DISSERTATION

MICROBIAL RESPONSES TO PLANT FUNCTIONAL TYPES AND HISTORICAL RESOURCES ADDITIONS IN THE SHORTGRASS STEPPE

Submitted by

Eliana E. Bontti

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY ELIANA E. BONTTI ENTITLED *MICROBIAL RESPONSES TO PLANT FUNCTIONAL TYPES AND HISTORICAL RESOURCES ADDITIONS IN THE SHORTGRASS STEPPE* BE ACCEPTED AS FULFILLING IN PARTIAL REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Dr William Lauenroth

Dr Mary Stromberger

Dr Joseph von Fischer

Dr Ingrid Burke Advisor

Dr N. LeRoy Poff Department Head/Director

ABSTRACT OF DISSERTATION

MICROBIAL RESPONSES TO PLANT FUNCTIONAL TYPES AND HISTORICAL RESOURCES ADDITIONS IN THE SHORTGRASS STEPPE

Nutrient addition in rangelands is an appealing way to increase plant biomass and quality, but little is known about the long-term effects of these additions on soil microbial activity and nutrient cycling. In addition, microbial activity may be affected by plant functional types (PFT) through influence on the levels of inorganic nitrogen (N) and labile carbon in the rhizosphere. This is particularly important in the shortgrass steppe (SGS), where plants with the C3 or C4 photosynthetic pathway differ in phenology, which affects the timing of maximum N uptake and root exudate production.

To understand the effect of PFT (C3 and C4 species) and historical nutrient additions on temporal patterns of N partitioning between microbes and plants, I estimated seasonal trends in plant biomass and N content, microbial N, and soil N availability. In addition, I evaluated monthly emissions of the greenhouse gases CO_2 and N_2O, discriminating between fungal and bacterial production through incubations of soils under the influence of different PFTs and historical N additions. Last, I tested the effect of biosolid application on CO_2 and N_2O emissions from fungi and bacteria in SGS soils.

Seasonal trends in plant and microbial N concentration indicated that the two were synchronous during most of the plant growing season and both strongly influenced by precipitation. Plant functional type did not explain differences in microbial N and available soil N, but historical N amendments increased plant N content , decreased microbial N, and had no detectable effect on soil available N.

Fungi showed higher emissions of CO_2 and N_2O compared to bacteria in the SGS, whereas there was no difference in emissions between the two groups in the historically N amended plots. There were no effects of PFT on bacterial and fungal emissions of CO_2 and N_2O but high historical N fertilization resulted in increased CO_2 and N_2O emissions from bacteria.

Fungal emissions of CO_2 were higher than bacterial emissions in SGS sites compared to biosolid amended sites, but I detected no differences between microbial groups in N_2O emissions. CO_2 and N_2O emissions were higher in biosolid treated sites than non-treated SGS sites even 20 years after amendments ceased. Biosolid treated sites dominated by forbs showed higher CO_2 emissions compared to sites dominated by C3 grasses, while C3-dominated sites with high available inorganic N had higher N_2O emissions than C4-dominated sites.

In summary, historical N additions had long lasting effects on SGS by increasing plant biomass and N. Given that N additions to ecosystems are increasing worldwide, it may be important to evaluate the impacts of these changes in processes on ecosystems services that grasslands provide. My results suggest that high levels of nutrient additions have unintended consequences such us increased CO_2 and N_2O emissions, and in particular carbon additions through

biosolids increase fungal activity, which is also conducive to N_2O production. These additions have a profound impact, since the elevated greenhouse gas emissions and changes in microbial communities last at least 20 years after the amendment was carried out.

Eliana E. Bontti

Graduate Degree Program in Ecology

Colorado State University

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CHAPTER I: INTRODUCTION

There is well-documented evidence of the limitation of nitrogen (N) on terrestrial ecosystems (Vitousek and Howarth 1991). Studies of N biogeochemistry are interesting both because it is important to address the effect of anthropogenic N deposition on ecosystem processes, and because N cycling may reveal underlying processes that control ecosystem structure and functioning.

Nitrogen cycling involves four microbiological processes: nitrogen fixation, mineralization, nitrification and denitrification. Nitrogen fertilizers influence these processes, especially nitrification and denitrification, and lead to increased production of N_2O (Davidson et al., 2000). The classical paradigm of nitrogen cycling in terrestrial ecosystems (Schimel and Bennett 2004) assumes that plant production occurs only under favorable conditions of N availability, meaning net N mineralization by microbes. However, this paradigm is being transformed in light of new observations, for instance the fact that N immobilization occurs concomitantly with plant production in temperate ecosystems, such that systems with high plant N demand also have high microbial immobilization (Augustine and McNaughton 2004, Barrett et al. 2002; Giblin et al. 1991; Nadelhoffer et al. 1984). Thus, there is potential for competition between plants and microbes (Kaye and Hart 1997) that is likely in ecosystems with N limitation. In general, microbes outcompete plants in the short term, but in some cases the capability of plants to absorb organic nitrogen makes them better competitors in the short term (Kielland 1994; Raab et al. 1996). In other situations plants store N from previous seasons, and this makes them better competitors in the long term (Chapin et al. 1988).

One of the approaches used to assess the potential for competition between plants and microbes analyzes temporal trends in microbial and plant uptake during the growing season (Jaeger et al. 1999). In some of these studies, competition between plants and microbes is not likely because microbial immobilization occurs earlier or later in the growing season with respect to plant growth (Jaeger et al. 1999). In other studies the asynchrony between microbial mineralization and plant uptake suggests that excess mineralized N might be lost from the system by leaching or in gaseous form.

Plant community composition can be an important determinant of soil microbial processes through its influence on the levels of inorganic N and labile C in the rhizosphere (Grayston et al. 2004; Kourtev et al. 2003). Plant species and functional types differ in phenology, nutrient use efficiency, litter quality, and root exudates. These differences might alter microbial decomposition activities, microbial biomass C and N, fungal and bacterial activities, and potentially, the rate of greenhouse gas emissions from microbes (Wardle 2002).

C4 and C3 grasses in the shortgrass steppe differ temporally in their influence on N retention and net N mineralization within a growing season (Epstein et al. 1998), since they differ in phenology, and therefore in the time of maximum growth (Monson et al. 1986; Tieszen 1970), nutrient use efficiency (Brown 1978), water use efficiency (Monson et al. 1986), litter quality (Murphy et al. 2002), and production of root exudates (Klein et al. 1988). These characteristics might affect spatial and temporal patterns of microbial activity throughout the growing season, greenhouse gas emissions, and fungal to bacterial ratio.

Nitrogen additions in many cases alter plant community composition and structure (Huenneke et al. 1990; Lauenroth et al. 1978; Wilson and Tilman 1991), changes that affect plant below- to aboveground allocation, and substrate availability for microbial activities (Grayston et al. 2004). These changes might also change soil microclimate (Eviner et al. 2006), likely modifying N partitioning between plants and microbes, as well as microbial respiration and N_2O production.

Experimental N and water additions in the shortgrass steppe affect plant community composition and structure (Lauenroth et al. 1978), changes that last at least 25 years after the cessation of resource additions (Milchunas and Lauenroth 1995). Previous studies on the effect of historic resources additions on N cycling indicate that forbs, which replace grasses, are less nutrient-use efficient, and have low C:N ratios that increase nitrogen mineralization, thus creating a positive feedback between high soil available nitrogen and colonization by plant species that are less nutrient use efficient (Vinton and Burke 1995). Nitrogen additions in grasslands might also change fungal:bacterial ratios towards more bacterial dominated communities in fertilized environments (de Vries et al. 2006; Wardle et al. 2004). In addition, changes in plant community composition in response to N additions may affect microbial community structure and function (Kourtev et al. 2003). All these changes potentially alter timing and level of N uptake by the microbial biomass, as well as greenhouse gases released by microbes, processes that are poorly understood in the shortgrass steppe .

Since semiarid grasslands are N limited, biosolids have been utilized at low to intermediate rates to dispose of the material in terrestrial locations while increasing

aboveground biomass and potentially improving soil structure and fertility (Cuevas et al. 2000). Consequences of high rate biosolids applications are nitrate pollution of ground water (Gaggiani 1991), increased P (Linderman and Davis 2004), increased heavy metals (Leyval et al. 1997), and changes in plant community composition (Sullivan et al. 2006).

Such biosolid amendments can also increase metabolic quotient, the amount of respiration per unit of microbial biomass carbon (Barbarick et al. 2004; Garcia-Gil et al. 2004), increasing emissions of CO_2 even when microbial biomass remains constant. Other consequences may be increased denitrifier populations with a concomitant rise in cumulative N_2O emissions (Paramasivam et al. 2008). Hence biosolids application in the shortgrass steppe is a good case study to understand the effect of resources additions on microbial activities leading to N mineralization and greenhouse gases emissions.

The general objective of this dissertation is to evaluate the effect of plant functional type and historical resources additions on microbial processes including temporal patterns of N uptake, CO_2 and N_2O emissions, and fungal and bacterial emissions of these gases.

Specifically, this dissertation has three objectives:

1) The first objective is to evaluate how plants and microbes differ in the timing of maximum N uptake and the potential for competition between plants and microbes. In addition, I evaluate whether different plant functional types (C3 and C4 grasses, and exotic forbs) show differences in the microbial communities growing in

soils under them that may reflect the timing and level of N uptake. Last, I evaluate whether C3 grasses that grow in control plots differ from the same plant functional type in historical resource addition plots, in terms of their timing and level of N uptake. I also compare microbial biomass N timing and level under C3 grasses in control plots, with the microbial N under C3 grasses in historical resources additions

2) The second objective is to evaluate N_2O and CO_2 emissions from fungi and bacteria. I seek to assess the response of fungal and bacterial emissions of these gases underneath C3, C4 grasses and forbs.

3) The third objective of this dissertation is to compare N_2O and CO_2 emissions from fungi and bacteria as well as potential N mineralization from native shortgrass steppe, and from former shortgrass steppe where biosolids were applied twenty years ago that currently differ in the dominant plant community and soils characteristics.

Each subsequent chapter in this dissertation addresses one of these specific objectives. The final chapter summarizes conclusions and discusses the important implications of this research, particularly with respect to global change scenarios that might alter fungal to bacterial ratios, and affect biogeochemical cycles.

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CHAPTER II: NITROGEN PARTITIONING BETWEEN MICROBES AND PLANTS IN THE SHORTGRASS STEPPE

Introduction

Nitrogen (N) limits plant production in most terrestrial ecosystems (Vitousek and Howarth 1991), particularly in grasslands (Hooper and Johnson 1999; Lauenroth et al. 1978). Water additions dramatically increase the effect of N additions on plant community composition and structure in dry grasslands (Lauenroth et al. 1978), suggesting that there are important interactions between water and N availability. While the interactions of water and N have been studied in long-term manipulative experiments (Lauenroth and Dodd 1979; Lauenroth et al. 1978; Milchunas and Lauenroth 1995; Vinton and Burke 1995), little is known about their dynamic interplay under the natural conditions of seasonality and pulse-precipitation events.

Nitrogen and water additions in the shortgrass steppe (SGS), the driest of the native grassland types in North America (Lauenroth et al. 2008), cause changes in plant community dynamics that alter the phenology of N and water demand by plants. Historical N additions have resulted in the replacement of dominant slow growing plants by fast-growing species that are less nutrient use efficient (Lauenroth et al. 1978; Milchunas and Lauenroth 1995). The litter of these fast-growing, often annual plants is low in C:N ratios, which appears to increase N mineralization, thus creating a positive feedback between high soil available nitrogen and colonization by plant species that are less nutrient use efficient (Vinton and Burke 1995). These

changes in plant community composition and N availability might change soil microbial community composition (de Vries et al. 2006; Porras-Alfaro et al. 2007) and functioning (Corkidi et al. 2002), but the effect of these changes on the seasonal timing of microbial N uptake are not clear. Plant community changes under N additions in the SGS have been reverted by adding labile carbon which increased microbial immobilization, therefore suppressing exotic species growth and favoring native species growth (Burke, unpublished data). Knowing the seasonality of both plant and microbial N uptake would help us to understand the time of maximum N demand by these groups, and would help optimize restoration practices such as the addition of labile carbon to alter plant competition.

Given the lack of information about timing of peaks of N uptake by different plant functional types (PFTs) and microbes in the SGS, my goals were to examine monthly patterns of N in plant and microbial biomass during the growing season, and to assess whether historical N additions alters the timing and magnitude of N uptake by microbes and plants.

The diversity of PFT's in the shortgrass steppe, and the prevalence of soil organic matter suggests that there may be very interesting and important patterns of N competition and nitrogen use among these organisms across seasons (Fig. 2.1), with strongest N uptake by C3 plants (grasses and forbs) during cooler seasons (spring and fall). C4 plants, most active during warmer seasons, might exhibit greater N content during the summer, at a time when soil microbes have the highest activity because summer is the wettest season in the shortgrass steppe. My working hypothesis is that the potential for overlapping N use is maximum between C4

grasses and microbes during midsummer (Figure 2.1). As a consequence, I expect that changing N availability would reduce this level of competition during the summer in response to the suppression of C4 species in N amended plots.

Specifically I address the following questions:

- 1) How does N in plant and microbial biomass vary through the growing season?
- 2) Do C3, C4 grasses and annual forbs differ in the timing of maximum N uptake peak? Do these patterns affect maximum microbial N uptake?
- 3) Do N additions affect the timing and magnitude of plant and microbial N uptake?

Methods

Study area

I conducted the research in the USDA-ARS Central Plains Experimental Range (CPER, 40° 49' N latitude, 104° 46' W longitude), a shortgrass steppe site in northern central Colorado that is a National Science Foundation Long-Term Ecological Research site. Mean annual precipitation at the CPER is 321 mm, with 83% falling between April and September, and mean annual temperature is 8.6 °C (Lauenroth and Sala 1992). The CPER is characterized by highly variable precipitation during the growing season, but with predictable peaks during May and June, and a late summer dry period (Sala et al. 1992).

The vegetation of the area is typical of shortgrass steppe and is dominated by the perennial bunch grass *Bouteloua gracilis* (H.B.K.) Lag (blue grama), a C_4 grass. Other common grass species include the perennial C_3 species *Stipa comata* (Trin and Rupr.) (needle-and-thread grass), and *Pascopyrum smithii* (Rybd.) (western wheatgrass). The vegetation also includes the half-shrubs *Gutierrezia sarothrae* (Pursh) Britt. & Rusby, and *Artemisia frigida* Willd.; the forb *Sphaeralcea coccinea* (Pursh) Rydb.; and the succulent *Opuntia polyacantha* Haw. Total vegetative basal cover is 25-35% (Milchunas et al. 1989).

I sampled plants and soils on an area that was protected from cattle grazing since 1968, and is part of a historical fertilization and irrigation experiment that ended in 1974 (Lauenroth et al. 1978). The original experiment was a factorial combination of four treatments with two replicates: control (no resource addition), water (W), nitrogen (N), and nitrogen+water (NW) additions. Each replicate was located on a 1-ha plot. I compared only the original NW, which I hereafter will call historical NW plots, and the control (C plots). I focused on the historical NW plots because after addition of resources stopped, the plant community in the treated plots reached a new state in terms of the relative abundance of species which persisted into the present time (Milchunas and Lauenroth 1995). Water and nitrogen were applied from 1970 to 1974. The amount of water added per year ranged from 458-707 mm and the addition of nitrogen ranged 100-200 kg ha^{-1} $year^{-1}$ (Lauenroth et al. 1978). After water and N additions stopped in 1974, except for some vegetation and soil sampling, the plots have been unaltered.

My plant functional type (PFT) treatments were patches of native C3 and C4 grasses in C plots; and patches of C3 grasses and invasive forbs in NW plots. My plot treatments were C plots and historical NW, so that I might assess the long term consequences of those treatments. The only PFT that grows in both plots is C3 grass, and therefore, when I compare plots, I am comparing patches of C3 grasses in C plots with patches of C3 grasses in NW plots. There were no significant differences in soil texture among plot treatments or patches (data not shown). From June to October 2006 I sampled three patches of each dominant functional type within each treatment replicate.

I took concurrent measurements of: 1) plant biomass and plant N content; 2) microbial biomass N, and 3) soil N availability as indicated by potential net N mineralization incubations and plant root simulator (PRS) probe–ion exchange membranes (Western Ag Innovations, Inc., Saskatoon, Canada), as described below.

These measurements allowed me to track the moment of maximum plant and microbial N, and observe if they occur at the same time during the growing season. If this occurs when available nitrogen is at minimum concentration in the soils, there might be potential for competition that should be further explored.

Plant biomass and N

I measured live aboveground biomass by clipping 0.25-m^2 quadrats under each plant functional patch in each treatment monthly from June to October 2006. I removed previous and current year's standing dead from current year's live material.

Biomass samples were dried at 50°C for a week, weighed, ball ground, and analyzed for total N content on a LECO CHN-1000 analyzer (St. Joseph, MI, USA). I combusted 0.2 g subsample in a muffle furnace at 600°C for 5 h to determine ash content (ranged from 8% to 14% of sample mass). The N content of the aboveground live biomass is presented on an ash-free basis (g N m^{-2}).

I obtained belowground biomass estimates by using a soil core of 5 cm diameter by 5 cm depth directly under the crown material of each plant functional patch in each treatment monthly from June to October 2006. I collected three cores per plant functional patch per treatment per month. I dried the collected samples at 50°C for a week to minimize decomposition during the time between collection and processing

I mixed dried samples with tap water and decanted floating roots into a 0.147-mm mesh sieve. I repeated the flotation procedure until no more visible roots floated to the surface. I thoroughly washed the material collected in the sieve with tap water, dried the sample at 50°C for 2 days, handpicked and removed any material clearly not root derived, and weighed the final product. I ball ground the samples and determined ash content and N concentrations by using the same procedures as for the aboveground live biomass samples. Ash content ranged from 30 to 90%. I calculated belowground biomass and N values in g m^{-2} by multiplying the ash corrected %N values by the ash-corrected belowground biomass values obtained from the total root sample weight and core size.

Microbial biomass N and N mineralization

I obtained soil samples by using a soil core of the same characteristics as for root sampling and I sampled under the crown of the plants that were clipped for aboveground biomass estimations. I collected three soil cores per plant functional type patch in each treatment immediately after plant clipping, monthly from June to October 2006. The three cores were combined and homogenized into a composite sample. I placed the soils immediately in coolers and once in the laboratory they were kept at 4°C for a week before use. I sieved fresh soils through a 2 mm mesh sieve to remove plant material and fragments greater than that size. I then weighed the soils, mixed them and subsampled for 3 analyses: soil moisture, microbial biomass C and N, and potential N mineralization.

I used a subsample of 10 g fresh soil for gravimetric determination of soil moisture content at the time of sampling, and a different subsample of 12 g dry soil for extractions with 60 ml of 2 N KCl for 30 minutes in an orbital shaker to measure initial inorganic N (Mulvaney 1996). The extracts settled for 10 minutes, after which I filtered them through Whatman # 40 paper and kept them frozen until analyzed for nitrate and ammonium on an Alpkem Autoanalyzer (Pulse Instruments Ltd., Saskatoon, SK). I followed the same procedure after the soil incubation described below, and I estimated potential N mineralization as the difference between initial and final inorganic N of the soil (Hart et al. 1994).

I used the substrate induced respiration-inhibition method (SIRIN) of Anderson and Domsch (1975) modified by Johnson et al. (1996) for dry soils to determine C in microbial biomass, with determinations of optimal soil moisture

content, glucose concentrations, and incubation time in preliminary incubations according to the criteria of Anderson and Domsch (1975). I placed 12 g of dry soil in small beakers, moistened to 60 % field capacity, mixed with 1 mg of glucose per gram of dry soil, and incubated for 12 hours in sealed incubation bottles in the dark at 25 °C. After the incubation air samples were extracted with 25 ml syringes and analyzed for CO_2 concentration on a Shimadzu GC14-B gas chromatograph (Shimadzu Scientific instruments, Columbia, MD) fitted with an FID and ECD (electron capture detector), with a temperature program of 325 °C detector, and 40 °C column. I used certified N_2O standards (Matheson TRI-GAS, Fort Collins, CO) for calibration.

Microbial biomass C was calculated according to the equation of Anderson and Domsch (1978) where: Biomass C (μg g^{-1} dry soil) = (μl CO_2 g^{-1} dry soil h-1) x 40.04. I estimated N in microbial biomass indirectly by estimating C:N ratio of 8.5 for the microbial community in the shortgrass steppe. I considered 75 % fungi and 25 % bacteria, percentages based on estimation of microbial biomass through PFLA (McCulley and Burke 2004), and I assumed that fungal C:N is 10, whereas bacterial C:N is 4 (Sylvia et al. 2004). I therefore divided the carbon values by 8.5.

Soil available N

I monitored monthly available mineral N under each PFT using Plant Root Simulator (PRS) probe–ion exchange membranes (Western Ag Innovations, Inc., Saskatoon, Canada), which measures N available for plants roots (http://www.westernag.ca/innov/prsprobe.php). I buried a pair of probes (one anion

and one cation probe) per plant patch per plot, which I left in place for 30 days, extracted them from the soil, extracted inorganic N from the probes, and regenerated them, after which I took them back to the plots, and placed them under the same patch for the following 30 days. I repeated this procedure from May 26 to October 27. I extracted the probes with 25 mL of 0.5 M HCl for one hour in a zip lock bag, in a shaker, and analyzed the extractant for NH_4^+ and NO_3^- using an Alpkem Autoanalyzer (Pulse Instruments Ltd., Saskatoon, SK). Results are reported as μg 10 cm^{-2} probe surface area over the 30 day burial period for each month.

Statistical analyses

I evaluated seasonal patterns of plant N (aboveground and belowground), soil N, and microbial biomass N using a mixed model analysis of variance in SAS statistical software (SAS Institute Inc 1989). I tested for differences among treatments (fix effects) of C and NW, and among PFTs (C3 grass, C4 grass, forbs). I ran the model multiple times, to test separate hypotheses. I used the same analysis with the same fix effects for root biomass and N, microbial N, soil N and potential N mineralization, with each variable analyzed separately. I incorporated a repeated measures design to handle the monthly sampling dates of data collected at the same sites, which allowed me to test for significant changes on N in the different pools through time (i.e. test whether observed peaks in N uptake represent significant changes in time).

First, I tested for the differences between C3 and C4 grasses by running the model for only C plots, using fixed effects of the two PFTs with two levels, C3 and C4 grass (recall that the NW plots did not contain C4 grasses).

Second, I tested for differences among the plant functional types in the NW plots, using the fix effects of PFTs with two levels, C3 grasses and forbs. Third, I tested the effects of NW and C treatments by using the fix effects of plot treatment with two levels, C plots and NW plots, and compared the only plant functional group that grows in both plots (C3 grasses).

I used a least significant difference mean separation test when significant main effects occurred. All data were transformed as needed to fit the normality and homogeneity of variance assumptions of the applied statistical test, and the level of significance of all tests was determined as a p-value < 0.05.

Results

Plant and microbial N: Seasonal patterns

When one compares the predicted trends in plant N (Figure 2.1) with the observed trends (Figures 2.2 and 2.3), it is possible to see that C4 grasses behaved as predicted in August, but contrary to predictions in June. C3 grasses showed the predicted peak in September, but they also peaked in July, which was not predicted, since these are cool season species.

Aboveground biomass and N of C4 species peaked twice during the growing season, first in late spring (June) and later in midsummer (August) (Figures 2.2a and 2.3a). C3 grasses did not show significant temporal differences in biomass (Figure 2.2a), but aboveground N showed peaks that mirrored those of C4 grasses, i.e. the significant peaks occurred in early summer (July) and late summer/early spring (September) (Figure 2.3a).

Belowground biomass was highly variable, therefore precluding any generalization about temporal trends during the growing season (Figure 2.4) but belowground N showed peaks that were different from predicted. C4 grasses and forbs did not show peaks of belowground N (Figure 2.5a and b). Belowground N of C3 grasses in C plots peaked in September, whereas it peaked in August and September in the historical NW plots (Figure 2.5c).

Microbial biomass N (Figure 2.6) did not fluctuate temporally as much as predicted (Figure 2.1). There was a significant increase in microbial biomass N in July, respect to June, and it was concomitant to increased precipitation (Figure 2.9).

Microbial N kept constant during August and September, and declined in October parallel to low temperatures.

Plant functional type influence on nitrogen partitioning

Even though PFTs differed temporally in above- and belowground biomass, as well as above- and belowground N (Figures 2.2 to 2.5), microbial biomass did not always responded to these variations (Figure 2.6). For example, in June C4's aboveground N peaked (Figure 2.3a), but microbial biomass N under this PFT was at its lowest level (Figure 2.6a). C3 grasses showed two peaks in aboveground N in C plots, parallel to high levels of microbial N, but in September, microbial biomass was high regardless of low C3's aboveground N (Figure 2.3a). Nevertheless, belowground biomass and N in September was high for this PFT, which might have influenced microbial N for that month.

Despite differences between PFTs in above- and belowground N, there were no differences in microbial biomass N that could be attributed to PFT. For example, C3 aboveground biomass and N was higher thanC4 grasses in July and September (Figures 2.2a and 2.3a), yet microbial biomass N under C3 did not differ from microbial biomass N under C4 patches (Figure 2.6a). Belowground biomass and N was higher for C3 grasses compared to C4 grasses in C plots in September, but no differences were detected between microbial biomass N under each of these plant PFTs.

Similar disparities occurred in NW plots, where aboveground biomass and N of forbs was higher than C3 grasses in July (Figures 2.2b and 2.3b), but there were no

differences in microbial biomass N that could be attributable to PFTs (Figure 2.6b). Belowground biomass and N of C3 grasses was higher than forbs from June to September (Figures 2.4b and 2.5b), but these differences were not observed in microbial biomass N (Figure 2.6b).

Even though I observed some differences in microbial biomass N under different PFT they were not the expected differences based on plant biomass and N. For example, in October C3 aboveground biomass and N were higher than C4's biomass and N (Figures 2.2a and 2.3a), but the opposite occurred in microbial N (i.e. it was lower under C3 than under C4 grasses, Figure 2.6a). There were no differences in aboveground biomass and N between C3 grasses and forbs in NW plots (Figures 2.2b and 2.3b); yet, microbial N under forbs was higher than under C3 grasses (Figure 2.6b).

In summary, there were no consistent differences in soil microbial N that can be attributed to PFTs. The timing and magnitude of microbial N seemed to be independent of plant trends; though some differences were evident in microbial N under different PFTs, they are not directly explained by PFTs trends.

Potential N mineralization rates varied between -0.3 g m^{-2} h^{-1} (net immobilization in October under C3 grasses in historical NW) to 0.15 g m^{-2} h^{-1} in C and historical NW plots (Figure 2.7). I did not detect differences in potential N mineralization that could be accounted for by plant effect, except in August, when it was higher in soils under C3 grasses respect to forbs in NW plots (Figure 2.7b). Despite the apparently homogeneous mineralization potential under different PFTs, soil available N as measured with the root simulator probes showed several differences attributed to

PFTs. In July and October, soil available N was higher under C3 grasses than under C4 grasses (Figure 2.8a), while in September it was higher under forbs than under C3 grasses (Figure 2.8b).

Effects of historical resources addition

Timing of production of aboveground biomass of C3 grasses in historical NW was different from C3 grasses in C plots (Figure 2.2c). There was no peak of aboveground biomass in C plots, whereas there were two peaks in historical NW plots, one in July and the other in September. Nevertheless, the historical resources additions had no effect on aboveground N dynamics, which peaked in C and NW plots in July and September.

Differences between C and NW treatments in plant biomass and N were consistent in July and September (Figures 2.2 c and 2.3c), when aboveground biomass and N were higher in NW than in C. Aboveground N was higher in NW plots with respect to C plots in July (Figure 2.3c), difference that was not observed in aboveground biomass. Belowground biomass was higher in historical NW respect to C in October (Figure 2.4c), and belowground N was higher in NW plots compared to C plots only in August (Figure 2.5c).

I did not detect peaks in plant belowground biomass, presumably because of high variability in the data (Figure 2.4c). Belowground N peaked in August and September in the historical NW and only in September in the C plots (Figure 2.5c). Microbial biomass N showed the same behavior in C and historical NW plots: there

was an increase from June to July, and a decrease in October with respect to previous months.

Microbial biomass N was lower in the historical NW plots compared to the C plots in June, July and August, a trend that is the opposite of plant N, in particular aboveground N (Figure 2.6c). Potential N mineralization was only different between treatments in October, when it was less negative in C compared to NW (Figure 2.7c), while soil available N was higher in C than in NW (Figure 2.8c) only in September.

Discussion

Plant and microbial N: Seasonal patterns

Unlike other ecosystems (Jaeger et al. 1999) plants and microbes had no distinct temporal pattern of nitrogen concentrations in the shortgrass steppe LTER. Most plant and microbial activities seemed to be triggered by an increase in precipitation, they continue throughout the warm wet months, after which they decreased, presumably in response to low temperatures in mid fall. It is important to have into account that a deeper analysis at wider spatial and temporal scales would be useful to extrapolate the trends observed in this study to the shortgrass steppe as an ecosystem.

Aboveground N of C3 grasses in the C plots and historical NW showed a minimum value in August, the same month when C4 grasses were at a peak of aboveground biomass and N, which indicates the ability of C4 grasses to cope with

high temperature in August, thus increasing biomass and N uptake, whereas C3 grasses and forbs decreased both in biomass and N uptake. Although aboveground N of C3 grasses showed a minimum in August, belowground N peaked during this month in the historical NW and C plots, and also in September in the NW only. This PFT seems to reallocate N to roots, as inferred by maximum aboveground N concomitant with minimum belowground N. This reallocated N seemed to be used in September in the C plots, when they showed a second peak of aboveground biomass and N. These temporal patterns of N allocation between plant parts seem to vary between years, because even though previous experiments showed a decreased in N allocation to shoots in C3 grasses, there were no increases in N allocation to roots or crown (Epstein et al. 1998).

Microbial biomass responded to increased precipitation in July, therefore immobilizing N, which seems to be the general pattern in temperate grasslands (Dell et al. 2005; Jackson et al. 1989). Microbes also seemed to have a rapid turnover, which was reflected in the peak of soil available N in July. During this month plant activity was still low, which might had kept soil N high, and in August available N rapidly decreased, when N mineralization was offset by plant growth and uptake up to September. Nitrogen immobilization in the fall might be in response to a change in carbon source from labile to more recalcitrant forms that might cause nitrogen limitation for microbes (Barrett and Burke 2000). The use of potential N mineralization could have led to an overestimation of mineralization because of controlled conditions in the laboratory, but Epstein et al. (1998) found similar

seasonal trends in N mineralization in the shortgrass steppe, even though they were measuring *in situ* net N mineralization.

The overall seasonal pattern indicated that water rather than nitrogen was a factor triggering most biological activities in the shortgrass steppe. The seasonal change in nitrogen peaks also supports the idea that there is a tight cycling among pools with rapid and intermediate turnover, with the soil and microbial biomass as the first retention pool and subsequent uptake by plants (Barrett and Burke 2002). The exception to this general trend were C4 grasses in control plots, as well as C3 grasses and forbs in NW plots that showed high levels of aboveground biomass and N even though soil inorganic N was low at the onset of the growing season. Clark (1977) and Epstein et al (1998) found that C4 species in the shortgrass steppe showed higher N allocation to crowns and roots compared to other grasses at the end of the growing season. The potential for N storage and for a rapid response to pulses of precipitation by active root growth (Lauenroth et al. 1987), gives an advantage over other PFTs, whereas historical resources additions might have determined high levels of N in C3 and forbs in NW plots.

Previous studies on N partitioning between plants and microbes aimed to test the hypothesis of potential for competition between plants and microbes. Competition is an interaction whereby two organisms are limited by and utilize the same pools of the same resource (Tilman 1982). My results did not support the idea that microbes would assimilate enough N early in the growing season to limit plant growth; on the contrary, C4 grasses showed a peak in aboveground biomass even when microbial N was low. The ability of plants to store N gives them an important competitive

advantage over soil microorganisms in storing N over the long term (Kaye and Hart 1997), and C4 trends in the shortgrass steppe suggest that there is low potential for competition for N between plants and microorganisms.

Different PFTs peaked in biomass and N at different times during the growing season (C3 grasses in July and September, forbs in July, and C4 grasses in June and August). This suggests that combining all PFTs together, there was overlap between plant and microbe N utilization. However, even when soil N was low from August to October, N peaks in plant biomass did not seem to have a negative impact on microbial biomass N.

The lower biomass of C4 in control treatments concomitant with biomass peaks of C3 grasses suggests that competition between plant PFTs rather than between microbes and plants might limit nitrogen available for plant production (Dell et al. 2005). The different temporal trend of C3 grasses in historical NW, in the absence of C4 grasses supports this idea.

Plant functional type influence on nitrogen partitioning

Unlike other grasslands (Bezemer et al. 2006), microbial biomass N did not respond to aboveground N differences among plant functional types, which supports the idea that in this semi-arid environment vegetation cover is more important than plant functional type for explaining spatial variability in nitrogen mineralization (Vinton and Burke 1995). Plant functional type affects the timing of certain soil processes, but they rarely affect the magnitude of microbial N, N mineralization, and soil N, trends that were found along different years and with different methodologies

in the shortgrass steppe (Epstein et al. 1998; Vinton and Burke 1995). My study attempted to characterize the whole growing season in terms of plant and microbial N concentrations, to detect potential changes not detected in previous studies due to sampling of one or some month during the growing season.

The only month when I detected differences in microbial N was October, when plants senesced. The higher microbial biomass N under C4 grasses respect to C3 grasses could have been due to enough N present in C4 residues for microbes to immobilize in proportion to their requirements and mineralize the rest. Other indices of above-and belowground litter quality such as Lignin:N may be important to explain these differences in potential net N mineralization rates.

Microbial biomass N was also higher under forbs compared to C3 grasses in the NW plots in October, which is not consistent with the low nutrient use efficiency of forbs, thus low mineralization due to high C:N is unlikely. The rapid N mineralization and biomass growth while forbs are active in July, might have gradually limited nitrogen available for microbes from August to October, after forbs senesced.

Effects of historical resources additions

Nitrogen additions affected plant biomass and N, but the effects were not consistent during the growing season. When differences between plots treatments were detected, plant biomass and N was higher in historical NW plots, which indicate the potential long-term effect of a disturbance like nitrogen addition in the shortgrass steppe LTER. This legacy effect is a consequence of a very high rate of

experimental addition, respect to the actual wet and dry deposition in this area which is lower than 4 Kg ha $^{-1}$ yr $^{-1}$ (Holland et al., 2005). Even the temporal patterns in biomass were affected by N additions since aboveground biomass was constant in C plots, whereas there were two peaks in historical NW plots in July and September. Nevertheless, control and historical resources additions had similar effects on temporal patterns of aboveground N, which peaked in July and September.

Even though there were differences in temporal patterns of plant biomass versus N, these differences did not affect temporal patterns of microbial biomass N, which were the same in C and historical NW plots: there was an increase from June to July, and a decrease in October with respect to previous months. Therefore, the potential for higher availability of N in NW did not change the time of maximum microbial activity, and rather precipitation in July triggered microbial activity.

Interestingly, C3 grasses showed higher aboveground biomass and N levels in historical NW compared to controls. These differences could potentially be a consequence of the suppression of C4 grasses in NW plots, and therefore the absence of competition between these two plant functional types. The peak of belowground N at historical NW plots in August that did not occur in C plots for the same month seems to support this idea.

Unlike the temporal trends, the level of microbial N differed between plot treatments. Historical resources addition showed lower microbial N than the control from June to August. Plant above- and belowground N was lower in C plots compared to historical NW plots, and C:N aboveground was three times higher in C plots than in NW plots (data not shown). These differences might have determined N

limitations for microbes in the C plots, therefore favoring N sequestration in these plots. Another potential explanation for these results is changes in microbial community composition that might lead to higher levels of N sequestration (de Vries et al. 2006).

Surprisingly, I did not find consistent differences between control and nitrogen treatments in terms of potential mineralization and soil available nitrogen, and the latter might be due to the active uptake by forbs which have low nutrient use efficiency and C3 uptake, especially in August and September. Potential N mineralization was only different between treatments in October, when it was less negative in C plots compared to NW plots, indicating less immobilization. Above- and belowground C:N ratio was higher in NW than in C plots in October (data not shown), which might have created N limitation conditions for microbes leading to potential N immobilization.

Soil available N was higher in C plots than in NW plots only in September, but net N mineralization was not different between treatments. In September C3 grasses were the only active plant functional group in C, whereas C3 grasses and forbs were active in the historical NW plots. Therefore these trends might be in response to low plant N uptake in C plots compared to NW plots in response to lower plant activity. It would be interesting to further study if there are changes in microbial community composition or activity that determine similar levels of N mineralization and biomass N uptake independently of the amount of available nitrogen and to complement this analysis with in situ N mineralization assay.

Conclusions

Nitrogen uptake by plants and microbes overlapped in the shortgrass steppe, suggesting a potential for plant-microbial competition. Plants showed the potential for reallocation from shoots to roots during the growing season, as well as the potential for growth even when soil available N was low, indicating that plant storage of N is strategy for competing with microbes. Microbes are water limited, as predicted, and as soon as microbes are activated by high precipitation levels, net N mineralization and N availability in soils increase, and that N is rapidly utilized by plants. There are no periods of N mineralization that are not accompanied by plant utilization, which suggests that the potential for losses of N from the system are low. Furthermore, there is potential for microbial retention of N when plants senesced, which makes loss of N from the system unlikely. Plant functional types showed no effect on microbial N immobilization, whereas historical resources additions showed long term effects on plant and microbial N, by increasing plant biomass and N and decreasing microbial N storage.

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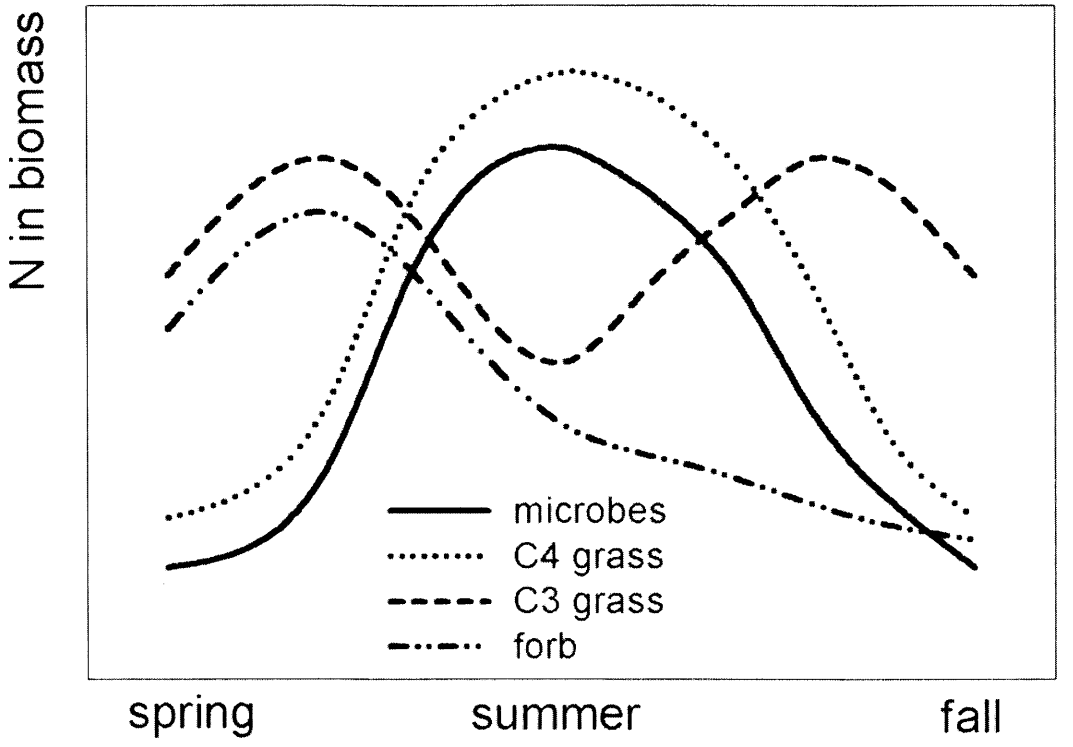

Figure 2.1: Conceptual diagram of expected temporal pattern in plant (for three different plant functional types) and microbial biomass N during the growing season in the shortgrass steppe based on temperature and moisture limitations.

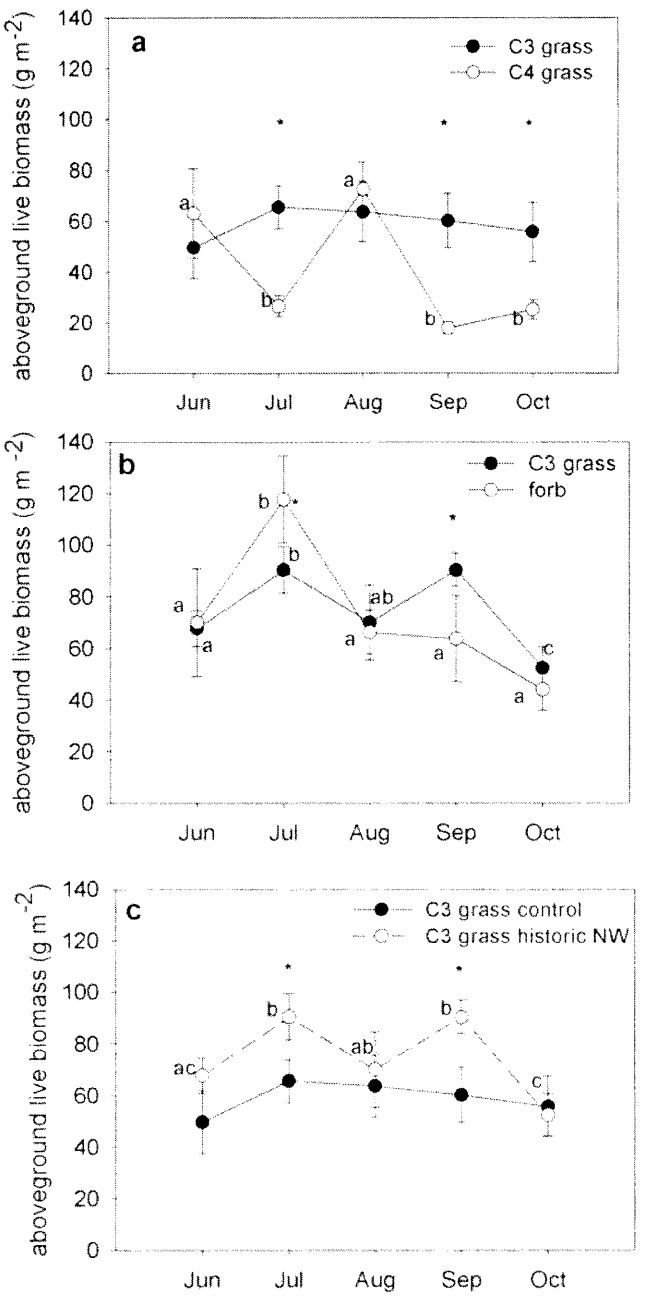

Figure 2.2: Monthly patterns of aboveground plant biomass in 2006 at the shortgrass steppe LTER of **a**: C3 and C4 grasses in control plots (C plots), **b**: C3 grasses and forbs in historical NW plots (historical nitrogen and water addition from 1970 to 1974), and **c**: C3 grasses in C plots compared to C3 grasses in NW plots. Vertical bars represent ± SE for n = 6. Asterisks mean significant differences between two plant functional types (a and b) or between treatments (c) within one month. Values designated with different letters indicate significant differences among months for a single plant functional type (repeated measures ANOVA). Comparisons were made through LSD multiple-range test ($p<0.05$).

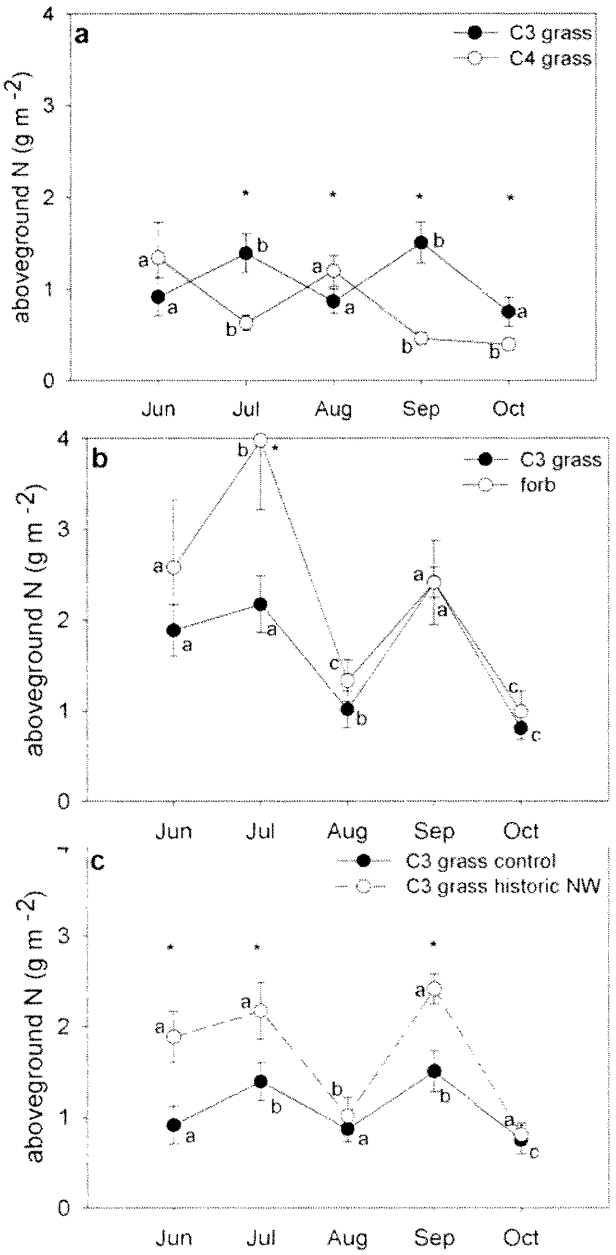

Figure 2.3: Monthly patterns of aboveground plant nitrogen at the shortgrass steppe LTER of a: C3 and C4 grasses in control plots (C plots), b: C3 grasses and forbs in historical NW plots (historical nitrogen and water addition from 1970 to 1974), and c: C3 grasses in C plots compared to C3 grasses in NW plots. Vertical bars represent ± SE for n = 6. Asterisks mean significant differences between two plant functional types (a and b) or between treatments (c) within one month. Values designated with different letters indicate significant differences among months for a single plant functional type (repeated measures ANOVA). Comparisons were made through LSD multiple-range test ($p<0.05$).

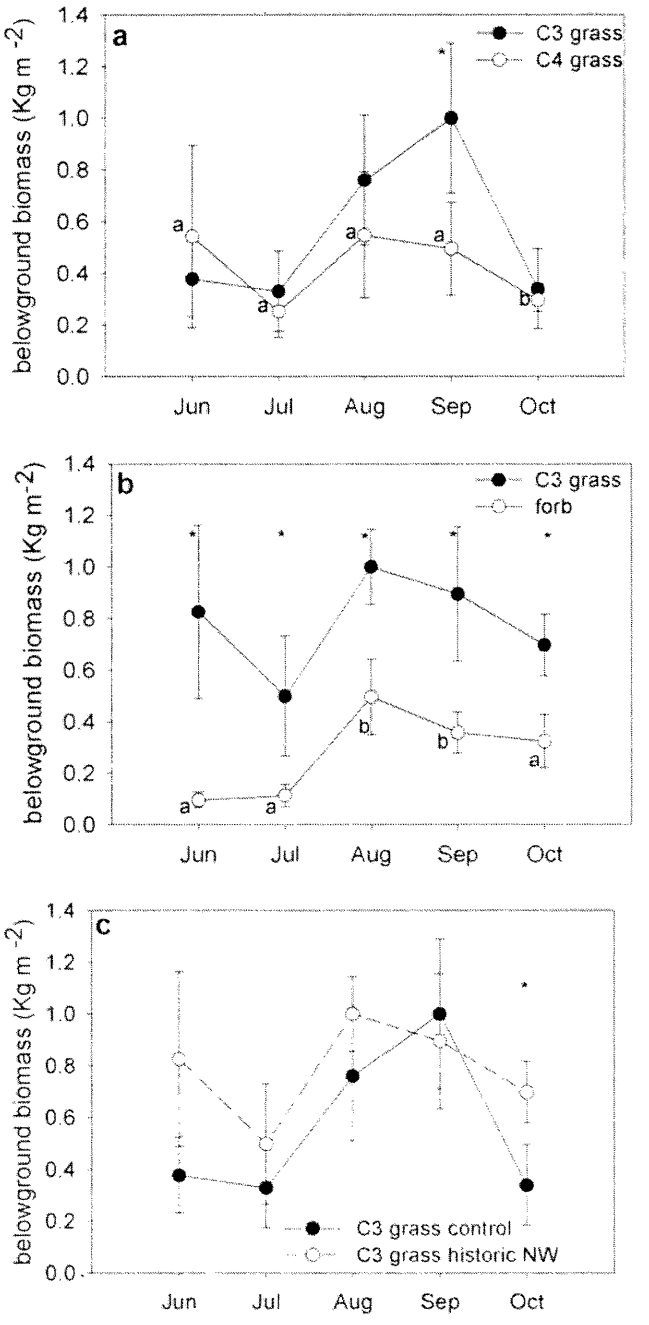

Figure 2.4: Monthly patterns of belowground plant biomass at the shortgrass steppe LTER of a: C3 and C4 grasses in control plots (C plots), b: C3 grasses and forbs in historical NW plots (historical nitrogen and water addition from 1970 to 1974), and c: C3 grasses in C plots compared to C3 grasses in NW plots. Vertical bars represent ± SE for n = 6. Asterisks mean significant differences between two plant functional types (a and b) or between treatments (c) within one month. Values designated with different letters indicate significant differences among months for a single plant functional type (repeated measures ANOVA). Comparisons were made through LSD multiple-range test ($p<0.05$).

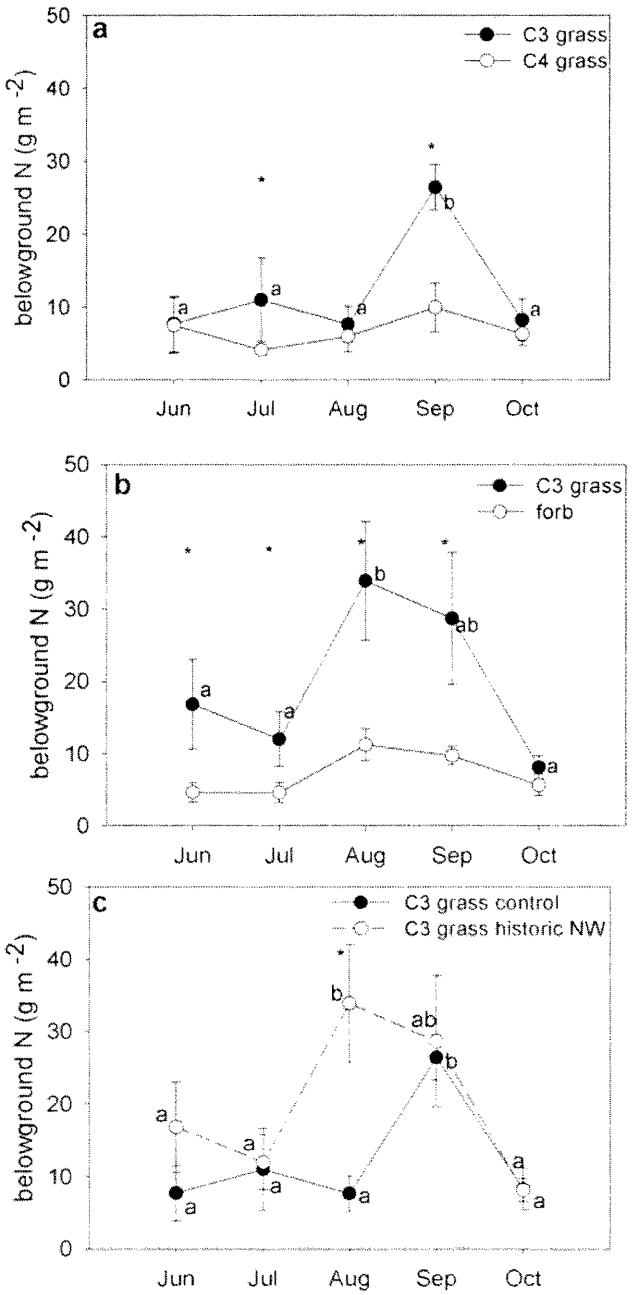

Figure 2.5: Monthly patterns of plant belowground total N content at the shortgrass steppe LTER of a: C3 and C4 grasses in control plots (C plots), b: C3 grasses and forbs in historical NW plots (historical nitrogen and water addition from 1970 to 1974), and c: C3 grasses in C plots compared to C3 grasses in NW plots. Vertical bars represent ± SE for n = 6. Asterisks mean significant differences between two plant functional types (a and b) or between treatments (c) within one month. Values designated with different letters indicate significant differences among months for a single plant functional type (repeated measures ANOVA). Comparisons were made through LSD multiple-range test ($p<0.05$).

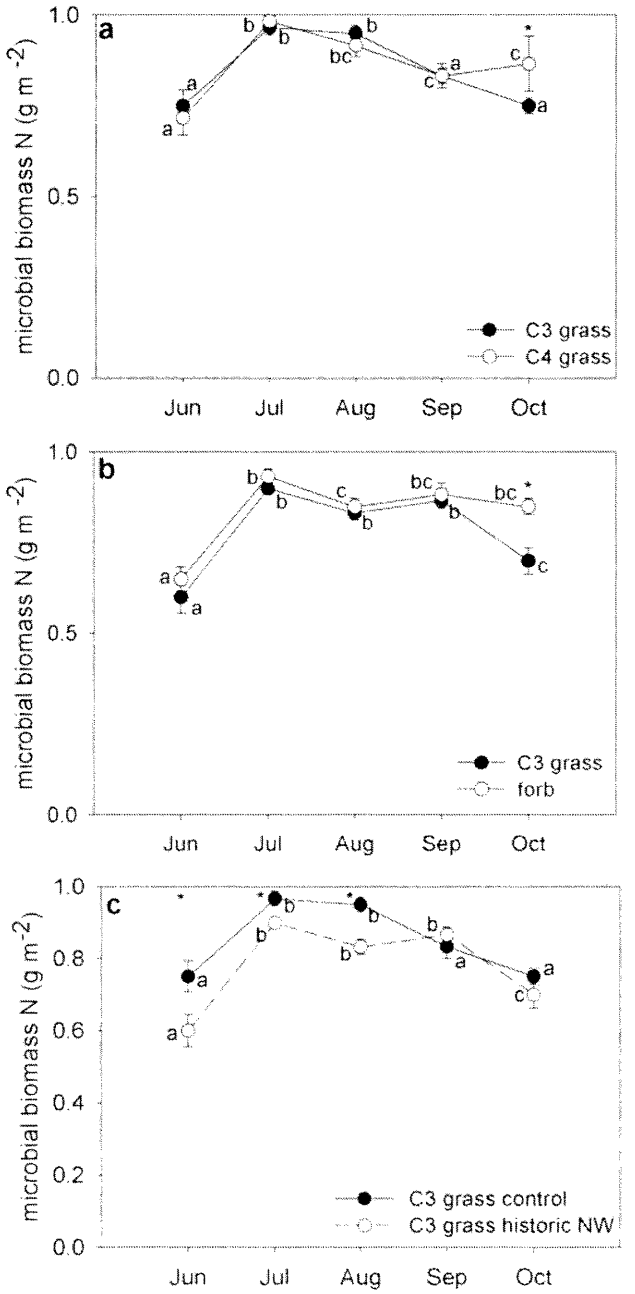

Figure 2.6: Monthly patterns of N in microbial biomass growing in soils of the shortgrass steppe LTER under a: C3 and C4 grasses in control plots (C plots), b: C3 grasses and forbs in historical NW plots (historical nitrogen and water addition from 1970 to 1974), and c: C3 grasses in C plots compared to C3 grasses in NW plots. Vertical bars represent ± SE for n = 6. Asterisks mean significant differences between two plant functional types (a and b) or between treatments (c) within one month. Values designated with different letters indicate significant differences among months for a single plant functional type (repeated measures ANOVA). Comparisons were made through LSD multiple-range test ($p<0.05$).

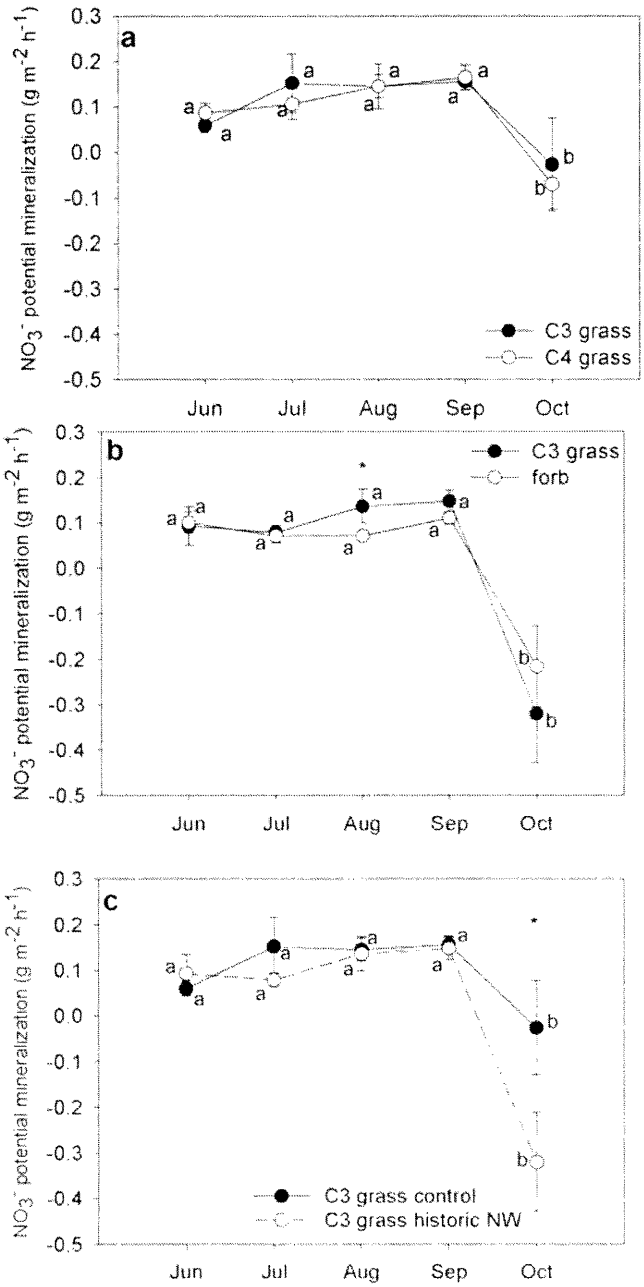

Figure 2.7: Monthly patterns of potential N mineralization in the shortgrass steppe LTER under a: C3 and C4 grasses in control plots (C plots), b: C3 grasses and forbs in historical NW plots (historical nitrogen and water addition from 1970 to 1974), and c: C3 grasses in C plots compared to C3 grasses in NW plots. Vertical bars represent ± SE for n = 6. Asterisks mean significant differences between two plant functional types (a and b) or between treatments (c) within one month. Values designated with different letters indicate significant differences among months for a single plant functional type (repeated measures ANOVA). Comparisons were made through LSD multiple-range test ($p<0.05$).

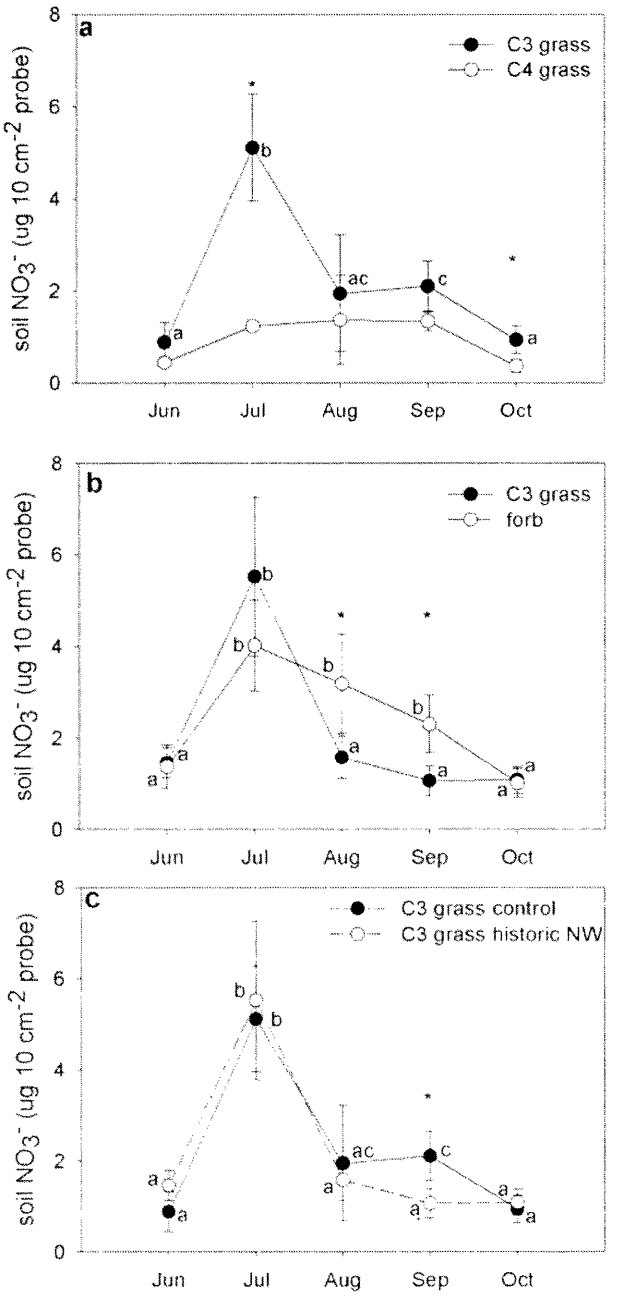

Figure 2.8: Monthly patterns of soil available N in the shortgrass steppe LTER under a: C3 and C4 grasses in control plots (C plots), b: C3 grasses and forbs in historical NW plots (historical nitrogen and water addition from 1970 to 1974), and c: C3 grasses in C plots compared to C3 grasses in NW plots. Vertical bars represent ± SE for n = 6. Asterisks mean significant differences between two plant functional types (a and b) or between treatments (c) within one month. Values designated with different letters indicate significant differences among months for a single plant functional type (repeated measures ANOVA). Comparisons were made through LSD multiple-range test ($p<0.05$).

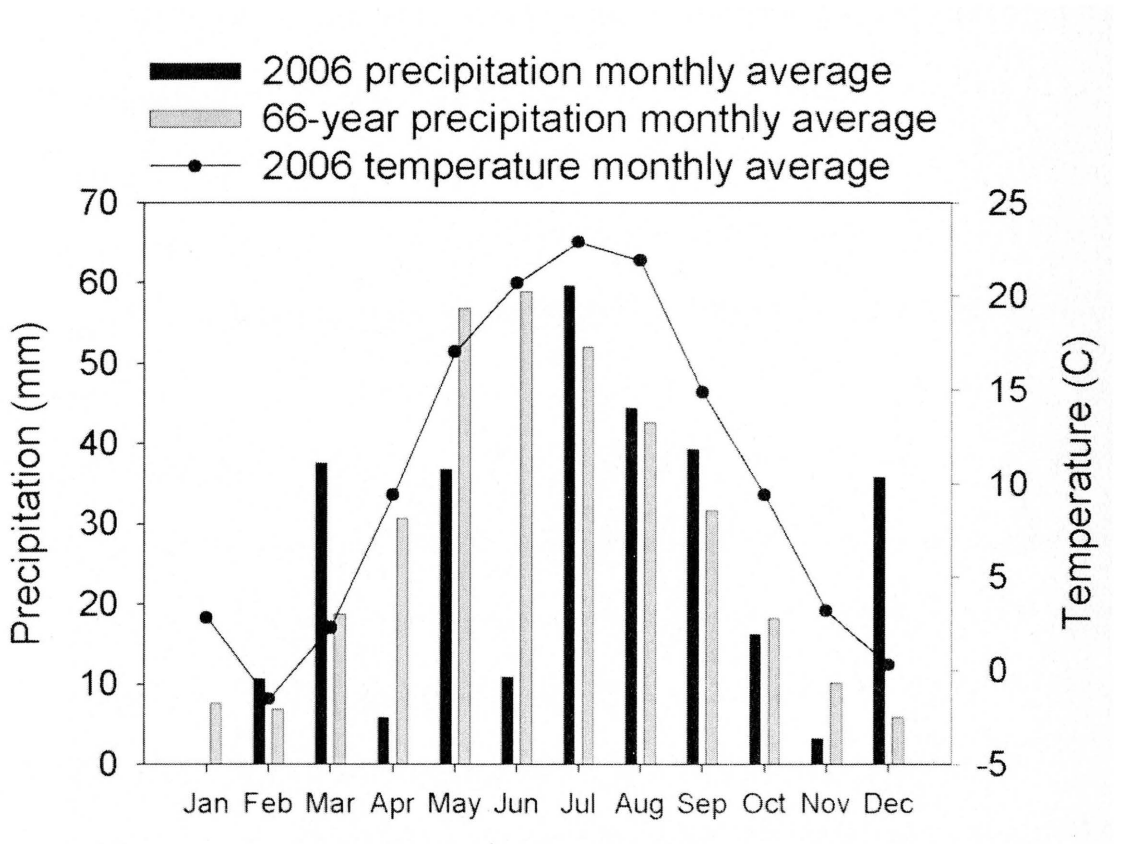

Figure 2.9: Monthly precipitation and temperature, and historical average precipitation in the shortgrass steppe LTER recorded in a weather station at the Central Plains Experimental Range headquarters.

CHAPTER III: POTENTIAL N_2O AND CO_2 EMISSIONS IN THE SHORTGRASS STEPPE SOILS: ARE FUNGI MORE IMPORTANT THAN BACTERIA?

Introduction

Greenhouse gases play multiple and important roles in the atmospheric system, and their increased concentration is associated with global climatic change. Nitrous oxide has a long mean residence time in the atmosphere, compared to CO_2, and influences both the stratosphere and the troposphere (Schlesinger, 1997). In the stratosphere, N_2O reacts with ozone, thereby decreasing its concentration, and in the troposphere, N_2O is a radiatively active trace gas (Smith, 1997). The concentration of N_2O in the atmosphere has increased continuously during the industrial era from approximately 275 ppbv in 1750 to about 311 ppbv in 1993 (Houghton et al., 2001), and it continues to increase at an annual rate of 0.3% (Khalil and Rasmussen, 1992). It is of special interest to accurately predict these emissions in grasslands since they cover more than 40% of the global terrestrial landscape (Chapin et al., 2001).

The main biogenic sources of N_2O are the microbial processes nitrification and denitrification, which together generate approximately 70% of the annual global N_2O budget (Mosier, 1998). These processes are complex, and our understanding of the organisms and of the controls over those organisms has changed through time. For instance, nitrification and denitrification were originally attributed to bacteria, but fungal production of N_2O through codenitrification and heterotrophic

nitrification has been found in pure cultures (Focht and Verstraete, 1977; Shoun et al., 1992) and in grassland soils (Crenshaw et al., 2008; Laughlin and Stevens, 2002; McLain and Martens, 2006). Archaea, in particular Chrenarchaeota are abundant ammonia-oxidizing prokaryotes in soils (Leininger et al., 2006), and might contribute to N_2O emissions as a byproduct of ammnonia oxidization (Hayatsu et al., 2008), other Archaea are capable of denitrification (Cabello et al., 2004) but a few studies have reported their effect in soil ecosystems (Hayatsu et al., 2008). Microbial processes conducive to N_2O emissions are controlled by soil moisture, soil temperature, soil O_2, and the availability of reactive substrates to microbes (Davidson et al., 2000; Hayatsu et al., 2008).

NH_4^+ availability in soils is a key control of nitrification (Davidson et al., 2000), whereas NO_3^- availability is an important control of denitrification (Hayatsu et al., 2008). Plant community composition can be an important determinant of these microbial processes through influences on the levels of inorganic N and labile C in rhizosphere (Grayston et al., 2004; Kourtev et al., 2003). Plant species and functional types differ in phenology, nutrient use efficiency, litter quality, and root exudates. These differences might alter microbial decomposition, microbial biomass C and N, fungal and bacterial activities, and potentially, the rate of GHG emissions from microbes (Wardle, 2002).

C4 and C3 grasses in the shortgrass steppe differ temporally in their influence on N retention and net N mineralization within a growing season (Epstein et al., 1998), since they differ in phenology, and therefore in the time of growth (Monson et al., 1986; Tieszen, 1970), nutrient use efficiency (Brown, 1978), water use efficiency

(Monson et al., 1986), litter quality (Murphy et al., 2002), and production of root exudates (Klein et al., 1988). These characteristics might affect spatial and temporal patterns of microbial activity throughout the growing season, including fungal to bacterial ratio and activity, likely affecting GHG emissions.

Nitrogen addition is another factor that alters biogeochemical cycles in the shortgrass steppe, leading to long lasting changes in plant community composition (Milchunas and Lauenroth, 1995) towards species that are less nutrient use efficient, whose litter is low in C:N ratios (Burke et al., 2008). High quality litter increases nitrogen mineralization, thus creating a positive feedback between high soil available nitrogen and colonization by plant species that are less nutrient use efficient (Vinton and Burke, 1995). In addition, exotic species that colonize fertilized plots can decrease fungi:bacteria ratio, increase N-related enzyme activity, and thus increase nitrification rate and available NO_3^- (Kourtev et al., 2003). These changes in labile substrate availability, and microbial community composition might alter GHG emissions.

Changes in N availability in arid environments leads to changes in soil microbial community composition (Porras-Alfaro et al., 2007), changes in enzymatic activities (Stursova et al., 2006), and increased emissions of GHG from fungi and bacteria (Crenshaw et al., 2008), but the seasonal trends on these emissions remain unclear. In other ecosystems, there are temporal changes during the growing season in maximum fungal and bacterial activities, with fungi being more active in winter, utilizing plant residues, and bacteria being dominant in summer, and utilizing

root exudates, hence the one-time estimations might underestimate microbial activity (Bardgett et al., 2005).

N_2O production in the shortgrass steppe is indirectly attributed to nitrification by - products, because soil water contents are usually low, therefore facilitating aerobic conditions that are conducive to nitrification (Parton et al., 1988). Fertilization in the shortgrass steppe leads to long lasting increase in N_2O emissions in this ecosystem (Mosier et al., 1996), and it is not clear whether these increased emissions are mainly driven by changes in microbial communities' composition or activity, and what are the potential seasonal changes in the relative emissions from these organisms. Furthermore, N additions are associated with decreased fungi:bacteria ratios (Bardgett et al., 1999; de Vries et al., 2006; Wardle et al., 2004), hence it would be important to understand the effect of these changes on GHG emissions.

To my knowledge, little is known about the relative role of fungi and bacteria on GHG emissions in the shortgrass steppe, which is particularly important because of the higher fungi:bacteria ratio compared to more mesic grasslands (McCulley and Burke, 2004) and recent evidence of the importance of fungi as mediators of biogeochemical cycles in semiarid soils (Collins et al., 2008).

In this study, I seek to characterize and find potential links between microbial groups and greenhouse gas emissions, with emphasis on plant functional type and nitrogen additions effects on microbial emissions. My objectives are: 1) to distinguish between N_2O and CO_2 released by fungi and bacteria and to detect patterns in their emissions throughout the growing season; 2) to compare fungal and bacterial

emissions from patches dominated by C_3 and C_4 grasses and forbs, and 3) to evaluate the effect of nitrogen availability on fungal and bacterial emissions of greenhouse gases.

Methods

Study area

I sampled soils at the USDA-ARS Central Plains Experimental Range (CPER, 40° 49' N latitude, 104° 46' W longitude), a shortgrass steppe site in northern central Colorado that is a National Science Foundation Long-Term Ecological Research site. Mean annual precipitation is 321 mm, with 71% falling during the May-September growing season (Lauenroth and Milchunas, 1991), and mean annual temperature is 8.6°C (Pielke and Doesken, 2008). The vegetation of the area is typical of shortgrass steppe and is dominated by the perennial bunchgrass *Bouteloua gracilis* (H.B.K.) Lag (blue grama), a C_4 grass. Other common grass species include the perennial C_3 species *Stipa comata* (Trin and Rupr.) (needle-and-thread grass), and *Pascopyrum smithii* (Rybd.) (western wheatgrass). The vegetation also includes the half-shrubs *Gutierrezia sarothrae* (Pursh) Britt. & Rusby, and *Artemisia frigid*a Willd.; the forb *Sphaeralcea coccinea* (Pursh) Rydb.; and the succulent *Opuntia polyacantha* Haw. Total vegetative basal cover is 25-35% (Milchunas et al., 1989).

In this site there is an experimental area that was protected from cattle grazing since 1968, and was part of a fertilization and irrigation experiment

conducted in the early 1970's (Lauenroth et al., 1978), that is still sampled today as part of the LTER monitoring. The original experiment was a factorial combination of four treatments with two replicates: control (C, no resource addition), water (W), nitrogen (N), and nitrogen+water (NW) additions. My plant plot treatments for this study were the C plots and NW plots since the main effect that perpetuated was increased N (Vinton and Burke, 1995). In this area I sampled three patches of each dominant plant functional type within each treatment replicate (two C plots and two NW plots) from June to October 2006. C_3 and C_4 species were dominant in the C plots, whereas annual forbs and C_3 species were the dominant functional types in the NW plots. There were no significant differences in soil texture among plot treatments, but patches dominated by forbs were sandy clay, whereas patches dominated by C3 and C4 species were sandy clay loam.

In the original experiment, the four 1-ha plots (2 C, and 2 NW) were randomly assigned within two blocks. Water and nitrogen were applied from 1970 to 1974. The amount of water added per year ranged from 458- 707 mm (Lauenroth et al., 1978). Nitrogen fertilizer (ammonium nitrate) was added to maintain a difference between the treatment and the control of at least 50 kg ha^{-1} of soil mineral nitrogen (NO_3^- + NH_4^+). Thus the addition of nitrogen consisted of 100-200 kg ha^{-1} $year^{-1}$ of nitrogen to the N treatment plots during four consecutive years. Water and N additions stopped in 1974 and since then, except for some vegetation and soil sampling, the plots have been unaltered. Although the addition of resources stopped 30 years ago, N availability remains high (Vinton and Burke, 1995), and changes in the plant community in the treated plots persist into the present (Milchunas and

Lauenroth, 1995). The treated plots are dominated by *Kochia scoparia* (L.) Roth and *Salsola iberica* (Sennen & Pau) Botsch. ex Czerep, species with low nutrient use efficiency, that are characterized by lower C:N and Lignin:N ratios compared to species native to the shortgrass steppe. Nitrogen mineralization is also high where these species are dominant, suggesting a positive feedback between these species with their high quality litter conducive to increased nitrogen mineralization, and increased soil available nitrogen that in turn facilitates further colonization of exotic species (Vinton and Burke, 1995).

Microbial biomass N and potential N mineralization

I sampled soils under crown of plants in patches dominated by C3 grasses, C4 grasses, and forbs. I collected three soils cores to a 5 cm depth per plant functional type patch in each treatment, monthly from June to October 2006. I am aware that a multiyear study would have been important to be able to analyze interannual differences in seasonal patterns of rainfall and would allow extrapolating to a wider temporal scale. The three cores were combined and homogenized into composite samples, stored at 4°C until processing, which occurred within a week. I sieved fresh soils through a 2 mm mesh sieve to remove plant material and fragments greater than that size. I then weighed the soils, mixed them and subsampled for 3 analyses: soil moisture, microbial biomass C and N, and potential N mineralization.

I used a 10 g fresh soil subsample to determine the moisture content gravimetrically at the time of sampling. A 12 g dry soil subsample was extracted with

60 ml of 2 N KCl for 30 minutes in an orbital shaker to measure initial inorganic N (Mulvaney, 1996). The extracts were allowed to settle for 10 minutes and then filtered through Whatman # 40 paper. The extracts were frozen until analyzed for nitrate and ammonium on an Alpkem Autoanalyzer (Pulse Instruments Ltd. Saskatoon, SK). I followed the same procedure after the soil incubation described below, and I estimated potential N mineralization as the difference between initial and final inorganic N of the soil (Hart et al., 1994).

I used the substrate induced respiration-inhibition method (SIRIN) of Anderson and Domsch (1975) modified by Johnson et al. (1996) for dry soils, to determine CO_2 and N_2O emissions. Optimal incubation conditions were determined in preliminary experiments according to the criteria of Anderson and Domsch (1975). I tested concentrations of glucose (0, 1, 3, 5, and 8 mg g^{-1} soil), streptomycin sulfate (2, 3, 6, and 8 mg g^{-1} soil), and cycloheximide (0, 1.5, 3, 6, 10, and 15 mg g^{-1} soil) in preliminary incubations to determine the optimal combination of antibiotics and incubation time when respiration stabilizes for these soils. I hereafter incubated the soils with glucose as a substrate (1 mg g^{-1} dry soil), cycloheximide (10 mg g^{-1} dry soil), as a fungal inhibitor; and streptomycin (3 mg g^{-1} dry soil) as a bacterial inhibitor.

Twelve grams of dry soil were placed in small beakers, moistened to 0.6 field capacity (field capacity for these soils is 25% moisture), and mixed with the antibiotics. Sixteen hours elapsed between addition of antibiotics and addition of C substrate and water for the stabilization of antibiotics in the soils. Samples were incubated for twelve hours in sealed incubation bottles in the dark at 25 °C. After the

incubation air samples were collected with 25 ml syringes and analyzed for CO_2 and N_2O concentration on a Shimadzu GC14-B gas chromatograph (Shimadzu Scientific instruments, Columbia, MD) fitted with an FID and ECD (electron capture detector), with a temperature program of 325 °C detector, and 40 °C column. I used certified N_2O standards (Matheson TRI-GAS, Fort Collins, CO) for calibration.

Soil available N

I monitored available mineral N in each treatment plot for each month using Plant Root Simulator (PRS) probe–ion exchange membranes (Western Ag Innovations, Inc., Saskatoon, Canada). I extracted the probes with 25 mL of 0.5 M HCl for one hour in a zip lock bag, on a shaker table, and the extractant was analyzed for NH_4^+ and NO_3^- using an Alpkem Autoanalyzer (Pulse Instruments Ltd., Saskatoon, SK). I buried a pair of probes (one anion and one cation probe) under each plant functional type patch. I left them in place for 30 days, then extracted them from the soil, extracted inorganic N as described above. i repeated this procedure from May 26 to October 27. Results are reported as μg 10 cm^{-2} probe surface area over the 30 day burial period for each month.

Statistical analyses

In this study I compared the emission of CO_2 and N_2O between fungi and bacteria, with respect to different plant functional types. Given that fungi and bacteria emissions were sampled under the influence of the plants, microbial and plant functional groups were related. Therefore, microbial groups and plant functional groups had to be included within the same analysis.

Since plot treatments led to different combinations of plant functional types (control plots: C3 and C4 grasses, and historical nitrogen plus water plots: C3 and forbs) the only way to compare plant functional types was to run separate ANOVAs for each plot treatment. I compared fungi and bacteria across treatment plots (i.e., C and NW) using C3 species because C3 plants were the only functional group common to all treatment plots.

All response variables were analyzed using a mixed model analysis of variance in SAS statistical software (SAS Institute Inc, 1989). The multiple sampling dates of data collected at the same sites were handled with repeated measures design. Separate ANOVA were conducted to test each hypothesis.

In order to test differences between microbial functional groups they were included as the fixed effects with four levels (all microbes, no microbes, fungi and bacteria), within plant functional type or plot treatment also as fixed effects in three separate mixed model analyses of variance describe below.

In order to test differences between plant functional types, they were included as fixed effects with two levels in two separate mixed model analyses of variance. The first one compared the plant functional types in control plots only, hence the fixed effect levels were C3 grasses and C4 grasses. The second one compared the plant functional types in historical NW plots, and the fixed effect levels were C3 grasses and forbs. To test differences between plot treatments I performed a mixed model analysis of variance where the fixed effects were plot treatment with two levels (control plots and NW plots).

I used a least significant difference mean separation test when significant main effects were found. All data were transformed as needed to fit the normality and homogeneity of variance assumptions of the applied statistical test, and the level of significance of all tests was determined as a p-value < 0.05.

Results

Fungal vs bacterial production of CO_2 and N_2O

Overall CO_2 and N_2O emissions significantly decreased when fungi were inhibited (Figures 3.1 to 3.3), demonstrating a major contribution of fungi to grassland these greenhouse gases emissions. This effect was especially clear in the C plots. The lowest emission of both GHG occurred when fungi and bacteria were inhibited, but there still were background emissions, indicating that microbial biomass inhibition was not complete (Figures 3.1 to 3.4).

CO_2 emissions from bacteria were always lower than total emissions (with no inhibitor), and in general bacterial CO_2 was lower than fungal CO_2, but there were variations in this trend in particular under forbs in NW plots (Figure 3.2 b). Fungal emissions of CO_2 contributed so much to the total that they were in general statistically equal to total emissions, and lower than total respiration only in October under C3 grasses in C plots (Figure 3.1a), and in July and August under C3 grasses and forbs in NW plots (Figure 3.2 a and b).

N_2O emissions from bacteria (when fungi were inhibited) were significantly lower compared to total emissions under native plant functional types in the C plots

(Figure 3.3). Fungal emissions of N_2O (when bacteria were inhibited) were slightly different or no different at all than total emissions (Figures 3.3 and 3.4). There were no differences between fungal and bacterial emissions of N_2O under C3 grasses and forbs in the historical NW plots (Figure 3.4).

Effects of Plant Functional Types

Fungal emissions of CO_2 showed no differences between C3 and C4 grasses in control plots (Figure 3.1), and neither were they different between C3 grasses and forbs in NW plots (Figure 3.2). Bacterial emissions of CO_2 did not differ between plant functional types in control and NW plots (Figures 3.1 to 3.4). Similarly, with the exception of soils under C4 grasses in October, when N_2O emissions were higher respect to C3 grasses in C plots ($p<0.01$, data not shown), there were no other differences between plant functional types in N_2O emissions from fungi and bacteria.

Effects of Long Term N Fertilization

Even though total CO_2 emissions did not differ between plot treatments (Figure 3.5a), fungal production of CO_2 was higher in C plots compared with NW plots in late spring and summer, after which, fungal CO_2 decreased up to be lower in the control plots than in NW in September and October (Figure 3.5b). There were no differences between plot treatments in bacterial CO_2 in June and October, whereas bacterial emissions were higher in NW than C from July to September (Figure 3.5c).

Contrary to CO_2, total N_2O did differ between plot treatments, and it was higher in the historical N-amended plots compared to the C plots from July to September (Figure 3.6a) mainly due to bacterial emissions. There were no

differences between plot treatments in June and October (Figure 3.6a). Overall, fungal emissions of N_2O did not differ between plot treatments, with the exception of September, when it was higher in the NW plots compared to the C plots (Figure 3.6b). Bacterial N_2O emissions were higher in the NW plots with respect to the C plots from June to August, and differences were not detected in September and October (Figure 3.6c).

In summary, the effect of N additions might not reflect in total CO_2 production, but differences were evident when discriminating between fungi and bacteria, and N additions increased total N_2O emissions that were mainly driven by increased bacterial emissions.

Monthly variation in CO_2 and N_2O flux

All treatments showed low CO_2 and N_2O flux in June (Figures 3.1 and 3.2), presumably in response to low precipitation (Figure 3.8) and low available N (Figure 3.7), followed by a significant ($p<0.05$) increase in July, after which they were relatively constant through September, decreasing in October (Figures 3.1 to 3.4).

Discussion

Fungal emissions of GHG were higher than bacterial emissions under the native plant functional types, especially in the C plots (Figures 3.1 and 3.3). There were no differences between fungal and bacterial emissions of GHG under exotic forbs in the NW plots (Figures 3.2 and 3.4), suggesting that fungi:bacteria ratio changed under historical resources additions, ultimately altering the relative emissions of GHG by fungi and bacteria.

Nitrogen additions in the shortgrass steppe have long lasting effects on GHG emissions, and even the same functional group, C3 grasses, undergoes changes in historically N-amended soils, that determine changes in substrate available for microbes, thus affecting microbial community composition that led to increased CO_2 and N_2O emissions from bacteria (Figures 3.5c and 3.6c). These results indicate that the decreased fungi:bacteria ratio observed in fertilized grasslands (Bardgett et al., 1999; de Vries et al., 2006; Wardle et al., 2004) also occurs under fertilization in the SGS and might change GHG emissions.

Fungal vs bacterial production of CO_2 and N_2O

The percentage of fungal biomass observed and calculated from the respiratory inhibitions ranged from 55 to 85 %, depending upon the plant type and date when it was estimated, which suggests that fungi dominate these soils, a common feature in temperate soils (Ruzicka et al., 2000) and consistent to the fungi:bacteria ratio estimated through PLFA in the short grass steppe (McCulley and Burke, 2004).

Fungi were the most important emitters of CO_2 and N_2O in the C plots (Figures 3.1 and 3.3), hence supporting that fungi are an important component of the soil microbial biomass, and an important source of GHG gases. Indeed fungi have mechanisms to resist drought, allowing them to translocate water and nutrients from nutrient rich microsites to nutrient poor microsites (Allen, 2007). Because of these characteristics fungi has been postulated to have an important role in

biogeochemical cycles in arid and semiarid soils (Collins et al., 2008) such as the shortgrass steppe.

A key assumption in this work was that most of the microorganisms inhibited by cycloheximide were fungi. However, the Crenarchaeota (Archaea) are widespread in terrestrial ecosystems and responsible for NH_4^+ oxidation (Leininger et al., 2006), such that they could play a role in N_2O emissions. Since cycloheximide targets protein synthesis, and ribosomal composition of Chrenarchaeota is similar to Eukariota, I could have overestimated the role of fungi by inhibiting Archaea. However I assumed that most of the organisms inhibited were fungi because cycloheximide has been proven to be an ineffective inhibitor of Crenarchaeota (Sanz et al., 1994). The use of inhibitors might also have altered competition between fungi and bacteria, therefore overestimating the role of fungi in GHG emissions.

The background emissions that I observed when I applied both inhibitors can be attributed to several factors. First there might be an antagonistic effect between antibiotics when both are applied together. Second, the inhibitors might suppress some microorganisms and decrease competition with other microorganisms that are not targeted by these specific antibiotics, therefore increasing their activity. Third, problems with the distribution of antibiotics, or adsorption to soil particles might difficult the inhibition.

Effects of Plant functional Types

Contrary to my expectations, there was no direct effect attributable to plant functional types on CO_2 and N_2O emissions from fungi and bacteria (Figures 3.1 to

3.4). Nevertheless, the lack of difference between fungi and bacteria in the NW plots under exotic forbs (Figures 3.2b and 3.4b) suggests that changes in fungi:bacteria ratios might occur that alters their relative production of GHG.

C3 plants initiate and maximize growth earlier in the growing season than do C4 plants (Dickinson and Dodd, 1976; Tieszen et al., 1997). C4 plants, on the other hand, are more active than C3 plants during warmer periods (Berry and Bjorkman, 1980). Therefore I expected that C3 grasses would favor microbial activity through root exudates early in the growing season, thus increasing the potential for respiration and N_2O emissions. I expected that C4 species, on the other hand would provide more substrate for microbial activity in July-August when temperatures are higher. Contrary to these predictions, I did not detect changes in fungal and bacterial emissions that can be explained by differences in phenology of various plant functional types.

Water availability affects the use of N in the shortgrass steppe (Lauenroth and Dodd, 1979), and therefore precipitation during this study seemed to regulate processes in the same way under the different plant functional types under consideration. Thus, GHG gas emissions were low in June, when precipitation was remarkably lower than the long-term precipitation data (Figure 3.8), while they significantly increased in July, when precipitation was higher than the long-term average for the shortgrass steppe. These emissions kept constant from July to September, after which they decreased presumably in response to low temperature, and to the lack of root exudates from all plant functional types that were senescent in October.

Effects of long-term N fertilization

Experimental N additions in the shortgrass steppe have dramatic and long lasting effects on plant community composition and structure (Milchunas and Lauenroth, 1995). This disturbance also increases N mineralization levels (Vinton and Burke, 1995), and N_2O emissions (Mosier et al., 1997). Consistent with these changes, I found that N_2O emissions from NW plots were 10-30% higher than those from C plots after 30 years of resources addition cessation. Fertilization changes plant community structure with a switch from belowground to aboveground biomass allocation. These changes likely influence the amount and quality of litter, and nitrogen mineralization, changes that perpetuate in time through positive feedbacks (Vinton and Burke, 1995). In this study leaf C:N of C3 grasses in NW plots was 2-4 times lower than C:N for the same plant functional type in C plots (data not shown). These changes in litter quality likely change microbial community composition and activity leading to changes in CO_2 emissions (Wardle, 2002).

Historical NW and C plots differed in the temporal patterns of bacterial and fungal GHG emissions through the growing season. Total microbial CO_2 (no inhibitor) was higher in NW plots than in C plots only in September (Figure 3.5a). Fungal CO_2 emissions (streptomicin inhibition) were higher in C plots than NW plots from June to August, whereas they were lower in C plots respect to NW plots in September and October (Figure 3.5b). An interesting question from these results is whether there are changes in fungal groups in NW plots whose activity changes throughout the growing season, thus increasing CO_2 emissions in fall. For example, dark septate fungi are associated with *Bouteloua gracilis* (Porras-Alfaro et al., 2008)

and they are more abundant than mycorrhizae fungi in aridlands; therefore, it would be interesting to explore the potential of these fungi to be less active during the spring and summer, and become more active in fall, presumably in response to changes in substrate quality in the rhizosphere of C3 grasses that grow in NW plots.

Bacterial CO_2 emission was equal in NW and C plots when precipitation (June) or temperature and presumably root exudates (October) were limiting, while under favorable temperature, moisture and root exudates (July to September) bacterial respiration was 20% higher in NW plots compared to C plots (Figure 3.5c). The increased CO_2 emissions from bacteria in NW plots may be a response to several factors. First, microbial communities of infertile ecosystems (represented here by the C plots) are frequently dominated by fungi and those of more fertile productive ecosystems (here represented by the historical NW soils), are primarily dominated by bacteria (de Vries et al., 2006; Wardle et al., 2004), likely increasing bacterial CO_2 in these plots. In addition, it has been postulated that more readily utilizable carbon is released into the rhizosphere of plant species that become dominant in fertilized grasslands, resulting in greater stimulation and carbon utilization by microbes. In contrast, the non-fertilized grasslands contain more recalcitrant compounds, the bulk of soil organic matter, which may explain the high fungal numbers and the lower metabolic activity found in non-fertilized grasslands, compared to those under resource additions (Grayston et al., 2004).

Historical resources additions have a long lasting effect by increasing N_2O emissions during most of the growing season in the shortgrass steppe LTER. This legacy effect is a consequence of a very high rate of experimental addition, respect

to the actual wet and dry deposition in this area which is lower than 4 Kg ha $^{-1}$ yr $^{-1}$ (Holland et al., 2005). Even though the shortgrass steppe is a source of N_2O, emissions are low in this ecosystem compared to other ecosystems (Mosier and Parton, 1998), therefore land use change that involves N additions should be carefully considered, since it changes ecosystem processes that lead to long lasting increase in GHG emissions.

Total N_2O emissions (no inhibitor) were higher in NW plots respect to C plots from July to September, whereas fungal N_2O was higher in NW plots only in September and there were no differences between plot treatments for the rest of the growing season. Bacterial N_2O emissions were also higher in the NW plots from June to August, and they were equal between treatments in September and October. These results suggest that the higher N_2O emissions in NW plots are mainly a result of bacterial activities. The peak of available NO_3^- in July, the higher potential mineralization from bacteria (Table 1), and the moisture conditions of the incubations all seem to favor nitrification. Therefore the higher N_2O emissions in NW plots are likely the result of bacterial nitrification. These results should be further explored since previous studies reported that microbial communities are more bacterial dominated in fertilized grasslands, but it is not clear which specific groups dominate the microbial communities and how they are related to nitrification and N_2O emissions (de Vries et al., 2006; Wardle et al., 2004). These results are also interesting in view that increased CO_2 leads to fungal dominated microbial communities (Kandeler et al., 2008), whereas fertilization leads to bacterial dominated microbial communities, therefore, it is important to determine how these

disturbances might counteract and which would be the net outcome of changes in microbial community composition.

It would be also interesting to further explore which groups of bacteria are responsible for aerobic and anaerobic process in different months during the growing season. These differences among microbial functional groups in the relative production of CO_2 and N_2O are important for better understanding biogeochemical processes and predicting changes under land use change or global climatic change.

Conclusions

This study indicates that N additions can have long lasting consequences in the shortgrass steppe, in particular by increasing GHG at least 30 years after the cessation of resources additions. These results should be taken into account when predicting and modeling the effect of N additions in this ecosystem. Soils under the same plant functional groups, C3 grasses, showed different responses under historical resources additions, with increased GHG emissions, in particular when soil moisture and temperature are not limiting of microbial grow.

Fungi are an important component of the microbial communities in the shortgrass steppe, and most of the greenhouse gas emissions are attributable to this microbial group. Therefore, future change scenarios like elevated CO_2 that increase the fungi:bacteria ratio may raise GHG emissions. The lack of difference in GHG emissions from fungi and bacteria under exotic forbs in the historical NW plots should be further analyzed, since it suggests that exotic forbs change the

microenvironment under their influence, ultimately changing microbial activities in fertilized plots that might increase bacterial emissions of CO_2 and N_2O.

The temporal changes of fungal and bacterial GHG emissions indicate that the one-time measurements of microbial emissions should be carefully taken into account to avoid underestimations of microbial activity.

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Table 3.1. Potential N (NO_3^--N and NH_4^+-N) mineralization rates for cycloheximide, streptomicin, and non inhibitor as determined by substrate induced respiration from June to October 2006. Values are mean (±SE, n = 6). Mean comparisons are among antibiotic treatments (among columns within plots treatment). Mean values followed by different letters are significantly different at 0.05 level. Incubated soils were collected under C3 grasses patches in the shortgrass steppe LTER.

	Control plots inorganic N ($g\ m^{-2}\ d^{-1}$)			NW plots inorganic N ($g\ m^{-2}\ d^{-1}$)		
	cycloheximide	streptomicin	no inhibitor	cycloheximide	streptomicin	no inhibitor
June	0.40 (0.11)	0.22 (0.03)	0.28 (0.05)	0.42 (0.06)a	0.21 (0.04)b	0.18 (0.04)b
July	0.40 (0.08)	0.30 (0.09)	0.25 (0.05)	0.29 (0.07)a	0.12 (0.04)b	0.14 (0.01)b
August	0.18 (0.12)	0.11 (0.10)	0.30 (0.05)	0.34 (0.11)	0.28 (0.14)	0.21 (0.04)
September	*0.20 (0.08)a*	*-0.09 (0.04)b*	*0.27 (0.03)a*	*0.01 (0.09)b*	*-0.11 (0.04)b*	*0.23 (0.03)a*
October	1.25 (0.76)a	-0.02 (0.06)b	0.04 (0.09)b	0.14 (0.08)a	-0.19 (0.09)b	-0.22 (0.13)b

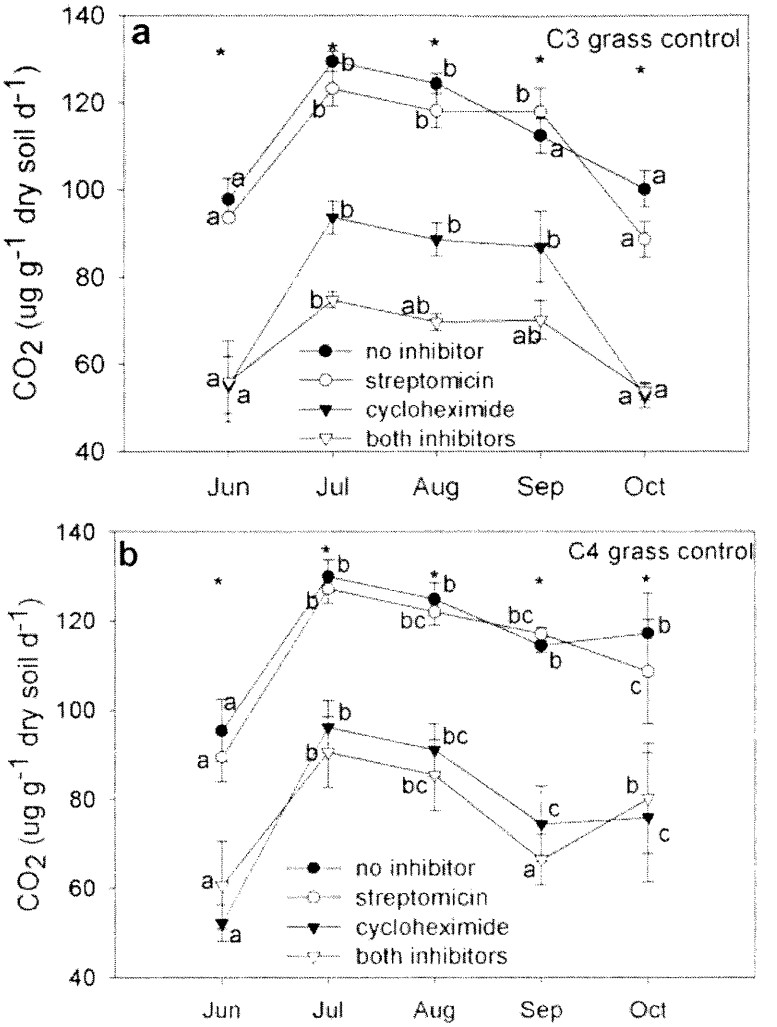

Figure 3.1: Monthly pattern of CO_2 emissions in the shortgrass steppe LTER soil comparing different antibiotic treatments in incubations of soils under two plant functional types (a:C3 grass and b: C4 grass) in control plots. Vertical bars represent ± SE for n = 6. Streptomicin inhibits bacteria and indicates fungal activity; cycloheximide inhibits fungi and indicates bacterial emissions. Asterisks mean significant differences between streptomicin and cycloheximide within one time point. Values designated with different letters indicate significant differences among months for a single antibiotic treatment (repeated measures ANOVA). Comparisons were made through LSD multiple-range test ($p<0.05$).

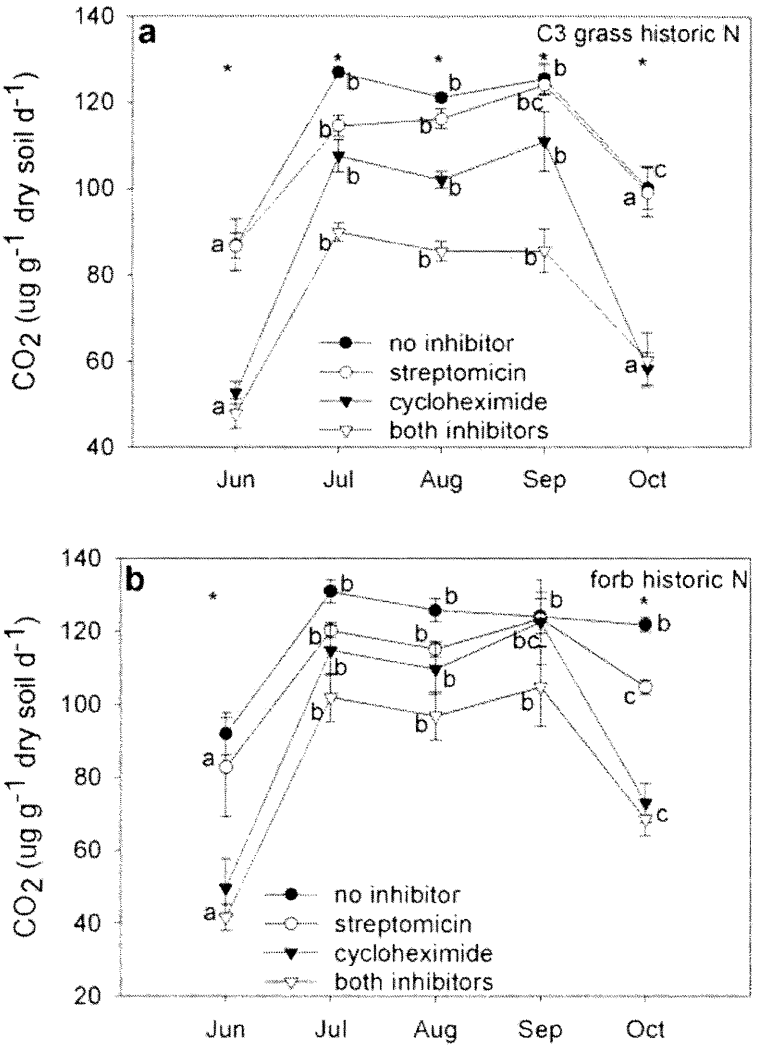

Figure 3.2: Monthly pattern of CO_2 emissions in the shortgrass steppe LTER soil comparing different antibiotic treatments in incubations of soils under two plant functional types (**a:** C3 grass and **b:** forbs) in historical nitrogen addition plots (1970-1974). Vertical bars represent ± SE for n = 6. Streptomicin inhibits bacteria and indicates fungal activity; cycloheximide inhibits fungi and indicates bacterial emissions. Asterisks mean significant differences between streptomicin and cycloheximide within one time point. Values designated with different letters indicate significant differences among months for a single antibiotic treatment (repeated measures ANOVA). Comparisons were made through LSD multiple-range test ($p<0.05$).

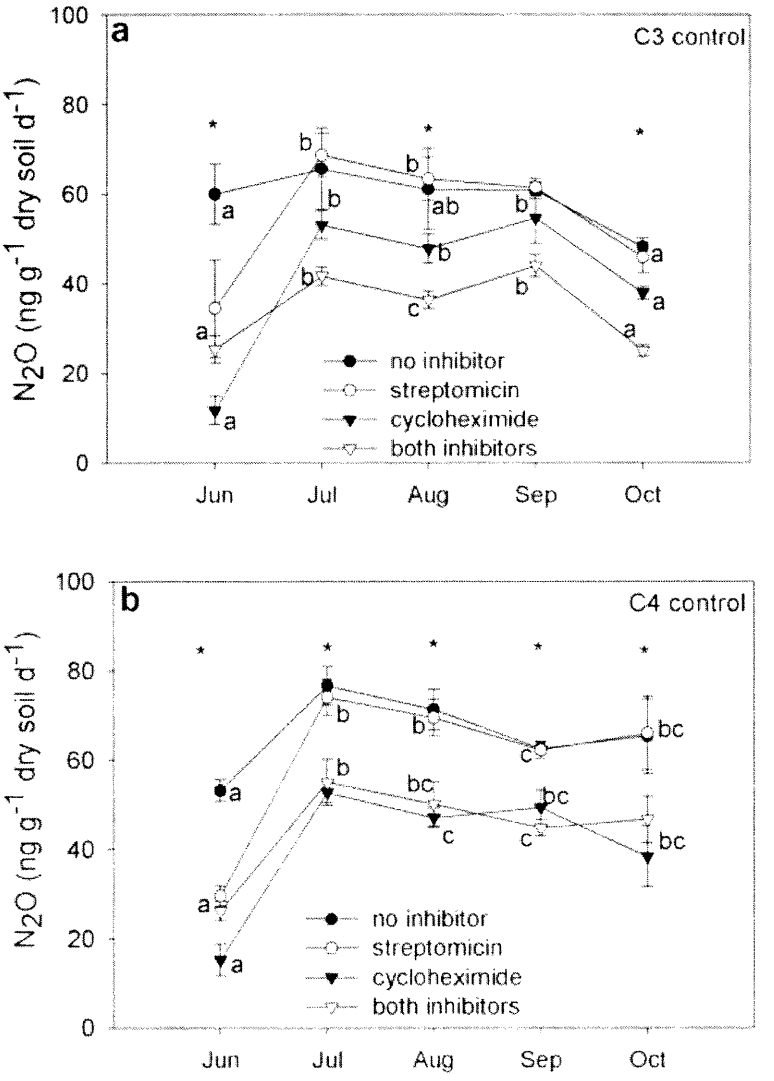

Figure 3.3: Monthly pattern of N_2O emissions in the shortgrass steppe LTER soil comparing different antibiotic treatments in incubations of soils under two plant functional types (a: C3 grasses and b: C4 grasses) in control plots. Vertical bars represent ± SE for n = 6. Streptomicin inhibits bacteria and indicates fungal activity; cycloheximide inhibits fungi and indicates bacterial emissions. Asterisks mean significant differences between streptomicin and cycloheximide within one time point. Values designated with different letters indicate significant differences among months for a single antibiotic treatment (repeated measures ANOVA). Comparisons were made through LSD multiple-range test ($p<0.05$).

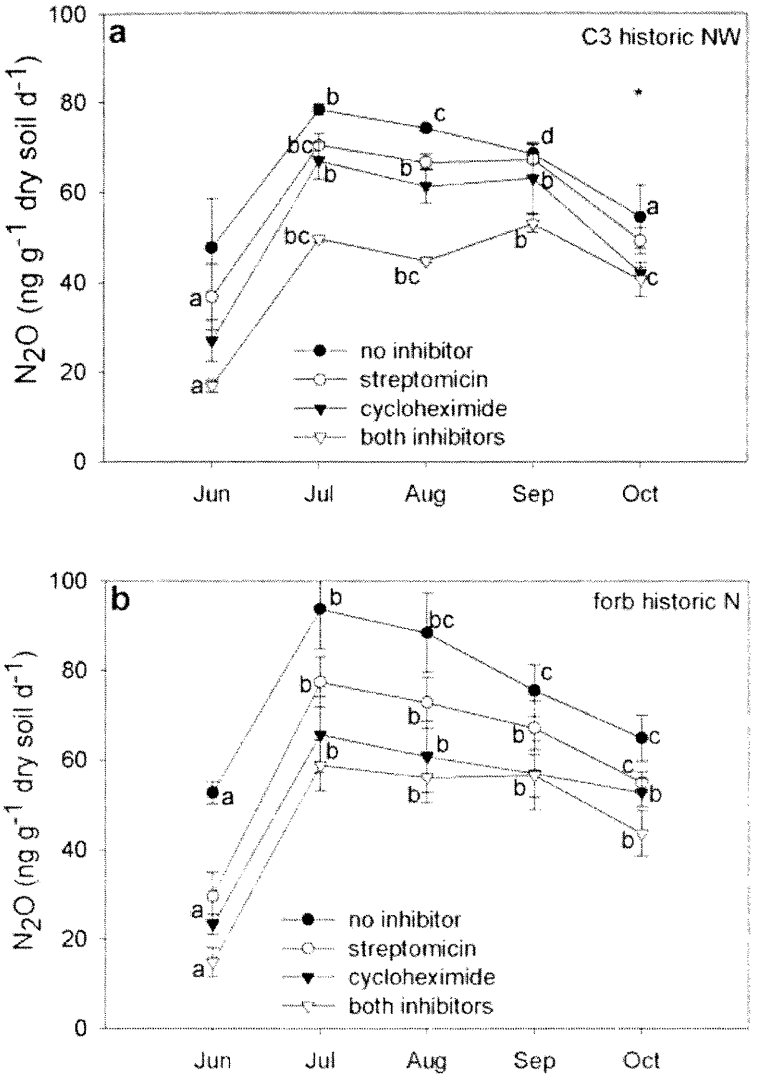

Figure 3.4: Monthly pattern of N_2O emissions in the shortgrass steppe LTER soil comparing different antibiotic treatments in incubations of soils under two plant functional types (**a:** C3 grasses and **b:** forbs) in historical nitrogen addition plots (1970-1974). Vertical bars represent ± SE for n = 6. Streptomicin inhibits bacteria and indicates fungal activity, cycloheximide inhibits fungi and indicates bacterial emissions. Asterisks mean significant differences between streptomicin and cycloheximide within one time point. Values designated with different letters indicate significant differences among months for a single antibiotic treatment (repeated measures ANOVA). Comparisons were made through LSD multiple-range test ($p<0.05$).

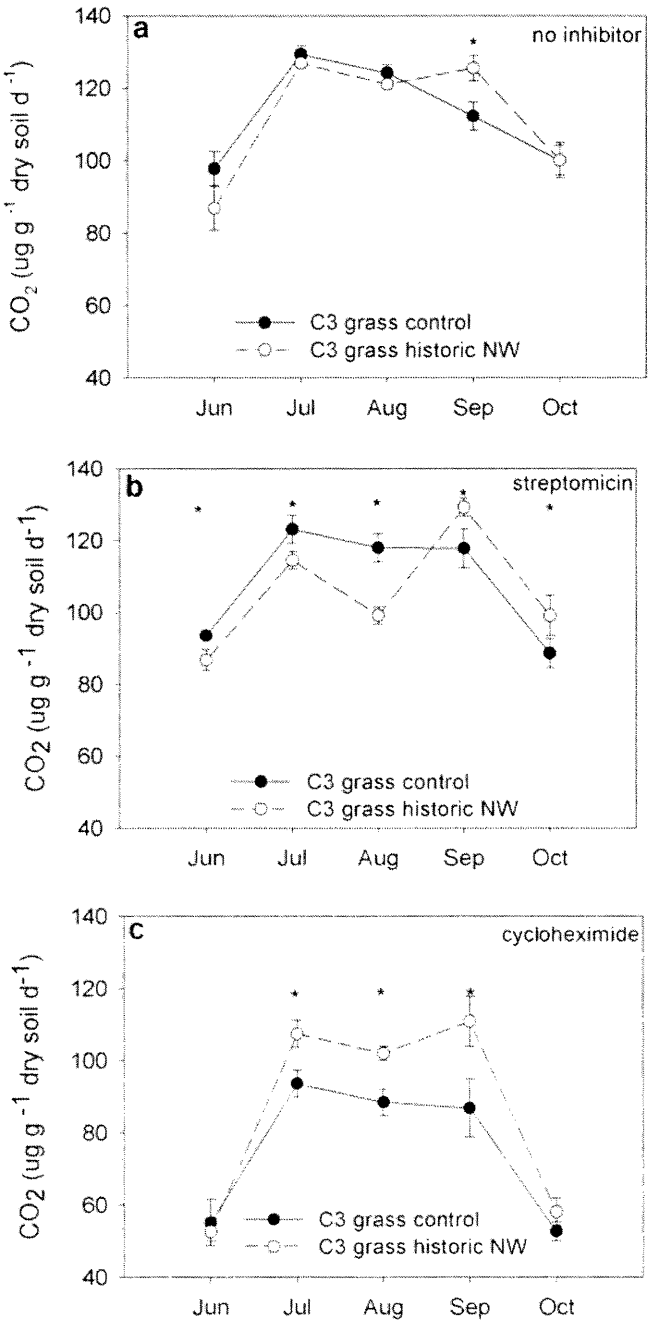

Figure 3.5: Monthly pattern of CO_2 emissions in the shortgrass steppe LTER soil comparing soils under C3 grasses in control and historical nitrogen addition plots (1970-1974). **a:** no inhibitor indicates total emissions, **b:** streptomycin means inhibition of bacteria and indicates fungal emissions, and **c:** cycloheximide inhibits fungi and indicates bacterial emissions. Vertical bars represent ± SE for n = 6. Asterisks mean significant differences between plot treatments within one time point. Comparisons were made through LSD multiple-range test ($p<0.05$).

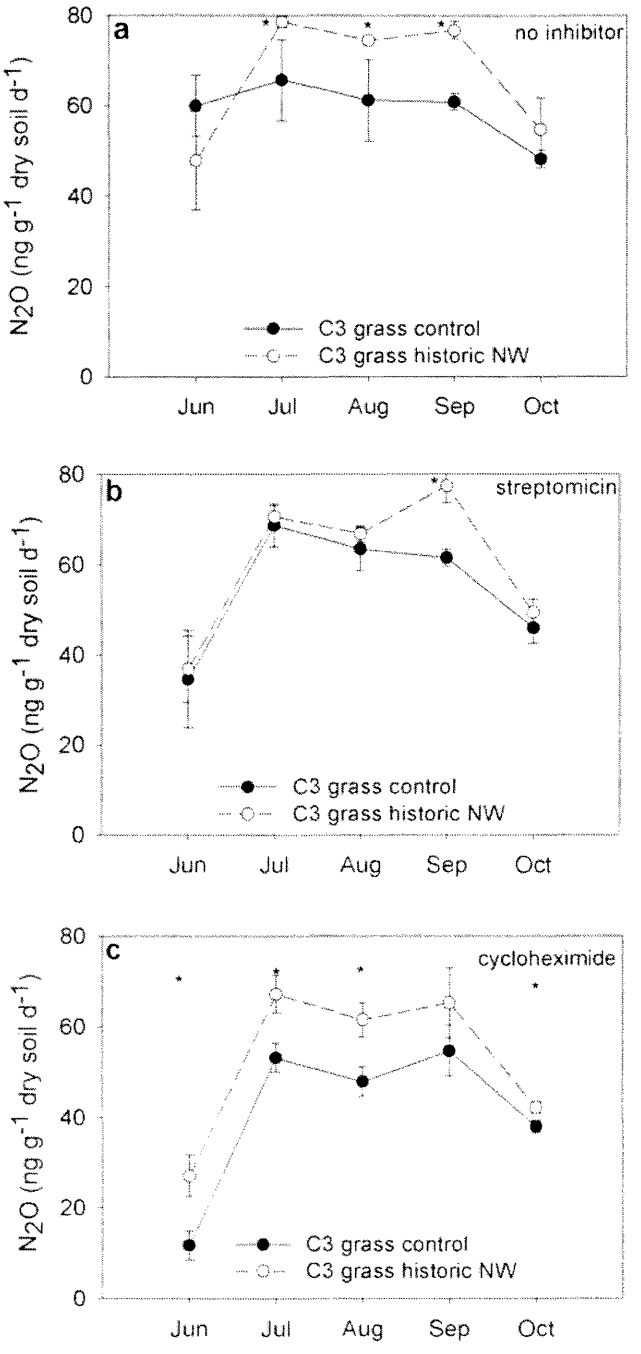

Figure 3.6: Monthly pattern of N_2O emissions in the shortgrass steppe LTER soil comparing soils under C3 grasses in control and historical nitrogen addition plots (1970-1974). **a:** no inhibitor indicates total emissions, **b:** streptomycin means inhibition of bacteria and indicates fungal emissions, and **c:** cycloheximide inhibits fungi and indicates bacterial emissions. Vertical bars represent ± SE for n = 6. Asterisks mean significant differences between plot treatments within one time point. Comparisons were made through LSD multiple-range test ($p<0.05$

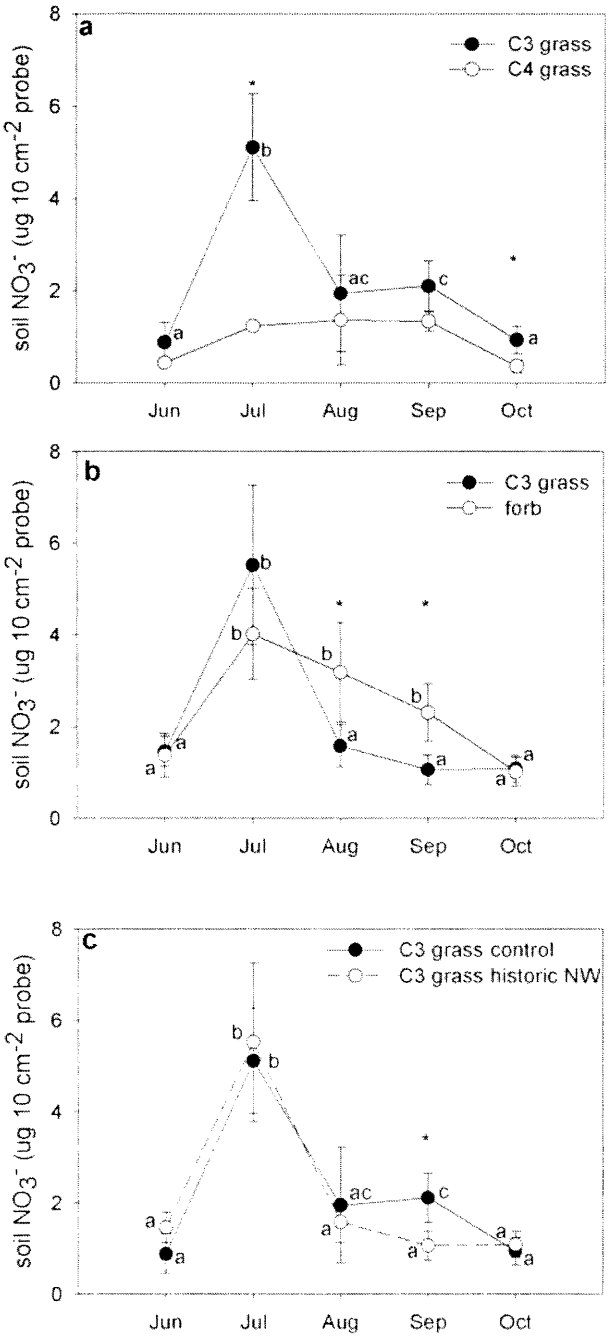

Figure 3.7: Monthly pattern of soil available N in the shortgrass steppe LTER soil under three plant functional types (a and b) in the short grass steppe in control and historic nitrogen addition (1970-1974) plots (c). Vertical bars represent ± SE for n = 6. Asterisks mean significant differences between two plant functional types (a and b) or between treatments (c) within one month. Values designated with different letters indicate significant differences among months for a single plant functional type (repeated measures ANOVA). Comparisons were made through LSD multiple-range test ($p<0.05$)

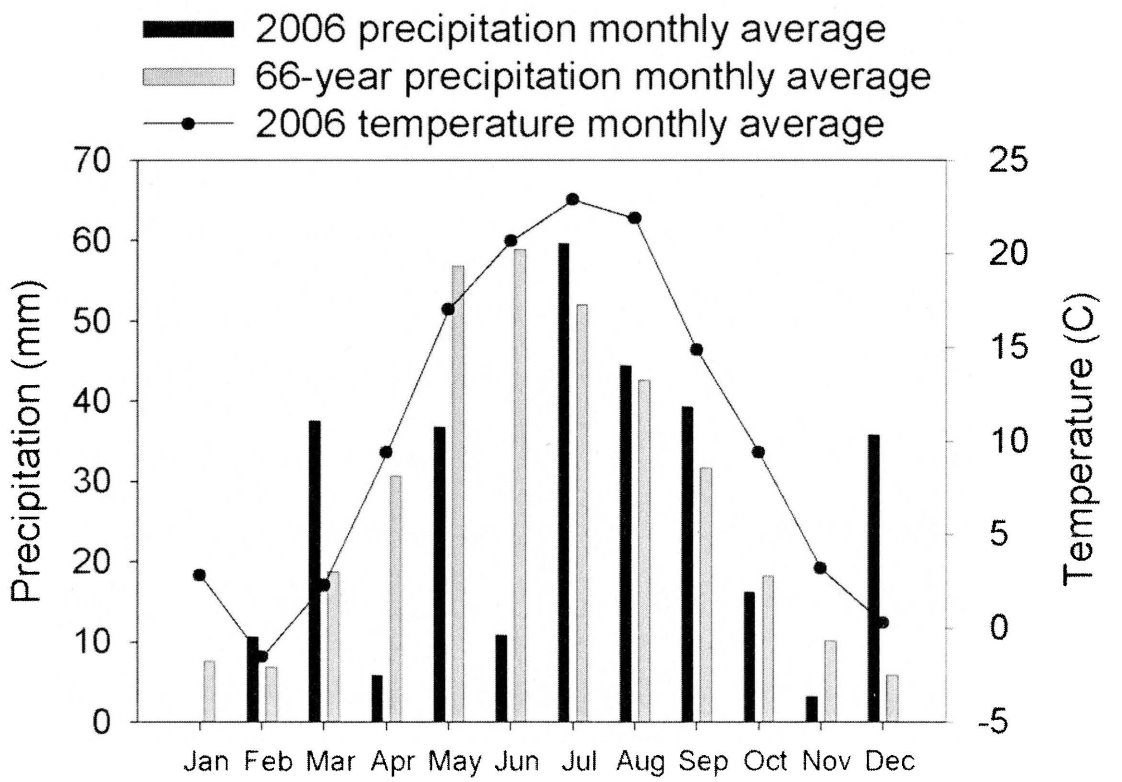

Figure 3.8: Monthly precipitation and temperature, and historic average precipitation in the short grass steppe LTER recorded in a weather station at the Central Plains Experimental Range headquarters.

CHAPTER IV: GREENHOUSE GAS EMISSIONS IN SHORTGRASS STEPPE SOILS TREATED WITH BIOSOLIDS.

Introduction

There is considerable evidence of the limitation of nitrogen (N) on terrestrial ecosystems (Vitousek and Howarth 1991), and of the anthropogenic influence that is greatly increasing nitrogen availability globally (Vitousek et al. 1997a; Vitousek et al. 1997b). As a result, the effect of N depositions on ecosystems and plant communities has received great interest during the last several decades (Perring et al. 2008; Pregitzer et al. 2004; Wedin and Tilman 1996).

Experimental N additions in the shortgrass steppe have been shown to be an important disturbance with long lasting effects on plant community composition and structure (Milchunas and Lauenroth 1995). Because many of the plant species that flourish under high N have N-rich litter, a self-perpetuating cycle begins, that lasts at least several decades (Vinton and Burke 1995). In addition, N fertilization increases N_2O emissions and decreases CH_4 uptake (Mosier et al. 1997).

Biosolids have been utilized at low to intermediate rates in semiarid grasslands as an alternative for dispose these products, rather than dispose them in land filling. Since semiarid grasslands are nitrogen limited, biosolids increase above-ground biomass, and improve soil structure and fertility (Cuevas et al. 2000). Biosolids are the residues that result from the treatment of domestic sewage sludge in treatment facilities. These products include nutrient-rich and organic compounds and therefore

they are useful to recycle and apply as fertilizers (http://www.epa.gov/owm/mtb/biosolids/index.htm).

In sites where application rates were high in the past there are unintended consequences that may include heavy metals, nitrate pollution of ground water (Gaggiani 1991) and increased production of greenhouse gases (Paramasivam 2008). Biosolids can negatively affect arbuscular mycorrhizal fungi in response to increased phosphorous (Linderman and Davis 2004), to increased heavy metals (Leyval et al. 1997), and to changes in plant community composition (Sullivan et al. 2006a). In addition, biosolid amendments increase the amount of respiration per unit of microbial biomass carbon (Barbarick et al. 2004; Garcia-Gil et al. 2004), which suggests increased emissions of CO_2 even when microbial biomass remains constant if increased plant biomass do not offset these emissions.

Despite the fact that 33% of biosolids currently produced in the USA is applied to land, and that projected land applications by 2010 are 3.72 million dry Mg per year (EPA 1999), knowledge about the effect of these applications on microbial processes leading to greenhouse gas emissions is not complete. The information available so far is variable, and consequences of biosolids applications appear to depend upon its composition (Inubushi et al. 2000) and the rate of application (Sullivan et al. 2006b). Most biosolids studies span a few years, but, given that additions of nutrients in the shortgrass steppe can have consequences that last for decades (Milchunas and Lauenroth 1995; Vinton and Burke 1995) more studies are necessary over longer, more ecologically meaningful time scales (Paschke et al. 2005).

Some of the long term studies reported greater soil total organic C, microbial biomass, basal respiration rate, and enzymatic activity even eight years after biosolids incorporation (Pascual et al. 1999). Increased bacterial biomass or decreased fungi:bacteria ratio is another response to fertilization (de Vries et al. 2006) or biosolids application (Sullivan et al. 2006b) in shortgrass steppe soils, but little is known about the effects of these changes on CO_2, and N_2O emissions, which are likely to occur given that carbon and nitrogen mineralization also increase in amended soils (Sullivan et al. 2006b), and N mineralization has been shown to be positively related to N_2O emissions (Davidson et al. 2000).

A site that is an interesting case study for the effect of high rates of biosolids application on microbial activities is the Aurora reservoir, an area approximately 15 miles Southeast of Denver, where the Metro Wastewater Reclamation District applied biosolids at an approximate rate of 20 Mg ha^{-1} yr^{-1} for 20 years, additions that stopped 20 years ago. One of the detrimental effects of this management was nitrate and nitrite leaching, leading to concentrations in wells in the area that exceed the U.S. Environmental Protection Agency recommended limit for drinking water (Gaggiani 1991). Excess available N might increase soil microbial activities, resulting in increased N_2O emissions (Chapuis-Lardy et al. 2007; Davidson et al. 2000).

The amended land shows a mosaic of two clearly different plant communities, one of them dominated by exotic forbs, and a different plant community dominated by C3 grasses. These changes have been related in other areas to differences in biosolid application rate (Paschke et al. 2005; Sullivan et al. 2006a) and biosolids quality and application form (i.e. topsoil vs subsoil) (Paschke et al. 2005). To my

knowledge there is no information available about differences in biosolids application rate or quality in the Aurora reservoir.

In this study, I address questions related to the effect of these biosolid treatments on trace gas fluxes. Specifically, I asked 1) are soil fluxes of N_2O and CO_2 from fungi and bacteria influenced by historical biosolids additions?; and 2) is potential N mineralization differentially affected by fungi and bacteria and by biosolids applications?

A secondary question is: Are there differences in N_2O and CO_2 and N mineralization from fungi and bacteria, as influenced by differences in plant community composition presumably due to non-uniform biosolids application?

Methods

Study area

I conducted the research in the Aurora reservoir (40° 49' N latitude, 104° 46' W longitude), a shortgrass steppe site in central Colorado that is currently proposed to become a recreation area. Mean annual precipitation is 392 mm with 69% falling during the May-September growing season and mean annual temperature is 10.3°C (http://www.srh.noaa.gov).

I sampled soils within the 6 km^2 area, in 3 patches per plant community type. Each patch was greater than 100 m^2, and all were located on level uplands. Each patch was approximately 0.8 km apart from the others. The relatively small area covered in my sampled was due to accessibility restrictions, and I am aware of the limitations of extrapolations from this study to wider scales. One of the plant

community types represented soils that have never been treated with biosolids (control, as native shortgrass steppe). The vegetation of the area is typical of shortgrass steppe (SGS) and is dominated by the perennial bunch grass *Bouteloua gracilis* f Lag (blue grama), a C_4 grass. Other common grass species include the perennial C_3 species *Stipa comata* (Trin and Rupr.) (needle-and-thread grass), and *Pascopyrum smithii* (Rybd.) (western wheatgrass). The vegetation also includes the half-shrub *Artemisia frigid*a Willd., the forb *Sphaeralcea coccinea* (Pursh) Rydb., and the succulent *Opuntia polyacantha* Haw.

The other two plant community types were treated with biosolids, at an application rate of approximately 20 Mg ha^{-1} yr^{-1} from 1969 to 1986, after which these areas have not been amended (Gaggiani 1991). The biosolids were incorporated to the soils either by burial (Gaggiani 1991) or by plowing the biosolids into the soil. Biosolids contained large concentrations of ammonia (NH_4^+), organic nitrogen, phosphorus, cadmium, chromium, copper, iron, lead, nickel, and zinc (Gaggiani 1991).

The two plant community types that had received applications of biosolids differed in the dominant plant functional type, with one of them dominated by exotic forbs in particular *Kochia scoparia* (L.) Schrad (kochia); and adjacent areas dominated by C3 grasses, mainly the perennial *Pascopyrum smithii* (Rydb.) A. Löve (western wheatgrass) and the annual *Bromus tectorum* L. (cheatgrass). To my knowledge, there is no apparent reason for the differences in plant community responses to biosolid applications, though preliminary data showed some differences among sites in terms of soil texture, and percent water filled pore space

(%WFPS) (Table 1). Presumably these changes are in response to differences in biosolids application rate or quality, or could be the consequence of differential disturbance when biosolids were applied.

Potential N mineralization

Within each of the sampling locations, I sampled soils under crown plants in three patches dominated by C4 grasses in the SGS sites, in three patches dominated by C3 grasses in one of the biosolids treated areas, and in three patches of exotic forbs in the other biosolids treated area. In late August 2007 I collected three soils cores at 5 cm depth per patch in each site. The three cores were combined and homogenized into composite samples, stored at 4°C until processing, which occurred within a week. I sieved fresh soils through a 2 mm mesh sieve to remove plant material and fragments greater than that size. I then weighed the soils, mixed them and subsampled for 3 analyses: soil moisture, potential N mineralization and SIRIN incubations.

I used a 10 g fresh soil subsample to determine the moisture content gravimetrically at the time of sampling. A 12 g dry soil subsample was extracted with 60 ml of 2 N KCl for 30 minutes in an orbital shaker to measure initial inorganic N (Mulvaney 1996). The extracts were allowed to settle for 10 minutes and then filtered through Whatman # 40 paper. The extracts were frozen until analyzed for nitrate and ammonium on an Alpkem Autoanalyzer (Pulse Instruments Ltd. Saskatoon, SK). I followed the same procedure after the soil incubation described below, and I

estimated potential N mineralization as the difference between initial and final inorganic N of the soil (Hart et al. 1994).

CO_2 and N_2O emissions

I used the substrate induced respiration-inhibition method (SIRIN) of Anderson and Domsch (1975) modified by Johnson et al. (1996) for dry soils, to determine CO_2 and N_2O emissions. Optimal incubation conditions were determined in preliminary experiments according to the criteria of Anderson and Domsch (1975). I tested concentrations of glucose (0, 1, 3, 5, and 8 mg g^{-1} soil), streptomycin sulfate (2, 3, 6, and 8 mg g^{-1} soil), and cycloheximide (0, 1.5, 3, 6, 10, and 15 mg g^{-1} soil) in preliminary incubations to determine the optimal combination and incubation time when respiration stabilizes for these soils. I incubated the soils with glucose as a substrate (1 mg g^{-1} dry soil), with cycloheximide (10 mg g^{-1} dry soil) as a fungal inhibitor, and with streptomycin (3 mg g^{-1} dry soil) as a bacterial inhibitor.

I placed 12 grams of dry soil in small beakers, moistened to 0.6 field capacity (field capacity for these soils: 25% moisture), and mixed with the antibiotics. Sixteen hours elapsed between addition of antibiotics and addition of C substrate and water for the stabilization of antibiotics in the soils. Samples were incubated for 12 hours in sealed incubation bottles in the dark at 25 °C. After the incubation I collected air samples with 25 ml syringes and analyzed for CO_2 and N_2O concentrations on a Shimadzu GC14-B gas chromatograph (Shimadzu Scientific instruments, Columbia, MD) fitted with an FID and ECD (electron capture detector), with a temperature

program of 325 °C detector, and 40 °C column. I used certified N_2O standards (Matheson TRI-GAS, Fort Collins, CO) for calibration.

Statistical analyses

To test for the effects of biosolids application on the three response variables CO_2 and N_2O emissions and potential N mineralization I utilized a mixed model analysis of variance in SAS statistical software (SAS Institute Inc 1989), with fixed effects as sites (represented by the patches of different plant communities), with two levels (SGS and biosolids), and antibiotic treatment with four levels (no inhibitor, both inhibitors, bacterial inhibitor: streptomicin, and fungal inhibitor: cycloheximide). This was an unbalanced model with SGS n = 3, and biosolids n = 6.

To test the secondary question about potential differences between biosolids sites dominated by different plant functional types, I utilized a mixed model analysis of variance in SAS statistical software (SAS Institute Inc 1989), with fixed effects as sites, testing for the effects of two levels (biosolids dominated by C3 grasses, and biosolids dominated by exotic forbs), and of antibiotic treatment with four levels (no inhibitor, both inhibitors, bacterial inhibitor: streptomicin, and fungal inhibitor: cycloheximide). This was a balanced model with n = 3 for each one of the plant community types

I used a least significant difference mean separation test when significant main effects occurred. All data were transformed as needed to fit the normality and homogeneity of variance assumptions of the applied statistical test, and the level of significance of all tests was determined as a p-value < 0.05.

Results

CO_2 and N_2O emissions: fungal vs bacterial emissions

CO_2 emissions were higher than N_2O released, irrespective of site and plant community type. Fungal emissions of CO_2 were equal to emissions with no inhibitors, whereas bacterial emissions of CO_2 were significantly lower than fungal emissions in the SGS sites (Figure 4.1).

No differences were detected between fungal and bacterial emissions of N_2O in any of the sites (Figure 4.2). Even though bacterial emissions in the biosolids sites were half of fungal emissions, the high variability of the data did not allow detecting those differences (Figure 4.2). I did not detect potential N mineralization differences that could be attributable to different microbial functional groups (Figure 4.3). Fungal emissions of CO_2 were equal to emissions with no inhibitors, whereas bacterial emissions of CO_2 were significantly lower than fungal emissions in the historic biosolids sites dominated by C3 grasses and forbs (Figure 4.4).

Effect of biosolids applications on GHG emissions and potential N mineralization.

The sites dominated by C3 grasses also showed finer texture (clay loam) compared to the SGS and the biosolids sites dominated by forbs (loam), and the sites dominated by forbs presented higher %WFPS compared to the SGS and the C3 dominated soils (Table 1). Previous analyses of these sites showed that C3 dominated sites had the lowest total nitrogen and total organic carbon compared to the other two sites, whereas they have the highest soil available N. In addition, the

SGS soils presented the highest C:N ratio compared to the biosolids sites (Holfelder unpublished data).

Biosolids application dramatically increased GHG emissions and N mineralization (Figures 4.1 to 4.3). The biosolids sites dominated by exotic forbs showed significantly greater CO_2 emissions compared to the biosolids sites dominated by C3 grasses (Figure 4.4). N_2O emissions were significantly higher in the sites dominated by C3 grasses, compared to the biosolid sites dominated by forbs (Figure 4.5). The biosolids sites dominated by exotic forbs showed significantly greater N mineralization compared to the biosolids sites dominated by C3 grasses (Figure 4.6).

Discussion

High level biosolids applications at this urban location are an example of the dramatic changes that resources additions can cause in semiarid ecosystems. Greenhouse gas emissions and potential N mineralization remained at least twice as high as native systems, even 20 years after soils were amended. As previously reported for the shortgrass steppe, N additions determined long lasting increase in N_2O emissions (Mosier et al. 1996).

In addition, it appears that there were changes in biosolids application rate or disturbance and/or differences in texture that resulted in differences in the dominant plant community composition, soil organic matter and nitrogen that ultimately affected the level of GHG emissions and N mineralization.

CO_2 and N_2O emissions: fungal vs bacterial emissions

Contrary to my expectations, fungal and bacterial emissions of GHG did not differ in all the sites and under all the community types. Bacterial CO_2 emissions were lower than fungal emissions in the SGS, results that are consistent with previous results in the SGS (Bontti, unpublished data), but no differences were detected in the biosolids sites when considered all together. When biosolids soils were contrasted i.e. C3 dominated soils vs forb dominated soils, in both sites bacterial CO_2 emissions were lower than fungal emissions. These results are the opposite as found in the shortgrass steppe under controlled N fertilization (Bontti unpublished data), and they are consistent with the idea that N additions alone decrease fungi to bacteria ratios (de Vries et al. 2006) whereas carbon additions, which was the case through biosolidis additions, increase fungi to bacteria ratios (Kandeler et al. 2008).

Microbial communities of infertile ecosystems (represented here by the SGS plots) are frequently dominated by fungi and those of more fertile productive ecosystems (here represented by the amended soils), are primarily dominated by bacteria (de Vries et al. 2006; Wardle et al. 2004). In addition, exotic species can decrease fungi:bacteria ratios, and increase N-related enzyme activity, changes that generally increase nitrification rates and available NO_3^- (Kourtev et al. 2003). My approach did not allow detecting those differences, and more detailed analyses would help clarify these discrepancies.

I detected no differences between fungal and bacterial emissions of N_2O in any of the sites, which was surprising since previous studies in semiarid soils

(Crenshaw et al. 2008; McLain and Martens 2006) and in the SGS showed that fungi are important components of N_2O production (Bontti, unpublished data). One of the problems for detecting differences between fungi and bacteria in N_2O emissions was the high variability in the data.

Effect of biosolids applications on GHG emissions and potential N mineralization.

Biosolid application was more important than microbial functional groups in accounting for patterns in GHG emissions and potential N mineralization. Both GHGs increased in response to biosolids application (Figures 4.1 and 4.2). The high GHG emissions and N mineralization from the biosolid sites may be a response to several factors. First, microbial biomass of fungi and bacteria increases linearly with rate of biosolids applications (Dennis and Fresquez 1989; Pascual et al. 1999), likely increasing carbon and N mineralization (Sullivan et al. 2006b). Second, the metabolic quotient, the amount of respiration per unit of microbial biomass carbon (Barbarick et al. 2004; Garcia-Gil et al. 2004) also increases in response to biosolids amendment.

Substrate quantity and quality are fundamental controls over decomposer and nitrifier activity, and the latter is strongly dependent on the nitrogen and lignin of plant tissue (Porazinska et al. 2003). Microbial community structure and activities might be significantly changed by the quality and quantity of substrate entering the soil (Hooper et al. 2000). The increased quality of biosolids substrate (Holfelder, unpublished data), and the increased plant tissue quality (i.e. lower C:N ratio) in

response to biosolids amendments (Fresquez et al. 1990), likely resulted in enhanced N mineralization in sites where biosolids were applied. These processes are conducive to positive feedbacks between plant litter quality and microbial N mineralization that perpetuate plant community change in time, even after decades that resources additions stopped (Vinton and Burke 1995). Furthermore, it has been postulated that more readily utilizable carbon is released into the rhizosphere of species that become dominant in fertilized grasslands, resulting in greater stimulation and carbon utilization by microbes. In contrast, the non-fertilized grasslands contain more recalcitrant compounds, the bulk of soil organic matter, which may explain the high fungal numbers and the lower metabolic activity found in non-fertilized grasslands, compared to those under resource additions (Grayston et al. 2004).

CO_2 emissions and potential N mineralization were significantly higher in the exotic forbs sites compared to the SGS and C3 species sites, and these differences were observed irrespective of the application of inhibitors. The sites dominated by forbs have the lowest bulk density, and highest total organic carbon and total organic N (Holfelder, unpubublished data), factors that might have been conducive to decomposition and nitrification.

Interestingly, N_2O emissions were significantly higher in sites dominated by C3 species compared to the forb dominated sites. I did not find higher N_2O emissions in sites under higher N mineralization potential, which suggests that N_2O was not the byproduct of nitrification. However, even though potential NO_3^- mineralization was one order of magnitude lower in the C3 grass dominated sites

compared to the exotic forbs sites, concentrations of NO_3-N in soils were 8-fold higher in the sites dominated by C3 grasses (Holfelder unpublished data), and available N is also positively related to N_2O emissions (Davidson et al. 2000). Furthermore, NO_3^- is more energetically convenient than nitrous oxide (N_2O) as an electron acceptor for denitrifiers (Chapuis-Lardy et al. 2007), thus increasing N_2O emissions. Therefore, incubations controlling N substrates should be used to assess the origin of N_2O.

The discrepancy between potential N mineralization and available soil N may be a response to differential uptake rate of the exotic forbs respect to C3 grasses or differential immobilization in organic matter in response to heterogeneous biosolids applications.

I found no differences in N_2O emissions that may be attributable to differences in % WFPS, which was unexpected, based on the conceptual model of Davidson et al. (2000), which postulates that in dry well aerated soils, the oxidative process of nitrification dominates, and the more oxidized gas, NO is the most common NOx emitted. In wet soils, where gas diffusivity is lower, and aeration is poorer, much of the NO is reduced, before leaving the soil, and the more reduced oxide, N_2O is therefore the dominant product. When the soil is even more water saturated, the N_2O is further reduced to N_2 by denitrifiers before it diffuses out of the soil. The soils in this experiment were at a level (14-40 %WFPS) that favors NO emissions, which I did not measure in this study, likely underestimating NOx emissions. Nevertheless, my results are in agreement with previous studies in the

shortgrass steppe (Martin et al. 1998) that showed complex relationships among soil WFPS and texture to explain NO_x production and diffusivity.

Conclusions

This study indicates that biosolid applications can have long lasting consequences, in particular by increasing GHG and available NO_3^- at least 20 years after the cessation of sludge additions. Fungi are an important component of soil respiration in SGS and in sites where biosolids application was accompanied by dominance of C3 grasses or forbs. Contrary to previous literature, I did not detect fungi as the main component of N_2O emissions, but these results should be further tested due to the high degree of variability in the data. Further work should examine the implications of biosolids application in particular for specific microbial groups that mediate nitrification and denitrification and might increase N_2O emissions in response to the high load of nutrients applied with biosolids. Contrary to predictions based on previous literature, my approach did not detect changes in fungi:bacteria ratio that might in turn explain differences in GHG emissions. Potential N mineralization was highest in sites that had received biosolids and that are dominated by exotic forbs, but this index of nitrogen availability should be complemented by other, *in situ* indices of available N, to give a better idea not only of N that is being mineralized, but N that is readily absorbed by plants.

CO_2 and N_2O released were dependent upon the plant community composition, which seems to reflect heterogeneity in the biosolids application rate and quality, because available NO_3^- and bulk density of soils also differ among these sites. This heterogeneity might be due to previous differences in soil texture as well.

As a consequence CO_2 was significantly higher in sites dominated by exotic forbs, and N_2O was significantly higher in sites dominated by C3 grasses.

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Table 4.1: Soil properties of three sites in the Aurora reservoir: shortgrass steppe native vegetation (SGS); historical biosolids application currently dominated by exotic forbs (BEF), and historical biosolids application currently dominated by C3 grasses (BC3G). %WFPS: percent water filled pore space. Values in brackets represent mean standard error of N = 3.

	SGS	BEF	BC3G
Sand (%)	46.3 (4)	36.3 (4)	32.8 (7)
Clay (%)	25.7 (5)	25.8 (5)	34.3 (6)
%WFPS	15 (8)	40 (16)	19 (3)

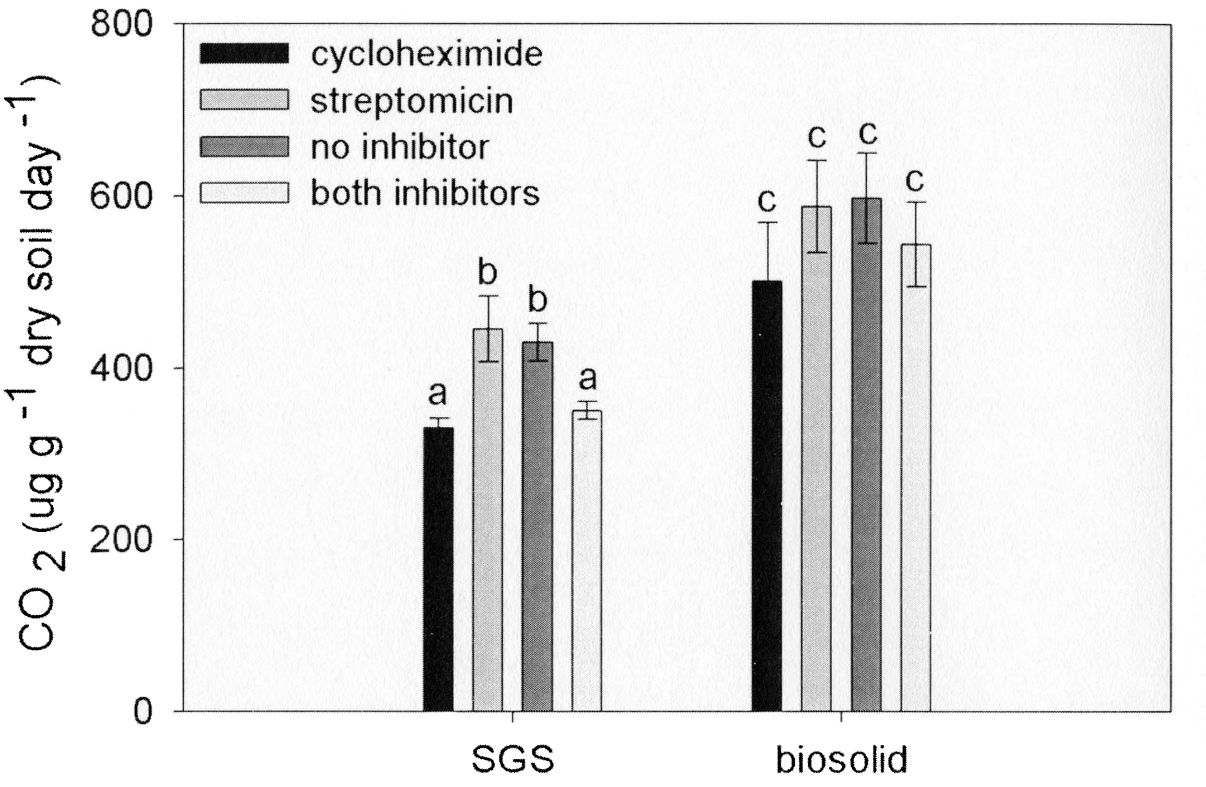

Figure 4.1: Carbon dioxide emissions (±SE) in two sites in the Aurora reservoir: shortgrass steppe native vegetation (SGS); and historical biosolids application. Cycloheximide indicates fungal inhibition and therefore Bacterial effect, and streptomicin determines fungal effect. Values designated with different letters indicate significant differences among antibiotic treatments within one site, and differences between sites when the same antibiotic was applied. Comparisons were made through LSD multiple-range test ($p<0.05$).

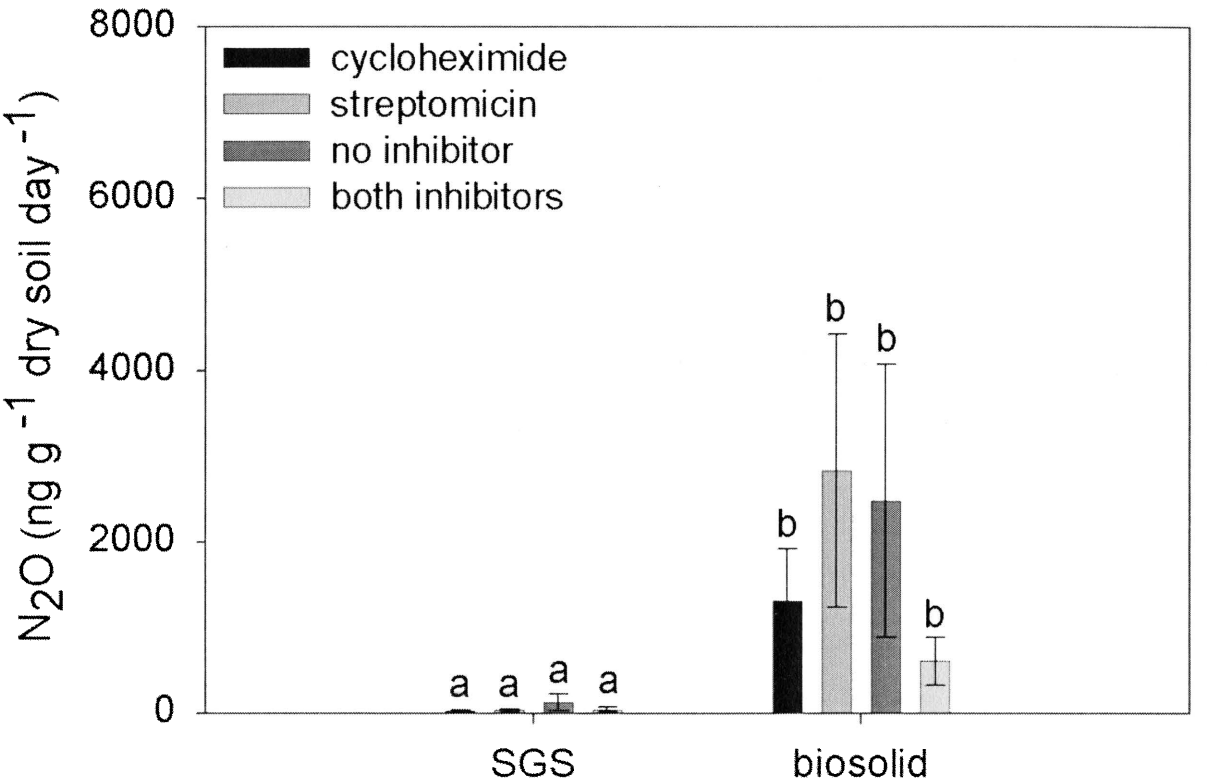

Figure 4.2: Nitrous oxide emissions (±SE) in two sites in the Aurora reservoir: shortgrass steppe native vegetation (SGS), and historical biosolids application. Cycloheximide indicates fungal inhibition and therefore bacterial effect, and streptomicin determines fungal effect. Values designated with different letters indicate significant differences among antibiotic treatments within one site, and differences between sites when the same antibiotic was applied. Comparisons were made through LSD multiple-range test ($p<0.05$).

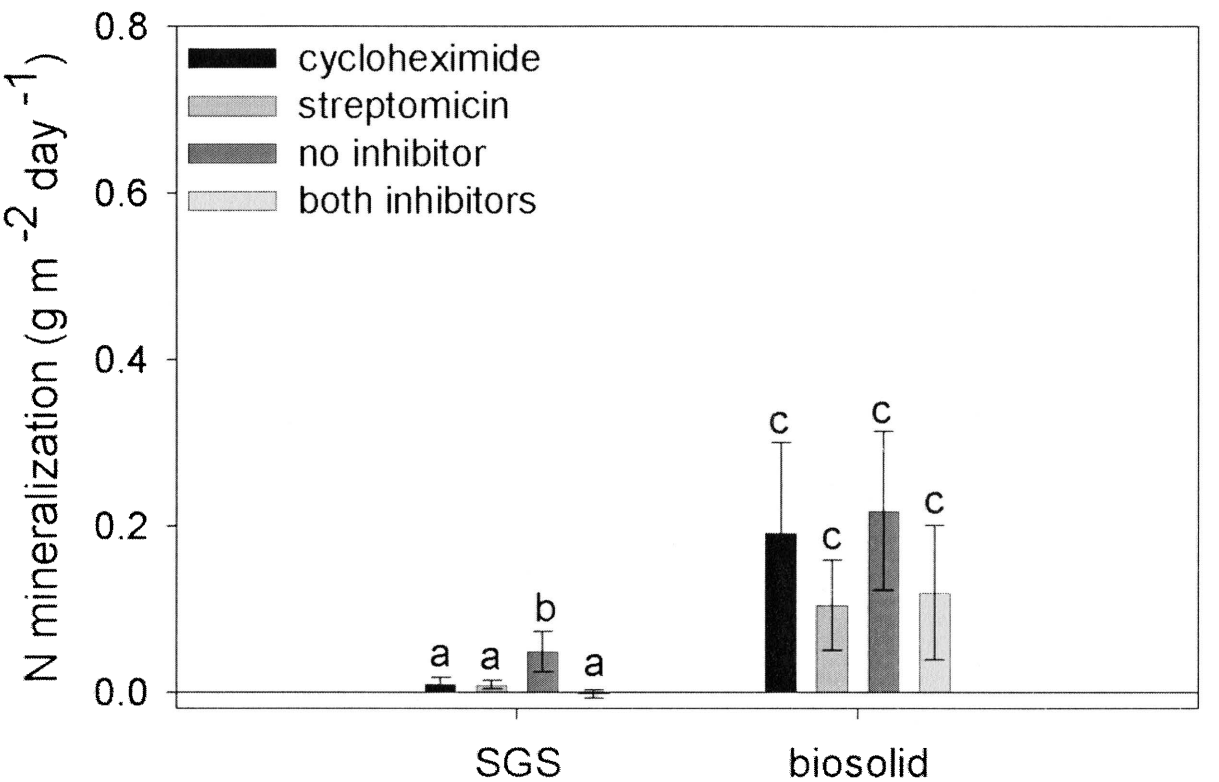

Figure 4.3: Potential nitrogen mineralization (±) in two sites in the Aurora reservoir: shortgrass steppe native vegetation (SGS); historical biosolids application. Cycloheximide indicates fungal inhibition and therefore bacterial effect, and streptomicin determines fungal effect. Values designated with different letters indicate significant differences among antibiotic treatments within one site, and differences between sites when the same antibiotic was applied. Comparisons were made through LSD multiple-range test ($p<0.05$).

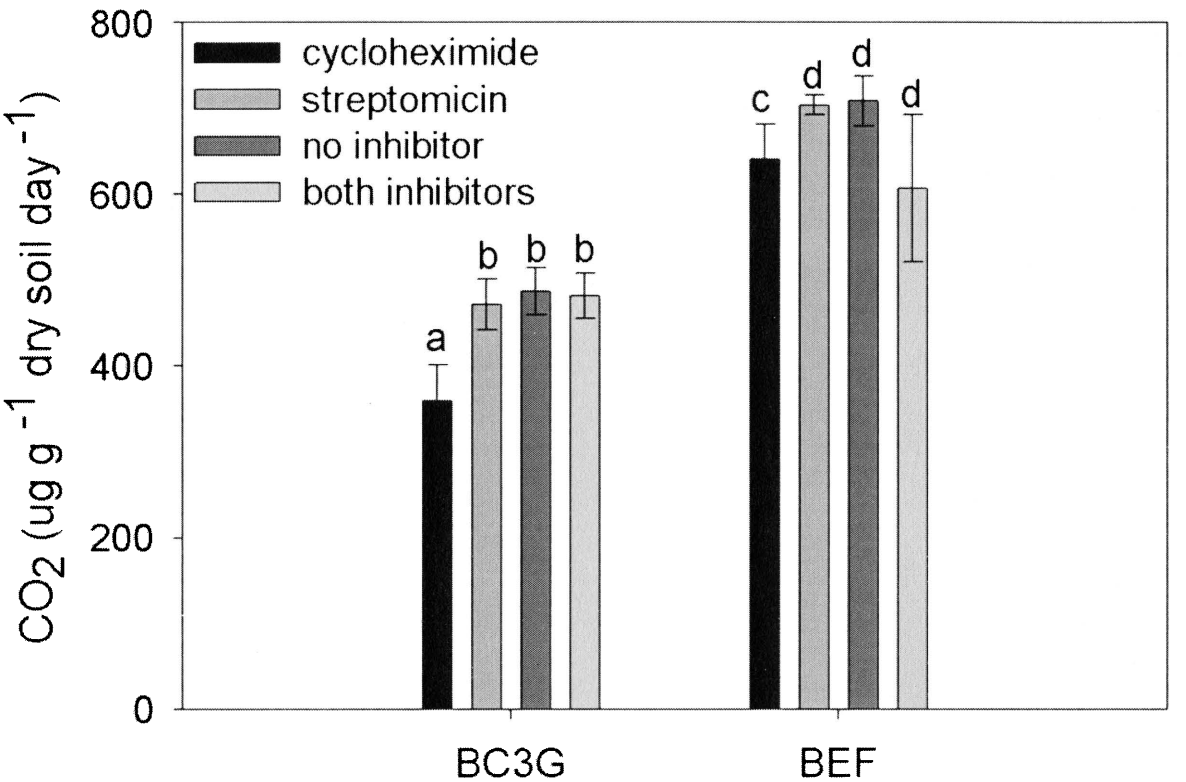

Figure 4.4: Carbon dioxide emissions (±SE) in two sites in the Aurora reservoir under historical biosolids application: currently dominated by C3 grasses (BC3G), and currently dominated by forbs (BEF). Cycloheximide indicates fungal inhibition and therefore bacterial effect, and streptomicin determines fungal effect. Values designated with different letters indicate significant differences among antibiotic treatments within one site, and differences between sites when the same antibiotic was applied. Comparisons were made through LSD multiple-range test ($p<0.05$).

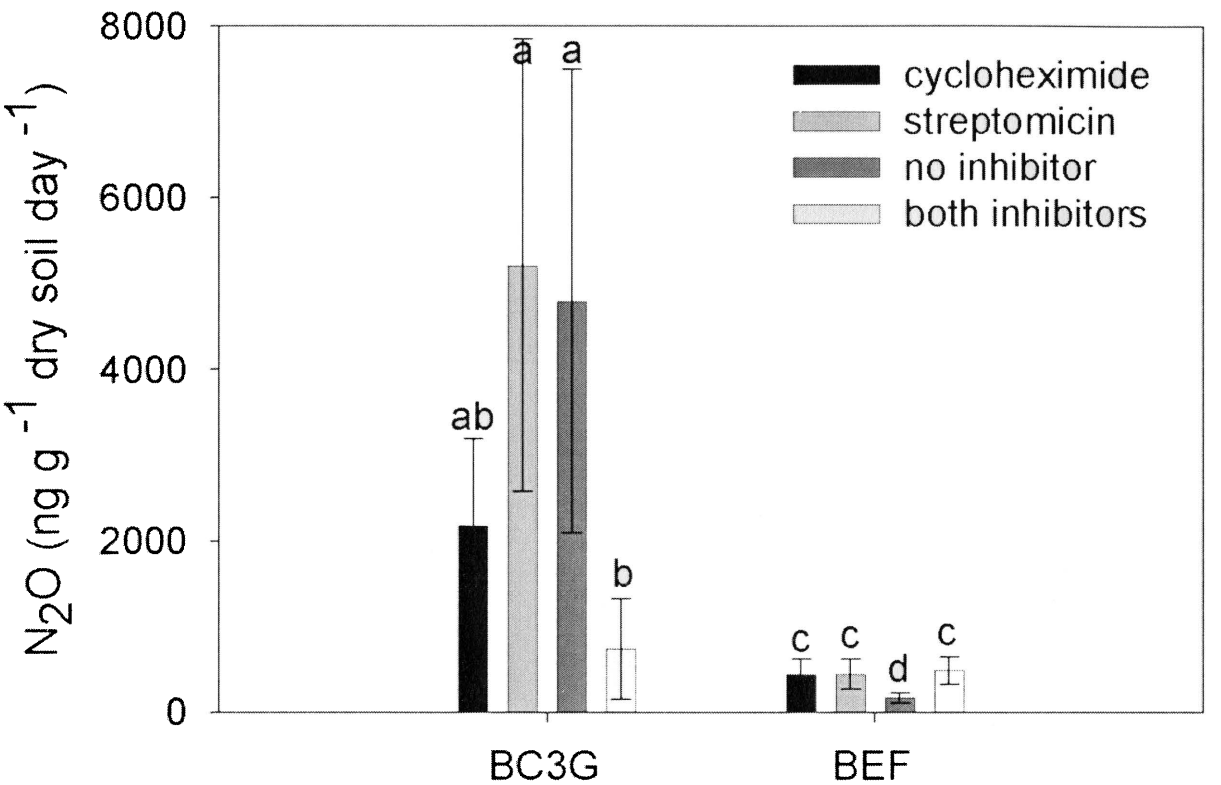

Figure 4.5: Nitrous oxide emissions (±SE) in two sites in the Aurora reservoir under historical biosolids application: currently dominated by C3 grasses (BC3G), and currently dominated by forbs (BEF). Cycloheximide indicates fungal inhibition and therefore bacterial effect, and streptomicin determines fungal effect. Values designated with different letters indicate significant differences among antibiotic treatments within one site, and differences between sites when the same antibiotic was applied. Comparisons were made through LSD multiple-range test ($p<0.05$).

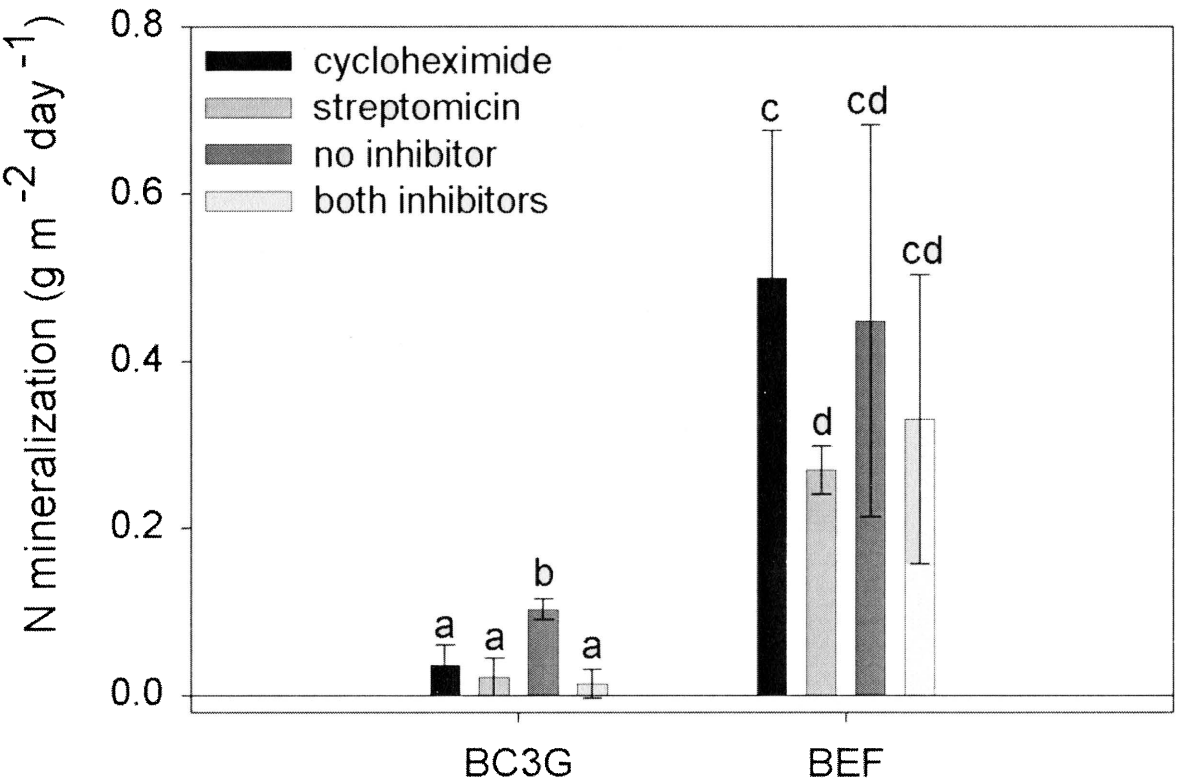

Figure 4.6: Potential nitrogen mineralization (±) in two sites in the Aurora reservoir under historical biosolids application: currently dominated by C3 grasses (BC3G), and currently dominated by forbs (BEF). Cycloheximide indicates fungal inhibition and therefore bacterial effect, and streptomicin determines fungal effect. Values designated with different letters indicate significant differences among antibiotic treatments within one site, and differences between sites when the same antibiotic was applied. Comparisons were made through LSD multiple-range test ($p<0.05$).

CHAPTER V: SUMMARY AND CONCLUSIONS

The general objective of this research was to evaluate the response of soil microbial processes to plant functional type and changes due to soil resource additions. I focused particularly on temporal patterns of microbial nitrogen (N), CO_2 and N_2O emissions, and discriminating between fungal and bacterial emission of these greenhouse gases.

In chapter II I found that aboveground plant N followed the predicted seasonal patterns of N uptake, based on phenological differences among plant functional types. Belowground N did not showed the predicted seasonal patterns of N uptake, which might reflect changes in N allocation from roots to shoots during the growing season. Microbial N followed the same temporal pattern and level of N uptake, independently of the plant functional type under which microbes were growing. Microbial N seemed to be driven by precipitation and temperature as well as available plant root exudates, as indicated by a rapid increase after a record high precipitation, and sharp decrease in fall when plants are senescent.

Microbial N was not out of phase with respect to plant uptake, considering all plant functional types together. These results have two main implications: First, there are no time points during the growing season where active N mineralization occurs out of phase with plant growth. This suggests that there are mechanisms for N retention. Second, in spite of overlapping temporal patterns of N uptake by plants and microbes, plants showed N uptake even though soil available N was low. Since the two native plant functional groups showed the potential for reallocation from roots to shoots during the growing season, and C4 grasses grew even when soil

available N was low, these plant functional groups are able to oucompete microbes over the long term (Kaye and Hart 1997).

Historical resource additions showed long lasting effects on plant and microbial N, by increasing plant biomass and N in C3 grasses, and decreasing microbial N sequestration. These differences were not evident in N mineralization and soil N availability, presumably because of rapid plant uptake. These changes should be taken into account when predicting the effect of N deposition, in particular the long lasting consequences, on the shortgrass steppe.

In Chapter III I found that fungi dominated CO_2 and N_2O emissions in the native vegetation of the shortgrass steppe (without N additions.) The temporal pattern of CO_2 and N_2O emissions suggests that these emissions increased in response to high precipitation, temperature and root exudates, and decrease by the end of the growing season, likely in response to low temperature and lack of available root exudates because of plant senescence. Plant functional type did not affect the level and temporal pattern of CO_2 and N_2O emissions from fungi or bacteria, although exotic forbs seemed to change the balance of fungal and bacterial emissions. Hence, further analyses would help to identify differences in microbial communities or microenvironmental characteristics that result in increased bacterial production of CO_2 and N_2O under exotic forbs. These mechanisms have important implications for predicting emissions under future scenarios because increased CO_2 (Kandeler et al. 2008), and nitrogen additions (de Vries et al. 2006) have antagonistic effects on fungal to bacterial ratios, and the interaction between these anthropogenic effects is not clear in the shortgrass steppe.

In chapter III I also found that, despite the lack of difference in total CO_2 emissions, historical resources additions decreased fungal CO_2 emissions, and increased bacterial CO_2 emissions. These results suggest that N additions changed the microbial community towards a more bacterial dominated one, as found in several previous works (de Vries et al. 2006; Grayston et al. 2004; Kourtev et al. 2003). Fertilization has a long lasting effect by increasing greenhouse gas emissions from bacteria in particular, and these long term consequences should be taken into account when predicting greenhouse gas emissions under land use change in the shortgrass steppe.

In chapter IV I found that biosolids amendments have long lasting effects in the shortgrass steppe, by increasing GHG emissions and nitrogen mineralization at least 20 years after the amendment cessation. Even though these trends are similar to expectations based on historical resource additions effects found in chapter III, there were differences in the level of CO_2 and N_2O released from different plant communities. Differences in plant community seemed to be in response to variability in soils characteristics presumably due to variations in biosolids application rate and quality. Thus soils dominated by forbs were high in total organic carbon, total nitrogen and had low bulk densities. CO2 emissions and N mineralization were higher in these soils compared to those currently dominated by C3 grasses. Sites dominated by C3 grasses had bulk density comparable to the native shortgrass steppe, and they were characterized by the highest N_2O emissions.

Chapter IV indicates that fungal CO_2 was higher than bacterial CO_2 in biosolids amended soils. These effects are particularly important in view of the

predicted increase of fungi under increased CO_2 scenarios, because the interaction between anthropogenic biosolids disposal, and increased CO_2 might further increase CO_2 emissions. N_2O and N mineralization also increased as a consequence of biosolids applications, but there were no differences between fungal and bacterial emissions of N_2O or N mineralization, and the N_2O results should be further tested due to the high variability in the data. N mineralization increased in sites dominated by exotic forbs, but available N was low in these sites, indicating rapid plant uptake or high immobilization due to high organic matter content. Incubations including different levels of water filled pore space, and different quantity and quality of substrate, would be helpful to elucidate which are the predominant processes conducive to N_2O emissions in the shortgrass steppe, when biosolids are applied.

In summary, seasonal patterns of microbial biomass N and GHG emissions are not influenced by plant functional type phenology, but by seasonal changes in rainfall and temperature. Unlike other ecosystems, plant and microbial N uptake is synchronous in the shortgrass steppe. Plant biomass and N increased, whereas total microbial N and fungal CO_2 emissions decreased in response to historical resources additions in the shortgrass steppe, independent of the level of soil available N. In addition, historical resources additions increased bacterial GHG emissions and blurred differences between fungal and bacterial emissions that occur under native plant functional types, where fungal emissions are higher than bacterial emissions. When N additions occur through biosolids amendments, there are no differences between fungal and bacterial emissions, but there is a long lasting increase on GHG emissions and N mineralization. The interpretation of these results

requires further study of complex interactions among several factors such as increased organic matter, total organic carbon and nitrogen, and increased soil disturbance through biosolids or N fertilizer application.

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