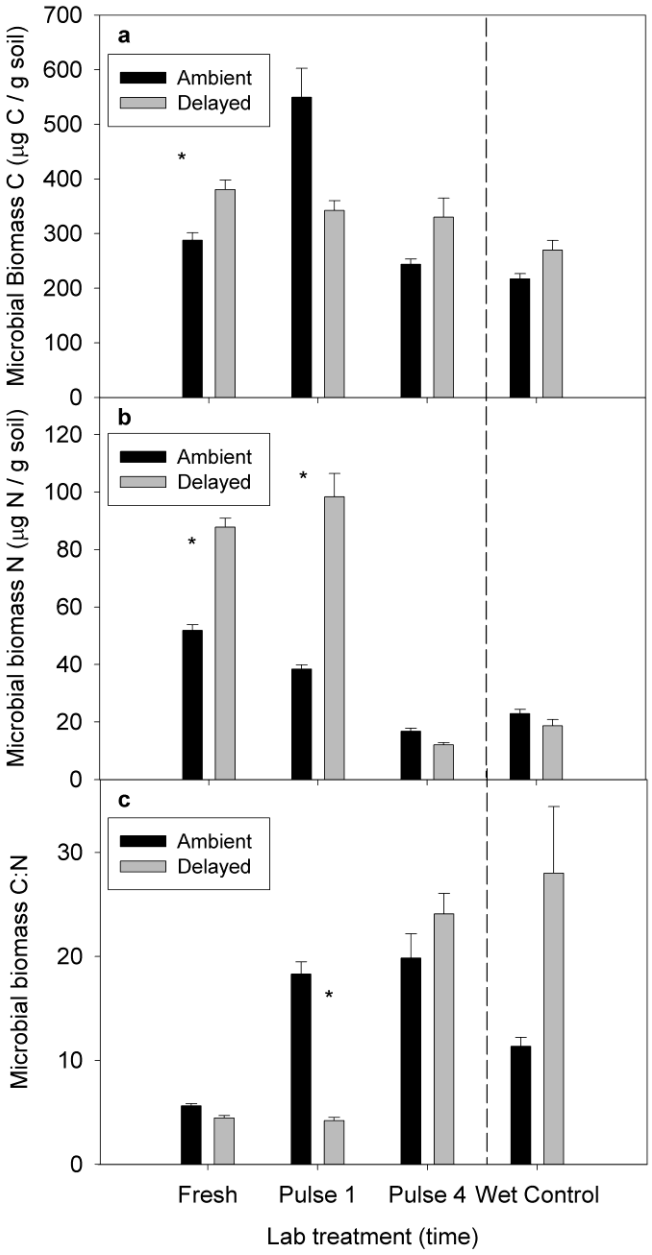

Figure 5.3. Microbial biomass carbon (a), nitrogen (b) and carbon:nitrogen (c) throughout lab treatment as determined by chloroform-fumigation extractions of wet soil 3 days after soils from two field treatments received a moisture pulse. *indicates a significant difference ($p<0.05$) between Ambient and Delayed treatments within that time point. Error bars are standard error for means (N=6)

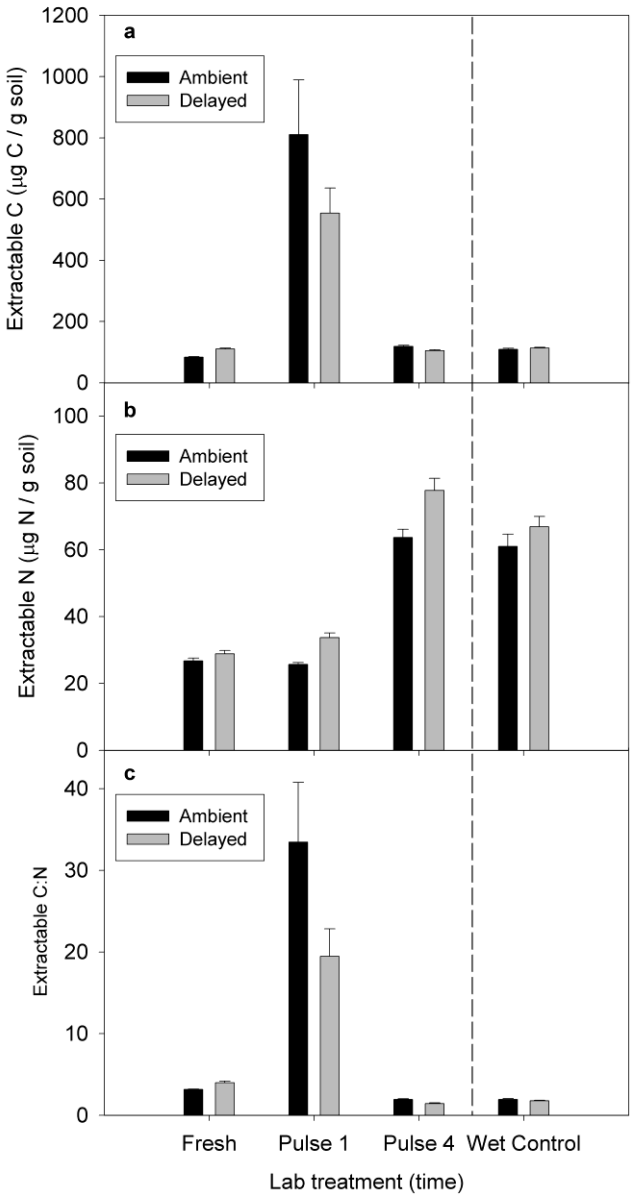

Figure 5.4. Mean extractable organic carbon (a) and nitrogen (b) throughout lab treatment. Error bars are standard error for means (N=6)

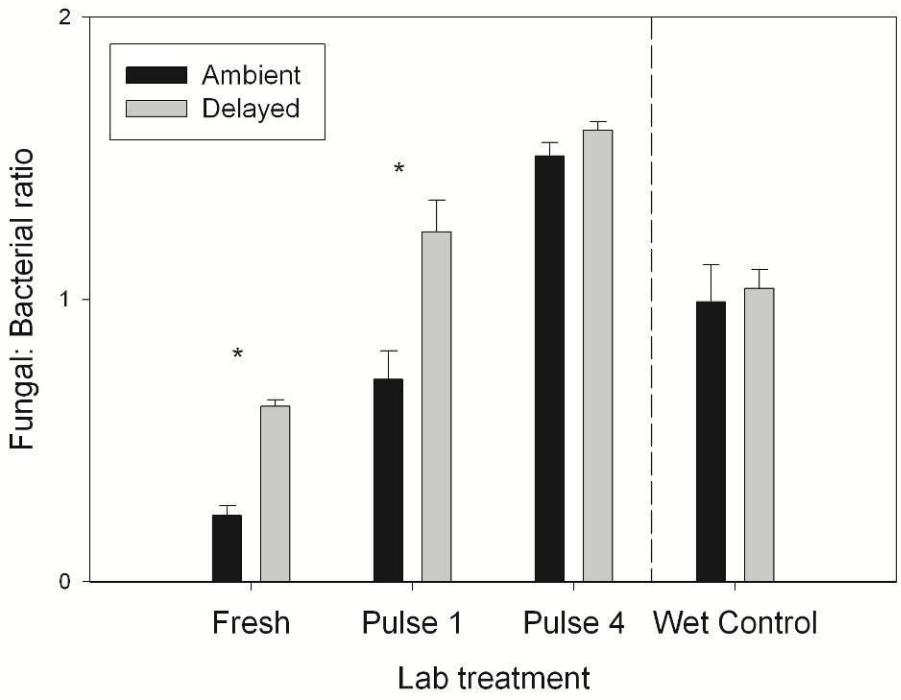

Fig. 5.5 Fungal to bacterial ratio as determine by quantitative PCR. *indicates a significant difference ($p<0.05$) between Ambient and Delayed treatments within that time point. Error bars are standard error for means (N=6)

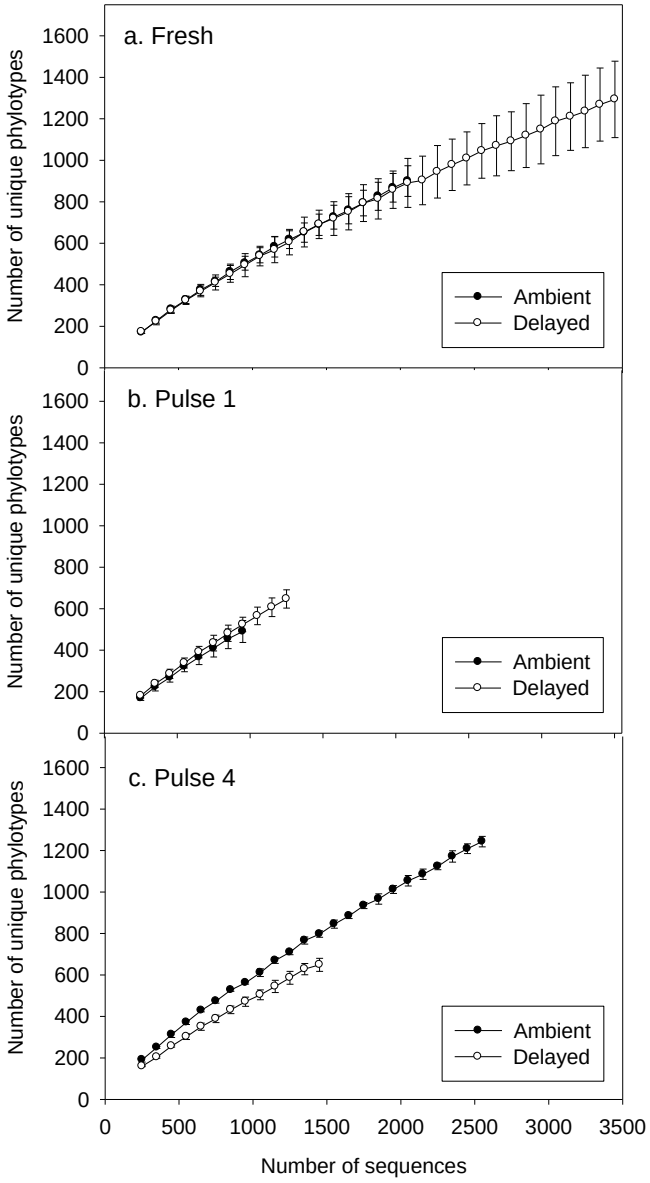

Figure 5.6. Rarefaction curves showing differences in Ambient and Delayed diversity in Fresh soil (a) after Pulse 1 (b) and after Pulse 4 (c) in the drying-rewetting lab incubation. Because many samples per group were averaged, the number of sequences per group was limited by the sample with the fewest number of sequences in that group

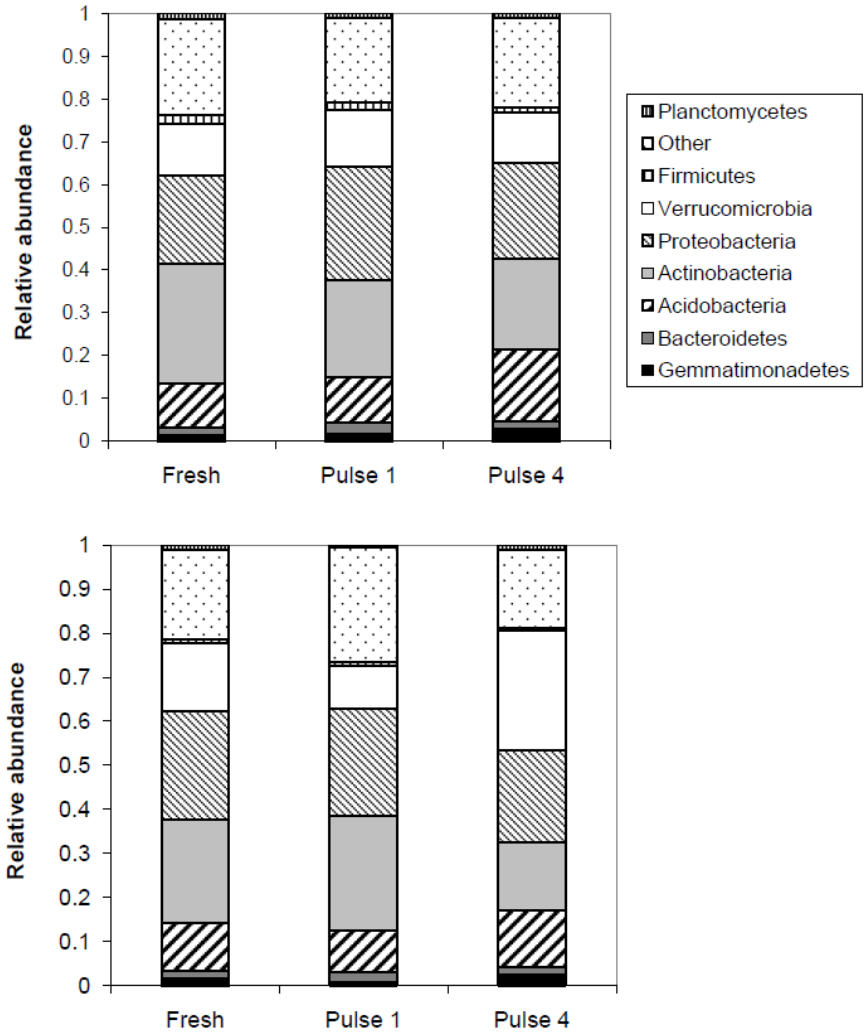

Figure 5.7. Relative abundance of the dominant Phyla in soils from Ambient (a) and Delayed (b) rainfall timing manipulations at different time points in a drying-rewetting lab incubation. Relative abundance is the abundance of a particular sequence relative to the total number of sequences in that sample.

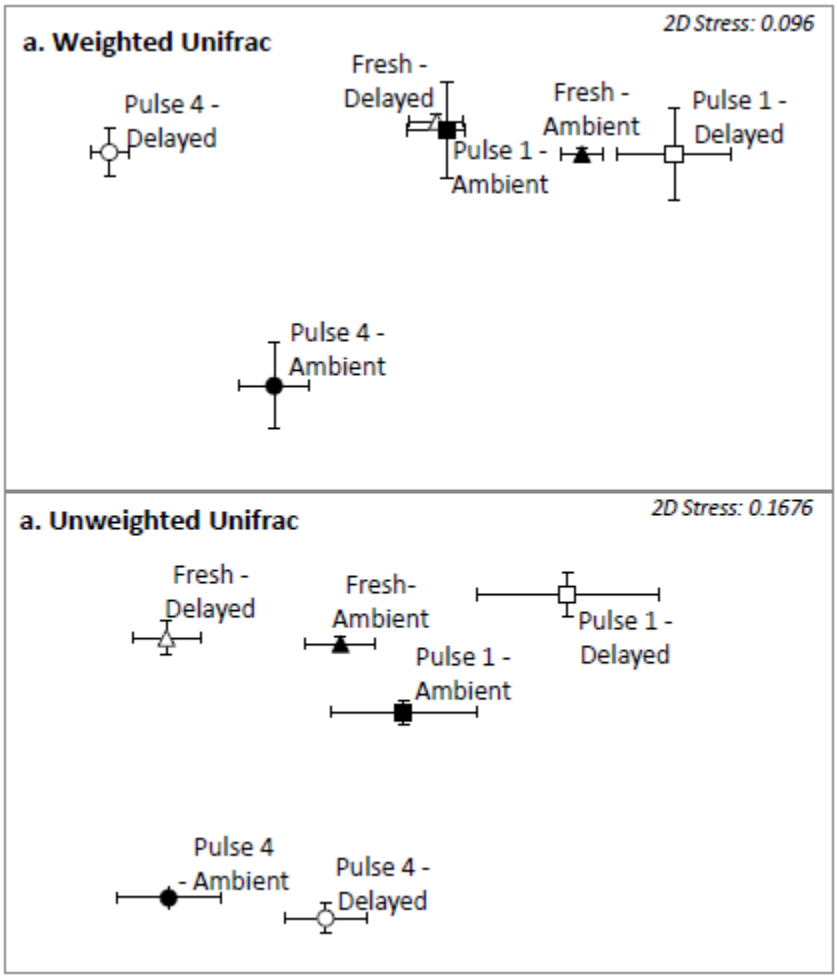

Figure 5.8. Bacterial community composition similarity among groups calculated from Weighted (a) and Unweighted (b) Unifrac distances by Non-metric multidimensional scaling. Symbol fill indicates field treatment (Ambient, filled and Delayed, open) and shapes indicate lab time point (Fresh, Pulse 1, and Pulse 4 as triangles, squares, and circles)

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Chapter 6: Summary and Conclusions

The primary objective of my dissertation was to improve understanding of how grassland ecosystems may respond to future precipitation regimes by examining the responses of biogeochemical cycles and microbial communities to shifts in rainfall. My major questions were

1. Are the relationships between soil carbon, soil nitrogen, and environmental factors the same across two similar environmental gradients in temperate grasslands of the US Great Plains and Inner Mongolia, China?
2. How are carbon and nitrogen linkages altered by long-term drought in the shortgrass steppe, and how does this affect drought recovery?
3. Does moisture niche partitioning drive shifts in microbial community composition under long-term drought in the shortgrass steppe?
4. Does a history of more extreme rainfall events in the tallgrass prairie alter the response of microbial communities to drying and rewetting?

In response to my first question, I found that a US grassland model overestimated soil carbon and underestimated soil nitrogen in Chinese grasslands. My results suggest that relationships among carbon, nitrogen, and environmental factors may differ across temperate grasslands. Specifically, these relationships were sensitive to changes in nitrogen deposition and historical land use, suggesting these or other factors may need to be considered to accurately describe biogeochemical dynamics in Chinese grasslands.

Second, I found that an 11-year drought can significantly alter biogeochemical and ecosystem dynamics in the highly drought-resistant shortgrass steppe. Soil inorganic nitrogen availability increased up to 4-fold in plots receiving 25% of summer precipitation. This accumulation of nitrogen under drought may explain the higher plant tissue nitrogen and N_2O flux observed under recovery. A more "open" nitrogen cycle that I observed following severe drought could affect the impact of drought on grassland ecosystems, as well as the timescale of recovery.

Soil microbial community composition was also altered by this 11-year drought manipulation in the shortgrass steppe, and these differences persisted even after communities were subject to the same moisture conditions for 36 hours in the lab. In this lab experiment, I also identified specific microbial groups that grew under a certain moisture levels, presenting evidence of moisture niche partitioning in microbial communities. However, this niche differentiation wasn't realized in the field; communities that grew under dry conditions in the lab were not similar to those that emerged under long-term drought plots. Overall, this work suggests that contrary to previous assumptions, microbial communities display legacies to long-term field treatments, and that although soil moisture has the potential to drive microbial community composition through niche partitioning, this factor may not always be the primary driver of long-term community composition.

In the tallgrass prairie, changes in the timing of precipitation also altered the composition of microbial communities, and precipitation history influenced how microbial communities responded to drying and rewetting pulses in the lab. Soils that had experienced more varied moisture regimes respired less than control soils under rewetting events, but the two soils functionally converged as they were subject to similar conditions. In contrast, microbial communities from the more extreme rainfall regime changed more with each moisture pulse, suggesting that a history of increased drying-rewetting did not make communities more resistant to this stress, and community composition did not provide a strong link to respiration.

Grasslands are strongly controlled by the availability of moisture, yet their fundamental properties have been shown to be resistant to drought stress. Despite this high tolerance to moisture stress, I have observed significant shifts in nitrogen pools and fluxes and microbial community composition under shifts in precipitation that mimic future climate scenarios. My results suggest that overall, changes in biogeochemical cycling as a result of shifts in soil moisture will play a large role in how grassland ecosystems will respond to precipitation variability, and the response of microorganisms to precipitation has the potential to influence these biogeochemical dynamics as well. These findings will be important for predicting grassland responses to new precipitation regimes. However, my work also shows that

precipitation is not the only factor controlling biogeochemical and microbial community dynamics in grasslands; microbial community composition was not primarily driven by precipitation changes under rainfall manipulations, and nitrogen deposition and historical land use played a greater role in biogeochemical dynamics in Chinese compared to US grasslands. Therefore, my work suggests that the impact of precipitation changes on grassland ecosystems will likely be influenced by interactions between precipitation and other environmental factors and by ecosystem legacies from previous precipitation regimes.