Fluxes of CH_4 and N_2O, soil N dynamics, and microbial communities in grassland grazing systems

by

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Abstract

The greenhouse gases (GHG) of nitrous oxide (N_2O) and methane (CH_4), from cattle grazing systems, need to be quantified to determine its environmental footprint. In grazed cattle systems, soil GHG fluxes dynamics respond to weather variations, soil cover, substrate availability, nutrient deposition, and land management. Soil microbial communities drive changes in carbon (C) and nitrogen (N). This study quantified N_2O and CH_4 fluxes, inorganic N dynamics, and the soil microbial community interactions in temperate and tropical grasslands.

The first study, located at Konza Prairie Biological Station, measured N_2O and CH_4 fluxes from annual and 3-yr patch burned sites from summer 2014 to 2017. Measurements included GHG fluxes from static chambers, air temperature, soil water, and soil inorganic N. Emissions of N_2O were relatively low and varied as a source or a sink. Fluxes of CH_4 were a net sink. Overall, the tallgrass prairie was a small source of N_2O ranging from 9.5 to 35.9 kg CO_2-eq ha^{-1} yr^{-1}, and a sink of CH_4 ranging from -8.0 to 51.8 kg CO_2-eq ha^{-1} yr^{-1}. During the 3-yr period, annual burning resulted in net emissions, and 3-yr patch burning GHG sink ranged from -1.7 to -4.2 kg CO_2-eq cow/calf land $unit^{-1}$ yr^{-1}. Annual grassland budgets differed by year, with lower net sink during years with relatively higher precipitation.

A second temperate prairie site involved a 28-d field study repeated for two years to determine the interaction between precipitation with urine and manure additions on GHG fluxes, inorganic N, and soil microbial communities. Higher N_2O (52.4 g N_2O ha^{-1} d^{-1}) fluxes occurred under the urine treatments and ambient conditions. The N_2O sink varied from -24.0 to -0.02 g N_2O ha^{-1} d^{-1} with no differences between treatments. Inorganic N from urine and feces reduced the CH_4 sink from -5.9 g CH_4 ha^{-1} d^{-1} to emissions up to 9.1 g CH_4 ha^{-1} d^{-1} under high precipitation. The soil microbial community decreased within the first seven days after the urine

and manure addition, and high precipitation and then increased by up to 45% within a 28 d period after the N addition or high precipitation. Overall, high precipitation and urine significantly altered soil N dynamics, causing temporary stress to the soil microbial communities, therefore altering N_2O and CH_4 fluxes. An automated static closed chamber system was used to determine the N_2O and CH_4 diurnal cycle, and the effect of sampling frequency on flux estimates. Daily average and cumulative flux are recommended to accurately estimate N_2O fluxes. The best sampling time for N_2O fluxes was between 6:00 to 12:00. During summer, biweekly sampling frequency overestimated cumulative N_2O flux. Furthermore, monthly sampling during the months of March through April overestimated CH_4 uptake. Daily, weekly, and biweekly sampling frequencies from 6:00 to 12:00 h was the most accurate method to estimate cumulative N_2O fluxes from March to August.

The third experimental site located at the International Center for Tropical Agriculture in Cali, Colombia studied the effect of urine on GHG fluxes, nitrification rates, and microbial community composition in *Brachiaria* pastures. Ammonia-oxidizing archaea (AOA) and bacteria (AOB) qPCR analysis were used to identify the impact on nitrifier populations. Soil emissions with urine applications on *Brachiaria* pastures ranged from 2.1 to 11.9 g N_2O ha^{-1} d^{-1}. Nitrification rates ranged between 0.72 to 4.5 mg $N\text{-}NO_3^-$ kg^{-1} soil d^{-1}. *Brachiaria humidicola* 16888 reduced nitrification and increased arbuscular mycorrhizal fungi and actinomycetes. Overall, grasslands provide a sink for CH_4 and are a small sink or source of N_2O depending upon the weather and management practices. These fluxes are governed by nutrient dynamics and soil water. As a result, cattle grazing systems have a lower environmental footprint than previously assumed.

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Table of Contents

List of Figures

List of Tables

List of Supplemental Figures

List of Supplemental Tables

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Dedication

In memory of Francisca Padilla, my great-grandmother.

1912-2018

For your work on the land, for sustaining a family as a woman in agriculture, and for all your love.

“Agriculture is the backbone of a country”

“La agricultura es la espina dorsal de un pueblo”

-Eugenio María de Hostos

“El gran ciudadano de las Américas”

Chapter 1 - Introduction

Grazed grasslands are among the largest ecosystems in the world with a total area estimation of 52.5 MM km^2 (Reynolds and Frame, 2005). Currently, research has focused on management of grazed grasslands to increase system resilience, reduce the environmental footprint, and offset greenhouse gas (GHG) emissions to meet the food, nutritional, and byproducts demand of the projected 9.6 billion people by 2050 (Foley et al., 2011; UN, 2013; Jat et al., 2014; Steiner et al., 2014; Nadeu et al., 2015; Rao et al., 2015; Tubiello et al., 2015). Grassland soils potential to mitigate GHG emissions through methane (CH_4) and nitrous oxide (N_2O) uptake have been extensively studied for understanding of the underlying mechanisms (Le Mer et al., 2001; Ciais et al., 2013; Polley et al., 2013). However, soil CH_4 uptake capacity and GHG emissions vary as a result of changes in soil nutrient availability, water holding capacity and structure resulting from changes in agricultural practices and weather conditions (Allard et al., 2007; McSherry and Ritchie, 2013; Russell et al., 2018). Also, temporal variations in GHG fluxes from grasslands respond to nutrient additions, carbon (C) availability, above and underground biomass, and weather conditions (temperature, precipitation) (Le Mer and Roger, 2001; Allard et al., 2007; Chapuis-Larfy et al., 2007; Conrad, 2009; Hayashi et al., 2015; Di and Cameron, 2016).

From a global perspective, the livestock sector contributes to 14.5 % of the total GHG emissions (Gerber et al., 2013). In the United States, the agricultural sector contributes 574.1 MMT CO_2, of the total CO_2 emissions (IPCC, 2014). During the last 15 yr, CH_4 and N_2O emissions from agricultural activities have increased by 12% and 6%, respectively (IPCC, 2014). Worldwide, the livestock sector represents approximately USD 1.4 trillion and employs 1.3 billion people (Thornton, 2010). Improving agricultural practices could potentially reduce CH_4

and N_2O emissions from livestock systems, and increase grasslands CH_4 capacity (Mosier et al., 1991; Allard et al., 2007; Chapuis-Lardy et al., 2007; Gerber et al., 2013). Improvement of agricultural practices can restore and maintain soil resources and production to an economical and environmental efficient grassland grazing system.

Soil organic C

Soils constitute the largest terrestrial organic C pool with 2,400 Pg C within the first 2 m (Batjes, 1996). Undisturbed lands, such as unmanaged grassland allocate biomass below ground achieving 0.5 to 2.0 Mg of C ha^{-1} yr^{-1}, which is considered a higher C sequestration rate than managed grasslands (Ogle et al., 2015). Therefore, practices that support the conversion of agricultural soils to native lands by agroforestry, grazed pastures, and no-till or reduced tillage practices are considered mitigation alternatives to reduce the agriculture's environmental footprint.

In agricultural systems, soil management practices define if an agrarian system is a source or a sink of C. Improved land use management by reducing soil disturbance and increasing nutrients inputs could potentially increase C storage capacity of soils (Balesdent et al., 2000; Dutaur and Verchot, 2007; Nadeu et al., 2015). Recent studies debate grazed grassland capacity for C sequestrations; perhaps most studies support the C sequestration theory under sustainable management practices (Balesdent et al., 2000; Powlson et al., 2011). In the U.S.A. grassland management practices include prescribed burning has a measure for control of vegetation and shrubs, control diseases, reduce wildfire hazard, improve wildlife habitat, improve plant productivity and quality, remove slash and debris, and facilitate grazing animal distribution (Fuhlendorf et al., 2011). Benefits from prairie burnings include the stimulation of mineralization, increased nutritional value of grasses, and nutrient recycling in the ecosystem.

However, appropriate conservation programs are vital for overseeing the dynamic role of burning practices in the ecosystem, including the ecological and social aspects (Fuhlendorf et al., 2011).

Under managed grasslands, the integration of biotechnology aspect by the use of plant species present a novel approach to adapt grazing systems to drought, high temperature, high precipitation, pest and diseases allowing higher resilience from the agricultural ecosystem (Steiner et al., 2014; Rao et al., 2015). Specific pastures traits are specific for increase root biomass, or use of pastures with deep roots systems are a mitigation practice to deposit C in deeper layers, improve soil structure, water dynamics, and recycle of leached nutrient.

Soil nitrogen dynamics

In soils, the nitrogen (N) cycle is a biochemical process carried by soil microorganisms and stimulated by N additions from organic and inorganic N additions. For a start, soil ammonium (NH_4^+) is electrostatically held by negatively charged clay surfaces and functional groups of soil organic matter. Once the NH_4^+ is transformed to nitrate (NO_3^-) by the nitrification process, N in the form of NO_3^- has less capacity to be bound to the soil and can be leached to deeper soil layers or further processed until lost to the atmosphere as N_2O emissions by denitrification (Di and Cameron, 2016). These losses of N through nitrification and denitrification in the form of N oxides contribute to environmental consequences through eutrophication and greenhouse gas emissions.

Major N biochemical processes in soils, such as N mineralization, N fixation, immobilization, ammonification, nitrification, and denitrification controls N availability for forage uptake altering forage quality, growth, and protein content. In grazing systems, the loss of N can occur in three main ways: ammonia (NH_3) volatilization, NO_3^- leaching, and N_2O

emissions. From animal urine depositions, the NH_3 volatilization results from the conversion of NH_4^+ to NH_3 under pH levels greater than 7, which will be a loss to the atmosphere. Among the main soil biochemical processes in the N cycle nitrification rates carried by *Nitrosomonas*, and *Nitrobacter* oxidizes NH_4^+ to NO_3^-. Soil N_2O emissions are further discussed in section Soil N_2O fluxes (Page 9). Furthermore, Crenshaw et al. (2008) concluded that denitrification by fungal and autotrophic nitrification by bacterial communities dominate N transformation in semiarid grasslands ecosystems. Overall, research on the N cycle suggests soil N and C availability and soil pH are the main predictors of soil microbial biomass, composition, and diversity (Nugroho et al., 2007; Hobbie and Hobbie, 2013; Zhalnina et al., 2015).

Soil physical properties on GHG emissions

Soil functions, and as a result, soil GHG fluxes, depend on three main components, physical, chemical, and biological. Soil physical properties include texture, structure, density, temperature, aeration, water flow, and redox conditions. Primary soil particles, sand, silt, and clay, its proportion in the soil defines soil texture (Osman, 2013). While, the geometric arrangement of the soil particles define soil structure (Osman, 2013). Soil texture and structure regulate essential functions such as soil aeration, water movement, and nutrient holding capacity; affecting soil microbial activity and dynamics (van Zwieten et al., 2009; Ball, 2013; Osman, 2013). For example, Ball (2013) poor soil structure reduces gas diffusion and restricts aeration resulting in N_2O emissions, depending on N availability. On another hand, the same study concluded that well-aerated soils favored CH_4 oxidation (Ball et al., 2013).

Microorganisms role on C

Recent studies evidence the microbial community role in C sequestration, as the result of the decomposition, and mineralization processes (Batjes, 1996; Grandy and Neff, 2008; Borken

and Matzner, 2009). Organic biomass supplied to microbes determine the microbial growth rate and the rate of nutrient release (Borken and Matzner, 2009; Hobbie and Hobbie, 2013; Zhalnina et al., 2015). Soil microbiota is responsible for organic matter decomposition and is part of biochemical reactions of nutrient transformation from organic to inorganic forms. Soil C dynamics occurs mainly by microbial activity. Species richness caused by increases in soil C through C sequestration can influence the microbial recovery after stressful events such as high precipitation and drought (Singh et al., 1997; Borken and Matzner, 2009; Butterbach-Bahl et al., 2013). Kallenbach et al. (2016) discussed how stabilized soil organic C comes from a microbial origin, rather than from plant origin; which supports the achieving a higher understanding in soil-plant-microbial relations, and its role in soil C sequestration.

Environmental drivers for soil microorganisms

Environmental conditions influence microbial responses, community diversity and composition, and its role in the ecosystem ecology (Balser and Firestone, 2005). The primarily known drivers of soil microbial activity are soil water, temperature, nutrient availability, pH, soil fauna and flora activities, and soil structure (Ludwig et al., 2001; Chapuis-Lardy et al., 2007; Dutaur and Verchot, 2007; Manzoni et al., 2012). Precipitation is associated with microbial stresses causing changes in soil C and N dynamics (Kieft et al., 1987; Manzoni et al., 2012). Microbes adapt to stress by allocating resources to survival pathways, change to anaerobic conditions survival pathways, move to a dormancy stage. When a microbe acclimates to stress, especially fungi, its energy and nutrients are redirected to synthesize chaperones to stabilize proteins and osmolytes to reduce water potential and maintain hydration (Csonka, 1989). Manzoni et al. (2012) indicate that in the future microbial activity will be limited by weather conditions, resulting in inactivity periods from decomposers and slower nutrient cycling in soils.

Soil water controls microbial activity and directly affects N_2O and CH_4 fluxes. Bacteria producing N_2O and CH_4 are triggered under anaerobic conditions (Bao et al., 2012); while CH_4 uptake occurs under aerobic conditions (Fiedler et al., 2005). Under drought conditions, nutrient movement through the soil becomes limited which limits biochemical processes and reduces GHG emissions. Harris (1981) explained how, during a drought, the microbial cell tends to accumulate solutes to reduce its internal water potential to avoid excessive dehydration. As a result, microbes can reduce by about 90% of their cellular C and N assimilate capacity (Killham, 1986; Sugai and Schimel, 1993). Stark and Firestone (1995) indicated that nitrifiers suffer dehydration under conditions below -0.6 MPa, while fungi are considered more tolerant than bacterial communities to drought conditions.

Microbial processes of nitrification and denitrification are the dominant sources of N_2O (Firestone and Davidson, 1989), and these soil microbial processes are subject to rapid responses to wetting and thawing events (Davidson, 1992). Rewetting induces pulses in heterotrophic respiration and nutrient mineralization affecting nutrient availability (Borken and Matzner, 2009). During rewetting, after bacteria accumulated osmolytes because of the drought, the microbes dispose of the osmolytes by respiring, polymerizing or transporting the osmolytes across the cell membrane.

Under anaerobic conditions, denitrifiers use alternative pathways producing N_2O (Holmes et al., 1996). Harris (1981) concluded that solute diffusion dominates microbial growth interaction under high water. Chimner and Welker (2005) described snow accumulation has the same effect as precipitation, causing an increase in anaerobic respiration.

Furthermore, soil amendments and fertilizer additions change soil pH and influence microbial activity. In the case of soil amendments such as lime, applications lead to a neutral pH

releasing CO_2 and triggering N_2O emissions (Nugroho et al., 2007; Cuhel et al., 2010). Minimum microbial activity and GHG emissions are expected from low pH (Dalal and Allen, 2008). Overall, abiotic factors trigger GHG emissions; however, oxygen, root activity, soil cover, and nutrient availability concentrations controlled the overall microbial dynamics and GHG fluxes (Butterbach-Bahl et al., 2013).

Greenhouse gas emissions from beef-cattle grazed pastures

Cattle (*Bos taurus*) management practices were the largest emitters of CH_4 enteric fermentation and manure management, representing 23.2% of the total CH_4 from anthropogenic activities (EPA, 2016). Specifically, emissions from beef-cattle grazing systems during 2014 were 116 MMT CO_2; which represent 71% of the total livestock emissions (IPCC, 2014). The management of animal residues, such as manure, represent 6,572 kt and 59 kt ha^{-1} yr^{-1} for CH_4 and N_2O emissions, respectively (IPCC, 2014). A portion of nutrients provided by manure and urine are taken up by the pastures and recycled back to the soil when the grazing animals consume the plants. An even distribution of manure and feces in grazing areas can result in better nutrient uptake by plants and reduce the presence of areas of the soil with high N concentration, and organic matter turnover; these areas are commonly known as “hotspots” (Petersen et al., 2004).

Additions of animal urine and feces have an essential role in soil nutrient cycling, especially C and N, in grazed pasture systems (Haynes and Williams, 1993). Biochemical processes generated by nutrient addition from animal feces and urine affect plant nutrient uptake, soil C sequestration, GHG emissions, and soil properties such as pH and soil moisture. The addition will also produce changes in soil pH, soil microbial community, nutrient concentrations,

nutrient cycling, and as a result in nutrient uptake by plants; these factors may result in changes in the microbial community and nutrient dynamics (Hobbie and Hobbie, 2013).

The contribution of urine to N_2O emissions are higher than the contribution from feces (Lee et al., 2014; Ussiri and Lal, 2013). In the case of urine, these spots create areas with high urea concentrations, which induce stress on soil microorganism (Bertram et al., 2012; Peters et al., 2013). Mulvaney et al. (2008) estimated N volatilization from grazing dairy cattle was 5.1 kg N cow^{-1} yr^{-1}. Also, Saarijärvi and Virkajärvi (2009) describe urine as a significant source of N losses in grazing systems by surface runoff, NH_3 volatilization, and denitrification. Depending on the animal diet and water uptake, the N loading rate of a cow urine patch can range between 700 and 1200 kg N ha^{-1} (Haynes and Williams, 1993; Jarvis et al., 1995). Chantigny et al. (2006) reported that soils with inputs near 300 kg N ha^{-1} yr^{-1} from manure or mineral fertilizer, produce N_2O emissions at a rate of 850 g N ha^{-1} yr^{-1}. However, Moir et al. (2011) indicated pasture N uptake from a urine patch ranged from 300 to 700 kg N ha^{-1} yr^{-1}.

Nutrient additions through animal excretion can affect soil properties, nutrient equilibrium, and soil microbial communities. Changes within the soil microbial community can significantly alter soil nutrient dynamics resulting in changes in the ecosystem (Bertram et al., 2012). Grassland N_2O uptake and fluxes will vary the response to weather condition, such as rain or drought, spatial variability from 'hotspots,' organic matter availability, and soil microbial activity (Butterbach-Bahl et al., 2013). Liebig et al. (2008) reported that cattle urine increase CO_2 fluxes by 46% than untreated soil in a mixed-grass prairie.

Soil CH_4 fluxes

Methane estimated global warming potential is from 28-36 times higher than CO_2 (EPA, 2017). Soil microbes produced CH_4 by methanogenesis under anaerobic conditions, and

consume CH_4 by methanotrophic microorganisms that use O_2 and CH_4 under aerobic conditions (Dutaur and Verchot, 2007). Oxidation of CH_4 is a metabolic process carried out by methanotrophs for energy generation and C assimilation (Singh et al., 1997). The methanotrophic process is the oxidation of CH_4 with O_2 to methanol, formaldehyde, and CO_2. Aerobic and anaerobic oxidation main controls are oxygen availability, soil water, organic matter mineralization, and heat transport, while the movement will mainly depend on the soil matrix, heat transport, and vegetation (Singh et al., 1997; Segers, 1998). Within the soil rhizosphere, roots affect CH_4 oxidation by transporting O_2 which suppresses CH_4 production, on the other hand, root decay and exudation promote CH_4 production (Segers, 1998; Philippot et al., 2013). Furthermore, Hayashi et al. (2015) explained how plants strongly affect CH_4 and N_2O dynamics through their root functions, such as growth and decay of roots, and O_2 consumption, labile organic C supply, and N uptake.

Methanotrophic activity can be categorized by high or low affinity for CH_4 concentration (Conrad, 2009). The aerobic CH_4 oxidation requires methane monooxygenase, for catalyzing the oxidation of CH_4 with molecular O_2 to methanol and water. This reaction requires additional electrons which are supplied by cellular redox carriers such as cytochrome C followed by the oxidation of methanol, formaldehyde, and formate where the electrons are donated back to cytochrome C. The electron flow through the membrane produces a proton motive force which is the cellular energy carrier ATP, by the ATPase enzyme complex, and O_2 as the terminal electron acceptor. The anaerobic oxidation of CH_4 occurs under anoxic marine environments and freshwater environments in sediments. In this case, the CH_4 is oxidized with terminal acceptors such as sulfate, nitrate (NO_2^-), nitrite (NO_3^-), iron (Fe), and manganese (Mn). For those reasons, three different anaerobic CH_4 oxidation processes occur, sulfate-dependent anaerobic

CH_4 oxidation, nitrate/nitrite-dependent anaerobic methane oxidation, and Mn_4^+/Fe_3^+-dependent anaerobic oxidation; while the aerobic methane oxidation requires CH_4 and O_2 (Hu et al., 2014).

Soil N_2O fluxes

The N_2O has a global warming potential from 265 to 298 times higher than CO_2. The microbial relations with N dynamics in managed and natural soils contribute to 70% of the global N_2O emissions (Braker and Conrad, 2011; Butterbach-Bahl et al., 2013). The soil N_2O dynamics are biochemical processes mediated by microbial activity. Butterbach-Bahl et al. (2013) described the known processes contributing to N_2O emissions were the chemical decomposition of hydroxylamine during autotrophic and heterotrophic nitrification. The chemodenitrification of soil nitrate, and abiotic decomposition of ammonium nitrate. Nitrifier-denitrification within the same nitrifying microorganism, or by a distinct microorganism (nitrite oxidizers and denitrifiers) and the denitrification using nitrogen oxides as an alternative electron acceptor under O_2 limiting conditions; where the water-filled pore-space is higher than 50% (Ussiri and Lal, 2013).

The soil N cycle is highly affected by soil practices, and have major environmental and ecological impacts (Di et al., 2010). Nitrification is a critical aerobic process produced by two groups of microorganisms, ammonia-oxidizing bacteria (AOB), and ammonia-oxidizing archaea (AOA). The AOB is constituted by *Nitrosomonas* and *Nitrosospira*. The process involves the microbial oxidation of NH_3 to NO_2^-, followed by further oxidation to NO_3^- from *Nitrobacter* or *Nitrospira*. On the other hand, the denitrification process allows heterotrophic bacteria to denitrify NO_3^- and produce nitrous oxide N_2O, which can be released to the atmosphere as a GHG. Soil water content is the main factor in N_2O fluctuations suggesting denitrification is the main source of the emissions. Belyaeva et al. (2016) indicated water-filled pore space <30% and

>65% indicate low and high N_2O emissions, respectively. Other factors such as inorganic N and labile C also affect N_2O fluxes. Peters et al. (2013) indicated N_2O emissions are projected to be four times greater than actual emissions, because of inorganic N demand.

Summary

Sustainable agricultural and land management practices improve soil health and increase agroecosystem resilience by reducing GHG emissions and in some scenarios promoting CH_4 and N_2O uptake. Decreasing the GHG emissions have a direct relationship with an increase in soil C sequestration, and conserving N, which enhances soil fertility and productivity, increase soil biodiversity, reduce soil erosion, runoff and water pollution, and offer a buffer to crops and pastures systems against climate change (Smith, 2012). Efforts on the inclusion of soil-centric mitigation projects to offset GHG emission, develop low-carbon markets, and increase C in agricultural soils may result in a global C sink of 1.2 Pg of C yr^{-1} (Paustian et al., 2016). Future assessments will close the economic gaps and environmental problems, by assessing ecosystem resilience to drought, heat, pest management, and a synergy of the agricultural field with other sciences for reduce GHG emissions, promote C sequestration, and promote environmental resilience.

Under a changing climate, it is essential to approach sustainable grazed grassland management and to understand its impact on climate change. This project aims to provide quantitative data of soil microbial community changes, inorganic N dynamics, and CH_4 and N_2O fluxes from grazed systems, urine and manure patches, and plant cover practices. Complete comprehension of the ecology of the system can be adapted to the animal spatial behavior to achieve an overall accumulation of GHG fluxes on grazing systems from temperate and tropical pastures. This general information, and emerging research from the field, laboratory, and

modeling efforts provide data to evaluate sustainable management of agricultural soils to develop new technologies, biotechnology, and policies.

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Chapter 2 - Management practices for minimizing the environmental footprint of beef cattle in tropical and temperate grazing systems

Abstract

Ruminant livestock provides meat and dairy protein that sustains health and livelihoods for much of the world's population and utilizes land that is otherwise unsuited to agriculture. Grazing lands that support ruminant livestock provide numerous ecosystem services, including provisioning of food, water, and genetic resources; regulating climate and water; supporting soil formation; nutrient cycling; and cultural services. There is a clear need to reduce the environmental impact, primarily by reducing the nitrogen (N) released to the environment, increasing carbon (C) sequestration, and improving the contribution of grazing systems to ecosystem services. Comprehensive environmental assessments are needed to develop the full range of options for extensive livestock production systems. Management options include plant management such as improved grass varieties/sward composition, e.g., deep rooting grasses, increased productivity, enhanced nutritional value, as well as improved nutrient management of the soil-plant-animal-environment system. Animal management includes appropriate breeds, stocking densities matched to land's carrying capacity, and improved grazing management. The intensity and timing of grazing influences plant growth, C allocation, and species composition of pastures, thereby affecting the amount of soil C. Soil C storage in pasture lands can be improved by promoting plant productivity. Reducing plant nutrient deficiencies increases photosynthesis, plant inputs of C into the system, and, hence, soil C storage. The influence of grazing intensity on the emissions of non-CO_2 gases is not well established. Adding N, however, may stimulate

nitrous oxide (N_2O) emissions and losses of N to water resources, thereby partially offsetting benefits. A total assessment of the net balances of water quality and greenhouse gas emissions is required to achieve sustainable livestock grazing systems.

Introduction

Global agriculture is at a critical nexus of balancing the need for production and the requirement to minimize the environmental footprint. Grasslands occupy 40% of the land surface (Blair et al., 2014). Cattle (*Bos indicus, Bos taurus*) grazing produce 24% of the total beef production (Boucher et al., 2012). The cattle industry often uses lands that are otherwise is unsuited for cropland, employs 1.3 billion people worldwide, and represent a substantial contribution for food required to sustain the global population (Thornton, 2010; Boucher et al., 2012; Herrero et al., 2012; UN, 2017; Steiner et al., 2018). The cattle industry also represents a significant environmental footprint through loss of biodiversity, loss of nutrients to water resources, and emissions of greenhouse gases (GHG) (Rao et al., 2014; Rao et al., 2015; Steiner et al., 2014; Steiner et al., 2018). Sustainable practices in beef cattle grazing systems need to be identified and implemented to intensify protein production, contribute to food security, and provide ecosystem services.

Beef is one of the commodities with the highest global economic value with the increasing demand of 16% by 2015 (OECD/FAO, 2016). Globally, an increasing middle class from developing countries creates a higher demand for meat-rich diets and increased pressure on ecosystems (Myers and Kent, 2004). As an example, the livestock sector in Latin America and the Caribbean (LAC) has a 3.7% growth rate. Meat exports have increased by 3.2% (FAO, 2012). The contribution of LAC to the global market is estimated to be 23% of beef and buffalo meat, and 11.2% of milk (FAO, 2012). In the United States, 76% of the livestock is cattle, and the grazing land area fluctuates around 808 M ha of which 14.8% are managed pastures, and 85.4% are rangelands or grazed forests (Musengezi et al., 2016). For 2014 in the United States,

beef exports produced $807 billion in revenue with 1.7 billion metric tons of beef and beef variety meat (NABI, 2018).

The livestock system has been affected by abrupt changes in weather conditions which create new challenges for the cattle industry (Calle et al., 2012; Gerber et al., 2013; Steiner et al., 2014; Angerer et al., 2016). McAlpine et al. (2009) reviewed the production of beef in Australia, Colombia, and Brazil concluding there is a need to increase governmental policy on the implications of cattle production on the environment and human health. While, Derner et al. (2018) projected that the expected changes in weather require adaptive approaches by the scientific community and ranch managers which vary by geographical regions.

The environmental footprint of beef cattle grazing systems can be reduced by the integration of practices that match animal density to carrying capacity, animal breeding, feed quality, feed supplements, forage, and soil management practices (Smith et al., 2013; 2014; Carvalho et al., 2017). This paper aims to summarize sustainable strategies for strengthening animal resilience, preserve biodiversity, reduce water use, and minimize the carbon (C) and nitrogen (N) footprint of beef cattle production in mesic and tropical environment.

Impact of beef cattle grazing systems on soil health, water quality and greenhouse gas emissions

Greenhouse gas fluxes

Globally, the livestock sector contributes 7.1 Gt CO_2-eq, which represents 14.5% of the total GHG emissions (FAO, 2016). About 342 kg CO_2-eq is produced per kg of protein produced (Gerber et al., 2013). Grazing systems are a primary contributor of GHG emissions, specifically CH_4, and N_2O (Caro et al., 2014; Smith et al. 2014; Steiner et al., 2014; Rao et al., 2015; FAO, 2016). Methane emissions are mainly from enteric fermentation and manure

management; while N_2O emissions are the result of direct and indirect management of animal wastes, fertilizer applications to grazed crops and forages (Eckard et al., 2010; Clark, 2013; Schils et al., 2013). Intake and nutritional value of the biomass consumed affect the total CH_4 from enteric fermentation (Eckard et al., 2010).

Grasslands can be either a source or a sink of GHG. For CH_4, grasslands are a potential sink (Mosier et al., 1991; Singh et al., 1997; Van den Pol-van Dasselaar et al., 1998). For N_2O, grasslands can be either a sink or source depending on nutrients, precipitation, temperature, soil characteristics, and management (Petersen et al., 2004; Mosier et al., 2004; Bertram et al., 2012; Laubach et al., 2013; Conant et al., 2017). Additional sources of CH_4 and N_2O fluxes are from C and N contained in the manure and urine patches (Sordi et al., 2014; Cai and Akiyama, 2016).

Manure, biosolids, and fertilizer management are associated with N_2O emissions (Caro et al. 2014; Cai and Akiyama, 2016; Nichols et al., 2016). Nitrogen inputs may stimulate N_2O emissions and losses of N to water resources, thereby offsetting some of the benefits. In grazing systems, urine patches contribute to greater N losses than dung (Laubach et al., 2013). Chantigny et al. (2006) reported that soils with inputs near 300 kg N ha^{-1} yr^{-1} from manure or mineral fertilizer produce N_2O emissions of 850 g N ha^{-1} yr^{-1}. Previous studies identified urine as the primary source of N losses, compare to manure, in grazing systems through surface runoff, ammonia volatilization, and denitrification (Saarijärvi and Virkajärvi, 2009; Ussiri and Lal, 2013; Lee et al., 2014; Cai and Akiyama, 2016; Nichols et al., 2016).

The resilience of beef cattle grazing systems is the result of management practices related to land, forage, and animal interrelationships to offset nutrient losses, maintain forage and animal productivity and achieve land conservation (Fig. 2-1, Table 2-1) (Conant et al., 2017). For grazing systems, excessive animal density for an extended period or lack of animal rotation

reduce the vegetation ability to recover in a timely fashion exposing the soil to erosion, and reducing water infiltration (De Oliveira et al., 2004; Smith et al., 2007; Steiner et al., 2014; Angerer et al., 2016; Eldridge et al., 2017; Pilon et al., 2017; Steiner et al., 2018). As a result, the ecosystem will increase soil erosion, surface runoff, sediment load to stream and rivers, reduce water storage, soil acidity, nutrient losses from animal deposition, and a decrease in soil microbial biomass (De Oliveira et al., 2004; Pilon et al., 2017; Steiner et al., 2018). Low water storage capacity reduces plant productivity and causes plant mortality thereby decreasing plant nutrient uptake, reducing forage quality, and decreasing animal yields (Raz-Yaseef et al., 2015; Steiner et al., 2018).

Sustainable intensification of beef-cattle grazing systems to improve soil health and water quality

In the United States, rangeland management includes prescribed grazing as a practice to improve or maintain ecosystem services such as desired species composition, quantity, and quality of forage, reduced soil erosion, improved water quality and quantity, and riparian and watershed functions while enhancing wildlife habitat (Briske et al., 2011). Grasslands also provide benefits to water infiltration and retention, nutrient cycling and supply, biological diversity, and good rooting habitat for plant productivity. Grazing intensity and rotational grazing affect plant productivity and species composition consequently impacting soil erosion and water quality (Pilon et al., 2017; Franzluebbers and Stuedemann, 2009; Franzluebbers et al., 2014). Overall, the interaction of sustainable management on grazed grassland is to preserve soil health, increase resilience to climate variability and to support ecosystem function and resilience (Bonaudo et al., 2014; Eldridge et al., 2017).

Plant breeding programs to develop high yielding and nutritious forage started in 1960 in tropical regions (Rao et al., 2015). Forages legumes are a high protein animal feed with the capacity for symbiotic N fixation and create and deep rooting systems that enhance drought tolerance, nutrient uptake, and to improve soil ecological diversity (Rao et al., 2015). Additionally, Peters et al. (2013) showed that managed grass-legume systems have the potential to reduce erosion and accumulate C in soils.

In the temperate and tropical zones, the integration of enhanced forages traits offers an opportunity for high-quality feed forages that contain tannins reducing CH_4 emissions (Herrero et al., 2013; Rao et al., 2015; Rojas-Downing et al., 2017). Animal breed improvement is another option to increase animal productivity while reducing the environmental impact per unit of production (Rojas-Downing et al., 2017). Dietary additives are another option to potentially decrease GHG emissions by providing antibiotics and propionate enhancers, while future microbial technology may be based on archaeal vaccines, methanotrophs, acetogens, defaunation of the rumen, bacteriophages and probiotics (Chantigny et al., 2006; Eckard et al., 2010; de Carvhalo et al., 2017; Thornton, 2010; Clark, 2013).

From a water quality perspective, pollution rates from livestock systems are a threat to human and environment health (Field and Samadpour, 2007; Conley et al., 2009; USEPA, 2012). The presence of microbial pollutants, such as fecal indicator bacteria, fecal coliform, and *Escherichia coli*, in public lands, constitute a threat to human health (Field and Samadpour, 2007). In terms of the environmental aspect, elevated N and phosphorus leaching cause eutrophication of aquatic ecosystems. Moreover, the consumption of water with high nitrate concentration leads to methemoglobinemia, colorectal cancer, thyroid disease, and neural tube defects in humans (Ward et al., 2018). The control of animal density and grazing time per area

to manage pastures could optimize nutrient uptake, protect soil cover, and decrease nutrient leaching and erosion.

Practices for environmental footprint mitigation

Conservation agricultural systems include those practices in which land, water, nutrient, and energy resources could be used more sustainably to maintain or restore agroecosystems (Franzluebbers, 2010; Keating et al., 2010). For example, in Eastern, Central, and West Africa, and U.S. temperate grasslands, native pastures and crop residues support cattle-grazing systems. In Latin America and the Caribbean, cattle-pasture systems include crop components as a "tropical forage-based system".

While the GHG mitigation potential varies among practices, adoption rates also vary depending on the ease of implementation and the time scale (Smith et al., 2014; Table 2-2). For example, improved pasture systems using adapted forages traits and controlling animal stocks density have a lower mitigation potential than another practice but is easier to implement and readily available (Rao et al., 2015). In contrast, manipulation of the rumen has a high mitigation potential but implementation may be difficult, and the technology needs further development (Smith et al., 2014). The mitigations practices for temperate and tropical regions provide further information and case scenarios examples about co-benefits of practices for reducing the overall environmental footprint of beef cattle grazing systems at larger geographical scales.

Environmental footprint mitigation in temperate regions

In 2015, the United States total beef consumption was 11.5 billion kg; with a retail equivalent value of $105 billion (ERS, 2019). Moreover, the Southern Great Plains is vital for the beef industry since is used for beef cattle grazing on introduced and native grassland pastures, rangeland forages, and crop grazing, particularly dual-purpose winter wheat (*Triticum*

aestivum L.). However, the beef cattle industry represents 71% of the total GHG emissions from the livestock sector in the United States (EPA, 2016). For this reason, during 2013 the Grazing Coordinated Agricultural Project (Grazing CAP) was established to better understanding grazing systems and to develop strategies for improving the resilience of beef grazing systems; and strengthen ecosystem services (Steiner et al., 2014).

Todd et al. (2016) reported enteric CH_4 emission factor for a cow with calf grazing early season tallgrass prairie of 306 ± 72 g d^{-1}. However, Shreck et al. (2016) found that protein supplements (chopped grass hay with a 3.9% crude protein), under confinement after the grazing period, decreased CH_4 emissions of steers per unit of dry matter intake by 30 g CH_4 d^{-1}. Also, McGee et al., (2016) suggested some strategies for mitigating environmental losses, such as leaching, and erosion, is possible by utilizing adjacent rangeland during winter, grazing wheat over spring, and confinement over strategic periods (high precipitation, or extensive droughts) can increase calf gains and reduce land area requirements.

Other practices such as prescribed burning, specifically patch burning, is a pasture management practice to manipulate grazing distribution, animal weight, parasite control, and natural resources conservation (Farney et al., 2016). Additionally, the burning of tallgrass prairie and associated biomass regrowth increases soil C sequestration (Rice and Owensby, 2000). Burning practices also improve the nutritional value of the regrowth with a higher crude protein and total digestible nutrients and reduce enteric CH_4 emissions per unit of beef produced, and it also increased animal productivity by 28% compared with unburned pastures (DeRamus et al., 2003; Moffet and Reuter, 2016). For a tallgrass prairie, the inorganic N demand from the soil is high and burning results in greater incorporation of C and N into the soil (Dell et al., 2005; Lee et al., 2014).

In USA temperate areas, restoration of degraded soil has been possible with planted forests, perennial pastures, use of mulch, and conservation tillage practices (Franzluebbers, 2005). Franzluebbers (2010) reported that pastures under conservation tillage increased the C sequestration capacity by 0.84 Mg C ha^{-1} yr^{-1} within a 16-yr duration. The use of deep-rooted plant species plays a significant role in C sequestration, soil water retention, and gas flux exchange (Hinsiger et al., 2009). For example, shortgrass prairie, the C sequestration capacity is 0.07 to 0.12 Mg C ha^{-1} yr^{-1} (Reeder and Schuman, 2002), northern mixed-grass prairie 0.30 Mg C ha^{-1} yr^{-1} (Frank et al., 1995), and Australian perennial grasses 0.35 Mg C ha^{-1} yr^{-1} (Young et al., 2009). Perennial pastures following conventionally tilled cropland increased SOC with 87% of sites achieving at least 0.10 Mg C ha^{-1} yr^{-1} to 30% achieving at least 1.00 Mg C ha^{-1} yr^{-1} (Franzluebbers, 2010). Related species richness with soil C and that a variety of species optimize ecosystem processes provides greater ecosystem services and returns the highest economic value (Conant et al., 2017; Hungate et al., 2017; Abdalla et al., 2018).

The Natural Resources Conservation Service (NRCS) agency efforts are focused on Conservation Activity Plan which includes a grazing management plan for ranchers to achieve an economically and environmentally efficient system (NRCS, 2019). The grazing plan includes expanding the variety of plant species and pastures depending on land use, the cattle breed, and the wildlife species in the area. Grazing distribution should consider weather conditions, including contingency plans for winter, drought, fire, flood, and mud. Also, site control by fencing, riparian herbaceous cover, stream habitat improvement, and streamline and shoreline protection. Overall, the project goal is to achieve the quality criteria of soil erosion control, water quality, fish and wildlife, rangeland health and productivity, and identify other resource concerns in rangelands.

Environmental footprint mitigation in tropical regions

Forage-based livestock systems in Latin American and the Caribbean (LAC) cover an area of 550.1 MM ha^{-1} (FAO, 2009). Currently, tropical forage systems in acid soils face challenging production conditions because the highly weathered soils consist of low natural fertility, low pH, and high Al saturation. About 75% of land in South America is degraded to some degree mainly because livestock densities exceed the carrying capacity of the pastures (Heerink et al., 2001). Also, in the Americas, cattle produced 75% of the total GHG emissions from the livestock sector (FAOSTATS, 2013). The primary sources of GHG emissions from agriculture in 2104 in LAC were 58% from enteric fermentation from ruminants, and 23% from manure in pastures.

The International Center for Tropical Agriculture (CIAT) has developed a strategy for eco-efficient agriculture as an agricultural approach integrates crops and livestock on grazing lands (Peters et al., 2013; Rao et al., 2015). Under eco-efficient agriculture, the goal is to achieve high agricultural outputs, regarding quality and quantity, under less input of land, water, nutrients, energy, labor, and capital. Land management systems with new pasture traits options can improve pastures of high productivity, nutritional quality, and tolerance to water stress and may prevent greater environmental degradation, and reverse land degradation.

One novel means to reduce GHG in tropical systems is the use of "biological nitrification inhibition" (BNI) of *Brachiaria* (Subbarao et al., 2009). *Brachiaria* spp. are the most widely planted tropical forages from Southeast Asia, Sub-Saharan Africa, Latin America, and the Caribbean. Certain species of *Brachiaria* reduce nitrification thereby enhancing N utilization and reducing nitrate leaching and N_2O emissions (Byrnes et al., 2017). *Brachiaria* pastures represent a mitigation potential of 29.8 Mt CO_2-eq yr^{-1} (Assad et al., 2013). Land use under

Brachiaria pastures had 15% greater soil C stocks than areas under native vegetation in Brazil (Assad et al., 2013). The integration of *Brachiaria* grasses in livestock systems could economically benefit farmers, increase land restoration, decrease agricultural N losses, reduce GHG emissions and soil acidity, and lead to higher soil C sequestration (Subbarao et al., 2009; Thornton, 2010; Rao et al., 2014; Rao et al., 2015).

Another effort in tropical regions is the silvopastoral systems that provide meat and wood, restores land, sequesters C, reduces GHG emissions and provides economic advantages (Assad et al., 2013, Conant et al., 2017). Silvopastoral systems are considered an agroforestry practice coupled with intensive cattle production which includes trees, improved forages, 40 to 50 d of resting land period, and 12 to 24 h rotational grazing (Murgueitio et al., 2011; Calle et al., 2012). In tropical systems, this system results in 5.8 times more protein per hectare than the traditional monoculture pasture system, 2.6 times higher stocking rates, reduces CH_4 emissions by 25 to 40%, and increases animal health (Campos Paciullo et al., 2012; Xóchitl and Solorio, 2013; Conant et al., 2017). Co-benefits of the silvopastoral systems coupled with forest and agricultural policies provide wildlife conservation and corridors; nitrogen fixation from legumes, and may reduce the need for chemical fertilization, increase in soil water relations, and promote C sequestration (Murgueitio et al., 2011; Boucher et al., 2012; Montagnini et al., 2013).

Integrating crop-livestock systems

The efficiency of integrated crop-livestock systems relies on minimizing soil and nutrients losses, restoring soil and water quality, use of renewable natural resources, and increasing ecosystem services of beef-cattle production (Franzluebbers et al., 2014). Bonaudo et al. (2014) summarized principles for managing integrated crop-livestock systems by considering production, immune and metabolic functions, tighten energy cycles with fewer losses, optimized

nutrient availability, and landscape management. High production forages can achieve closing energy and input cycles, agroforestry, substitution of natural inputs for chemical fertilizer inputs, use of animal and green manure, maintain soil cover with mulch, and integrated pest management. Landscape management may require diversification and biotechnology for efficient crop varieties and animal breeds to reach sustainability.

Diversification and biotechnology in beef cattle grazing

Eisler et al. (2014) listed eight strategies to reduce economic costs and environmental impacts, and boost yields and qualityof beef production systems. The first strategy was to feed animals less human food. Currently, up to two-thirds of cereal production is used for animal feed (Erb et al., 2016). Enhancing pasture productivity increase rotational systems and reduce confinement production, while releases land for crops production. Strategy two recommended regionally appropriate animal breeds and genomics to boost production and animal resilience. Strategy three focused on animal health by identifying risky pastures and controlling animal infections. Strategy four proposed adoption of supplements able to manipulate the rumen microbiome for energy, and nutrient efficiency. The fifth strategy recommended dietary quality over quantity for humans. Balanced diets based on high-quality nutritional foods would improve human development and reduce illness. Strategies six and seven tailored practices to local culture and tracked costs and benefits. Strategy eight suggested studies to quantify agricultural, economic and environmental impacts of systems for the adoption of sustainable practices.

Table 2-3 summarizes the co-benefits associated with livestock grazing systems. Some mitigation efforts lead to greater ecosystem services and thus reduced the environmental footprint of beef cattle grazing systems. Sustainable grazing systems can enhance livestock resilience to extreme weather events, drought, and floods, reduce productivity losses associated

with heat stress, host-pathogen interactions, and reduced feed quality and quantity (IPCC, 2014). Beef cattle systems in grasslands or land not suitable for crop cultivation can minimize the competition with crop production in arable lands because of the ability of cattle to utilize feed that humans cannot utilize and convert it into high-value protein (Suttie, 2005).

Conclusion

Mitigation activities proposed by the previous case studies discussed the management of grazed pastures while reducing nutrient losses and the conservation of water, land and existing C stocks for increasing the resilience of beef cattle grazed grasslands (Peters et al., 2013; Steiner et al., 2014; Rao et al. 2015, Abdalla et al., 2018; Rojas-Downing et al., 2018). Moreover, these practices emphasize maintaining soil covers and increasing soil organic matter as a critical factor to optimize nutrient cycling, soil aggregation, and improve water holding capacity to support grassland pastures productivity for use as natural forage system. Furthermore, this higher understanding of GHG emissions from grazed pastures denotes the importance of synergy between disciplines and the critical role of biotechnology on animal and pastures resilience.

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Table 2-1. Specific management practices which mitigate GHG emissions from the beef-cattle sector. Source: adapted from (Steiner et al., 2014)

Objective	Management Practices	
Enhance beef cattle productivity and profitability	Animal	Dietary additives, and feed supplementation
		Genetic selection
		Grazing management (stocking density, duration, timing)
		Increase digestibility of feed
		Nutrient utilization timing
		Weaning time
	Plant	Breeding and genetic technology
		Increase in nutritional value
		Shift in forage species, and incorporate legumes
Enhance soil health and water quality	Soil, water, and nutrient	Agro-forestry
		Conservational tillage
		Riparian buffer strips
		Patch burning of grasslands
		Plant residue and animal waste management
		Rate, source, and timing of nutrient application
		Rotational grazing
		Silvopastoral system
		Use of cover crops

Table 2-2. Co-benefits and trade-offs of mitigation in grazing systems. The "+" means the practice is beneficial (benefit), while the "-" means there is a negative effect (trade-off). Source adapted from: Smith et al., 2007.

Measure	Practice	Water Quality	Water conservation	Soil quality	Air quality	Bio-diversity, wildlife	Energy conservation	Conservation of other biomes	Aesthetic/amenity value
Grazing land management/pasture improvement	Grazing intensity			+		+			+
	Increased productivity (e.g. fertilization)	+							
	Nutrient management	+/-	+	+		+	-	+	+/-
	Fire management (e.g. patch burning)	+			-	+/-			+/-
	Species introduction (including legumes)			+			+		
Restoration of degraded lands	Erosion control, organic amendments, nutrient amendments	+		+		+		+	+
Livestock management	Improve feeding practices				+/-			+	
Manure/biosolid management	Improve storage and handling	+/-		+	+/-				
	Anaerobic digestion				+		+		
	More efficient use as nutrient source	+		+	+		+		

Table 2-3. Technical mitigation potential in grazing systems. The "+" means difficult, "++" means easy, and "+++" means universal applicability. Source adapted from: IPCC WGIII AR5.

Category				Practices and impacts	Technical mitigation potential	Ease of Implementation	Timescale
Livestock	Grazing Lands	Plant management		Improved grass varieties and composition. improved grazing management forage production, and plant diversification	+	+++	+++
		Herd management		Appropriate stocking densities.	+	++	++
		Fire management		Appropriate use of fire for sustainable grassland management.	+	++	++
		Feeding		Improved feed and dietary additives to reduce emissions from enteric fermentation; including improved forage quality, dietary additives to manipulate the rumen microbiome.	++	++	++
		Breeding and other long term management		Improved breeds with higher productivity or with reduced emissions from enteric fermentation; microbial technology such as archaeal vaccines, methanotrophs, acetogens, defaunation of the rumen, bacteriophages and probiotics.	++	+	+

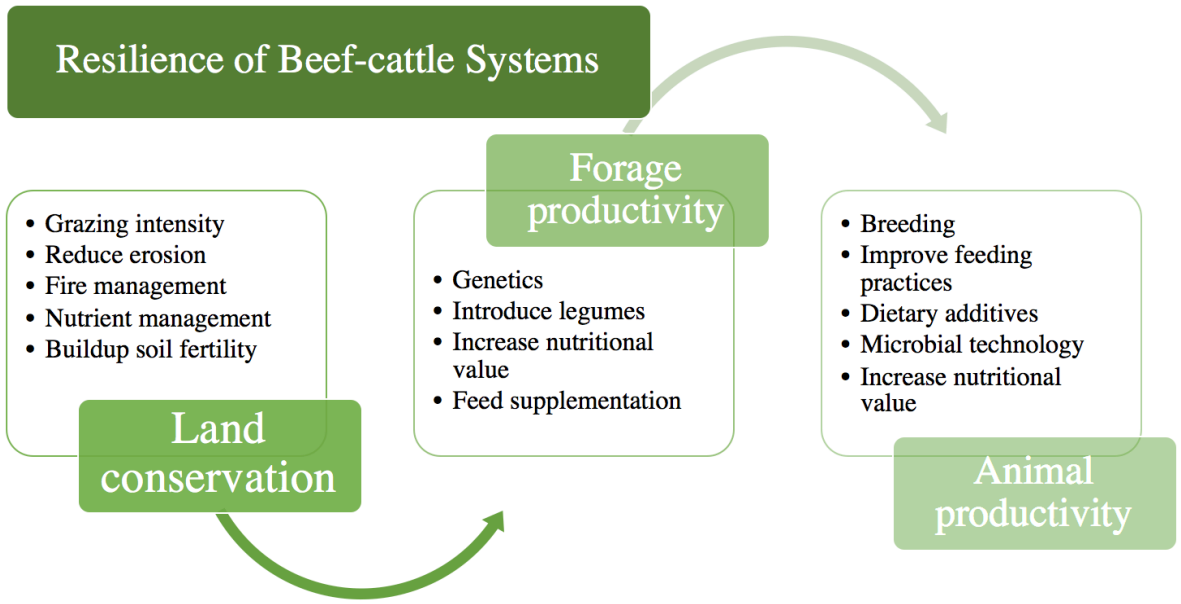

Figure 2-1. Improving the resilience of beef-cattle systems through interrelated land, forage, and animal management practices and technologies.

Chapter 3 - Greenhouse gas emissions from beef-cattle grazing systems in a temperate grassland

Abstract

Soil methane (CH_4) and nitrous oxide (N_2O) emissions from beef cattle and prescribed burning of grasslands are targeted environmental problem in the cattle industry. The use of grasslands for animal grazing represents an opportunity for controlling vegetation and serves as an agricultural system for beef production. This research aims to described CH_4 and N_2O dynamics in a temperate grassland. The research started in summer 2014 to December 2017. Gas samples were collected and analyzed for CH_4 and N_2O concentration from three grazing areas under three different burning regimes at the temperate grassland of Konza Prairie Biological Station in Kansas. Burning regimes included one site burned annually, and two sites patch burned every three years on offset years. Each site had five replications with four sampling points in a 15 min interval (0, 15, 30 and 45 min period). Gas samples were collected on a weekly to biweekly basis using the static chamber method. Soil N_2O flux varied from an emission of 8.9 g N_2O-N ha^{-1} d^{-1} to a sink of -11.2 g N_2O -N ha^{-1} d^{-1}. Soil CH_4 flux fluctuated from emissions of 12.3 g CH_4-C ha^{-1} d^{-1} and a sink of -10.8 g CH_4-C ha^{-1} d^{-1}. Precipitation events and increased soil NO_3^- during fall increased soil N_2O emissions. Low soil water content increased soil CH_4 sink while decreasing N_2O emissions. The 3-yr GHG balance budget estimations indicate N_2O and CH_4 sink capacity of temperate grasslands can partially or entirely offset N_2O and CH_4 emissions during a 3-yr period, with higher sink capacity under patch burning. Overall, this study and previous studies provide evidence temperate grassland as a CH_4 sink, and the sink can be enhanced by 3-yr patch burning.

Introduction

In North America, prairies, which occupy 27% of the total land area, are a terrestrial sink of methane (CH_4) (Mosier et al., 1991; Guo et al., 2006). Prairies are considered grasslands which constitute up to 40% of the Earth's terrestrial surface (Blair et al., 2014). Temperate prairies in the U.S.A evolved from fire-grazing interactions which control vegetation while serving as an agricultural system for beef production (Fuhlendorf et al., 2009; Blair et al., 2014). Also, prairie soils provide invaluable ecosystem services such as carbon (C) sequestration, CH_4 sink, and wildlife and vegetation richness and diversity (Blair et al., 2014). As a result of weather, fire, grazing and soil microbial interactions, grazed grasslands soils can be a source or a sink of greenhouse gases (GHG) (Mosier et al., 1991; Van del Pol-van et al., 1998; McSherry and Ritchie, 2013; Blair et al., 2014).

Temporal variations of nitrous oxide (N_2O) and CH_4 fluxes occur in response to nutrient availability, weather conditions, and microbial activity which regulates GHG production and consumption (Ravishankara et al., 2009; Butterbach-Bahl et al., 2013; Gerber et al., 2013; EPA, 2014). Over the last decades, atmospheric concentrations of N_2O and CH_4 have dramatically increased, since preindustrial times, atmosphere N_2O and CH_4 concentrations have more than doubled reaching 329 and 1,853 ppb, respectively (WMO, 2017). Furthermore, from the cattle grazing perspective, studies have targeted livestock systems as a primary contributor to GHG emissions, principally CH_4, and N_2O (Gerber et al., 2013; EPA, 2016). Globally, beef-cattle systems are responsible for 65% of GHG fluxes from the livestock sector (Gerber et al., 2013; EPA, 2016). In the U.S.A., emissions from beef-cattle grazing systems during 2014 were 116 MMT CO_2; representing 71% of the total livestock emissions (EPA, 2016). As previously mentioned, beef-cattle grazing, and prescribed burning are essential for reducing woody

vegetation, and increasing grazing areas which helps animal resilience by maintaining healthy pastures during abnormally dry years and consequently maintaining animal weight gains (Fuhlendorf and Engle, 2004; Hinsinger et al., 2009; Singh et al., 2010; McSherry and Ritchie, 2013; Allerd et al., 2014).

Moreover, prescribed burning create patches areas with different vegetative states of recovery creating different vegetative composition and structures, known as shifting mosaic vegetation, across the landscapes and resulting in habitat heterogeneity (Fuhlendorf and Engle, 2004). Fuhlendorf and Engle (2004) reported a 75% more evenly grazing in recently burned patches (<2 yr), compared with an annually burned system; however, cattle gains were not affected by burning frequency. Burning regimes and animal grazing are also known to influence the grasslands N budget (Hobbs et al., 1991). Moreover, Dell et al., (2005) described the immobilization of N within the root zone increased with prescribed annual burning. After prescribed burning during early spring, the low-intensity fire chemically converts nutrients bound in dead plant tissue to more available forms increasing mineralization rates and soil microbial activity (Schoch and Binkley, 1986; Turner et al., 1997). Burning of tallgrass prairie removes N through volatilization but the post burning effect on N_2O and CH_4 emissions need to be better quantified.

Soil microbial processes involved in the N cycle are sensitive to changes in soil vegetative cover and weather patterns (Van der Putten et al., 2013; Zeglin et al., 2015). Moreover, changes in temperature, CO_2, and water availability influence seasonal shifts in plant competition altering plant species, soil N dynamics, and microbial dynamics (Thornton et al., 2008; Thornton et al., 2009, 2015; IFAD, 2010; Polley et al., 2013). For example, freeze-thaw conditions during winter impose physiological limitations on microbial cells thus reducing soil

microbial activity. A similar effect can be expected from wet-dry cycles (Bardgett et al., 2005, Zeglin et al., 2013; Russell et al., 2018). Carson and Zeglin (2018) indicated soil microbial populations were lower under annually burned compared to unburned sites on the Konza tallgrass prairie (Carson and Zeglin, 2018). The same study reported that bacteria populations were ten times greater during summer with no differences between annually burned and unburned sites (Carson and Zeglin, 2018).

The objectives of this study were to quantify CH_4 and N_2O fluxes from grazed tallgrass prairie and to determine the effect of burning regimes comparing annual burning to a 3-yr patch burn on CH_4 and N_2O fluxes. The goal was to provide estimates of annual CH_4 and N_2O emissions and the CH_4 balance between CH_4 sink and enteric fermentation from a cow-calf operation. The resulting data will improve the knowledge of GHG fluxes from temperate grasslands as a CH_4 sink and N_2O emissions.

Materials and methods

Experimental site

The study site was located at the Konza Prairie Biological Station located in the Flint Hills of northeastern Kansas (39°05’ N, 96°35’ W). The dominant soil was Benfield series (Fine, mixed, superactive, mesic Udertic Argiustolls). Soil chemical properties are summarized in Table 3-1. Elevation ranged from 320 to 444 m above sea level. Mean annual precipitation was 811 mm and mean annual temperature fluctuated from 6.6°C to 19.4°C (Table A-1, A-2, A-3, and A-4). The study location was in watersheds C1A (39° 4' 40.08" N, 96° 32' 36.6" W), C3A (39° 5' 40.2" N, 96° 32' 45.24" W), and C3B (39° 5' 25.8" N, 96° 32' 41.28" W) of the Konza Prairie Biological Station (Fig. A-1). Grazed watershed units were stocked with cow/calf pairs from approximately May 1 until September 1 at a stocking density of 3.2 ha per cow/calf. Konza

prairie predominant vegetation is perennial, warm-season grasses such as big bluestem (*Andropogon gerardii),* little bluestem (*Schizachyrium scoparium*), indiangrass (*Sorghastrum nutans*), and switchgrass (*Panicum virgatum*); annual burning watersheds are expected to have a decrease in species diversity (Joern, 2018). Scheduled prescribed burning occurred annually during March for watershed C1A and every 3 years for watersheds C3A and C3B. The C3A site was burned during March in 2016, and the C3B site was burned during March in 2014 and 2017.

Inorganic N

Monthly soil samples were taken from 0-5 cm depth from June 2016 to July 2017. Three areas near the gas chambers were randomly chosen from each watershed, and each sample was composed of five subsamples. Soil inorganic N, ammonium (NH_4^+), and nitrate (NO_3^-), was extracted by adding 100 mL 1M KCl to 25g of moist soil. The samples were shaken for 60 min on an orbital shaker at 300 rpm and filtered through Whatman No. 42 filter paper (MilliporeSigma, St. Louis, MO) into a 20 mL scintillation vial. Extracts were analyzed for NH_4-N and NO_3-N by colorimetric analysis (Gelderman and Beegle, 1998) at the KSU Soil Testing Lab. For each sample, gravimetric soil water content was determined by oven drying a 10 g sample at 105^{o}C for 48 h.

Soil water, air temperature and precipitation.

Soil water content was measured using a POGO® (Stevens Water Monitoring Systems, Inc., Oregon, USA) at each gas sampling date. Air and soil temperature data was gathered with a digital thermometer during each gas sampling date. In the case of missing or inaccurate air temperature data, air temperature was obtained from the LTER Network Data Portal for Konza Prairie LTER at Konza Headquarters (https://climhy.lternet.edu).

Methane and nitrous oxide emissions

Fluxes of CH_4 and N_2O were measured for each watershed C1A, C3A, and C3B from July 2014 to December 2017 using static, vented polyvinyl chloride (PVC) chambers (7.5 cm high x 20 cm in diameter) using the method described in Hutchinson and Mosier (1981). In each watershed, 5 PVC anchors were placed over the landscape and inserted 15 cm into the soil. During the burning season (March), chambers were removed and re-installed to the same position 1-2 days after burning, if needed.

Samples were collected weekly during the growing season and once every two to four weeks during the remainder of the year. Additional samples were taken following a precipitation event. The gas samples were collected by placing a closed vented chamber over the buried anchor and taking a 25 ml gas sample using a syringe. The gas samples were transferred to a 20 mL (22x75 mm) glass vial (Wheaton, New Jersey, USA), closed with a 20 mm gray butyl stopper (Labco Limited, Wales, UK), and sealed with a 20 mm unlined seal open top aluminum (Labco Limited, Wales, UK). The sampling began by placing a chamber over the PVC core and sealing it with a rubber strap. Once the chamber was sealed, the first sample (0 min) was taken, and then successive samples were taken 15, 30, and 45 min after sealing.

Gas samples were analyzed for CO_2, CH_4 and N_2O using a Bruker Scion 456 Gas Chromatography (Scion Instruments©, Austin, TX). The gas chromatography was calibrated daily using analytical-grade standards containing 0.2, 0.512, 3.5, and 15.3 µL N_2O L^{-1}, 4.0 µL CH_4 L^{-1}, and 495, 800, and 993 µL CO_2 L^{-1}. The concentration of N_2O and CH_4 in each sample was converted to µg N_2O-N m^{-2} and CH_4-C m^{-2} using:

$$\mu g\ N_2O - N\ m^2\ or\ CH_4 - C\ m^2 = \frac{CPVM}{ART}$$

where *C* was the volumetric concentration of N_2O (μL N_2O L^{-1}), *P* was the atmospheric pressure at 304.8 m above the sea level (0.965 atm), *V* was the chamber volume (L), *M* was the mass of N in N_2O (28 μg N μmol^{-1} N_2O), *A* was the chamber surface area (m^2), *R* was the Universal Gas Constant (0.08206 atm μL μmol^{-1} K^{-1}), and *T* was the chamber headspace temperature (K) at the time of sampling. Results were analyzed by the Hutchinson-Mosier method and linear equations (Pedersen et al., 2010). Soil CH_4 and N_2O sink were quantified by a negative flux in soils using the static chamber method by Bogner et al. (1997).

Annual budget

The CH_4 and N_2O annual budgets were calculated by the cumulative values of CH_4, and N_2O over the year in terms of CO_2 equivalent; considering a value of 298 CO_2-eq for N_2O, and 25 CO_2-eq for CH_4 (Forster et al., 2007). Total annual flux was estimated using linear interpolation between sampling points and calculation of the area under the curve using:

Equation 3-1:

$$\text{Cumulative (g } N_2O \text{ or } CH_4\ ha^{-1}) = \sum_i^n \left(\frac{F_i + F_{i+1}}{2}(t_{i+1} - t_i)\right)$$

where F_i and F_{i+1} were the N_2O or CH_4 fluxes (g ha^{-1} d^{-1}) at sampling points *i* and *i+1*; t_i and t_{i+1} were the sampling dates (Julian date) at sampling points *i* and *i+1*, and *n* was the number of sampling points taken in a given year. Accumulative N_2O or CH_4 for 2014 was calculated for six months, and 2015, 2016 and 2017 for the annual cycle.

Cumulative values were calculated for January to December during 2015, 2016, and 2017; cumulative value for 2014 was estimated but was not presented since it included only a partial data from June to December 2014 (Table A-5a, and Table A-5b). For the linear interpolation, daily values 2 times higher or lower than the standard deviation of the mean were consider outliers and, therefore were not considered for the summation of the total annual

budget. Annual grassland balance was considered as the net value of soil uptake and emissions of CH_4-C and N_2O-N in CO_2-eq. Grassland balance from a cow/calf unit was calculated by the accumulative values of the grassland CH_4-C and the CH_4-C emissions from the cow/calf pair, considering a total emission of 7.6 CO_2-eq kg cow/calf^{-1} per land unit year^{-1} (Todd et al., 2016). The CH_4 flux was converted to kg of CO_2-eq per cow/calf unit of 3.2 ha^{-1} following the animal stock density of the Flint Hills region of Kansas.

Statistical analysis

Statistical Analysis System (SAS) 9.3 was used to analyze results using the Proc Mixed model with repeated measurements over time, and the analysis of variance (ANOVA) method (α=0.05). Soil CH_4 and N_2O fluxes were analyzed over time for the effect of time, burning regimes, and the interaction. Fluxes were analyzed separately by year to identify trends within a year. A complete analysis of soil CH_4 and N_2O fluxes from June 2014 to December 2017 (n=81) was also analyzed (Appendix 8). Analysis of regression was performed to identify the correlation of N_2O and CH_4 with soil water and temperature. The correlations were not significant (R^2<0.04) (Data not shown). Similarly, inorganic N concentrations, NH_4^+ and NO_3^-, were analyzed using a Proc Mixed model with repeated measurements over time for the effect of burning regimes, time, and the interaction. Differences between the accumulative annual N_2O and CH_4 emission results were analyzed for the effect of watershed, year, and the interaction using the Proc Glimmix model; significant differences were determined using lsmeans ($p<0.05$).

Results

Methane fluxes

During 2014, there was a significant interaction within dates (p=0.0153), but no differences were observed between burning regimes (Table 3-2). Grassland CH_4 uptake ranged

from -3.6 to -7.5 g CH_4-C ha^{-1} d^{-1} (Fig. 3-1). In 2015, there was a significant interaction between burning regimes and time ($p<0.0001$). Emissions ranged from 0.2 to 2.3 g CH_4-C ha^{-1} d^{-1} during summer for annually burned (C1A) and patch burned (C3A burned in 2013). During early fall, CH_4 emissions were 12.3 g CH_4-C ha^{-1} d^{-1} for C3A (burned in 2013). Soil CH_4 sink ranged from -5.5 to -10.8 g CH_4-C ha^{-1} d^{-1} for the C1A and C3B, respectively.

During the three studied years, 2015 had the highest precipitation (1,000 mm) and CH_4 emissions were significantly different between burning regimes. There were no significant differences of N_2O fluxes between days, burning regimes, and the interaction for 2016 ($p=0.7543$). The 2016 year (993 mm) also had higher precipitation, like 2015, compared with 2017 (726 mm). Our results registered an increase in CH_4 sink in the C3A site, which was burned that same year ($p=0.6433$). During 2017, there were no significant differences in the interaction between burning regimes and dates ($p>0.5887$). However, during 2017, the C3B site (burned in 2014 and 2017) had lower CH_4 uptake than the C3A site (burned in 2016) ($p=0.0596$).

Nitrous oxide fluxes

During the year 2014, there was a significant effect on N_2O fluxes between the day of the year, burning regimes, and the interaction ($p=0.4317$). During Fall and Winter, N_2O emissions were as high as 1.4 and 1.5 g N_2O -N ha^{-1} d^{-1} for C1A and C3B, respectively, both sites burned in 2014 (Fig. 2). While that same year (2014), N_2O emissions in the C3A watershed averaged 1.75 g N_2O-N ha^{-1} d^{-1}. During the summer of 2014, there was N_2O uptake (-0.43 to -0.05 g N_2O-N ha^{-1} d^{-1}) with no significant differences between burning regimes. In 2015 ($p=0.9681$) and 2016 ($p=0.8473$), the same years with the highest precipitation of 1,000 and 993 mm, respectively; there was no significant interaction between burning regimes and time. Soil N_2O uptake were consistent in the watershed C3A during spring to summer 2015, and summer 2016, however, the

N_2O uptake was not significantly different. During 2017 ($p=0.0234$), higher N_2O emissions from the watersheds C3B (burned in 2014 and 2017) and C1A did not show specific trends as a result of burning. However, watershed C3A maintained soil N_2O uptake during summer 2017. Recent burning of patch burned sites increased N_2O fluxes within the burning regimes, especially during high precipitation periods.

Inorganic N

Soil inorganic N from summer 2016 to summer 2017 was used to understand CH_4 and N_2O flux behavior over time. There was no significant change in NH_4^+ concentration over time or within the studied sites during the studied months ($p=0.5504$). However, NO_3^- concentrations were significantly variable over the year but no differences were identified within the sites ($p<.0001$) (Fig. 3-3, Appx. 8). Significantly higher values were measured during summer and early fall 2016 with values ~3.1 mg NO_3^--N kg^{-1} soil, followed by a peak during early winter of 1.7 NO_3^--N kg^{-1} soil. During 2017, the highest NO_3^- concentrations were measured over summer with values ~1.2 NO_3^--N kg^{-1} soil, but concentrations decreased during early fall to ~0.3 NO_3^--N kg^{-1} soil which were the same concentrations during late fall.

Annual budget

The N_2O budget was significantly different between the burning regimes ($p=0.0641$) (Fig. 3-4, Table 3-3). Total N_2O ($p=0.0231$) and CH_4 ($p=0.0062$) emissions were significantly different between the years (2015-2017) (Table 3-4). Overall, C3A (burned in 2016) had 59% lower emissions than C3B (burned in 2014 and 2017), and C1A (annually burned) (Fig. 3-5). The lowest N_2O emissions occurred in with a mean of 32.2 g N_2O ha^{-1}, followed by 68.7 g N_2O ha^{-1} during 2016, and 94.5 g N_2O ha^{-1} during 2017 (Fig. 3-5). Higher CH_4 uptake occurred in 2015,

the same year with the lowest N_2O emissions and precipitation. Total CH_4 uptake during 2015, 2016, and 2017 were -1,628, -895, and -666 g CH_4 ha^{-1}, respectively (Fig. 3-5).

To understand the temperate grassland sink in relation with the GHG emissions from grazing cattle, soil CH_4 and N_2O fluxes were compared to CH_4 emissions from the cow/calf pair. Todd et al. (2016) estimated a CH_4 emission of 7.6 kg CO_2-eq kg $cow/calf^{-1}$ land unit $year^{-1}$ in temperate grasslands of Texas. Using the data from Todd et al. (2016) and this three year soil CH_4 and N_2O fluxes study, the CH_4 sink of the tallgrass prairie soil ranged from -1.8 to -8.6 kg CO_2-eq cow/calf per land unit yr^{-1}, and total emissions ranged from 2.3 to 5.2 kg CO_2-eq kg cow/calf per land unit yr^{-1}, when no sink occurred (Table 3-5). Furthermore, this study considered each burning regime offsetting as the difference between the total soil CH_4 sink and the annual cow/calf CH_4 emissions. An overall the net sink for the 3-yr budget study reports a GHG balance of grassland resulting in neutral emissions from C1A (annually burned). Moreover, watersheds under patch burning were able to offset the 7.6 kg CO_2-eq kg $cow/calf^{-1}$ land unit $year^{-1}$ and an additional 4.1 kg CO_2-eq kg cow/calf per land unit for C3A, and 1.8 kg CO_2-eq kg cow/calf per land unit for C3B during the 3 yr period.

Discussion

In this experiment, winter and spring CH_4 uptake were similar to Singh et al. (2010). Daily soil CH_4 uptake ranged from -3.6 to -7.5 g CH_4-C ha^{-1} d^{-1} similar to that reported by Mosier et al. (1991) for a Colorado shortgrass prairie which ranged from -3.6 to -6.3 g CH_4-C ha^{-1} d^{-1}. Low precipitation and recent burning appear to increase CH_4 uptake. During 2017, the lower CH_4 sink of C3B after the 3-yr patch burning compared to C3A (burned in 2016) could be the result of higher inorganic N after the burning in C3B site. However, there was no trend

during the 3-yr patch burned of C3A during 2016, probably as a result of high precipitation which would inhibit CH_4 sink (Fig. 1, Appx. 3) (Hu et al., 2014).

Soil microbial activity and net CH_4 uptake are strongly influenced by soil water (Singh et al., 2010). Also, high soil water content reduces diffusivity of CH_4 into the soil thus inhibiting oxidation by the methanotrophs (DelGrosso et al., 2000). However, N_2O and CH_4 fluxes were weakly correlated with soil water content and the temperature. Overall, CH_4 uptake was constant except for some scenarios of decrease in CH_4 uptake and net CH_4 emissions during high precipitation and times of vegetative growth.

The N_2O fluxes in this study ranged between 1.3 to 1.8 g N_2O-N ha^{-1} d^{-1} similar to that reported by Mosier et al. (1991) for a Colorado unfertilized shortgrass prairie of 1.8 to 3.0 g N_2O-N ha^{-1} d^{-1}. As expected increases in N_2O emissions occurred after high precipitation events (Fig. 3-2). High temperatures during summer 2016 and the high precipitation patterns during the season maintained high soil water content causing peaks in N_2O emissions. During 2015 lower N_2O emissions because how high precipitation produced N losses by leaching and erosion, therefore, reducing N_2O production (Bijoor et al., 2008).

Furthermore, the differences on N_2O fluxes over time and by burning regimes suggests the emissions are related to additional factors such as oxygen, C availability, pH and temperature, and the amount of burned biomass and consequently the mineralization rates after a burning episode (Wallenstein et al., 2006). Moreover, the increase in NO_3^- concentrations during fall can be explained because of higher decomposition of organic matter after warm periods combined with precipitation event (Table A-2) and translocation of nutrients by rhizodeposits and root exudates causing a shift in microbial community and its activity (Kuzyakov and Xu, 2013; Hayashi et al., 2015; Armstrong et al., 2016). Similarly, Mosier et al. (1996) suggested that N_2O

emissions were controlled by N turnover coupled with precipitation, rather than by bulk soil mineral N. Additionally, the low N_2O emissions and the N_2O uptake during summer may result from low N mineralization rates (Dijkstra et al., 2013; Mosier et al., 2002). Increased N_2O emissions was related to the precipitation and NO_3^- (Appx. 3) (Bardgett et al., 2005, Chimner and Welker, 2005; Zeglin et al., 2013; Russell et al., 2018). However, further research could be done to identify the role of the flora phenological changes during Spring and Fall and its relationship with N_2O emissions. As prairie pass by seasonal phenology changes ranging from flora during the hot-warm period and shifts to flora during cold-wetter periods (Knapp, 1998; Kuzyakov and Blagodatskaya, 2015).

Grasslands can be a minor source or sink of N_2O (Chapuis-Lardy et al., 2007; EPA, 2014). The N_2O sink of the C3A watershed (burned in 2016) could be explained as the result of low soil water and low soil inorganic N therefore enhancing N_2O diffusion from the atmosphere to the soil.

Annual budgets for CH_4 differed between years, with lower net sink during high precipitation years (2015 and 2016). During 2015, highest CH_4 uptake was from 3 yr- patch burning with a total consumption of -10.0 to -51.8 kg CH_4-CO_2 eq ha^{-1} yr^{-1} (-0.4 and 2.01 kg CH_4 ha^{-1} yr^{-1}). Our results are similar to the -1.74 kg CH_4 ha^{-1} yr^{-1} grassland uptake reported by Dutaur and Verchot (2007). Uptake was lower than the highest to CH_4 uptake from savanna's and tropical forest ecosystems which are known for high uptake rates ranging from -0.04 to -27.7 kg CH_4 ha^{-1} yr^{-1}.

Conclusion

This research examined the N_2O and CH_4 dynamics from a grazed tallgrass prairie as a function of weather, fire, and grazing. Precipitation and higher soil NO_3^- during fall increase soil N_2O emissions. Low precipitation patterns increased soil CH_4 uptake and reduced N_2O

emissions. The 3-yr patch burning resulted in lower N_2O uptake and increased CH_4 uptake compared to the annual burning. The 3-yr GHG estimates indicate N_2O and CH_4 sink of temperate grasslands was higher with patch burning.

Considering CH_4 emission from enteric fermentation of a cow-calf operation the soil CH_4 sink can partially or completely offset the CH_4 footprint from 33% to 212% considering a cow/calf emissions of 17.65 kg cow/calf land unit^{-1} yr^{-1} (Todd et al., 2016). Overall, this study and previous studies evidence Konza temperate grassland capacity as a CH_4 sink is improve by 3-yr patch burning.

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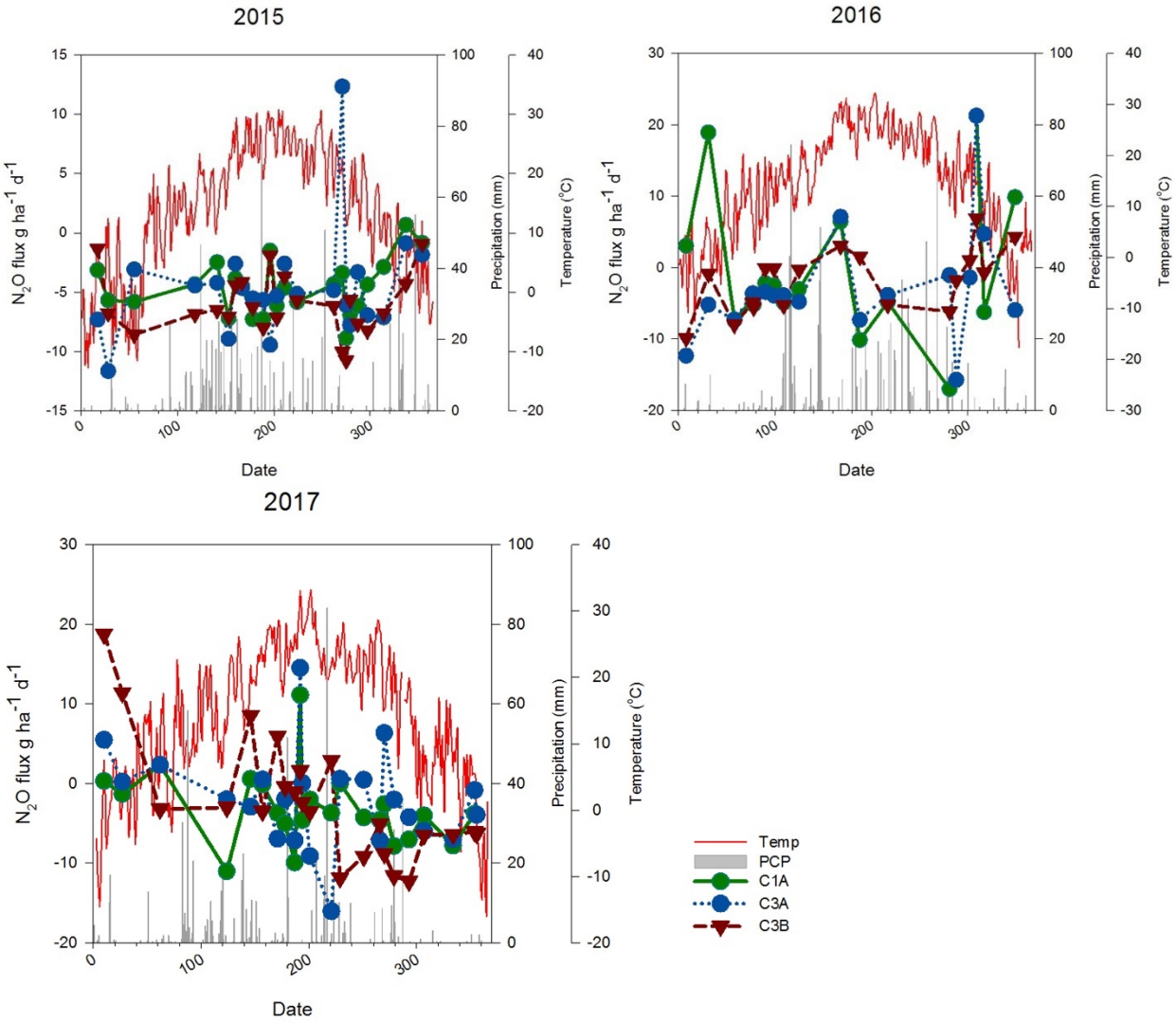


Figure 3-1. Nitrous oxide (N_2O) emissions, temperature (Temp), and precipitation (PCP) during 2015, 2016, and 2017 on the watersheds C1A (annually burned), C3A (3-yr patch burned in 2016) and C3B (3-yr patch burned in 2014 and 2017) at Konza prairie.

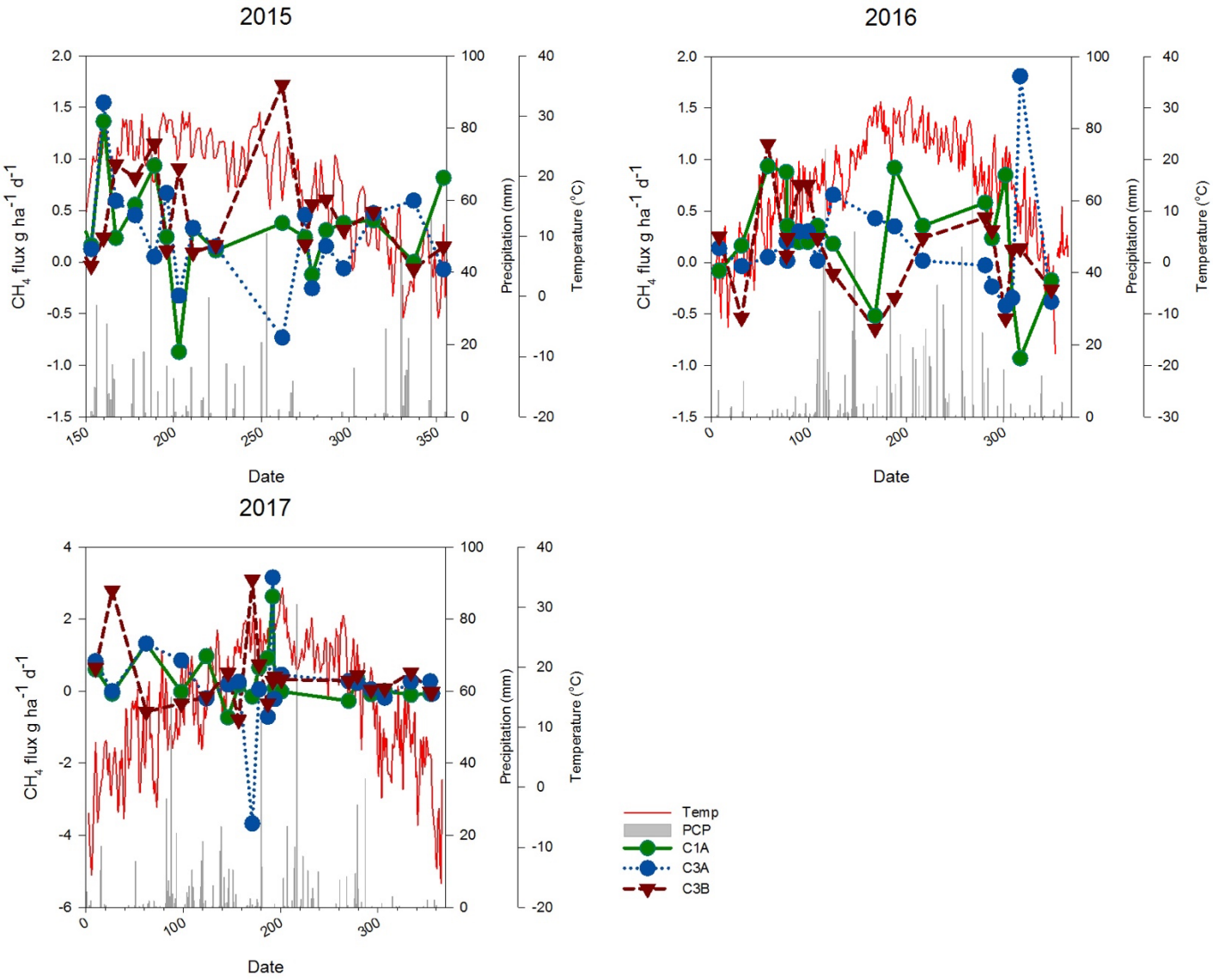


Figure 3-2. Methane (CH_4) emissions, temperature (Temp), and precipitation (PCP) during 2015, 2016, and 2017 on the watersheds C1A (annually burned), C3A (3-yr patch burned in 2016) and C3B ((3-yr patch burned in 2014 and 2017) at Konza prairie.

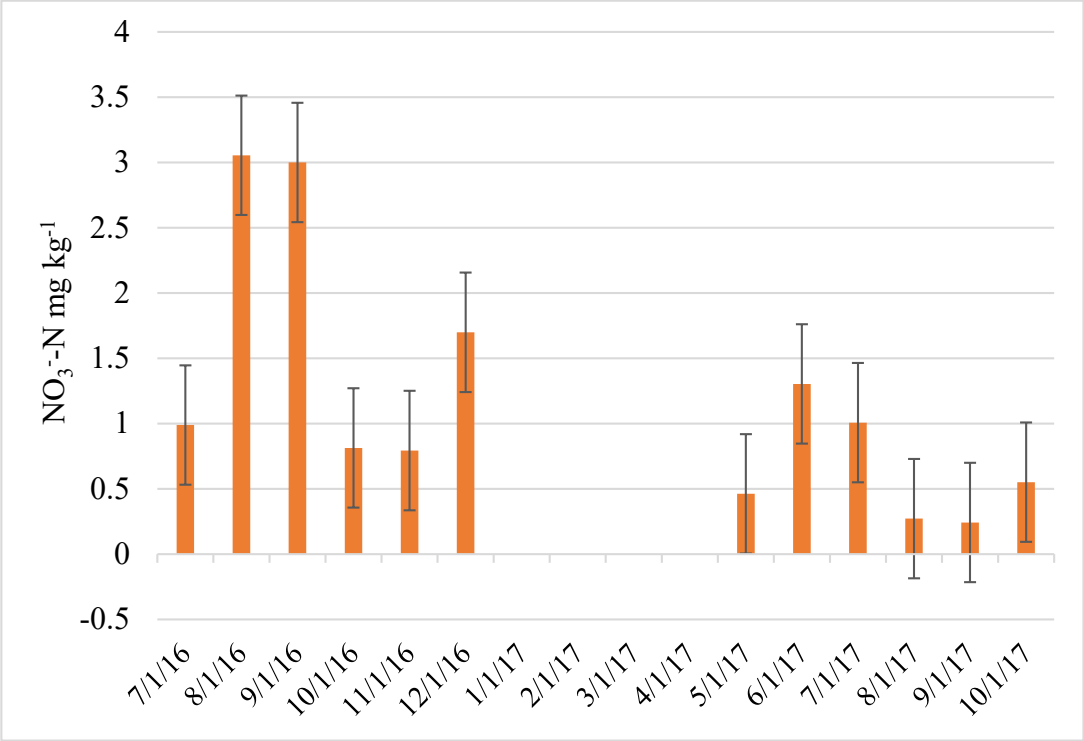


Figure 3-3. Nitrate (NO_3^-) concentration during summer 2016 to winter 2017 in the watersheds C1A, C3A, and C3B at Konza prairie. Errors bars indicate the standard deviation of the mean.

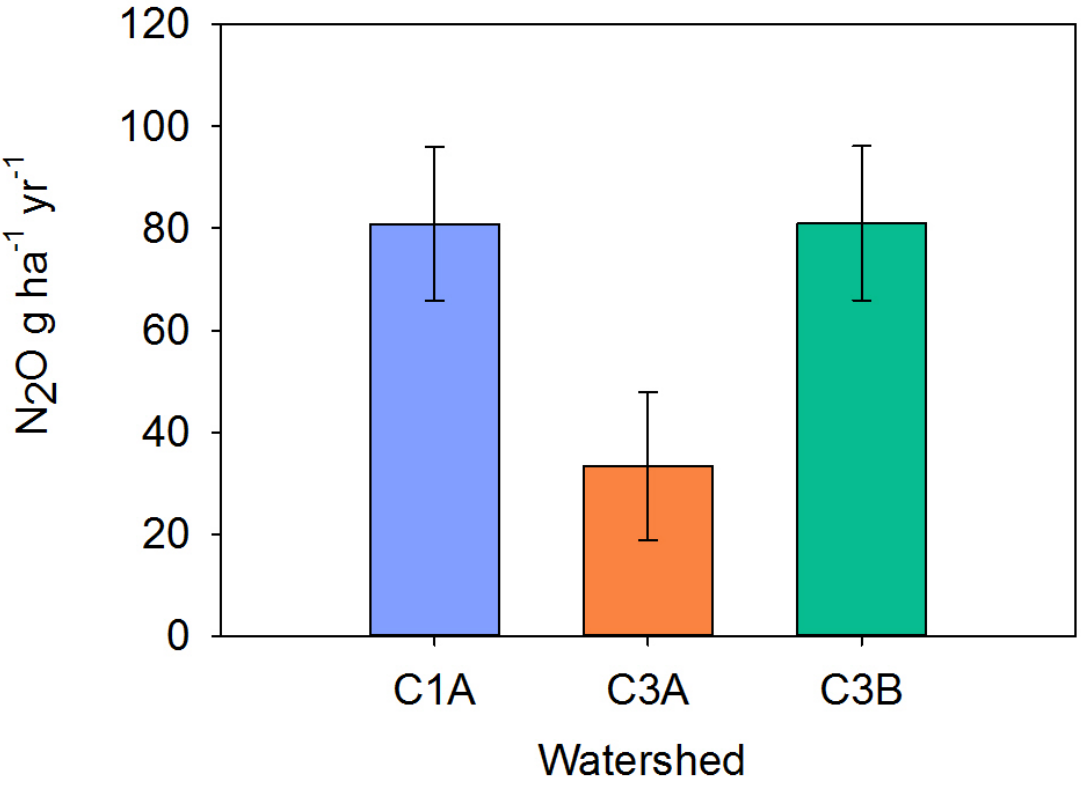
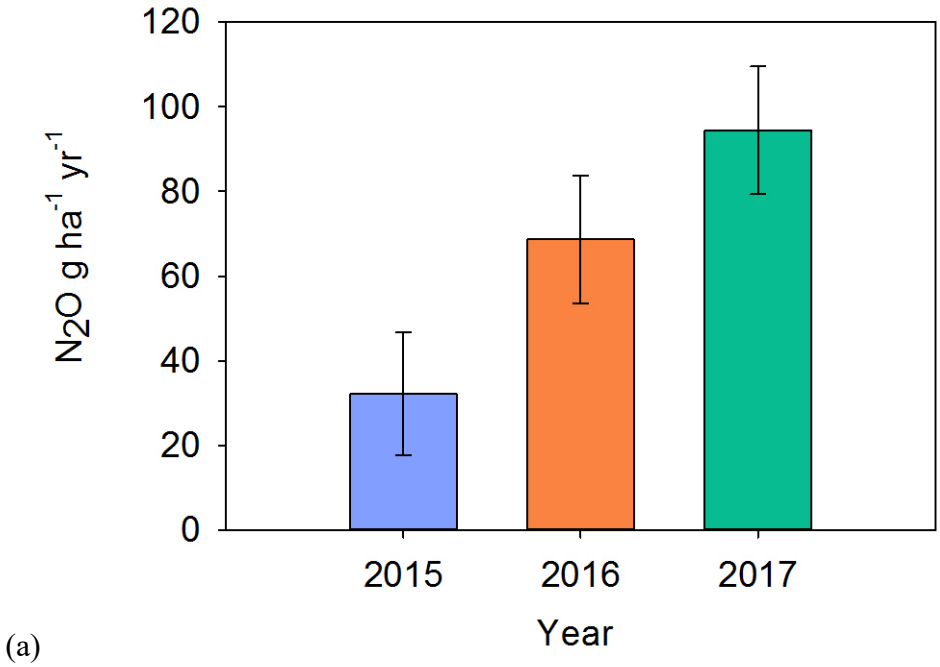
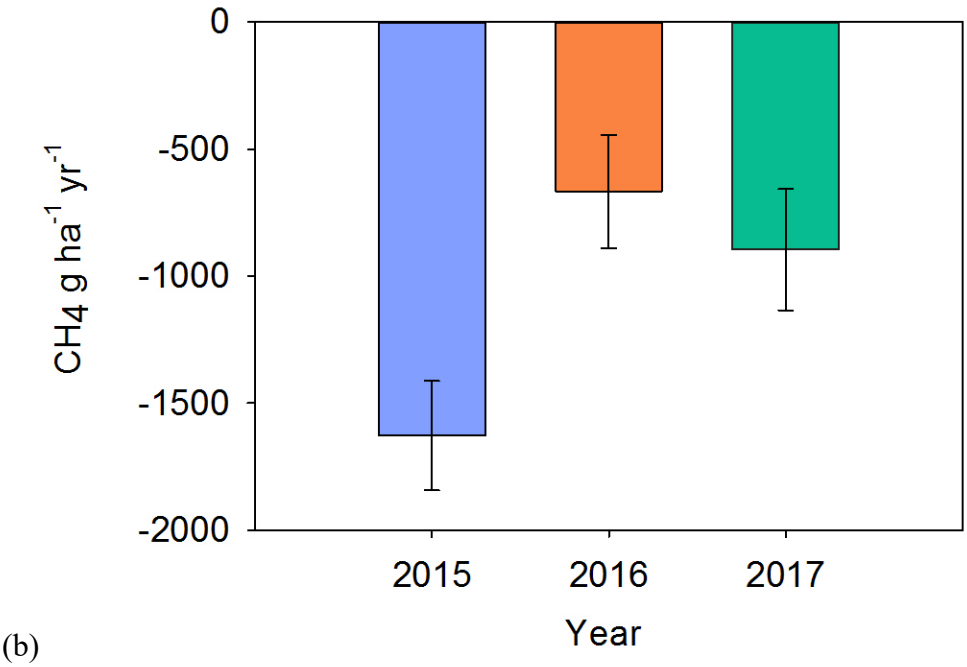


Figure 3-4. Mean N_2O annual budget during 2015 to 2017 on the watersheds C1A (annually burned), C3A (3-yr patch burned in 2016) and C3B (3-yr patch burned in 2014 and 2017) at Konza prairie. Errors bars indicate the standard deviation of the mean.



(a)



(b)

Figure 3-5. Total nitrous oxide (N_2O) (a) and methane (CH_4) (b) annual budget across the watersheds during 2015, 2016, and 2017. Errors bars indicate the standard deviation of the mean.

Table 3-1. Average soil chemical properties status in the watersheds C1A, C3A, and C3B within the first 0-5 cm depth. Values correspond to the average of four soil samples gathered from four sampling transects on each watershed. Soil sampling was performed during 2015 (O'neal, 2018).

Watershed	pH [1]	Mehlich-P [2]	K [3]	Ca [4]	Mg [4]	Na [4]	NH_4^+-N [5]	NO_3^--N [5]	Total N [6]	Total C [6]
		----------μg g^{-1} soil----------							------- g kg^{-1} -------	
C1A	6.5	5.0	459	2263	338	10	7	3	3	46
C3A	6.5	3.6	404	2295	320	8	5	4	3	42
C3B	6.6	4.2	395	2289	331	8	6	2	3	50

[1] Soil pH was determined using a 1:1 soil:water method.

[2] Samples were analyzed by Mehlich 3 Phosphorus for P (Lachat Quickchem 8000, Loveland, CO, USA).

[3] Ammonium Acetate extraction for K.

[4] DTPA extraction for Cu, Fe, Mn, and Zn both analyzed by a Inductively Coupled Plasma Spectrometer.

[5] KCl extrancion for inorganic nitrogren (N), NH_4^+ and NO_3^-.

[6] Total C and N content analysis by dry combustion method using a C N analyzer (Flash EA 1112 Series, Thermo Scientific, Waltham, MA).

Table 3-2. The p-values for CH_4 and N_2O fluxes during July to December 2014, all year of 2015 and 2016, and 2017, and for inorganic N (NH_4^+ and NO_3^-) from July 2016 to July 2017 for the effects of date (D), burning regimes (B), and the interaction. Significance difference was calculated by Proc Mixed over time ($p<0.05$).

Factors	**----------CH_4----------**				**----------N_2O----------**				**NH_4^+**	**NO_3^-**
	2014	**2015**	**2016**	**2017**	**2014**	**2015**	**2016**	**2017**		
Date (D)	0.0002	<.0001	0.2425	0.0994	0.0001	0.0923	0.5844	0.4526	0.1496	<.0001
Burning regimes (B)	0.2380	0.0009	0.6433	0.0596	0.4812	0.6002	0.8195	0.0528	0.3618	0.6291
D*B	0.0153	<.0001	0.7543	0.5887	0.4317	0.9681	0.8473	0.0234	0.5504	0.4721

Table 3-3. The p-values for CH_4 and N_2O budget for the effects of date, burning regimes, and the interaction. Significance difference was calculated by Proc Mixed over time ($p<0.05$).

Factors	**CH_4**	**N_2O**
Year (Y)	0.0062	0.0231
Burning regimes (B)	0.8930	0.0641
Y*B	0.1274	0.1924

Table 3-4. Grassland balance as the total C footprint CH_4-C, and N_2O-C CO_2 equivalent for the different watersheds C1A, C3A, and C3A watersheds. Significance difference was calculated by Proc Glimmix ($p<0.05$).

Watershed	CH_4	N_2O	Grassland Balance
	---------kg CO_2-eq ha^{-1} $year^{-1}$----------		
2015			
C1A*	-35.3	13.7	-21.6
C3A	-35.0	-5.2	-40.2
C3B	-51.8	20.3	-31.6
2016			
C1A*	-8.0	35.7	27.7
C3A*	-32.0	9.5	-22.5
C3B	-10.0	16.2	6.2
2017			
C1A*	-30.3	22.9	-7.3
C3A	-19.8	25.6	5.8
C3B*	-17.1	35.9	18.8

*means watershed was burned that year

Table 3-5. Cattle grazing system CH_4-C balance as the annual CH_4-C budget from a grazed temperate grassland in the C1A, C3A, and C3A watersheds considering a cow/calf unit area of 3.2 ha, and a total emission of 7.6 CO_2-eq kg cow/calf^{-1} per land unit yr^{-1} (Todd et al., 2015). Significance difference was calculated by Proc Glimmix ($p<0.05$).

Year	C1A	C3A	C3B
	CO_2-eq kg cow/calf^{-1} land unit year^{-1}		
2015	-3.4	-3.3	-8.6
2016	5.2	-2.3*	4.5
2017	-1.8	1.5	2.3*

*means watershed was burned that year

Chapter 4 - Effects of beef-cattle urine and manure depositions on greenhouse gas emissions, soil nitrogen dynamics, and soil microbial community in a temperate grassland

Abstract

On grazed pastures, animal depositions of manure and urine create soil "hotspots" which alters soil microbial dynamics causing nutrient losses by nitrogen (N) leaching, and production of greenhouse gases (GHG), methane (CH_4) and nitrous oxide (N_2O). A field study was conducted to determine the effect of precipitation patterns, cattle urine and manure on the soil N_2O and CH_4 emissions, inorganic N dynamics, pH, and the microbial community composition over time in a grazed tallgrass prairie. A 28 d field trial with four replications tested the following treatments: high precipitation, drought, and ambient as the main treatments and additions of cattle manure, cattle urine, and control (no N addition) as sub-treatments. Higher N_2O emissions occurred under the urine treatments and high precipitation conditions. Inorganic N from urine and feces reduced CH_4 uptake, and increased N_2O emissions. Hotspots from manure and urine had higher N_2O fluxes than no manure patches and no urine patches. The total soil microbial community did not change significantly to precipitation conditions, urine or manure addition over the incubation period.

Introduction

Grazed grasslands can be a source or sink of greenhouse gases (GHG) including nitrous oxide (N_2O) and methane (CH_4). While grasslands can be a sink for CH_4 and potentially of N_2O, cattle manure and urine create "hotspots" of high nitrogen (N) concentration in soil altering soil microbial dynamics and causing changes on CH_4 and N_2O fluxes (Haynes and Williams, 1993; Saarijärvi and Virkajärvi, 2009; Ussiri and Lal, 2013; Sordi et al., 2014; Cai and Akiyama, 2016). Previous studies identify cattle feces as a source of ammonia (NH_3) and N_2O emissions, and urine as a major source of N losses by surface runoff, NH_3 volatilization, and denitrification (Saarijärvi and Virkajärvi, 2009; Ussiri and Lal, 2013). Additionally, precipitation and drought conditions control microbial activity rates, therefore, controlling N dynamics and rate of N losses in the soil.

From an environmental perspective, the carbon (C) and N additions from cattle manure and urine result in a saturation of the area causing CH_4 and N_2O emissions, and N leaching (Haynes and Williams, 1993; Petersen et al., 2004; Hobbie and Hobbie, 2013; Ussiri and Lal, 2013; Lee et al., 2014; Sordi et al., 2014). Nevertheless, some benefits from manure and urine patches, under controlled animal density, is the use as a source of nutrients for plant uptake and to increase the soil C storage (FAO, 2011; Petersen et al., 2004). Allard et al. (2007) reported that the role of livestock in soil C sequestration of unfertilized grasslands outweighed their CH_4 and N_2O emissions.

Under grazing systems, soil microbial stresses are induced by anaerobic conditions, changes in pH, and increases in N concentrations (Baatout et al., 2007; IPCC, 2014). Urine and manure patches trigger microbial responses through changes in water availability, nutrients, and pH. High N concentrations from the urine and manure patches create N hotspots that exceed soil

N retention and plant N uptake, potentially increasing N losses (Moir et al., 2007). Another factor that contributes to the "hotspots" is the stress from the salt concentration in the urine and the manure (Rath et al., 2016). For example, fungi are reportedly more tolerant than bacteria to acute salt exposure since fungal cell walls provide greater resistance to water loss (Rath et al., 2016). As a result, fungi should be more resilient to changes in soil water content (Six et al., 2006; Gordom et al., 2008; Waring et al., 2013).

Soil microbial communities control soil N processes and are crucial for determining ecosystem services, including the supply of nutrients, soil water regulation, and soil structure (De Ruiter et al., 1993; Gordom et al., 2008; Kibblewhite et al., 2008; Waring et al., 2013). Several studies highlight the importance of bacteria and fungi for their role driving organic matter decomposition, C sequestration, and nutrient cycling. Under drought conditions, the soil water content limits nutrient movement and reduces mineralization rates. On the other hand, as precipitation increases, increased soil water content promotes anaerobic conditions and enhance denitrifiers activity which produces N_2O emissions (Linn and Doran, 1984; Xu et al., 2004; Hagerty et al., 2014).

Studies on GHG emissions and N dynamics from grazed systems are necessary to gain a better understanding of the contribution of cattle to grazed temperate grassland GHG budgets. Additionally, identifying soil microbial communities role in the N cycle , and GHG emissions provide a greater understanding of what management practices, such as animal density, animal rotation, and plant cover, could be manipulated to reduce the environmental footprint of grazing systems. The objectives of this study were to quantify CH_4 and N_2O emissions, inorganic N concentrations, and the microbial community response to the additions of cattle manure and urine under differing precipitation patterns in a temperate grassland.

Materials and Methods

Experimental Site

The experimental site was located in Pottawatomie County, Kansas (39° 15' 25.4736" N, 96° 29' 14.0784" E) with an elevation of 413 m (Appx. B). The soils of the experimental site were Tully (Fine, mixed, superactive, mesic Pachic Argiustolls) (NRCS, 2006) and Clime (fine, mixed, active, mesic Udorthentic Haplustolls) (NRCS, 2013); corresponding to 60% and 40% of the area, respectively. The experimental site was in tallgrass prairie. Basic soil properties are provided in Table 1. The experiment was conducted during the summer of 2016 (June 27 to July 20) and 2017 (June 12 to July 10). Mean temperature during the field experiment was 26ºC, and 30ºC for 2016, and 2017, respectively. Total precipitation during the 28 d experiment was 106 mm and 160 mm for 2016 and 2017, respectively.

Experimental Setup

The experiment was a strip-plot block arranged with four replications. Each block was composed of three plots of 6 m x 1 m. Each plot was composed of three sub-plots of 2 m x 1 m. Each sub-plot had four polyvinyl chloride (PVC) collars with 21.6 cm in diameter and 15 cm in height. The PVC collars were inserted into the soil to a depth of 5 cm at each plot. Urine and manure were applied to the soil inside the collar. The main factor simulated three precipitation conditions: (1) high precipitation, (2) drought, and (3) ambient. High precipitation was simulated with a weekly addition of 2.54 cm of water for a 1 h period about 1 h before GHG sampling. No water was applied to drought treatments during the 28 days. A 6-mm standard clear greenhouse film (Item no. GF-6MC, Greenhouse Mega Store) was positioned over the drought plots to minimize natural rainfall during the time period. Temperatures under the greenhouse film were ~2ºC higher than the treatments with no shade. The ambient treatment

was the local precipitation regime during the 28-d period. The sub factor was (1) urine or (2) manure and a control.

The amount of manure and urine applied to each treatment was calculated based on a single event of urination and defecation. The covered area by urine and feces by event was calculated following the method from Saarijärvi and Virkajärvi (2009). They used 0.075 m^2 and 0.353 m^2 for the manure and the urine patch, respectively. Fresh cattle urine was frozen to a temperature of -30ºC until addition, while manure was collected early in the morning of the day of addition and saved in Ziploc sealed bags until applied (1-2 h after collection). Manure applied was 0.55 kg with a total N content of 109 g N per plot, while the urine applied was 1 L for a total N content of 8.47 g N m^{-2} (Table B-1).

Inorganic N and soil pH

Soil samples were taken from 0-5 cm depth at 4, 7, 10, 17, 24, and 28 days. Soil samples were passed through a 4.0 mm sieve to homogenized the soil. Soils were analyzed for inorganic N (NH_4^+-N and NO_3-N) by extraction of 25 g of moist soil with 100 mL 1 M KCl solution. The slurry was shaken for 60 min on an orbital shaker at 300 rpm, and filtered through Whatman No. 42 filter paper (MilliporeSigma Corporate Offices, St.Louis, MO) into a 20 mL scintillation vial. The extractions were frozen at 4 ºC until analyzed for NH_4^+-N and NO_3-N by colorimetric analysis (Gelderman and Beegle, 1998) at the Kansas State University Soil Testing Laboratory. For each sampling time, soil water content was measured gravimetrically by oven drying a 10 g sample at 105ºC for 48 h. Soil pH was determined at 14 and 21 d using a 1:10 soil:water ratio mixed until forming a paste measured with an OrionStar A111 pH meter (ThermoFischer Scientific, Waltham, MA).

Soil water and air temperature

Soil water content and air temperature were collected from each treatment within 2 h after urine and manure application, and 4, 7, 10, 17, 21, and 28 after application from 11:00 to 15:00 h. Soil water content was measured during each GHG sampling using a POGO® (Stevens Water Monitoring Systems, Inc., Portland, Oregon, USA). Specific soil water sampling occurred 2 h and 4, 7, 10, 17, 21 and 28 d after application. Precipitation data was obtained from the National Weather Service Forecast Office (http://w2.weather.gov/climate/index.php?wfo=top).

Phospholipid fatty acid analysis

Phospholipid fatty acid analysis (PLFA) was performed in 2017 7 and 28 d after the urine and manure application to compare the difference in microbial community composition between treatments. About 25 g of soil from the top 0-5 cm soil was sampled and immediately frozen at -30 °C, and later freeze-dried for 72 h using a FreeZone 6 (LABCONCO, Kansas City, MO). Total lipids were extracted using a modification of the Bligh and Dyer (1959) extraction (White and Rice, 2009). The PLFA were separated from the total lipid extract using silicic acid chromatography, the fatty acids were cleaved from the glycerol backbone using KOH saponification, and the harvested fatty acids were methylated to form fatty acid methyl esters (FAME). The resulting FAME was analyzed using a Thermo Scientific Trace GC-ISQ mass spectrometer equipped with a DB5-MS column. The FAME peaks were identified by comparison with the bacterial acid methyl esters mix. Fatty acids were grouped into gram-positive bacteria (i15:0, a15:0, i16:0, i17:0, a17:0), gram-negative bacteria (16:1w7c, cy17:0, cy19:0. 18:1w7, 2-OH 10:0, 2-OH 12:0, 3-OH 12:0, 2-OH 14:0, 3-OH 14:0, 2-OH 16:0), actinomycetes (10Me 16:0, 10Me18:0), arbuscular mycorrhizal fungi (16:1w5c), and fungi (18:2w9,12c , 18:2w6,9,12) (White and Rice, 2009).

Methane and nitrous oxide emissions

Fluxes of CH_4 and N_2O were measured using static chambers (7.5 cm high x 20 cm in diameter) as described in Hutchinson and Mosier (1981). A PVC collar was inserted 10 cm into the soil until 5 cm of the anchor remained above the soil surface. Gas samples were taken 2 h and 4, 7, 10, 17, 21 and 28 d after urine and manure addition, from 11:00 to 15:00 h. The gas samples were collected by placing a closed vented chamber over a buried anchor and taking a 25 ml gas sample using a syringe, and transferring the gas sample to a 20 mL (22 x 75 mm) glass vial (Wheaton, New Jersey, USA) closed with a 20 mm gray butyl stopper (Labco Limited, Wales, UK), and sealed with a 20 mm unlined seal open top aluminum (Labco Limited, Wales, UK). The sampling began by placing a chamber over the PVC core and sealing it with a rubber strap. When the chamber was sealed the first sample (0 min) was taken and then sample at 15, 30, and 45 min after sealing.

Gas samples were analyzed for carbon dioxide (CO_2), CH_4 and N_2O using a Bruker Scion 456 Gas Chromatography (Scion Instruments©, Austin, TX). The GC was calibrated daily using analytical-grade standards containing 0.2, 0.512, 3.5, and 15.3 μL N_2O L^{-1}, 4.0 μL CH_4 L^{-1}, and 495, 800, and 993 μL CO_2 L^{-1}. The concentration of N_2O and CH_4 in each sample was converted to μg N_2O m^2 or CH_4 m^2 using:

Equation 4-1

$$\mu g\ N_2O\ m^2, \text{or } \mu g\ CH_4\ m^2 = \frac{CPVM}{ART}$$

where C is the volumetric concentration of N_2O (μL N_2O L), P is the atmospheric pressure at 304.8 m above sea level (0.965 atm), V is the chamber volume (L), M is the mass of N in N_2O (28 μg N μmol^{-1} N_2O), A is the chamber surface area (m^2), R is the Universal Gas Constant (0.08206 atm μL^{-1} $\mu molK^{-1}$), and T is the chamber headspace temperature (K) when the

sample was taken. Results were analyzed with the Hutchinson-Mosier method and linear equations (Pedersen et al., 2010) (Table B-2).

Statistical analysis

Statistical Analysis System (SAS) 9.3 was used to analyze results using a Proc Glimmix analysis of variance (ANOVA) (α=0.05) of repeated measurements over time. Results were compared to determine significant interactions among the interactions of the factor precipitation conditions, and cattle waste, and time on inorganic N, soil pH, GHG fluxes, and soil microbial communities. A Proc mixed method (α=0.05) was used to determine the effect of precipitation patterns, cattle urine and manure and the interactions on accumulative N_2O, and CH_4 flux over the incubation time.

Results

Nitrous oxide and methane dynamics

For 2016, N_2O emissions were significantly affected by the interaction of precipitation, urine and manure, and time (p=0.001) (Fig. 4-3). Under ambient conditions and the control (no urine or manure addition), N_2O emissions were highly variable over time ranging from -1.7 to 5.1 g N_2O ha^{-1} d^{-1}. The N_2O emissions from ambient with manure decreased with time from 2.3 to -0.1 g N_2O ha^{-1} d^{-1} by the end of the experimental period, while a peak of N_2O at 10 d was associated with a precipitation event of 39.6 mm (Fig.4-1a). The highest N_2O flux occurred from the ambient conditions under urine patches with a peak N_2O flux of 52.4 g N_2O ha^{-1} d^{-1} at 10 d, same day with precipitation of 39.6 mm (Fig.4-1a). Under drought, N_2O fluxes reached the lowest values, especially under control treatment ranging from 1.1 to -0.5 N_2O ha^{-1} d^{-1}. The highest flux from the drought conditions was from the urine with 37.0 g N_2O ha^{-1} d^{-1} 10 d after addition probably as a result of lateral water movement from the rainstorm (Fig.4-1a). Fluxes

from drought conditions and manure treatment ranged from 4.2 to -0.6 g N_2O ha^{-1} d^{-1}. As expected, high precipitation with no N additions from urine and manure did not increase N_2O flux with values ranging from 1.7 to -1.8 g N_2O ha^{-1} d^{-1}. With high precipitation, N_2O emissions from the manure were 1.7 to -1.0 g N_2O ha^{-1} d^{-1}. With high precipitation, the highest and lowest N_2O emissions from the urine patches were 17.6 g N_2O ha^{-1} d^{-1} and -0.2 N_2O ha^{-1} d^{-1} during 20 and 4 d after urine addition, respectively.

In 2017, N_2O emissions were not significantly different for the precipitation treatments, the addition of urine and manure, time, and the interactions ($p>0.05$) (Fig. 4-3). However, time and cattle urine and manure were significantly different at $p < 0.1$. For the control (no manure or urine), N_2O fluxes ranged between -24.0 to 2.7 g N_2O ha^{-1} d^{-1}; for the manure treatment, N_2O emissions ranged between -6.8 to 28.8 g N_2O ha^{-1} d^{-1}; and urine treatment values ranged between -7.6 to 4.5 g N_2O ha^{-1} d^{-1}. Overall, N_2O fluxes varied with time with no specific pattern, with highest N_2O emissions from urine patches.

Soil CH_4 emissions were statistically affected by the interaction of precipitation with cattle urine and manure during 2016 ($p=0.020$) (Fig. 4-4). Emissions over time ranged from 13.9 to -5.9 g CH_4 ha^{-1} d^{-1}. Higher values occurred from urine, with values from 9.1, 7.7 and 6.0 g CH_4 ha^{-1} d^{-1} from high precipitation, drought, and ambient, respectively. Under manure, emissions were -5.9, 1.6, and 8.5 g CH_4 ha^{-1} d^{-1} from high precipitation, drought, and ambient, respectively. Under control treatment (no manure or urine addition) CH_4 consumption was -1.7, and -5.9 g CH_4 ha^{-1} d^{-1} for ambient and drought, respectively; while for high precipitation, the flux was 13.9 g CH_4 ha^{-1} d^{-1}. By the end of the 28-d study, the urine treatments had higher CH_4 uptake with a mean of -13.6 g CH_4 ha^{-1} d^{-1}. Manure had the highest CH_4 emissions of 8.9 and

8.4 g CH_4 ha^{-1} d^{-1} 2 h and 4 d after manure application; while uptake varied from -4.7 to 7.5 g CH_4 ha^{-1} d^{-1}.

During 2017 CH_4 emissions were significantly affected by the interaction of precipitation, and urine or manure over time ($p=0.001$) (Fig. 4-4). High precipitation and urine registered the highest emissions ranging from 22.6 to 6.9 g CH_4 ha^{-1} d^{-1}; while manure varied from 11.4 to -5.8 g CH_4 ha^{-1} d^{-1}. The high precipitation-control treatment (no manure or urine) had the highest CH_4 sink with a mean of -11.8 g CH_4 ha^{-1} d^{-1}. Under drought conditions, urine CH_4 flux varied from 14.5 to -18.5 g CH_4 ha^{-1} d^{-1}; while manure treatment ranged from 30.5 to -7.8 g CH_4 ha^{-1} d^{-1}. Drought conditions under control treatment (no manure or urine), ranged from 15.5 to -5.5 g CH_4 ha^{-1} d^{-1}. Overall, under drought conditions, manure had the greatest CH_4 emissions, and urine the greatest sink. During both years, cumulative CH_4 fluxes were not significantly different for precipitation patterns, urine and manure application, or the interaction ($p<0.05$) (Table B-3). Overall, CH_4 dynamics were highly variable with no specific trends except for consistent consumption of CH_4.

The correlation of N_2O and CH_4 with soil water content, inorganic N, and temperature was not significant ($R^2<0.001$) (Data not shown). The high variability in CH_4 fluxes impeded our ability to conclude CH_4 trends over time. Further studies should consider edaphic factors such as soil bulk density, since highly grazed areas are expected to affect soil bulk density and will possibly cause higher N_2O, NH_3 losses, and leaching.

Cumulative N_2O losses with high precipitation were the highest under urine averaging 90.8 g N_2O ha^{-1} during the 28 d period. Under drought conditions, urine also had the highest cumulative emissions averaging 68.5 g N_2O ha^{-1} during the 28 d study. Cumulative CH_4

emissions from manure averaged 51.9, and 90.4 g CH_4 ha^{-1} during the 28 d of the study under the high precipitation and drought, respectively.

Soil inorganic N and pH

Soil inorganic N, NH_4^+ and NO_3^-, was significantly affected by the interaction of precipitation, and urine and manure during 2016 ($p<0.0001$) (Table 4-2, Fig. 4-5, and Fig. 4-6). Soil NH_4^+ was significant over the 28 d study ($p<0.0001$). In 2016, inorganic N increased after urine addition. Similarly, during 2017 there was a significance difference between precipitation, urine or manure addition, and time for NH_4^+ and NO_3^- concentrations (Table 4-5, and Table 4-6). Overall, as expected, NH_4^+ concentrations decrease over time the NO_3^- increase towards the end of the experiment. During both years, 2016 and 2017, soil pH fluctuated over time (Fig. B-1).

Soil microbial communities

Soil microbial biomass and actinomycetes were not affected by precipitation, urine or manure application, and time with an average of 121.3, and 7.1 2 nmol PLFA/g soil, respectively ($p>0.05$) (Table 4-3). Precipitation caused significant differences in gram-positive bacteria under high precipitation ($p<0.05$) (Fig. 4-7). Considering the ambient condition under control treatment (no manure or urine), the gram-positive bacteria was 30.2 nmol PLFA g^{-1} soil. When comparing the ambient-control (no manure or urine)with the high precipitation conditions our results registered a decrease in gram-positive bacteria within the first 7 d after urine or manure application. As a result of the high precipitation stress, gram-positive composition was 24.1 nmol PLFA g^{-1} soil and then recovered to 30.2 nmol PLFA g^{-1} soil as the rest of the treatments (Fig. 4-7). The interaction between precipitation, manure and urine patches, and time was significant for gram-negative bacteria ($p<0.05$) (Fig. 4-8). Fungi were significantly different between precipitation and time ($p<0.05$) (Table 3) (Fig. 4-9). The fungal community increased

by about 45% after 7 d of the study. In all the treatments, actinomycetes increased about 12% from day 7 to day 28 of the study; this coinciding with a decrease in precipitation over time which could have affected the soil water content in all the plots and experiment (Fig. B-2).

Discussion

Nitrous oxide and methane dynamics

As previously discussed by Mosier et al. (1991), N fertilizer additions in temperate grasslands can increase N_2O production. In this study, N_2O and CH_4 fluxes significantly increased by the additions of urine and manure. However, the lack of significant differences on N_2O and CH_4 during 2017 was possibly due to N loss as a result of 106 mm of precipitation during the first week of the experiment. The precipitation during the first 10 d of the study during 2016, and first 7 d of the study during 2017 potentially cause leaching and lateral movement of NO_3^-. The precipitation events could also explain the decrease in NH_4^+ and NO_3^- and the N_2O peak from the high precipitation-control treatment (no manure or urine).

The N_2O emissions were coupled with a decrease in CH_4 uptake (Fig. 4-3, and Fig. 4-4). Low N_2O emissions and soil inorganic N from manure were expected since the nutrients were mainly insoluble and the release occurs over time through decomposition, compared to the urine were the N is immediately available for microbial use (Whitehead, 2000; Cai and Akiyama, 2016). Cattle manure had lower inorganic N due to organic N in manure being unavailable and requiring more time to undergo mineralization. Wachendorf et al. (2008), who studied N_2O and N dynamics from urine and manure patches in a grassland sandy soil, reported about 51% and 2.5% of applied ^{15}N was found in leachate as inorganic N, and N_2O losses were 0.5% and 0.33% from urine and manure, respectively. Overall, manure patches were a source of CH_4 and affect N_2O emissions as a result of gas flow restriction from the physical barrier of the dung.

External factors not considered in this study, such as plant N uptake, are also responsible for changes in soil inorganic N availability. Saarijärvi and Virkajärvi (2009) identified the three significant N losses from urine applications as immediately after urine application by surface runoff and volatilization, immobilization and denitrification, and leaching. Similarly, Bowatte et al. (2018) found that two weeks after urine application N exceeded plant uptake resulting in N_2O emissions. The same study examined the N_2O response from urine patches and found that peak emissions occurred 7 d after urine application (Bowatte et al., 2018).

Furthermore, N competition from plants and microbes discussed by Dell and Rice (2005) suggest that under this low N environments the N availability cause an energy focus for N acquisition instead of C leading to N immobilization. Adittionally, Nichols et al. (2016) described plant composition as another factor controlling GHG fluxes with a mean flux of 0.64 and 0.30 kg CH_4-C ha^{-1} yr^{-1} under C3 and C4 pastures, respectively. Dell et al. (2005) discussed how the incorporation of organic matter increases microbial activity and mineralization rates, in this case manure. Overall, further studies should considered plant cover and root activity causing changes in inorganic N concentrations, pH, and actinomycetes over time (Hinsinger et al., 2009).

Urine and manure additions under ambient conditions resulted in a net sink of -1.2 g CH_4, ha^{-1}, and -17.9 g N_2O ha^{-1} during the 28 d period, respectively (Table B-3). Moreover, considering 152 d during the grazing season coupled with 12 urinations and 10 defecation events per cow a day the urine and manure patches covered 0.22% and 0.19% of the total area. Ramirez et al. (2012) hypothesized that N from animal depositions does not exceed plant N uptake.

Soil microbial communities

The reduction in gram-positive bacteria and AMF with high precipitation, and urine or manure additions were the result of the release of organic compounds from microbial cells to

counteract and stabilize soil osmotic changes (Halverson et al., 2000). Salazar-Villegas et al. (2016) indicated dominant microbial populations remained similar under short term changes in environmental conditions. Gram-negative bacteria increased over time under drought conditions with manure; suggesting the addition of nutrients and physical barrier from the manure create suitable conditions for gram-negative bacteria (Byrne-Bailey et al., 2009).

Gordom (2008) reports that fungal rich microbial communities retain more nutrients, under drought and rewetting conditions, which explains why fungi were significantly different over time. Simillarly, Rath et al. (2016) indicated that fungi are more tolerant to acute salt and drought exposure since the chitinous cell walls on fungi are highly protective against water loss (Strickland and Rousk, 2010). Rath et al. (2016) found that salt addition affects the soil microbial community within a 2 h period, and the fungal community was least affected by salt additions. The effect may even last for 48 h before the microbes recover from salts in the added urine.

Conclusion

Results support our hypothesis that the urine and manure patches creating high N and microbial activity "hotspots". In this study, higher N_2O emissions occurred under the urine treatments and high precipitation conditions. An increase in inorganic N from urine and feces reduced CH_4 uptake, and increase N_2O emissions. The absence of significant changes in the total soil microbial community by precipitation conditions, and urine or manure addition over the incubation period suggested temporal strength of soil microbes to excessive N and water additions. The increase over time from the gram-positive bacteria, gram-negative bacteria, and fungi communities describes an adaptation capacity to precipitation and nutrient additions.

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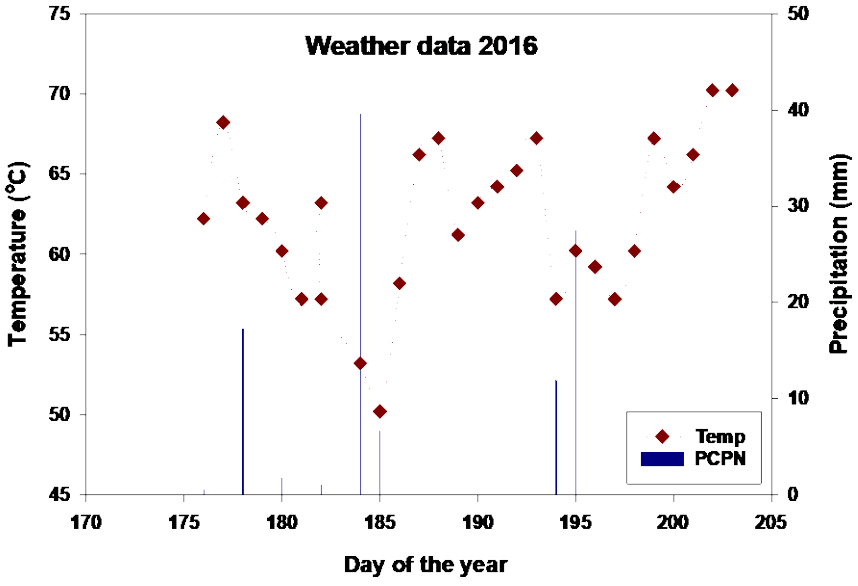


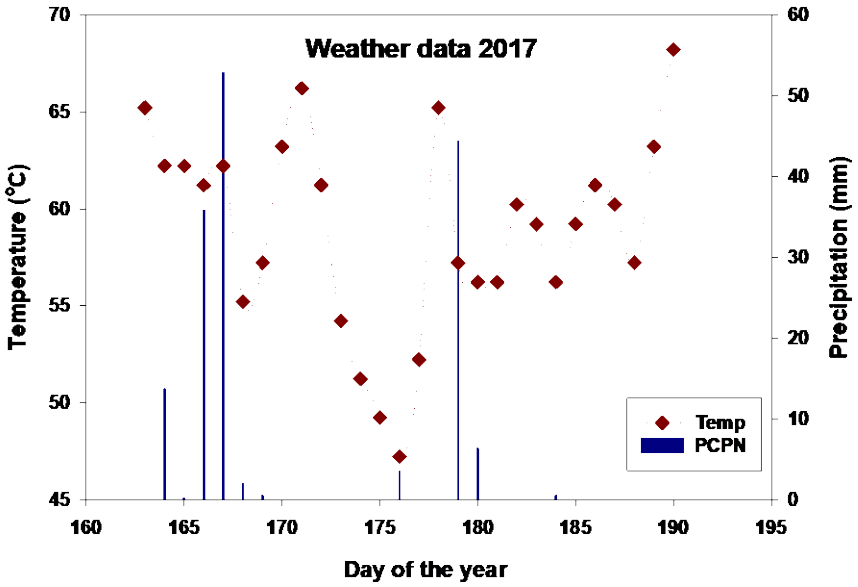


Figure 4-1. Mean daily temperatures and precipitation during the 28-d experiment during the summer 2016 and 2017 in Pottawatomie County, Kansas (39° 15' 25.4736" N, 96° 29' 14.0784" E)

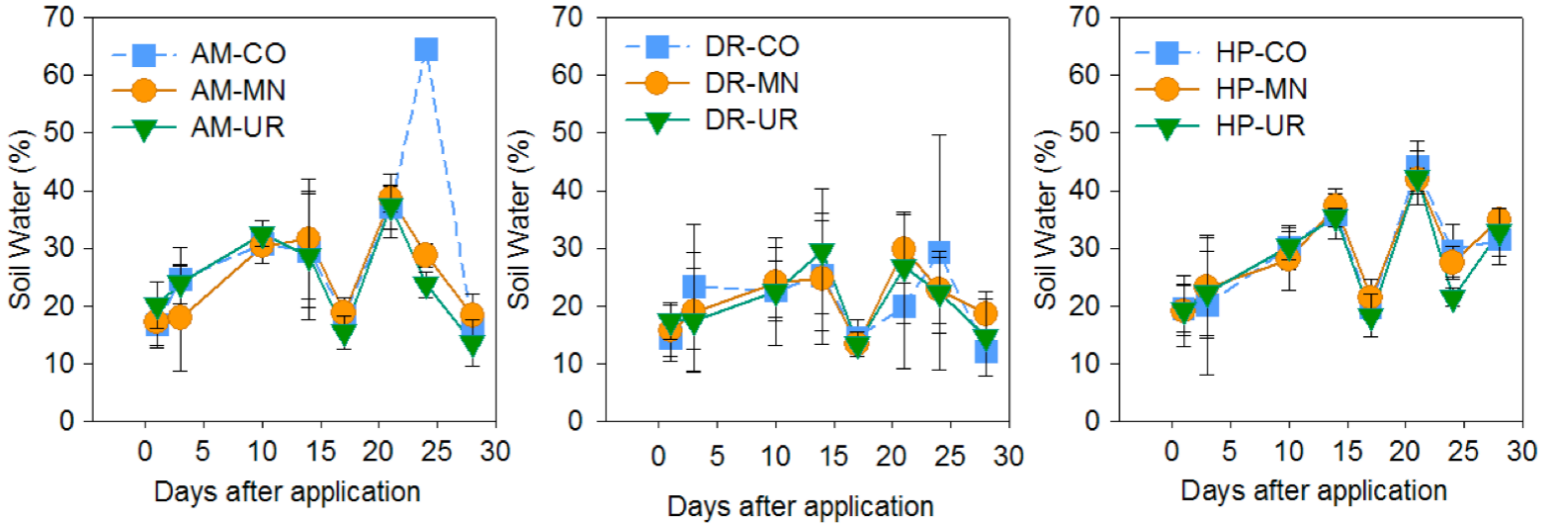
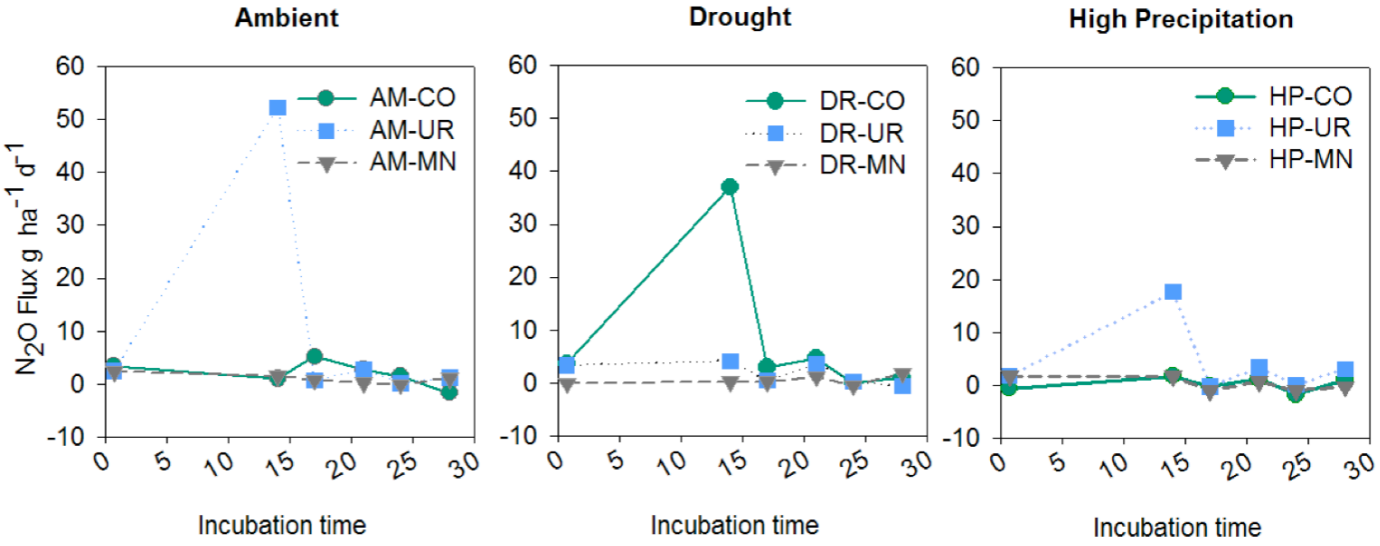
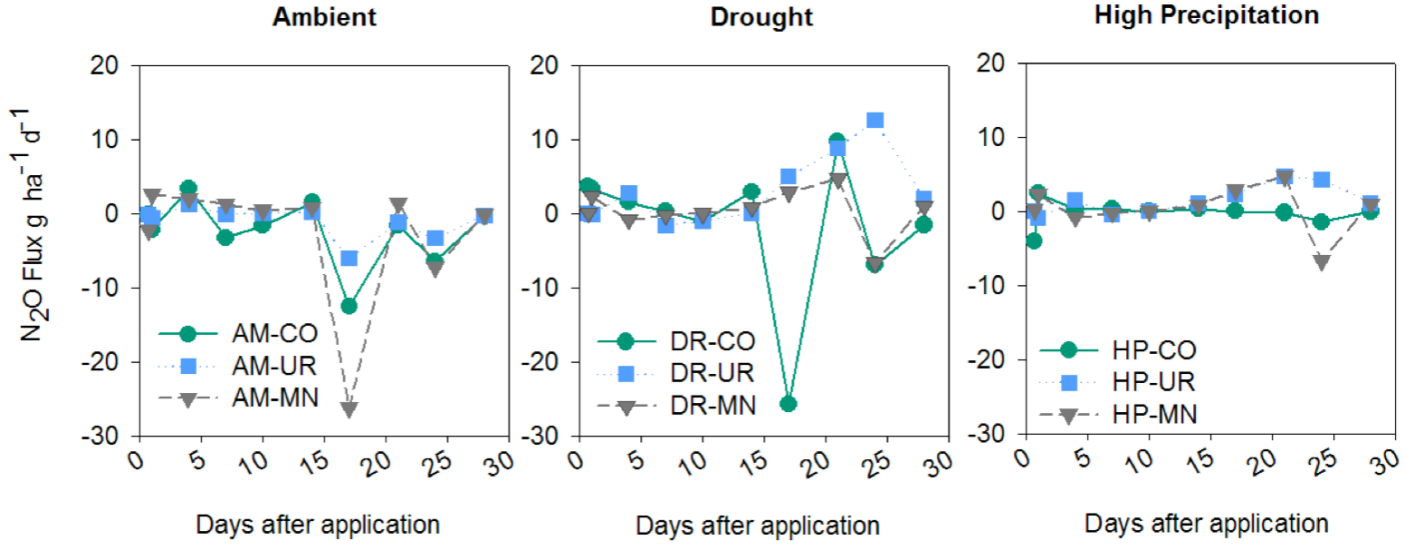


Figure 4-2. Soil water content during 2016 for the precipitation patterns: high precipitation (HP), drought (DR), and ambient (AM) conditions under control, and urine (UR) and manure (MN) patches. Error bars represent standard deviation of the mean.

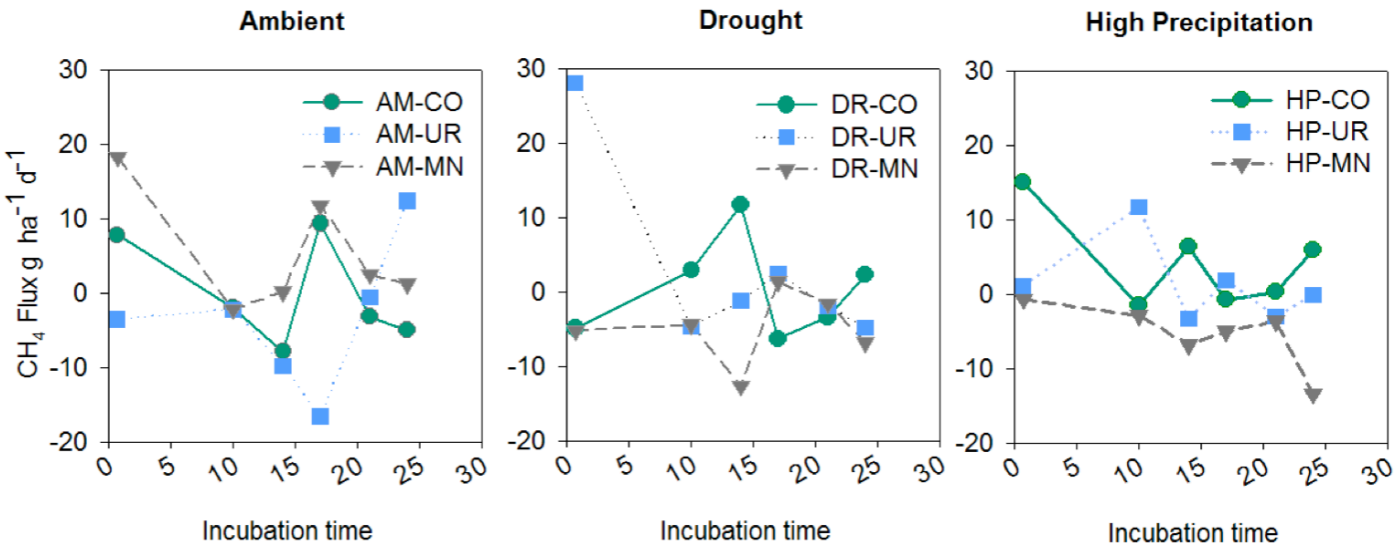


(a)

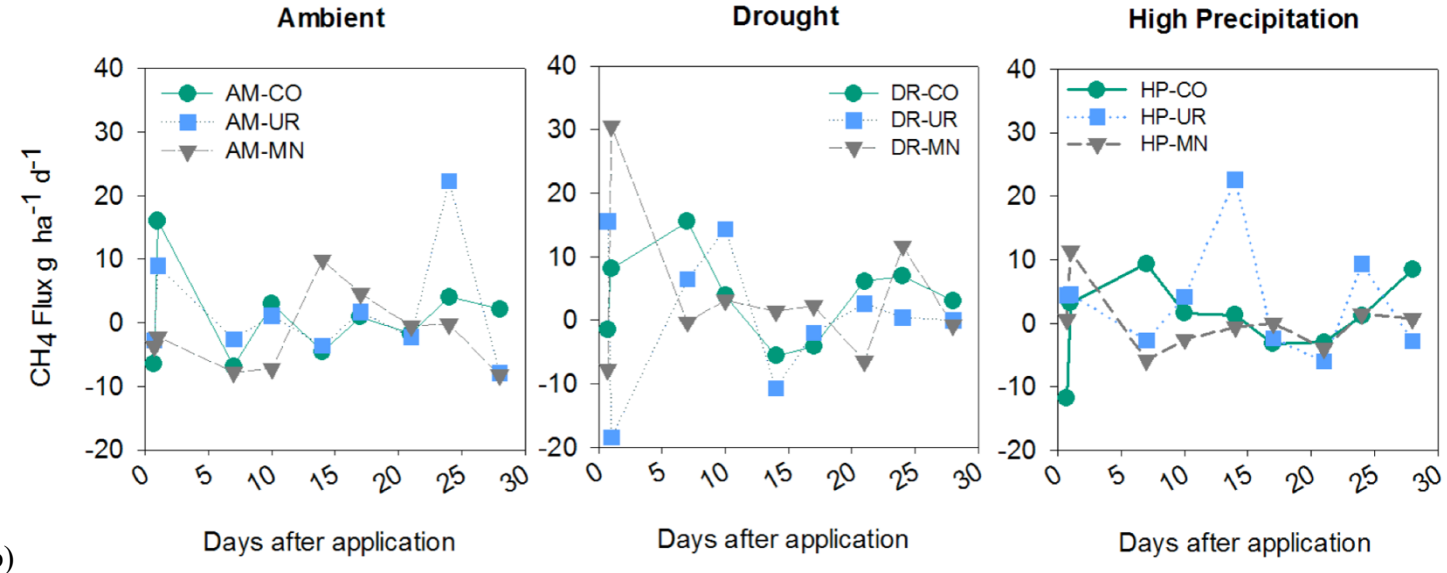


(b)

Figure 4-3. Fluxes of N_2O for (a) 2016 and (b) 2017 as a result of the precipitation patterns: high precipitation (HP), drought (DR), and ambient (AM) conditions under control, and urine (UR) and manure (MN) patches.



(a)



(b)

Figure 4-4. Fluxes of CH_4 during (a) 2016 and (b) 2017 as a result of the precipitation patterns: high precipitation (HP), drought (DR), and ambient (AM) conditions under control, and urine (UR) and manure (MN) patches.

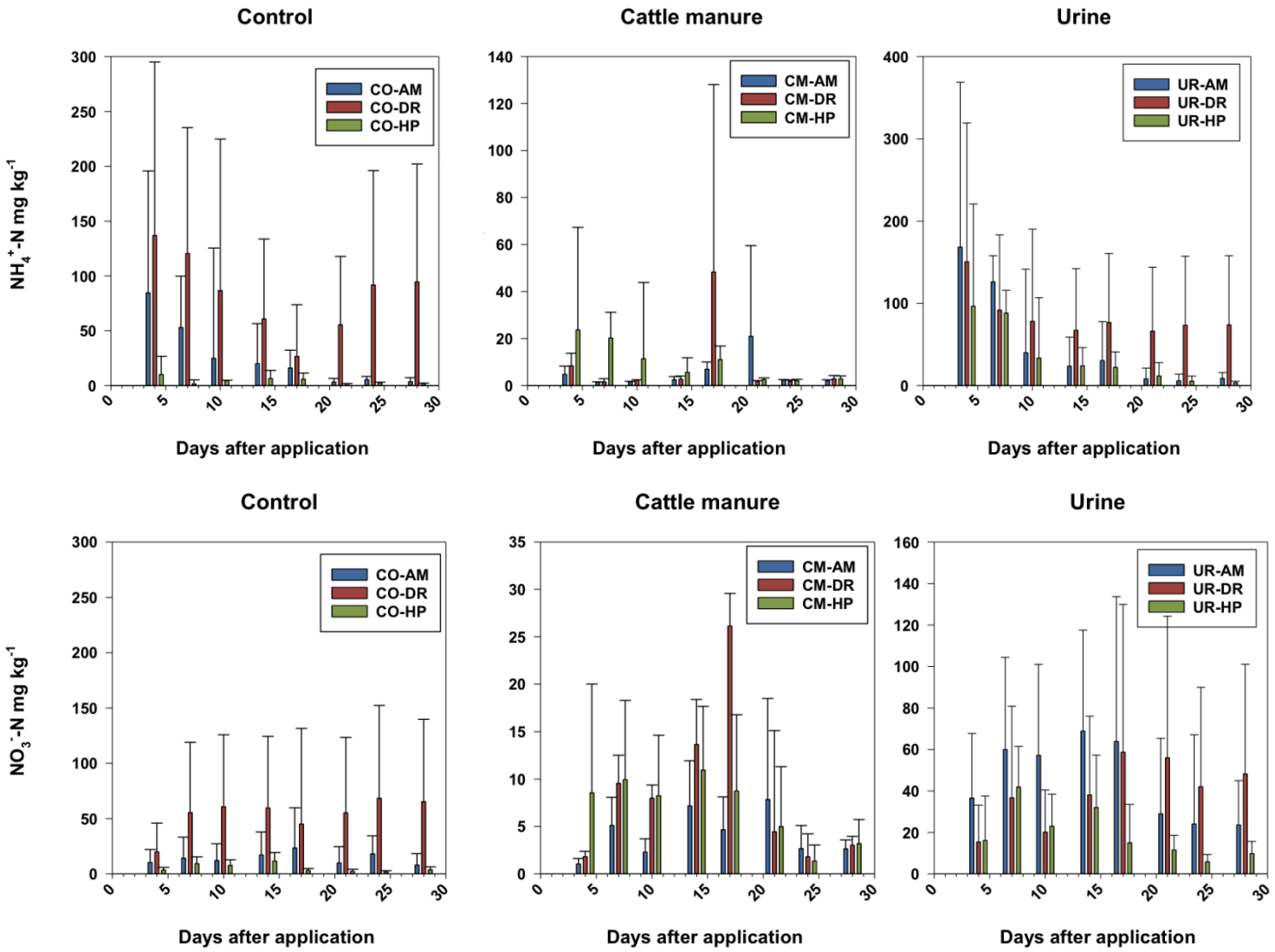
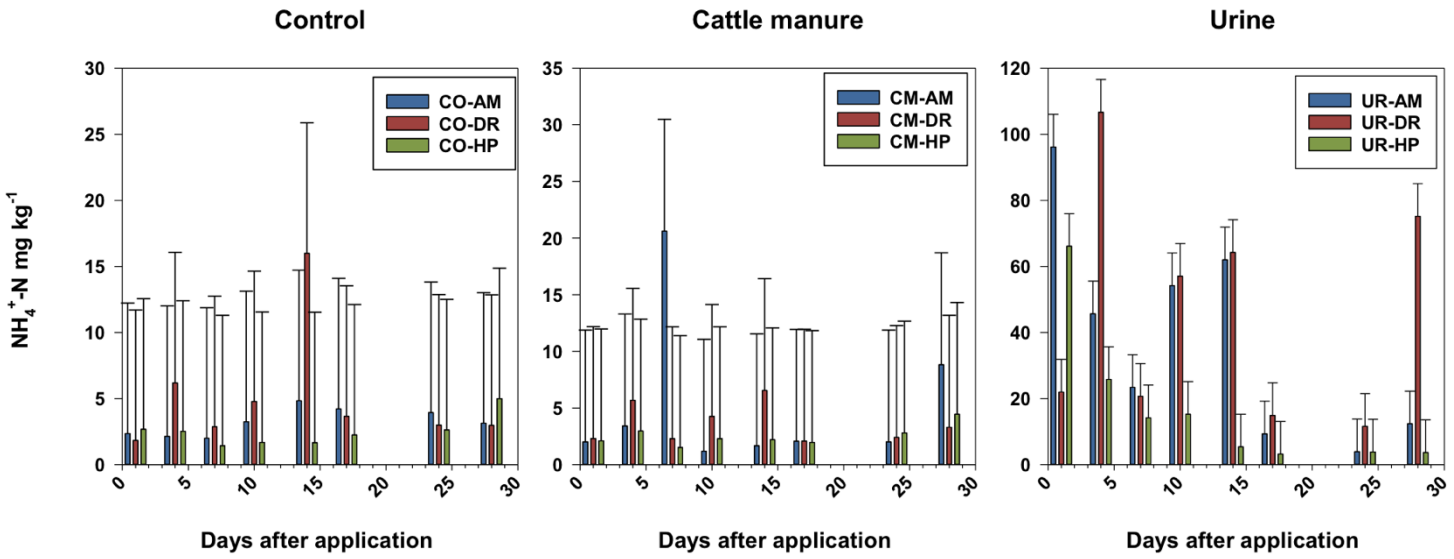
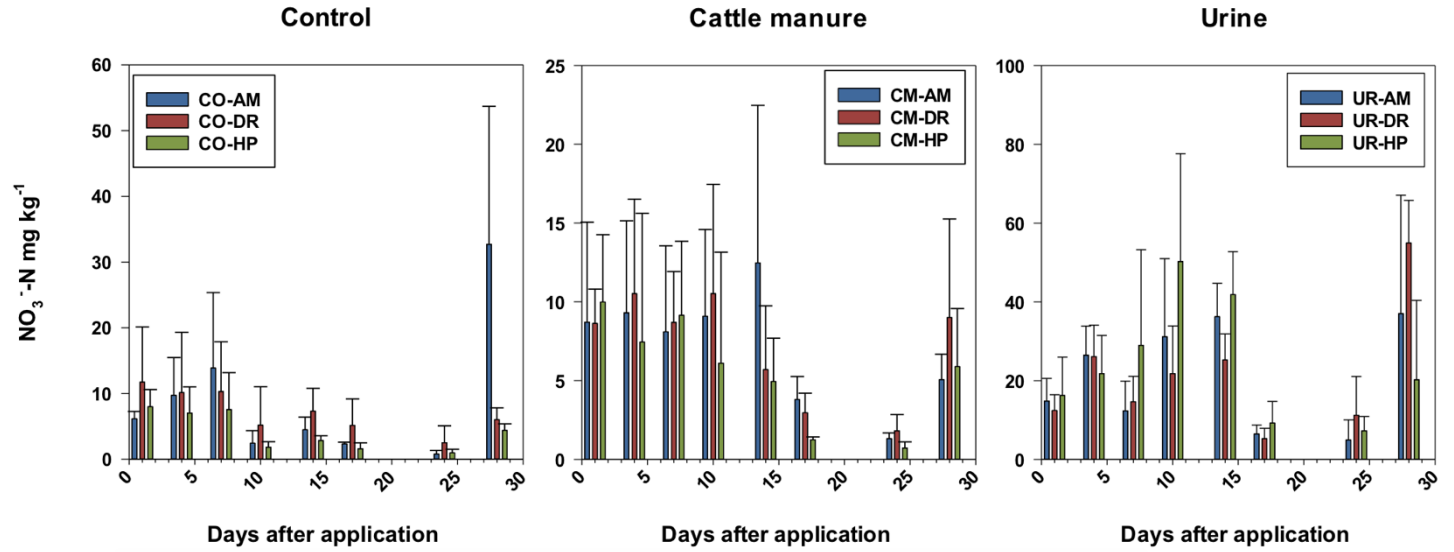


Figure 4-5. Soil ammonium (NH_4^+) and nitrate (NO_3^-) dynamics during 2016 affected by urine (UR), and manure (MN) patches and the control (CO) treatment under the preciptation conditions: high precipitation (HP), drought (DR), and ambient (AM). Error bars represent standard deviation.



(a)



(b)

Figure 4-6. Soil NH_4^+ dynamics during 2017 affected by urine (UR), and manure (MN) patches and the control (CO) treatment under the preciptation conditions: high precipitation (HP), drought (DR), and ambient (AM). Error bars represent standard deviation.

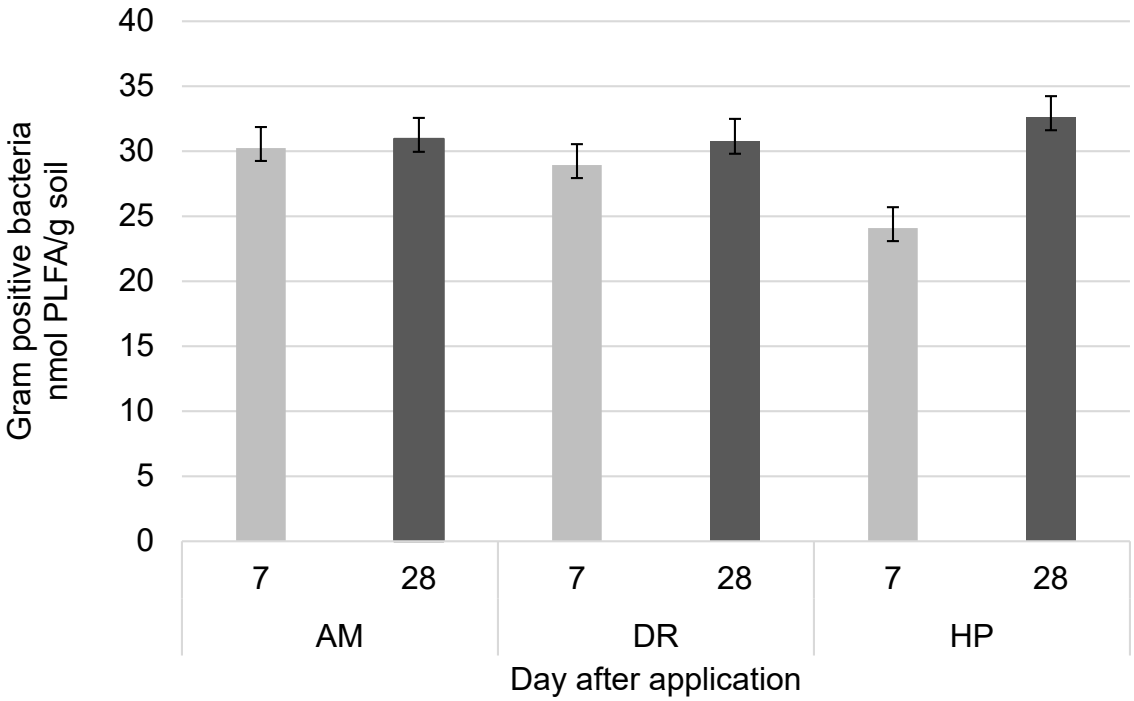


Figure 4-7. Mean (n=4) change in the group abundance of gram-positive bacteria at 7 and 28 d after application of urine and manure under the high precipitation (HP), drought (DR), and ambient (AM) conditions. Overlapping standard error bars are not significantly different (LSD protected, $p<0.05$).

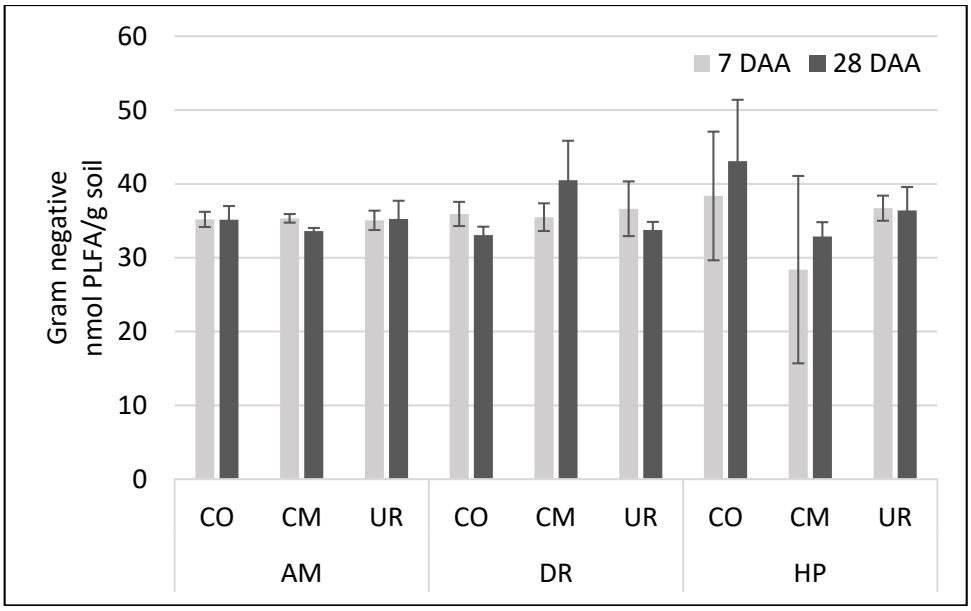


Figure 4-8. Mean change in the group abundance of gram-negative bacteria at 7 and 28 d under the cattle urine (UR), and manure (MA) patches and the control (CO) treatment under the precipitation conditions of high precipitation (HP), drought (DR), and ambient (AM) to compare the difference in the microbial community composition. Overlapping standard error bars are not significantly different (LSD protected, $p<0.05$).

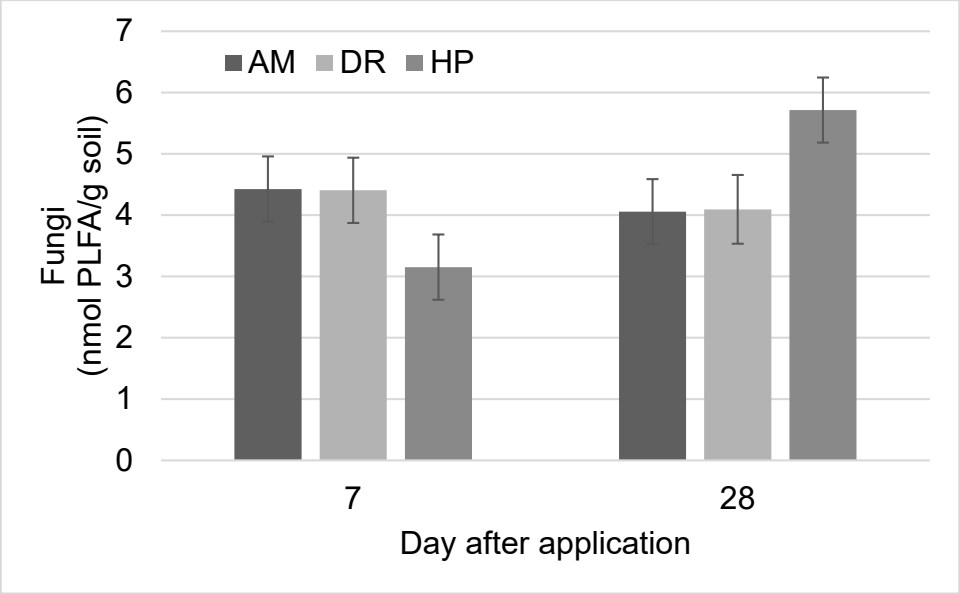


Figure 4-9. Mean (n=4) changes in the fungi group abundance at 7 and 28 d after urine and manure application under the high precipitation (HP), drought (DR), and ambient (AM) conditions. Overlapping standard error bars are not significantly different (LSD protected, $p<0.05$).

Table 4-1. Soil chemical characteristics of the experimental site located at Pottawatomie County, Kansas.

Organic Matter[1]	Total C	Total N	NH_4^+-N[2]	NO_3^--N[2]	Ca	Cu[3]	Fe[3]	K[3]	Mg	Mn[3]	Na	P-Melich[4]	Zn[5]
----------g/kg----------			----------mg/kg----------										
77.6	41.6	3.3	0.41	0.17	3,178	1	102	374	460	21	59	4	4

[1] Organic matter analysis by loss on ignition method
[2] KCl extrancion for inorganic nitrogen (N), NH_4^+ and NO_3^-.
[3] DTPA extraction for Cu, Fe, Mn, and Zn both analyzed by a Inductively Coupled Plasma Spectrometer.
[4] Samples were analyzed by Mehlich 3 Phosphorus for P (Lachat Quickchem 8000, Loveland, CO, USA).
[5] Ammonium Acetate extraction for K.

Table 4-2. p-values for pH, soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations, and nitrous oxide (N_2O) and methane (CH_4) for the effects of weather conditions (WC), cattle urine and manure (CR), time (T), and the interactions between during the 28 d period during summer 2016, and 2017.

Factor	pH	NH_4-N	NO_3-N	N_2O	CH_4	Soil Water
----------2016----------						
Precipitation (P)	0.524	0.004	0.004	0.387	0.560	<.0001
Cattle urine and manure (CR)	0.190	<.0001	<.0001	0.044	0.238	0.0599
TIME (T)	<.0001	<.0001	0.230	<.0001	0.014	<.0001
P X CR	0.422	0.001	<.0001	0.043	0.020	0.5755
P X T	0.382	0.985	0.794	0.961	0.969	0.0006
CR X T	0.168	0.056	0.970	0.051	0.051	0.7162
P X CR X T	0.561	0.989	0.993	0.002	0.002	0.7402
----------2017----------						
Precipitation (P)	0.549	0.061	0.868	0.992	0.491	
Cattle urine and excreta (CR)	0.888	<.0001	<.0001	0.196	0.759	
TIME	<.0001	<.0001	<.0001	0.999	0.0207	
P X CR	0.965	0.0001	0.166	0.594	0.021	
P X T	0.408	0.002	0.011	1.000	0.487	
CR X T	0.995	<.0001	<.0001	0.055	0.284	
P X CR X T	0.910	0.0001	0.0001	0.930	0.030	

Table 4-3. p values for precipitation regimes, cattle amendments and time and their interactions on phospholipids fatty analysis results for gram-positive bacteria (gram-pos), gram-negative bacteria (gram-neg), actinomycetes (actino), fungi, and total microbial communities during the 28 d incubation in 2017.

Factors	Total	Gram-pos	Gram-neg	Fungi	AMF	Actino
Precipitation (P)	0.8848	0.4786	0.9124	0.9209	0.4200	0.456
Cattle urine and manure (MN)	0.1686	0.2048	0.8100	0.6069	0.3374	0.2229
TIME	0.2668	0.0022	0.0202	0.1649	0.2643	0.0027
P X MN	0.9706	0.9751	0.0703	0.9427	0.5021	0.7367
P X T	0.2698	0.0135	0.0043	0.0155	0.5886	0.1030
MN X T	0.2685	0.2416	0.5933	0.9221	0.4113	0.1510
P X MN X T	0.2094	0.5452	0.0379	0.6151	0.4855	0.2197

Chapter 5 - Characterization of soil nitrogen, nitrous oxide fluxes and soil microbial dynamics as influenced by biological nitrification inhibition from *Brachiaria* grasses

Abstract

The use of *Brachiaria* cultivars as grazed pastures represents an opportunity to increase the sustainability of livestock production systems. The biological nitrification inhibition (BNI) capacity of *Brachiaria* grasses and their dense root biomass and rapid root turnover contribute towards decelerating the soil nitrogen (N) cycle, and increasing soil carbon (C) accumulation. The roots of several *Brachiaria* cultivars exudate nitrification inhibitors which increase N use efficiency and mitigate N losses in grazed pastures. To achieve a better understanding of the direct effect of BNI in pastures and soil, we studied the soil N dynamics and microbial communities in high N hotspots created by cattle urine patches. The study was conducted on a long-term (12 years) trial located at the International Center of Tropical Agriculture (CIAT) in Cali, Colombia. Through a 48-d *in situ* incubation, we tested two *Brachiaria* cultivars (*Brachiaria* humidicola 16888 and *Brachiaria* hybrid cv mulato 1) and a bare soil (a proxy for degraded pasture) as a control. Soil nitrous oxide (N_2O) and methane (CH_4) fluxes were determined during the incubation period, soil inorganic N was monitored at 5 cm intervals to a 30 cm depth. Soil samples (5 cm depth) were collected and analyzed for soil nitrification capacity, pH, ammonia-oxidizing bacteria, ammonia-oxidizing archaea, and microbial composition by phospholipid fatty acid analysis. Plant biomass and N uptake were also determined on a 15-d basis. High ammonium (NH_4^+) suggest suppression of nitrifier activity by *Brachiaria* grasses following urine application resulting in lower nitrate (NO_3^-) and N_2O

production compared to the bare soil control. *Brachiaria* grasses, especially *Brachiaria humidicola 16888*, increased soil microbial diversity, and decreased N_2O emissions and inorganic N leaching. We conclude that the adoption of *Brachiaria grasses* conserves N and increases N uptake, especially, *Brachiaria* humidicola which had higher capacity to reduce N losses.

Introduction

The cattle (*Bos indicus, Bos taurus*) grazing industry serves as a source of economic progress and food security for tropical regions (Miles et al., 2004). Nonetheless, in Latin America cattle production is a major driver of deforestation, soil erosion, and other detrimental environmental impacts (Gloor et al., 2012; Braz et al., 2013; FAO, 2015). Loss of nitrogen (N) from grazing systems, especially from animal urine, occurs as nitrous oxide (N_2O) emissions, nitrate (NO_3^-) leaching, and soil erosion (Saarijärvi and Virkajärvi, 2009; Herrero et al., 2013; Selbie et al., 2015; Pilon et al., 2017, Steiner et al., 2018). In tropical systems, the use of *Brachiaria* cultivars provide an opportunity to increase the sustainability of livestock systems by providing quality forage, conserving N, and restoring degraded soils (Byrnes et al., 2017).

In grasslands, the presence of grass is critical for regulating soil N dynamics, animal productivity, and ecosystem services. Grasses influence the soil N cycle and consequently influence the soil-nutrient-plant interactions, which are key to achieve higher yields in livestock production systems. About 75% of the grazed pastures in South America are reported to be in some form of degradation because of poor management of soils, which reduces plant growth (Heerink et al., 2001). An additional driver of soil degradation is high livestock densities which leads to a decrease in soil cover and, consequently, high soil erosion rates (Heenrink et al., 2001; Rippstein et al., 2001). Sotelo et al. (2016) described Colombia pastures as extensive and in need of forages that could be used as mono-crops or within silvopastoral systems to achieve sustainability. Several research efforts have focused on *Brachiaria* cultivars which have the capacity to exude nitrification inhibitors (BNI) in the presence of ammonium (NH_4^+), and thus increase plant N use efficiency and mitigate greenhouse gas (GHG) emissions in tropical

pastures (Miles et al., 2004; Chanchila et al., 2008, Subbarao et al., 2009; Braz et al., 2013; Mateus et al., 2013; Byrnes et al., 2017; Nuñez et al., 2018).

The high adaptability of *Brachiaria* grasses, under tropical conditions, make them an option for restoring degraded grasslands and increasing animal productivity. For example, Mateus (2013) conducted research on acid soils where *Brachiaria* grasses were highly adaptive, increased animal yield, and provided tolerance to insect pests (plant produces chemicals that repels insect pests). Chanchila et al. (2008) reported 50% soil cover after 138 d of transplant on a highly compacted, low fertility, and high acidity soils in the southern regions of Colombia. For monoculture systems with a low BNI capacity *Brachiaria* grass, animal gains averaged 371 kg ha^{-1} yr^{-1}; while with a high BNI capacity *Brachiaria* grass gains varied from 540 to 840 kg ha^{-1} yr^{-1}, especially in association with legumes (Peters et al., 2011; Gutiérrez et al., 2016).

The BNI capacity inhibit nitrification rates, controls soil microbial processes, and reduces N losses in the system (Subbarao et al., 2009; Rao et al., 2015; Byrnes et al., 2017). Soil microbial functions regulate soil diversity and richness, recycle and stabilize the terrestrial C stocks, and control N cycling (Gray et al., 2011; Schmidt et al., 2011). Ammonia-oxidizing bacteria (AOB), is a biomarker of *Nitrosomonas*, and ammonia-oxidizing Archaea (AOA) dominate soil ammonia oxidizers, which are key microbes in the soil N cycle (Martens-Habbena et al., 2009; Verhamme et al., 2011; Taylor et al., 2012). More specifically, AOA is associated with low NH_4^+ concentration, while AOB has higher activity under high NH_4^+ (Verhamme et al., 2011). For instance, Byrnes et al. (2017) found no correlation between AOA abundance and NO_3^- production. The BNI activity provides an environment of higher N utilization by reducing soil microbial activity and keeping inorganic N at shallow depths allowing for higher N uptake by the crop (Subbarao et al., 2009; Karwat et al., 2018).

Several soil-plant interactions have been found between *Brachiara* cultivars and soil biochemical processes (Subbarao et al., 2009; Byrnes et al., 2017; Nuñez et al., 2018). This research was focused on the soil-plant-microbial relations and the environmental impact on tropical pasture systems by investigating the potential of *Brachiaria* grasses to close the N cycle, and suppress N_2O emissions. We also characterized the soil microbial communities under two *Brachiaria* cultivars. *Brachiaria humidicola* 16888, and *Brachiaria mulato* hybrid 1 forages with low and high BNI capacity, respectively, were used to document *Brachiaria* benefits in livestock production systems by supporting high pasture productivity, soil fertility, forage nutritional quality, and restoration of the degraded pastures. We hypothesized soil covers with *Brachiaria* grasses, will have lower N_2O emissions, and greater microbial activity; while we expected higher plant N uptake from *Brachiaria humidicola* (with high BNI capacity) when compare with *Brachiaria mulato* hybrid 1.

Materials and methods

Experimental site

The study was conducted on a 12-year old long-term field experiment at the International Center for Tropical Agriculture (CIAT) at Palmira, Valle of Cauca (3°30′7″N 76°21′22″W and approximately 1,000 m above sea level) in Colombia. The site had a mean annual temperature of 24^{o}C and a mean annual rainfall of 894 mm. During the 48-d period (February 4 to April 4), mean temperatures fluctuated between 26.8 to 39.0^{o}C, and total precipitation was 326 mm. The long-term field experiment was established in 2006, to evaluate the BNI potential of five tropical forage grass cultivars. For this study, we used two forages: *Brachiaria humidicola* 16888 (BH), *Brachiaria mulato* hybrid 1 (BM), and bare soil (BS) control. Soil at the experimental site was classified as a Vertisol (Typic Pellustert) with a silty clay loam texture with a clay content of 40

to 60% in the soil 0-25 cm layer. Soil bulk density was 1.19, 1.45 and 1.62 g cm^{-3} for BH, BM, and BS, respectively.

The experiment was a completely randomized block design with three replicates per main treatment (*Brachiaria* cultivar or bare soil). Each block had individual plots for each main treatment BH, BM and the bare soil as a control. Each plot of 10 m × 10 m, had two sub-plots of 1 m x 1 m with the secondary treatment control and urine (15.7 g N L^{-1}). Within each sub-plot two, polyvinyl chloride tubes as static chambers with a 26-cm internal diameter and 20 cm height were established, and five areas were also delineated for soil sampling 2 hours, 2, 8, 28 and 48 d after urine application. Bovine urine was collected from cows, sealed, and stored at 5 ^{o}C until application to the soil. Collected urine was mixed and applied at a rate of 1 L within the demarcated area (0.123 kg N m^2). Prior to application to the experimental area, a sample of the collected urine was analyzed for N content.

Methane and nitrous oxide emissions

Gas samples were measured 2 h, and 1, 2, 3, 7, 10, 14, 24, 28, 35, 44 and 49 d after urine application. All gas samples were collected from static chambers (area 16 cm^2, volume 1.78 L) placed in each experimental unit. For each gas sampling, pre-evacuated vials (Labco, 5.9 ml Soda Glass Vial Flat bottom) were used to store the gas samples. On each gas sampling day, chamber covers were connected to chamber bases and sealed with a rubber strap. The first sample was taken immediately after connecting chambers to chamber bases (0 min) and then at 15, 30 and 45 min after sealing. The concentrations of CO_2, CH_4, and N_2O in each collected sample were quantified using a Shimadzu GC-2014. Results were analyzed with the Hutchinson-Mosier method and linear equations (Pedersen et al., 2010).

Soil properties

Soil samples was collected on 7-d intervals; specifically, at 2 h, 2, 8, 28, and 49 d after urine application. Soil samples were taken at depth intervals of 0-5, 5-10, 10-15, 15-20 and 20-30 cm. Each sample was characterized for inorganic N, pH, nitrifiers activity, PLFA and qPCR for AOA and AOB. Soil inorganic N (NH_4^+, and NO_3^-) were extracted from soil by adding 50 mL of 1 M KCl to 5 g of the soil samples and shaken on a rotary shaker for 30 min. The extract was filtered and stored in the freezer until the spectrophotometric determination of inorganic N.

For each sample, soil water content was measured gravimetrically after oven drying a 10 g sample at 105ºC for 48 h. Soil pH analysis was measured 2 h, 7, 28 and 48 d after urine application. The pH was determined from air-dried soil using 1:10 mix of soil:deionized water.

Nitrifier activity

Nitrifier activity was measured 2 h and 7, 28, and 48 d after urine application. Nitrification rates, in these soils, were determined through a soil incubation assay as described by Subbarao et al. (2006). Soil from 0-5 cm depth was collected and air-dried at room temperature for 2 d, and then passed through a 2-mm mesh sieve. Homogenized soil (5 g) was supplemented with 1.5 mL of ammonium sulfate (27 mM), to maintain the soil at 60 % field capacity and three replicates were used per incubation time. The NO_3^- concentration was determined using an autoanalyzer as described by Subbarao et al. (2006).

The nitrification rate (NR) was expressed as a rate of NO_3^- production per kilogram of soil per day according to:

$$NR = \frac{T_4\ N - NO_3 - N - NO_3\ basal) - (T12N - NO_3 - N - NO_3\ basal)}{Incubation\ days}$$

Phospholipid fatty acid

For phospholipid fatty acid (PLFA) analysis the 0-5 cm soil layer of each treatment was sampled after 2 h and 7 and 35 d after urine application. Soil samples were freeze-dried through lyophilization for 72 h and analyzed in the Soil Microbial Ecology Laboratory at Kansas State University. Total lipids were extracted from samples using a modification of the Bligh and Dyer (1959) extraction (White and Rice, 2009). The PLFA was separated from the total lipid extract using silicic acid chromatography, the fatty acids were cleaved from the glycerol backbone using KOH saponification, and the harvested fatty acids were methylated to form fatty acid methyl esters (FAME). The resulting FAME was analyzed using a Thermo Scientific Trace GC-ISQ mass spectrometer equipped with a DB5-MS column. The FAME peaks were identified by comparison with the bacterial acid methyl esters mix. Fatty acids were grouped into gram-positive bacteria, gram-negative bacteria, actinomycetes, arbuscular mycorrhizal fungi and fungi (White and Rice, 2009).

qPCR- AOA and AOB

The ammonia-oxidizing archaea (AOA) and bacteria (AOB) analysis was performed at 2 h, and 7, 35, and 48 d after urine application. qPCR was used for the amoA gene marker (Subbarao et al., 2009) in the 0-5 cm depth. The DNA was isolated from 500 mg fresh soil using the Fast DNATM SPIN Kit for Soil (MP Biomedicals, Solon, Ohio, USA) according to the manufacturer's instructions with modification during the washing steps. Before washing with SEWS-M buffer, two washing steps using 500 uL of guanidine thiocyanate (5 M) were applied to reduce the co-extraction of PCRs inhibitors such as humic acids. The mix for qPCR contained 10 ng of DNA, 10 µL of brilliant sybr mix (promega), primers (0.5 µM) amoA-1F/amoA-2R for AOB (Rotthauwe and Witzel, 1997) and amoA19F/amoA643R for AOA (Leininger et al., 2006)

according to the methods of Rasche et al. (2011). The 7-point standard curve ranged from 10^3 to 10^8 molecules using a dilution series of a known amount of plasmids DNA (pGEM-T Easy Vector System I, Invitrogen) containing the specific PCR product amplified from soil AOA and AOB amoA gene, using the primer sets. Gene copy numbers and reaction efficiencies were obtained using the Q-Rex software (QIAGEN).

Vegetative biomass

During the study period, the grass was cut on a 15 d interval to a height of 10 cm simulating animal grazing. The fresh weight of the sample was recorded, and moisture measured by weight loss of a subsample at 60ºC. Samples were ground and analyzed for total N by the total C and N method by LECO TruSpec CN combustion analyzer (LECO Corporation, MI, USA). Crude protein in biomass was calculated by multiplying the total N content by a correction factor of 6.25, which is the established factor for forages.

Statistical Analysis

Statistical Analysis System (SAS) 9.3 was used to analyze results using a Proc Mixed analysis of variance (ANOVA) method (α=0.05) of repeated measurement over time. Analysis results were conducted for the effect of the soil cover (primary factor) from *Brachiaria humidicola* 16888, *Brachiaria mulato* hybrid 1, and bare soil, the effect of N (secondary factor) application from urine application, and control (no N), and the effect of days of incubation during the 48 d incubation, and the interaction of the 3 factors. Under the GHG emissions, the analysis was performed for N_2O and CH_4 gases (12 data points). Soil samples were analyzed for inorganic N (5 data points), pH (4 data points), and nitrifier activity (4 data points). The PLFA analysis (3 data points) was analyzed for total microbial biomass (total), gram-positive bacteria (Gram +), gram-negative bacteria (Gram -), actinomycete (act), arbuscular mycorrhizal fungi

(AMF), fungi, and the fungal:bacteria (Ratio). From the qPCR procedure AOA, and AOB was analyzed (4 data points). The *Brachiaria* grasses aboveground biomass (kg ha^{-1}), N in the biomass (kg N ha^{-1}), and crude protein content have 4 data points.

Results

Nitrous oxide and methane fluxes

The interaction between soil cover, urine application, and time significantly affected N_2O ($p=0.0001$), and CH_4 ($p=0.0020$) soil fluxes over the 48-d period. For all soil covers with urine (UR) application, N_2O emissions occurred 2 h, and 1 and 2 d after urine application (Fig. 5-2). The BS-UR had emissions of 39.8, 6.4, and 3.2 g N_2O ha^{-1} d^{-1} 2 h and 1 and 2 d after urine application, respectively. Under *Brachiaria* grasses, emissions were lower than bare soil with values of 1.7, 3.1 and 2.1 g N_2O ha^{-1} d^{-1} under BH-UR, and 11.9, 5.6, and 3.5 g N_2O ha^{-1} d^{-1} under MH-UR 2 h and 1 and 2 d after urine application, respectively. By the end of the 48 d, BS-UR, BH-UR, and MH-UR emitted a total of 49.6, 7.1, and 21.8 g N_2O ha^{-1} d^{-1}, respectively. Increase of N_2O emissions, for all treatments, at the end of the 48 d were related to a precipitation event.

Emissions of CH_4 were significantly affected by the interaction of soil cover and UR application over time ($p=0.0020$). Soil CH_4 fluxes 1 d after incubation were -0.8, 0.4, and 1.1 g CH_4 ha^{-1} d^{-1} for BS-UR, BH-UR, and MH-UR, respectively (Fig. C-1).

Soil pH and inorganic N dynamics

Soil pH was significantly different between soil cover ($p=0.0003$) and time ($p=0.0030$). Soil pH averaged 6.1, 6.4, and 5.8 for BS, BH, and MH, respectively. For all soil covers, pH increased during the first week with no significant differences by the end of the 48 d period

(Appx. C-1, and C-5). Higher soil pH from BH coincided with the low concentrations of NO_3^- under this soil cover compared to MH, and BS (Appx. C-3b).

The interaction between soil cover, urine application, and sampling date significantly influenced soil NH_4^+ and NO_3^- dynamics ($p=0.0001$). Soil NH_4^+ increased immediately after urine application and then decreased over time (Appx. C-2). The BS treatment had the greatest increase in NH_4^+ (Fig. 5-4a). Soil NO_3^- increased 8 d after application in the BS-UR treatment (Fig. 5-4b). There were no statistical differences between inorganic N for both *Brachiaria* grasses. For bare soil, NO_3^--N ranged from 28.2 to 67.6 mg NO_3^--N kg^{-1} soil. Movement of NO_3^--N was highest under the BS-UR treatment with 27.0 mg NO_3^--N kg^{-1} soil observed at 20 to 25 cm depths 10 d after urine application ($p<0.05$). While *Brachiaria* grasses had the highest amount of NH_4^+-N observed at 20-25 cm was 10.6 mg NO_3^--N kg^{-1} ($p<0.05$). The amounts of NO_3^--N from *Brachiaria* grasses varied from between 5.0 to 15.8 NO_3^--N mg kg^- for MH and from 2.5 to 3.4 mg NO_3^--N kg^{-1} for BH during 10 and 28 d after urine application (Fig. 5.4 c,d,e,f).

Nitrification rates

Nitrifier activity was significantly altered by the combination of soil cover and urine application over time (Appx. C-1). Nitrification rates between both *Brachiaria* grasses ranged between 0.72 mg NO_3^--N kg^{-1} soil d^{-1} with an increase of 4.5 mg NO_3^--N kg^{-1} soil d^{-1} over time. A significant reduction of 3.5 mg NO_3^--N kg^{-1} soil d^{-1} was observed at the end of the 48-d period (Fig. 5-4). In the case of BH, nitrification rates after urine application was 2.64 mg NO_3^--N kg^{-1} soil d^{-1}, followed by a reduction of 0.9 mg NO_3^--N kg^{-1} soil d^{-1} and an increase to 3.8 mg NO_3^--N kg^{-1} soil d^{-1} in nitrification rates 28 d after urine application. Nitrification rates under urine patches in the BS treatment were almost double those observed in the *Brachiaria* grasses; rates

decreased over time from 11.3 to 6.1 mg NO_3^--N kg soil d^{-1} after the urine application to 48 d later.

Soil microbial communities

Total microbial biomass increased over time (p=0.0332) with an increase of 40% in the first week with no significant differences between soil cover, urine addition or the interaction (Fig. 5-7, Appx C-3). For all treatments, by day 35, the average microbial biomass was 36.1 nmol PLFA g $soil^{-1}$ (Fig. 5-7). The abundance of gram-positive (p=0.0001) and gram-negative (p=0.0036) bacteria significantly changed with soil cover over time. Overall, gram-positive and gram-negative bacteria under *Brachiaria* grasses were 82% and 77%, respectively, higher than those observed in the BS treatment (Appx. C-6, and C-7). Gram-positive bacteria ranged from 9.1 to 27.9, and 6.9 to 23.7 nmol PLFA g $soil^{-1}$ for BH and MH, respectively. Gram-positive bacteria increase 71%, and 67%, BH and MH, respectively during 7 d after incubation; but decrease by the end of study. Values for gram-negative bacteria ranged from 2.9 to 12.5, and 1.8 to 11.0 nmol PLFA g $soil^{-1}$ for BH and MH, respectively. Similarly to gram-positive bacteria, gram-negative bacteria increased 7 d after application by 77%, and 84%, respectively. However, MH maintained a gram-negative bacteria community at the end of the study period.

Actinomycetes significantly changed over time (p=0.001) from 2.6 to 3.6 nmol PLFA g $soil^{-1}$ for BH, and 2.9 to 4.0 nmol PLFA g $soil^{-1}$ for MH. Actinomycetes under BS increased over time from 0.41 to 4.13, (p=<.0001) (Appx. C-8). Fungi and AMF were significantly affected by the interaction between soil cover and time (p=0001) (Appx. 9). Fungi increased with time, especially within the first week under *Brachiaria* grasses with values of 1.67 to 2.3, and 0.9 to 1.8 nmol PLFA g $soil^{-1}$ for BH, and MH (Appx. C-10). The AMF biomarker increased in BS over time with an increase from 0.17 to 1.5 nmol PLFA g $soil^{-1}$ from 2 h to 48 d.

The AMF community was 32% higher under *Brachiaria* grasses, with an average of 1.5 nmol PLFA g soil^{-1}. The fungal:bacterial ratio changed with time (p=0.0216) as the ratio was reduced by 41% after 7 days compared to 2 h after urine application (Fig. 5-6).

AOA and AOB

Interactions between soil cover and urine (p=0.004), and urine application over time (p=0.0289) caused significant changes in the AOA communities (Appx. C-3). High AOA suppression of the BH pastures was constant over the period. Urine application increased NH_4^+ causing a reduction in AOA for a week from 4.7E+07 to 5.4E+06 (Fig. 5-7). Overall, AOA was higher, averaging 1.25E+08, when NH_4^+ was low. The interaction between soil cover, urine application and time was significant for AOB (p=0.0001). A reduction in AOB occurred during the 48-d period, while MH increase AOB at 28 d (Fig. 5-8). The increase in AOB under MH was coupled with an increase in nitrification rates, and N_2O and CH_4 emissions by the end of the 48 d period.

Brachiaria grasses biomass production

Brachiaria biomass production was significantly influenced by soil cover (p=0.0193), and time (p=0.0001), and N concentration in biomass by N cover (p=0.0109) and time (p=0.0001) (Appx. C-1). Biomass production over the 48-d period was 954 and 735 kg ha^{-1} for BH and MH, respectively (Fig. 5-9). Biomass production increased under urine patches with 1,295 and 1,001 kg ha^{-1} for BH, and MH, respectively. The *Brachiaria* grasses with urine treatment had a 30% increase in biomass N. Biomass crude protein was significantly higher 28 d after the urine application with 18.6% compared to 12.1% from the rest of the 48 d period, while the grasses in the control treatment had 7.4% of crude protein (Fig. 5-10). Crude protein in

biomass was significantly different between *Brachiaria* grasses, with 11.5% in MH compared to 9.7 in BH.

Discussion

As hypothesized, *Brachiaria* grasses showed the capacity to suppress N_2O fluxes during the first 35-d of incubation. After urine application, N_2O emissions were expected from anoxic conditions, and the increase of inorganic N. For BH, and MH, N_2O suppression overlapped with low nitrification rates (Fig. 5-2, and 5-6a). The abrupt increase in soil water and NH_4^+ from the urine in BH-UR, and MH-UR can potentially explain the co-occurrence of N_2O and CH_4 from denitrification and methanogenesis, respectively (Appx. C-4). A possible reason why BS-UR did not affect CH_4 emissions, compared to BH-UR, and MH-UR, could be the immediate loss of N as N_2O (Fig. 5-1, Appx. C-4). The CH_4 emissions from BH-UR, and MH-UR after urine additions were expected since methanogenesis is an indicator of anoxic conditions created by the increase in soil water and soluble C from urine (Yamulki et al., 1999). However, under low N, more specifically NH_4^+, CH_4 emissions were reduced for the remainder of the 48-d. Brewer et al. (2018) previously explained the relation between methanogenesis and NH_4^+ with a positive correlation between methanogenesis and NH_4^+, and a negative correlation with NO_3^-. During the remainder of the incubation, CH_4 uptake by aerobic methanotrophs assimilated methane as a source of C and energy (Jiang et al., 2010).

Addition to the BNI, the lower soil pH from MH, compared to BH, could also affect nitrification rates (Fig. 5-4), N_2O emissions (Fig. 5-2), and the decrease in microbial communities compared with BH (see PLFA and qPCR section). Nitrogen additions from urine increased emissions of N_2O but BNI activity of *Brachiaria* inhibited nitrification rates thus reducing N_2O emissions. As described by Subbarao et al. (2009) the mechanism for BNI release

occurs in the presence of NH_4^+ by the roots which delivered the BNI to nitrifiers sites. During this study, the BNI activity was reduced after 28-d as nitrification rates and N_2O emissions increased for the urine treatments under BH and MH. The increase in NO_3^- paralleled an increase in nitrifier activity during a precipitation event at the end of the measurement period (Fig. 5-1). Nitrification rates were not significantly different between the two cultivars. Long-term establishment of *Brachiaria* grasses could reach maximum nitrification inhibition by building up BNI capacity (O'Sullivan et al., 2016; Nuñez et al., 2018; Karwat et al., 2018).

This study provides an insight to the soil microbial dynamics under the BH and MH traits for their capacity to decrease in N losses (Mateus, 2013; Byrnes et al., 2017; Karwat et al., 2018). Gram-positive bacteria, actinomycetes, fungi, and AMF were enhanced with *Brachiaria* traits. Microbial communities are key components for soil and plant functions by protecting them from abiotic and biotic stressors, promoting nutrient cycling and maintaining soil N and C dynamics (Subbarao et al., 2009; Gray et al., 2011; Karwat et al., 2018, Nuñez et al., 2018). In addition, AMF fungi are key to soil C storage and soil aggregation (Brundrett, 2009; Wilson et al., 2009).

The specific biomarkers AOA and AOB confirm previous studies where BH had higher BNI capacity. Our results support Nuñez et al. (2018) observation that *Brachiaria* grasses with high BNI potential had low nitrification rates and a reduction in AOA abundance. During this study, the AOA suppression capacity of *Brachiaria* grasses is the result of the BNI exudations by the roots which are triggered by the presence of NH_4^+ from the urine in the rhizosphere. As expected, AOA abundance was reduced for both *Brachiaria* grasses; the abundance increased in relation with the nitrification rates (Subbarao et al., 2009; Gubry-Rangin et al., 2017; Byrnes et al., 2017; Hink et al., 2017). Reduction in AOA under high NH_4^+ was expected since previous

studies have related AOA abundance with a decrease in pH, and an increase in nitrification activity in soils (Prosser and Nicol, 2008; Verhamme et al., 2011). Overall, the AOB coincided with nitrification rates and N_2O emissions (Subbarao et al., 2007; Proser and Nicol, 2008; Di et al., 2009; Di et al., 2010b). In addition, Teutscherova et al. (2018) reported AOB community symbiosis with AMF are a key component in soil agroecosystems. Changes in AOA and AOB community confirm that BNI activity was the mechanism regulating NH_4^+ dynamic in the rhizosphere of BH (Subbarao et al., 2007).

Contrary to what was expected, urine patches did not affect plant biomass production between both *Brachiaria* cultivars, but it did affect protein content. Bowatte et al. (2018) and Abalos et al. (2018) reported plants with high N uptake results on low N_2O emissions as a result of a reduction in the mineral N pool and in substrate for denitrifiers. In this study the increase in N uptake from *Brachiaria* grasses was the result of inorganic N retention in the surface soil because of the reduced nitrification rates, NO_3^- leaching, and N_2O losses (Subbarao et al., 2009; Di et al., 2010a). The MH had higher crude protein than BH (Fig. 5-10). Results coincide with Nuñez et al. (2018) who indicated one of the unfavorable characteristics of BH was its low nutritional value. Overall, N from cattle urine deposition encouraged plant growth and protein content for both *Brachiaria* grasses.

Significantly higher biomass production from BH might explain the lower N availability from BH compared to MH (Fig. 5-3 and Fig. 5-9a). Over time, both *Brachiaria* grasses significantly increased N uptake when nitrification rates were reduced (Fig. 5-4 and Fig. 5-9a). Furthermore, BH was more efficient reducing nitrification rates thus resulting in lower N_2O emissions and increase in N uptake (Fig. 5-4, and 5-9b). The establishment of *Brachiaria* on grazed pastures for improve soil health and agroecosystem resilience has been previously

mention (Herrero et al., 2013; Schils et al., 2013; Rao et al., 2015), and this study provides more information on biomass yields, and protein content for BH and MH.

Conclusion

Results support our hypothesis that the use of *Brachiaria* grasses reduce N_2O emissions from livestock and closed the N cycle. Under the studied conditions, 'hotspots' from cattle urine caused changes in N dynamics and a reduction of soil microbial communities for 28 d, and affected N_2O and CH_4 dynamics for over 48 d. Between both *Brachiaria* grasses, *Brachiaria* humidicola 16888 produced more biomass, lowered nitrifications rates, and created soil conditions for higher population of arbuscular mycorrhizal fungi and actinomycetes. Long-term use of *Brachiaria* grasses significantly increase soil microbial communities. Our conclusions recognize BNI from *Brachiaria* grasses, especially high activity from *Brachiaria humidicola* 16888, capacity to increase soil microbial communities and close the N cycle.

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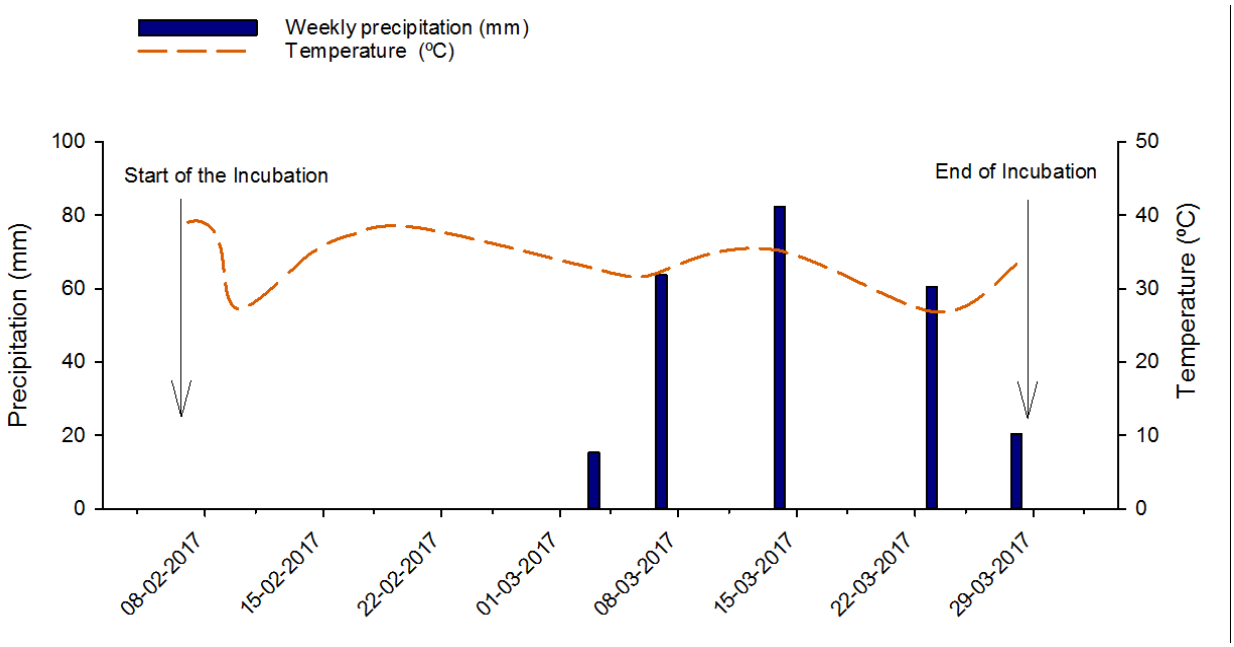


Figure 5-1. Precipitation and temperature during the 49 d of the experiment.

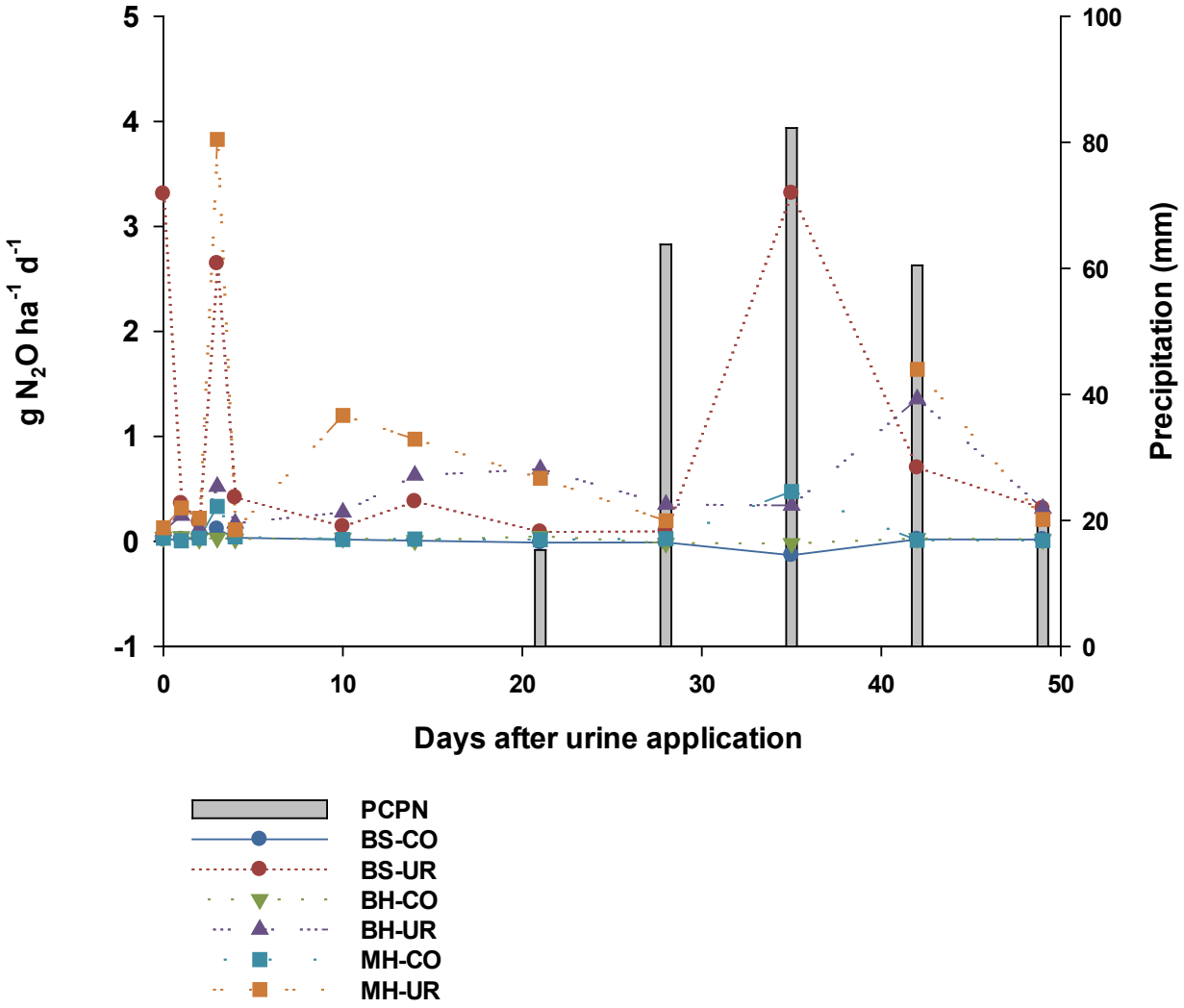


Figure 5-2. Nitrous oxide (N_2O) emissions during the 48 d of the experiment. Six treatments were studied: bare soil (BS), *Brachiara humidicola* 16888 (BH), and *Brachiaria mulato* hybrid 1 (MH) each soil cover had a secondary treatment which was the application of bovine urine (UR), and the control (CO (no nitrogen)).

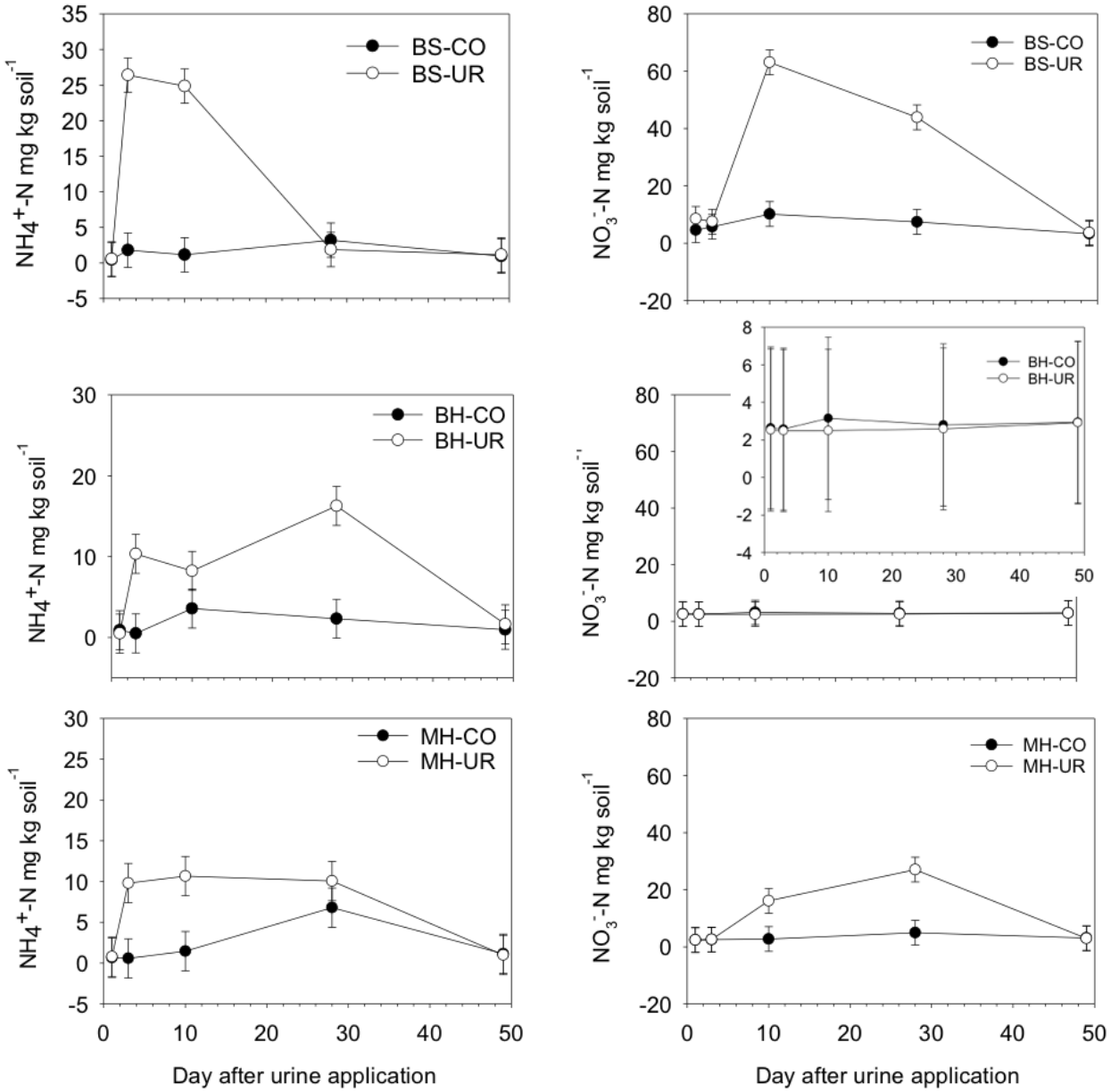


Figure 5-3. Response of inorganic N (ammonium (NH_4^+) and nitrate (NO_3^-)) during the 49 d of the experiment. Six treatments were studied: bare soil (BS), *Brachiara humidicola* 16888 (BH), and *Brachiaria mulato* hybrid 1 (MH) each soil cover had a secondary treatment which was the application of bovine urine (UR), and the control (CO (no nitrogen)). Soil samples were taken 2 h, and 8, 28, and 48 d after urine application. Significance differences were calculated by Proc Mixed $p>0.05$, errors bar indicate standard deviation.

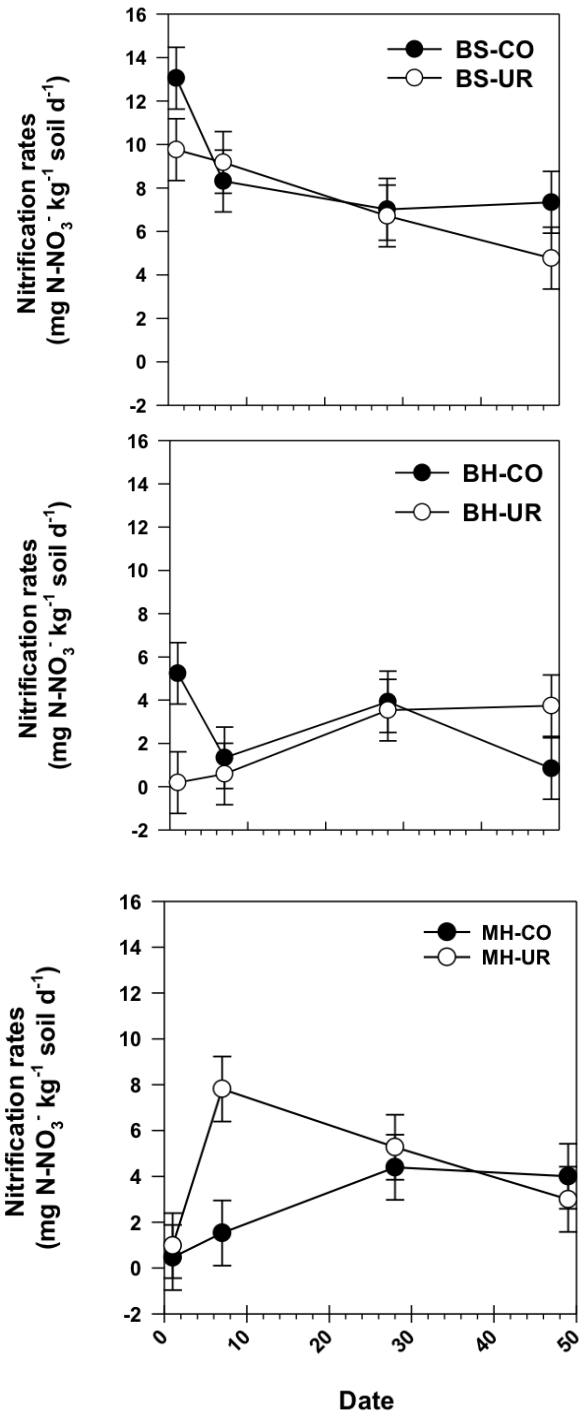


Figure 5-4. Nitrification rates during the 49 d of the experiment. Six treatments were studied: bare soil (BS), *Brachiara humidicola* 16888 (BH), and *Brachiaria mulato* hybrid 1 (MH) each soil cover had a secondary treatment which was the application of bovine urine (UR), and the control (CO (no nitrogen)). Soil samples were taken 2 h, and 2, 8, 28, and 48 d after urine application. Significance differences were calculated by Proc Mixed $p>0.05$, errors bar indicate standard deviation.

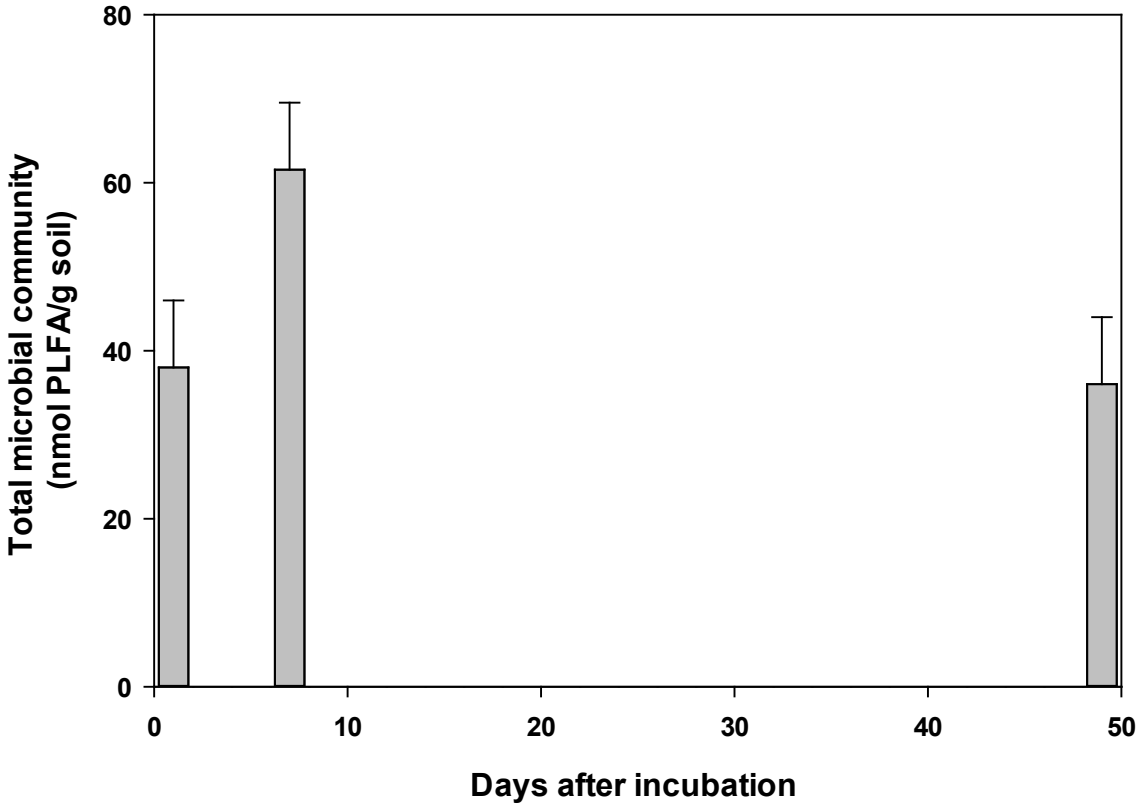


Figure 5-5. Total microbial biomass dynamics during the 48 d of the experiment. Soil samples were taken 2 h, 7, and 35 d after urine application. Significance differences were calculated by Proc Mixed $p>0.05$, errors bar indicate standard deviation.

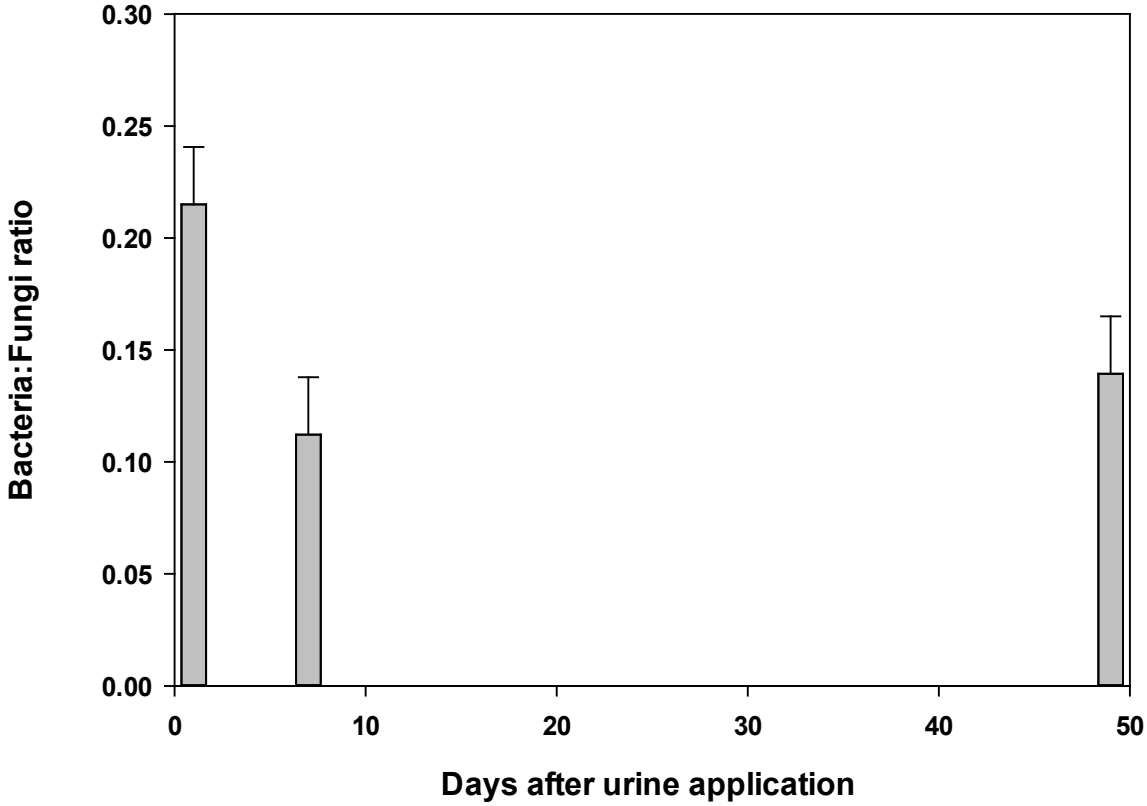


Figure 5-6. Bacterial:Fungal ration during the 48 d of the experiment. Soil samples were taken 2 h, 7, and 35 d after urine application. Significance differences were calculated by Proc Mixed $p>0.05$, errors bar indicate standard deviation.

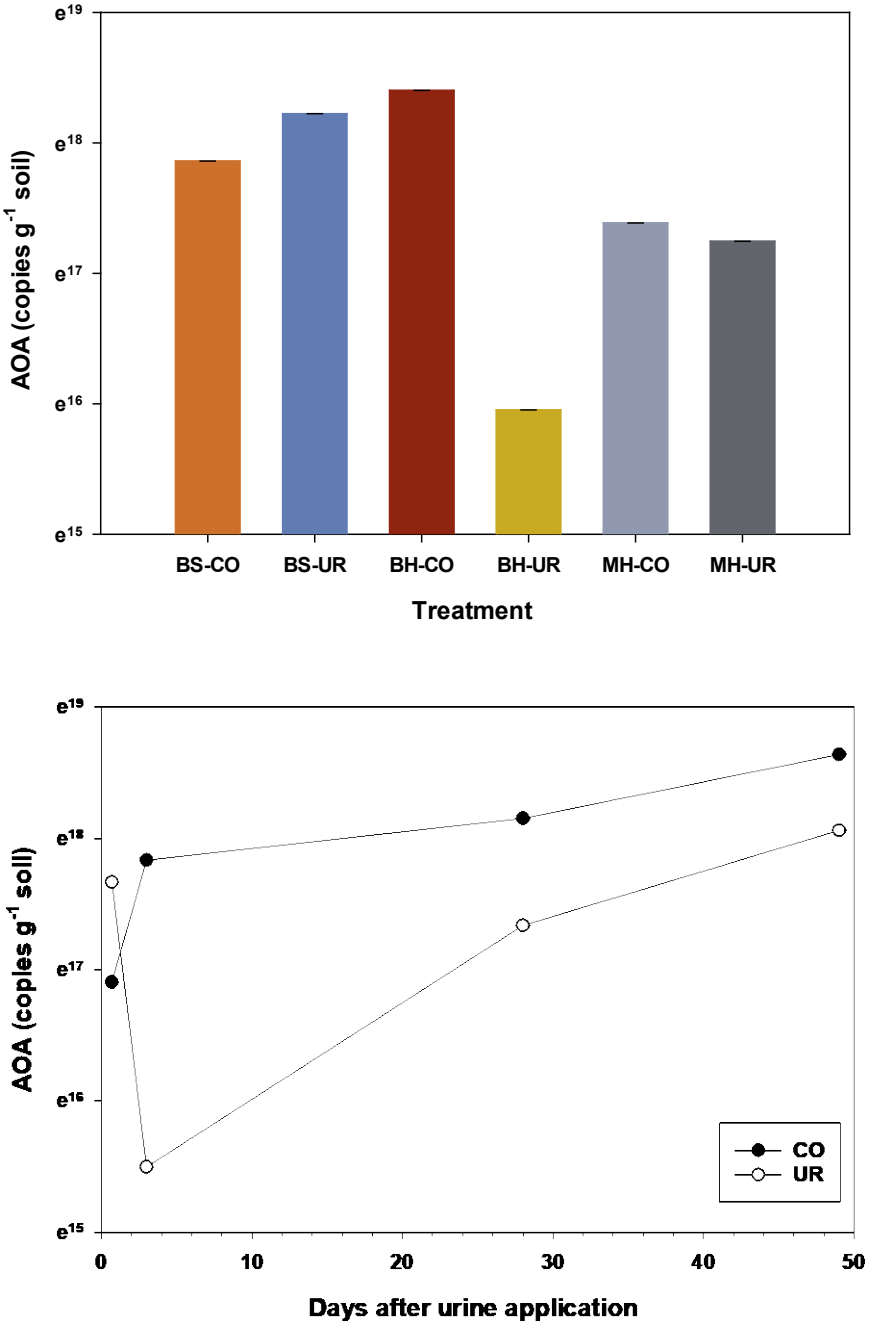


Figure 5-7. (a) Ammonia oxidizing-archaea (AOA) dynamics in the six treatments studied: bare soil (BS), *Brachiara humidicola* 16888 (BH), and *Brachiaria mulato* hybrid 1 (MH) each soil cover had a secondary treatment which was the application of bovine urine (UR), and the control (CO (no nitrogen)). (b) Abundance of AOA during the 49-d experiment. Samples were taken 2 h, 7, 35 and 48 d after urine application. Significance differences were calculated by Proc Mixed $p>0.05$, errors bar indicate standard deviation.

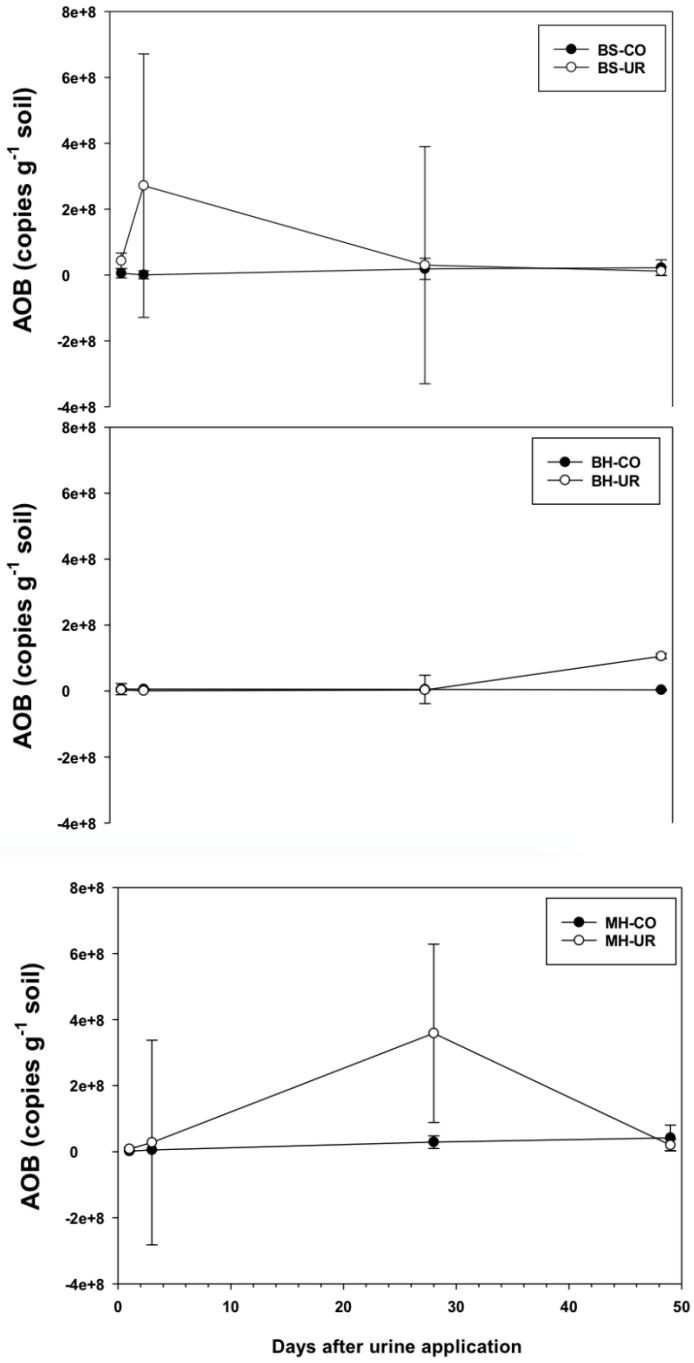
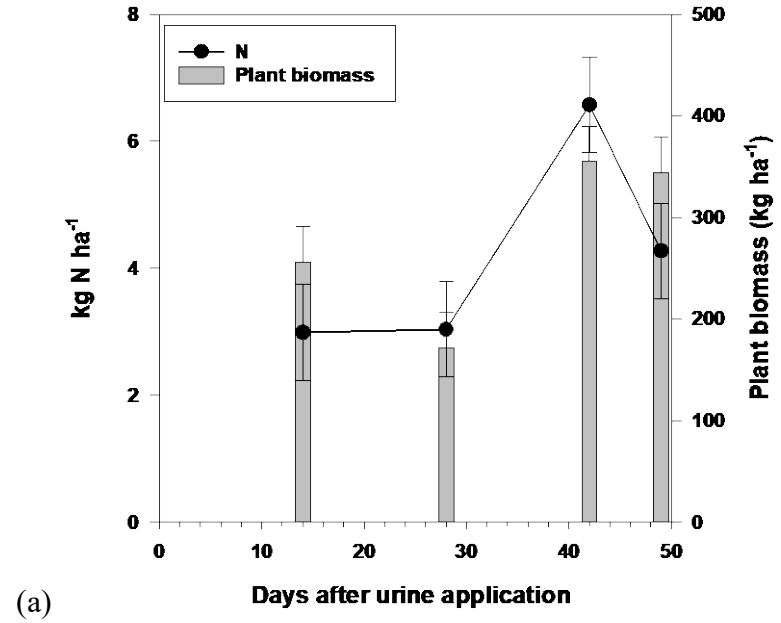
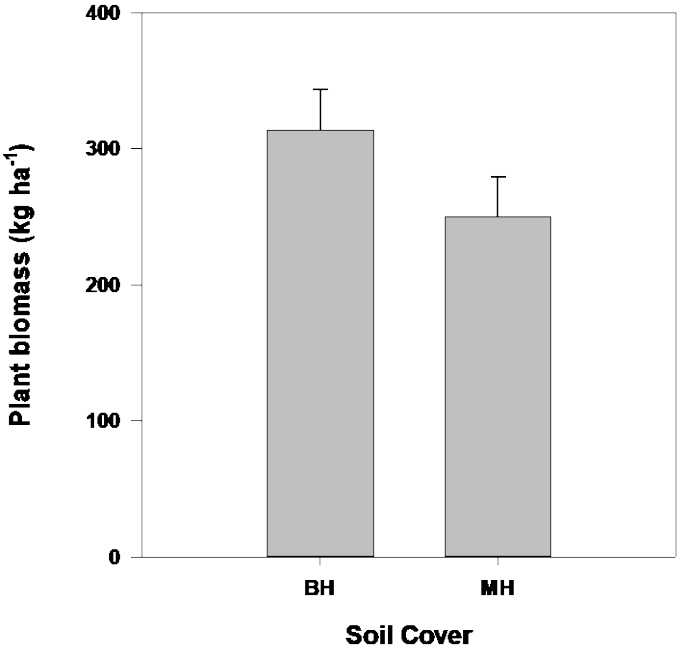


Figure 5-8. Abundance of ammonia oxidizers bacteria (AOB) according to the six treatments studied during the 48-d experiment. The six treatments studied were: bare soil (BS), Brachiara humidicola 16888 (BS), and Brachiaria mulato hybrid 1 (MH) each soil cover had a secondary treatment which was the application of bovine urine (UR), and the control (CO (no nitrogen)). Samples were taken 2 h, 3, 28, and 49 d after urine application. Significance differences were calculated by Proc Mixed $p>0.05$, errors bar indicate standard deviation.

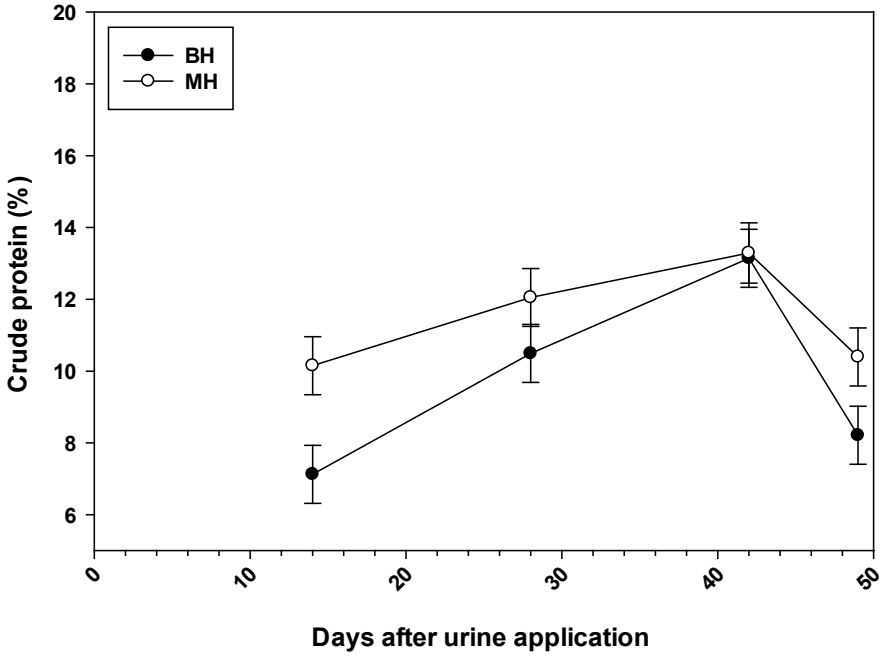


(a)



(b)

Figure 5-9. (a) Nitrogen (N) content in biomass and aboveground biomass (kg N ha^{-1}) from *Brachiaria* humidicola 16888 (BH), and *Brachiaria* mulato hybrid 1 (MH) in 15-d laps during the 48-d of the experiment. (b) Total biomass production of *Brachiaria* humidicola 16888 (BH), and *Brachiaria* mulato hybrid 1 (MH) during the 48-d on incubation. Significance differences were calculated by Proc Mixed $p>0.05$; bars represent the mean value and error bar indicate the standard deviation.



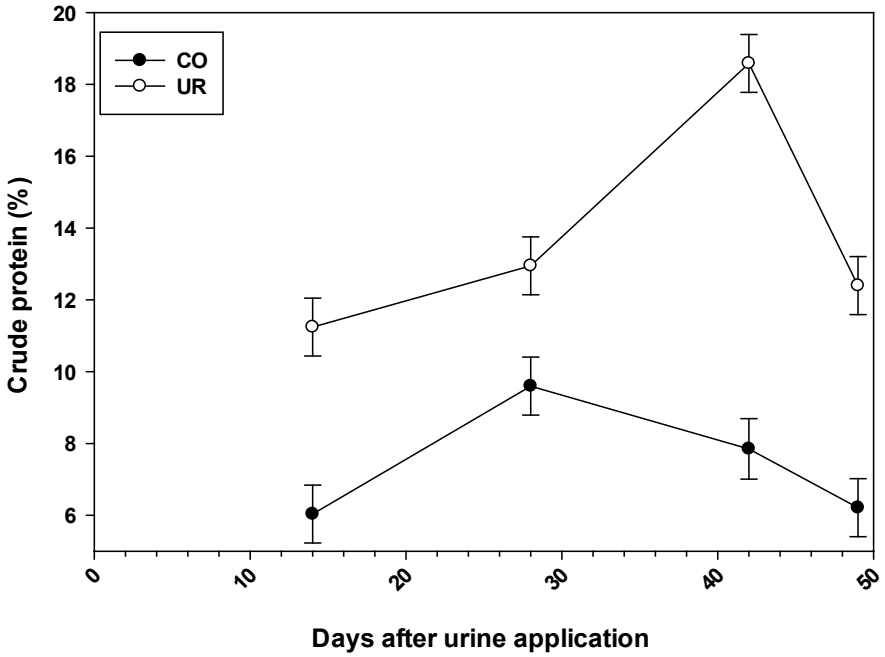


Figure 5-10. (a) Crude protein (%) from *Brachiaria humidicola* 16888 (BH), and *Brachiaria mulato* hybrid 1 (MH) in 15-d laps during 48-d of the experiment. (b) Crude protein (%) from urine (UR) patches and control ((CO) no nitrogen) areas in 15-d laps during 48-d of the experiment. Significance differences were calculated by Proc Mixed $p>0.05$; errors bar indicate the standard deviation.

Table 5-1. p- values for the variables forage biomass, nitrogen (N) in forage, and crude protein in forage, soil pH, nitrifier activity, nitrous oxide (N_2O), and methane (CH_4) for the effects of soil cover (SC), nitrogen (N) deposition, sampling date (T), and the interactions.

Effect	**Forage Biomass**	**N in forage**	**Crude Protein**	**pH**	**Nitrifier activity**	**N_2O**	**CH_4**
SC	0.0193	0.7192	0.0007	0.0003	<.0001	<.0001	0.0021
N	0.0749	0.0109	<.0001	0.9088	0.774	<.0001	0.2826
T	<.0001	<.0001	<.0001	0.0030	0.3267	<.0001	<.0001
SC X N	0.7223	0.5576	0.0115	0.0822	0.0297	<.0001	0.1119
SC X T	0.6346	0.5288	0.2083	0.7828	<.0001	<.0001	0.0011
N X T	0.5765	0.6622	<.0001	0.6916	0.0093	<.0001	0.8989
SC X N X T	0.8542	0.9056	0.9570	0.2301	0.0356	<.0001	0.0020

Table 5-2. p- values for the variables ammonium (NH_4^+) and nitrate (NO_3^-) for the effects of soil cover (SC), nitrogen (N) deposition, depth (D), sampling date (T), and it interactions.

Effect	**NH_4^+**	**NO_3^-**
SC	0.2410	0.0325
N	<.0001	<.0001
D	0.1530	0.9397
T	<.0001	<.0001
SC X N	0.0222	<.0001
SC X D	0.9945	0.9942
SC X T	<.0001	<.0001
N X D	0.1991	0.9998
N X T	<.0001	<.0001
SC X N X D	0.9680	0.9791
SX X N X T	<.0001	<.0001
SC X D XT	0.9984	1.0000
N X D X T	0.2008	0.9997
SC X N X D X T	0.9999	0.9997

Table 5-3. p-values for the soil microbial variables from the ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), total microbial biomass (total), gram-positive bacteria (Gram +), gram-negative bacteria (Gram -), actinomycete (act), arbuscular mycorrhizal fungi (AMF), fungi, and the fungal:bacteria (Ratio) for the effects of soil cover (SC), nitrogen (N) deposition, depth (D), sampling date (T), and the interactions.

Effect	Total	Gram+	Gram-	Act	AMF	Fungi	Ratio	AOA	AOB
SC	0.2657	<.0001	0.5089	0.0003	<.0001	<.0001	0.9669	0.1124	0.001
N	0.4060	0.0799	0.8335	0.9197	0.2420	0.4401	0.3367	0.1622	0.000
T	0.0332	<.0001	0.0088	0.0005	<.0001	0.0014	0.0216	0.0099	0.002
SC X N	0.3201	0.9696	0.2062	0.9832	0.5073	0.2919	0.6418	0.004	0.039
SC X T	0.0745	<.0001	0.0036	<.0001	<.0001	<.0001	0.4148	0.2677	0.007
N X T	0.6750	0.6123	0.2691	0.4681	0.6622	0.7431	0.1840	0.0289	0.495
SC X N X T	0.6418	0.1079	0.6332	0.1186	0.6544	0.5039	0.5133	0.292	<.0001

Chapter 6 - Temporal dynamics and sampling frequency on methane and nitrous oxide flux estimates using an automated greenhouse gas sampling system

Abstract

Weather variations in temperate grasslands affect greenhouse gas (GHG) fluxes of nitrous oxide (N_2O) and methane (CH_4). This study was to characterize N_2O and CH_4 fluxes temporal dynamics using high-resolution flux data and to determine the effect of sampling frequency on flux estimates. Temporal variations in CH_4 and N_2O fluxes were measured from Spring to Summer 2016 from a tallgrass prairie near Manhattan, Kansas using an automated greenhouse gas sampling system. Daily CH_4 and N_2O fluxes were estimated by different sampling frequencies of one a day, six to eight times per day, weekly, biweekly, and monthly. Daily values were calculated by considering one single sample during noon, the sum of six to eight samples per day, the average of six to eight samples per day, or the cumulative value of six to eight samples per day by linear interpolation. CH_4 and N_2O fluxes were significantly affect by landscape position and burning. The N_2O daily values by the four different estimations were not significantly different. However, CH_4 daily estimations using a single data point during the day, and the average of 6 to 8 daily samples resulted in underestimations of soil uptake potential. Seasonal estimation using the mimic of the static chamber method resulted in a decrease in N_2O sampling frequency of less than twice per week is expected to increase underestimations. The recommended CH_4 sampling frequency is from daily, twice a week, and biweekly basis. Our data confirm that temperate grasslands are a minimal sink of N_2O and sink of CH_4.

Introduction

Soil chambers are widely used to determine greenhouse gas (GHG) dynamics at ecosystems (Parkin and Kaspar, 2006; Mishurov and Kiely, 2011; Rowlings et al., 2012). However, the typical daily or longer sampling frequencies in static chamber studies raise concerns about how accurate or representative is the one daily sample compare to the real daily flux, and how sampling frequency affects estimations of total GHG emissions. Automated chamber systems provide high-temporal resolution data (~ 3h) GHG flux data, therefore, increasing the accuracy on GHG estimations (Rowlings et al., 2012).

Continuous flux measurements provide a more efficient and accurate interpretation of the underlying processes influencing the GHG fluxes (Livingston and Hutchinson, 1995; Kutzbach et al., 2007; Pihlatie et al., 2013). Soil fluxes are bi-directional depending on the circumstances soil fluxes can change from production (source) to consumption (sink). Temporal variation in the production and transport of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) fluxes occurs in response to abiotic factors, soil microbial activity, spatial variability, and substrate availability which regulates GHG production, consumption, and emissions from soil (Allard et al., 2007; Chapuis-Lardy et al., 2007; Conrad, 2009; Reay et al., 2012; Butterbach-Bahl et al., 2013; Hayashi et al., 2015; Di and Cameron, 2016).

Extensive amount of research on GHG emissions has been done to understand GHG dynamics from agricultural systems (Allard et al., 2007; Kutzbach et al., 2007; Pihlatie et al., 2013; Gerber et al., 2013; van Delden 2018). Yet, our current knowledge on the drivers of GHG flux temporal and spatial variability is still limited. Automated GHG chamber systems provide a chance to measure and monitor GHG emissions at short time (<day) scales, a daily basis, under real field scenarios by using technology approaches to biological systems and providing high

resolution data for accurate estimations on GHG fluxes, and C sequestration (Parkin and Kaspar, 2006; Mishurov and Kiely, 2011; Savage et al., 2013; van Delden et al., 2018).

In the United States, temperate grasslands under native pastures are key for the cattle-grazing systems, industry supporting the consumption of 11.5 billion of beef cattle products during 2015 (ERS, 2019). In temperate grasslands, N_2O and CH_4 fluxes are expected to vary driven by edaphological factors, changes in weather, shifts in soil cover within the seasons, and nutrient additions (Borken and Matzner, 2009; Ball, 2013; Hayashi et al., 2015). Additionally, GHG fluxes are affected by prescribed burning which is a common practice used on grazed pastures to manipulate grazing distribution, parasite control, animal productivity, and natural resources conservation (Fuhlendorf et al., 2011; Mishurov and Kiely, 2011; Ferrea et al., 2012). Additionally, from a GHG perspective under grasslands, burning regimes accelerate soil mineralization rates, therefore, increasing CH_4 and N_2O soil nutrient recycling in the ecosystem (Fuhlendorf et al., 2011).

The CH_4 dynamics is the result of methanogenesis and CH_4 oxidation. The oxidation occurs as a microbial metabolic process carried by methanotrophs for energy generation and C assimilation (Fenchel et al., 2012; Butterbach-Balh et al., 2013). On the other hand, soil methanogenesis is the use of H_2 for the reduction of CO_2 producing CH_4 and H_2O (Fenchel et al., 2012). The N_2O dynamics is the result of nitrification and denitrification soil potential (Firestone and Davidson, 1989; Di and Cameron, 2016). Nitrification is the oxidation of NH_4^+ to NO_3^- by *Nitrosomonas*, and *Nitrobacter*. While, denitrification is the reduction of nitrite (NO_2^-) or nitrate (NO_3^-) to molecular nitrogen (N_2) and then to N_2O (Firestone and Davidson, 1989; Butterbach-Balh et al., 2013).

Understanding the drivers of biochemical processes and the spatiotemporal dynamics of GHG fluxes is needed for developing GHG inventories at regional scales, and developing strategies for mitigating GHG emissions from natural and managed ecosystems. This research aims to 1) improve the current understanding CH_4 and N_2O flux variations in short timescales (< 1 d), 2) to compare N_2O and CH_4 budget estimates using the automated chamber system with the ones obtained using one sampling point per day to estimate sampling frequency needed for improving CH_4 and N_2O seasonal budget estimations. Further understanding of the quantification of GHG emissions from grassland soils could potentially increase accuracy on GHG budget estimations which is necessary to reach knowledge on grassland ecosystem services, and the improve adaptation of cattle grazing systems.

Materials and methods

Research Site

The research site was established at the Lazy N Ranch in Pottawatomie County, Kansas (39° 15' 25.4736'' N, 96° 29' 14.0784'' W, 413 m a.s.l.). The soil series at the experimental site were Tully (Fine, mixed, superactive, mesic Pachic Argiustolls) (NRCS, 2006) and Clime (fine, mixed, active, mesic Udorthentic Haplustolls) (NRCS, 2013); corresponding to 60% and 40% of the area, respectively. Mean temperature from March to August 2016 was 20.1° C, and mean annual precipitation is 89 cm mainly in late spring to early summer. Prominent grass species an the site are big bluestem (*Andropogon gerardi*), little bluestem (*Schicachyrium scoparium*), switchgrass (*Panicum virgatum),* Indian grass (*Sorghastrum nutans*), and brome grass (species of the genera *Bromus*). Soil nutrient availability is described in Table 6-1. The site was grazed by cattle from April to August (5 months).

Experimental Setup

Soil gas fluxes were measured utilizing an automated chamber based on the static closed chamber technique (non-steady-state, non-through-flow) (Livingston and Hutchinson, 1995) and using the same automated gas sampling system described in detail by Rowlings et al. (2012). The automated chamber system consisted of twelve pneumatically operated measuring chambers linked to a sampling control system and a gas chromatograph located in a field shed. The chambers were divided in groups of four for a total of three plots (1) annually burned summit (AB), (2) annually burned in a terrain slope (ABS), and (3) unburned summit (NB).

The chambers were made of transparent acrylic glass chambers and covered a surface area of 0.25 m^2 (50 cm × 50 cm) with a total headspace of 150 mm. The chambers were not thermally insulated. Chambers were located 2-3 m apart from each other within the same plot, and 55 m apart to the field shed. The chambers were placed over stainless steel bases inserted into the soil to a depth of 10 cm. Chambers were connected to the sampling unit, located within a distance of 50 m from the chambers, by a 3.2 mm non-reactive Teflon-coated sample line, and two pneumatic airlines which were used for opening and closing the chamber lids. A flow meter was used to control the flow of air through the automated chamber sampling line.

Each air stream was drawn to an injector valve using a suction bump within the three-minute sampling time, to minimize chamber air dilution. The recommendation of Rowlings et al. (2012) was to determine the volume in the sample divided by the chamber volume to maintain chamber air dilution less than 5%. The minimum sample flow rate was calculated by dividing the length of the sampling line by the three min of the sampling, which provided a value of mL min^{-1}. The recommended value, from Rowling et al. (2012), for flow rate was between 200-300 mL min^{-1} under ambient conditions, during this study we used the maximum of 300 mL min^{-1}.

After three minutes the injector valve switched to allow a carrier gas, in this study high purity N, to move the air sample from the sampling loop into the GC separation column. Each sample passed through an in-line column filter containing sodium hydroxide coated in silicate for the removal of any presence of CO_2 and H_2O to avoid sample contamination. From there, the sample passed through the electron capture detector (ECD) and then the flame ionization detector (FID) analysis. During this time, each chamber was sequentially sampled for 3 min followed by a known calibration standard (0.5 ppm N_2O, Air Liquide, Houston, TX, USA). A full measurement cycle for flux determination commenced with lid closure and finished when the lids were opened 48 min later. Chambers then remained open for a period of 96 min before the commencement of the next sampling cycle, allowing 10 individual N_2O fluxes to be calculated daily. The N_2O and CH_4 concentrations were determined using a gas chromatograph (SRI GC8610, Torrance, CA, USA) equipped with a ^{63}N Electron Capture Detector.

The chamber was completely sealed except for the opening and closing of the lids controlled by the automated system, and the pressure gage. Any leak in the sampling box was considered as an underestimation of the actual concentrations values of the samples over time and it was not considered (Livingston and Hutchinson, 1995). The increase in CO_2 concentration over time in the chamber allowed verifying the presence of leaks since once the chamber close the CO_2 concentrations are expected to increase over time providing an R^2=0.95.

Additionally, a wired tipping bucket rain gauge (Davis Instruments Corp. CA, USA) was connected to the system to keep the chamber lids open during rainfall events. The rain gauge was installed at three meters from the field shed away to obstacles that could influence the precipitation measurement. Also, each plot had one external sensor, and one sensor inside one of the chamber for measuring environmental data including temperature, soil temperature, and soil

water content using a 5TN soil moisture sensors (Decagon Devices, Inc. WA, USA). In the case of soil water the sensor was buried at depths of 5 and 10 cm.

Control of Abiotic and biotic factors

There is a substantial reduction on convective transport of heat between the soil surface and atmosphere once the chamber is closed. Under high solar radiation conditions, the temperature within the chamber may increase therefore possibly harming the vegetation inside the chamber. The chambers contained an internal temperature sensor receiving temperature data every 15 minutes and control the chambers; which was connected to a controller that automatically opened the chambers if the internal temperature exceeded 55 ^{o}C to avoid heat damage to pasture, and allow air circulation. Grass inside the chamber was maintained at a height of 5-10 cm from the soil to mimic animal grazing and to confirm the chamber was completely sealed during the gas sampling.

During the course of the study, temporary fences were used to preventing cattle to approach the area where the chambers were installed. During burning, fences and chambers were moved out of the field and re-installed 1-2 days after the burning. In addition, the sampling lines, pneumatic tubing and temperature probes lines were buried to avoid damage to the lines.

N_2O and CH_4 flux analysis

The GC uses 1-3 ml of air samples during each sampling time. Once in the GC, the different compounds in the gas sample are separated on an analytical column which passes to the ECD and FID detectors. The ECD was used for N_2O, this detector operates between 330-350°C. Argon was used as the carrier gas during the sampling (Wang et al., 2010). The FID was used for CO_2 and CH_4 analysis. Chromatograms outputs from the FID and ECD are recorded in millivolts by the free software PeakSimple (SRI Instruments, Torrance, CA, USA). The system

was connected with a secondary GHG system software, which controls the sampling box, sampling times, opening and closing of the chambers and records CO_2, soil temperature, air temperature, and precipitation signals. Any trouble with the system was recorded in a Troubleshoot file in the PeakSimple software and later used for data analysis.

The CH_4 and N_2O fluxes were calculated from the linear increase of the gas concentration in the chamber area by the software FluxNet version 3.3 (Baldochi et al., 2001) (Appx D, Equation D-1). Fluxes were calculated based on the slope of the linear increase of the four N_2O and CH_4 concentration in the chamber headspacing taken at 15 min intervals. Fluxes were discarded if the N_2O and CH_4 concentrations over the 45 min period were lower than $R^2 < 0.85$, using Pearson's correlation coefficient (r^2). The flux rate was calculated and corrected for air temperature during measurement and site pressure using the procedure outlined by Barton et al. (2008). When temperature data was missing the gas concentration was calculated using a standard value of 25ºC.

Daily N_2O and CH_4 fluxes

The effect of sampling frequency within the daily period for calculating the daily flux was studied by comparing a single daily flux, the average of the daily fluxes, and the cumulative of the fluxes during 1 d period. A total of 20 d for N_2O and 26 d for CH_4 with 6 to 8 samples per day were chosen for the sum, average, and linear interpolation techniques. For single daily value was the sample from 9:00 to 12:00; in case of missing samples the samples from time 6:00 to 9:00, or 12:00 to 15:00 were considered as the daily value. In addition, the cumulative flux was calculated using a modification of the linear interpolation technique described by McGowan et al. (2018) using the linear interpolation technique between sampling points every three hours during the day, and calculating the area under the curve using the equation:

Equation 6-1

$$\text{Cumulative} = \sum_{i}^{n} \frac{(F_i + F_{i+1})}{2} (t_{i+1} - t_i)$$

where Fi and $Fi+1$ were the N_2O or CH_4 fluxes (g m^{-2} h^{-1}) at sampling points I and $i+1$, t_i and $ti+1$ were the sampling times during the day (hour) at sampling points I and $i+1$, and n was the number of sampling points taken in a day. Time during the day was distributed as 00:00 to 03:00, 03:01 to 06:00, 06:01 to 9:00, 9:01 to 12:00, 12:01 to 15:00, 15:01 to 18:00, 18:01 to 21:00, and 21:01 to 24:00. In order to calculate the linear interpolation we used the time 3:00; 6:00, 9:00, 12:00, 15:00, 18:00, 21:00 and 24:00.

N_2O and CH_4 diurnal cycle

The diurnal cycle was considered the average N_2O and CH_4 flux over eight different times on a 24 h d period. Sampling times during the day were distributed as 00:00 to 03:00, 03:01 to 06:00, 06:01 to 9:00, 9:01 to 12:00, 12:01 to 15:00, 15:01 to 18:00, 18:01 to 21:00, and 21:01 to 24:00. Studied N_2O values were calculated by the ensemble of 35, and 45 sampling dates for spring and summer, respectively. Studied CH_4 values were calculated considering 70 sampling dates during spring.

Sampling frequency effect on cumulative N_2O and CH_4 fluxes

Sampling frequency effect on flux estimations was studied by comparing five different sampling frequencies: daily, twice a week, weekly, biweekly, and monthly. Mimicking the manual sampling of the static chamber method, we utilize one daily flux per day and calculate CH_4 and N_2O flux estimations over time. Daily values were considered by using daily reading from 9:00 to 12:00 from the automated chambers systems; in case of missing values the samples

from time 6:00 to 9:00, or 12:00 to 15:00 was considered as the daily value. Also, for N_2O values, samples with hourly values outside a -20 to 20.0 g N_2O-N or CH_4-C ha^{-1} h^{-1} were considered outliers and not considered for the cumulative estimation. Daily frequency of N_2O fluxes were calculated for two months period during spring (April and May, 61 days) and summer (July and August, 62 days). As a result of sampling technical and weather difficulties, from April to May we gathered 26 daily values, and from July to August a total of 29 samples were gathered. For CH_4, accumulative fluxes were calculated for 122 days March to June. The cumulative flux was estimated using the linear interpolation between days and calculating the area under the curve as described by McGowan et al. (2018) using the equation:

Equation 6-2

$$\text{Cumulative} = \sum_{i}^{n} \left(\frac{F_i + F_{i+1}}{2}(t_{i+1} - t_i)\right)$$

where F_i and F_{i+1} were the N_2O or CH_4 fluxes (g m^{-2} d^{-1}) at sampling points I and $i+1$; t_i and t_{i+1} were the sampling dates (day of the year) at sampling points I and $i+1$; and n was the number of sampling points taken in a given season.

Statistical analysis

The statistical package Statistical Analysis System (SAS, v9.3) was used for all statistical analysis. Baseline N_2O and CH_4 dynamics values were analyzed for the effect of burning regime annually burned flat surface, annually burned in a slope, and unburned, and the time of the day (1 to 8) in a 24 h d period, and its interaction using a Proc Glimmix analysis of variance (ANOVA) method (α=0.05). Differences in the effect of sampling frequency within the daily period for calculating the daily N_2O and CH_4 flux was studied by comparing a single daily flux, the average

of the daily fluxes, and the cumulative of the fluxes during 1 d period were analyzed using a Proc GLM ANOVA method (α=0.05). A Proc GLM ANOVA method (α=0.05) was used to identify significant differences in CH_4 budget under different sampling frequencies within 122 d (Mar-Jun). A Proc GLM ANOVA method (α=0.1) was used to identify significant differences in N_2O seasonal budgets between sampling frequency, season, and its interaction.

Discussion of results

N_2O and CH_4 flux baseline data

Soil N_2O emissions revealed significant differences for the interaction of time of the day, day of the year, and burning regime ($p<0.0001$). Mean daily N_2O fluxes ranged from 5.2, 4.7, and 2.8 g N_2O m^{-2} h^{-1} for annually burned, annually burned in a slope, and unburned, respectively (Fig. 6-1). Over the day, hourly fluxes ranged from -1.5 to 2.8 g N_2O m^{-2} h^{-1}. Highest hourly emissions were from the annually burned site with a mean of 0.85 g N_2O m^2 h^{-1}. Under the annually burned in a sloping site, the mean N_2O fluxes ranged from -0.2 to 2.7 g N_2O m^{-2} h^{-1}. Contrary to the burned sites, the unburned site N_2O fluxes from 12:00 to 15:00 had a mean of 0.72 g N_2O m^{-2} h^{-1}; while during the rest of the day gathered data resulted in N_2O uptake with an average of -0.6 g N_2O m^{-2} h^{-1}.

Similarly to N_2O, soil CH_4 fluxes were significantly affected by time of the day, day of the year, and burning regime ($p<0.0001$). An increase in soil CH_4 sink ranged from -6.5 to -6.0 g CH_4 m^{-2} h^{-1}during the month of June (Fig. 6-1). During spring (March to May) CH_4 fluxes ranged from -4.6 to 1.7 g CH_4 m^{-2} h^{-1}. Highest CH_4 uptake was observed from unburned and annually burned in a terrain slope with an hourly mean of 1.9 g CH_4 m^{-2} h^{-1} for both sites. Annually burned sites CH_4 uptake was slightly lower with a mean of 1.2 g CH_4 m^{-2} h^{-1}. A decrease in daily CH_4 uptake occurred at the annually burned site. Moreover, the CH_4 uptake

under the unburned and annually burned in the sloping site decreased from 9:00 to 15:00, and higher CH_4 uptake was measured from 18:00 to 06:00. Significant differences between practices have been previously reported (Mosier et al., 1991; Hammes et al., 2008). More specifically, Mosier et al. (1991) reported variations in an unfertilized pasture with a mean of -5.3 g CH_4-C ha^{-1} d^{-1}, and 2.5 g N_2O-N ha^{-1} d^{-1}; while midslope fluxes were -6.3 g CH_4-C ha^{-1} d^{-1}, and 1.8 g N_2O-N ha^{-1} d^{-1}.

Daily flux

The N_2O daily values were significantly different when compared to a single daily flux, the average of the daily fluxes, and the cumulative of the daily fluxes during 1 d ($p=0.0482$) (Fig. 6-3, Table 6-3). A single value around noon, from 6:01 to 9:00, 9:01 to 12:00, or 12:01 to 3:00, increased the daily value estimations by 61% (Fig. 6-3). Daily flux considering the daily average or the cumulative were not significantly different with a mean daily flux of 23.8 g N_2O m^{-2} d^{-1}; compared with the single daily value of 62.0 g N_2O m^{-2} d^{-1}. Furthermore, CH_4 daily values were not significantly different between daily technique ($p>0.05$) (Fig. 6-4, Table 6-3).

Temperature variation affects the diurnal flux. A correction to the daily flux is possible using the average daily temperature for adjusting the measured flux (Parkin et al., 2003). Additionally, Perez-Quesada et al. (2016) studied the daily estimates of soil CO_2 respiration with 24 sampling points per day. Perez-Quesada et al. (2016) indicated that ≥2 per samples day were necessary to improve accuracy, particularly if one of the samples was during night.

N_2O and CH_4 diurnal cycle

The N_2O diurnal cycle was significantly different from the interaction of time of the day and burning regime ($p=0.0416$) (Table 6-4). Data from the unburned and the annually burned in slope sites did not show significant differences in N_2O emissions between the time of the date or

burning regime with values ranging from 2.6 to -1.4 g N_2O-N ha^{-1} h^{-1} (Fig. 6-5). Lower soil inorganic N was likely due to plant competition and lower mineralization rates in the unburned site. The annually burned at the summit was significantly greater for N_2O emissions from 6:00 to 12:00 h, followed by 6:00 to 9:00 h, and 21:00 to 24:00 h with N_2O fluxes ranging from 9.0 to 5.2 g N_2O-N ha^{-1} h^{-1}. The diurnal cycle of soil CH_4 fluxes was not significantly different between burning regimes or time of the day ($p>0.05$) (Table 6-4).

Overall, the results from the diurnal cycle of N_2O and CH_4 fluxes supports the sampling recommendations from mid-morning and noon (Parkin et al., 2003; Pedersen et al., 2010).

Mosier (1989) and Parkin et al. (2003) observed diurnal variations in soil gas flux following changes in daytime temperatures and by seasons, rainfall, free-thaw events, fertility, and soil disturbance. Dong et al. (2010) reported N_2O and CH_4 fluxes, with a daily mean of 0.10 and -0.39 mg m^{-2} h^{-1}, respectively with a strong diurnal cycle. Parkin (2003) recommendation is to measure the gas fluxes at a time close to the daily average temperature.

Sampling frequency effect on cumulative N_2O and CH_4 value

Cumulative N_2O flux resulted in significant differences from the interaction of sampling frequency and season of sampling ($p=0.0164$) (Table 6-5, Fig. 6-7). Seasonal estimations for N_2O emissions during Jul-Aug were significantly higher than emissions during Mar-Apr. Differences over seasons are expected since high precipitation patterns, and changes in temperatures patterns affect soil water content and soil microbial activity resulting in higher N_2O dynamics (Lloyd and Taylor, 1994; Mishurov and Kiely, 2011; Reay et al., 2012). Moreover, during Jul-Aug twice a week sampling frequency registered 57% significantly higher N_2O flux estimation compared with weekly, biweekly, daily and monthly estimations (Fig. 6-7). During Mar-Apr twice a week was 95% significantly lower compared with monthly sampling frequency.

Since months during spring have lower variability on N_2O fluxes, a decrease in sampling frequency increases the chances of missing peaks on N_2O fluxes resulting in underestimations.

Cumulative CH_4 estimations were not significantly different between sampling frequency ($p=0.8474$) (Fig. 6-8, Table 6-5). It is important to notice that the CH_4 budget was estimated from March to June and we hypothesized there will be higher variability between sampling frequency if the cumulative CH_4 estimation occurs later during summer and early fall season. Net sink of N_2O and CH_4 in soils have been previously discussed (Paekin et al., 2003; Dong et al., 2010). Nevertheless, the sampling frequency is a factor to consider when estimating N_2O and CH_4 uptake capacity of grasslands (Chapuis-Lardy et al., 2007; Schlesinger, 2009). Simillarly, Barton et al. (2015) reported measurement frequency could be decreased by considering environmental factors. However, sampling more than once a week is still required to achieve accuracy when considering annual N_2O fluxes (Barton et al., 2015). Parkin et al. (2003) also recommended three to four samplings per week all year long to reduce temporal variability.

The underestimations and the decrease in sampling frequency in this research are in agreement with Kutzbach et al. (2007) and Pihlatie et al. (2013) who reported the linear regression method increases uncertainty when compared to the exponential flux calculation method. Adittionally, Mishurov and Kiely (2011) recommended the gap-filling techniques for achieving higher accuracy on annual N_2O flux budgets. Similarly, Parkin and Kaspar (2006) recommend using the "flux vs. temperature" relationship to minimize under and overestimations on N_2O fluxes. Is important to understand the studied grassland have low N concentrations and the results should be more variable under perturbed soils such as rainfall, tillage, and fertilization.

Conclusion

Annually burning of temperate grassland resulted in significant changes in CH_4 and N_2O fluxes. Daily average and cumulative daily are recommended methods for accurate estimations of N_2O fluxes. While one single value around noon, daily average, and cumulative were able to accurately estimate CH_4 fluxes. There were no significant differences the diurnal N_2O cycle. In the case of CH_4, the diurnal cycle was not affected by burning regimes. We conclude best practices for increase accuracy on N_2O fluxes requires sampling between 6:00 to 12:00 to catch mean daily values. A sampling frequency of daily to biweekly basis is recommended during the summer season. With spring having the lowest N_2O fluxes, a decrease in sampling frequency of less than twice per week would result in underestimates. The recommended CH_4 sampling frequency is from daily, twice a week, and biweekly. Furthermore, the monthly sampling during Mar-Apr resulted in underestimates of CH_4 flux. Daily, weekly, and biweekly sampling frequencies from 6:00 to 12:00 h is the recommended sampling time to achieve accuracy in cumulative N_2O fluxes from March to August.

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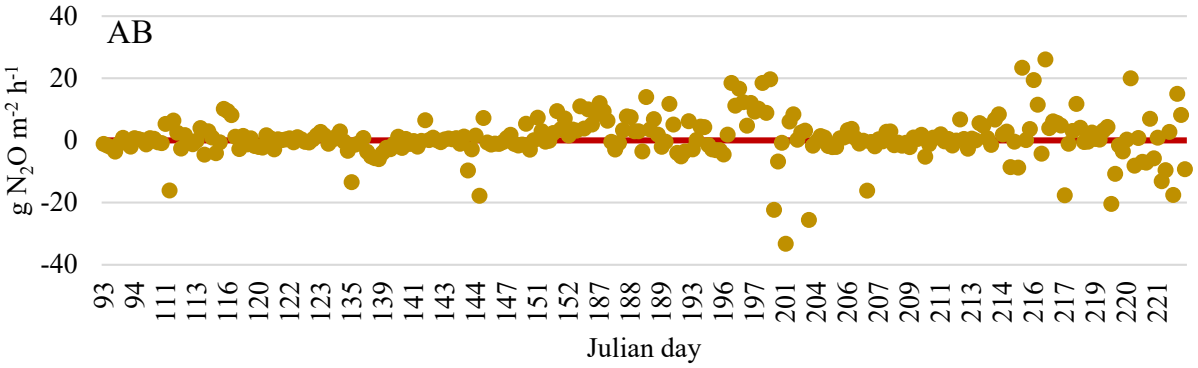

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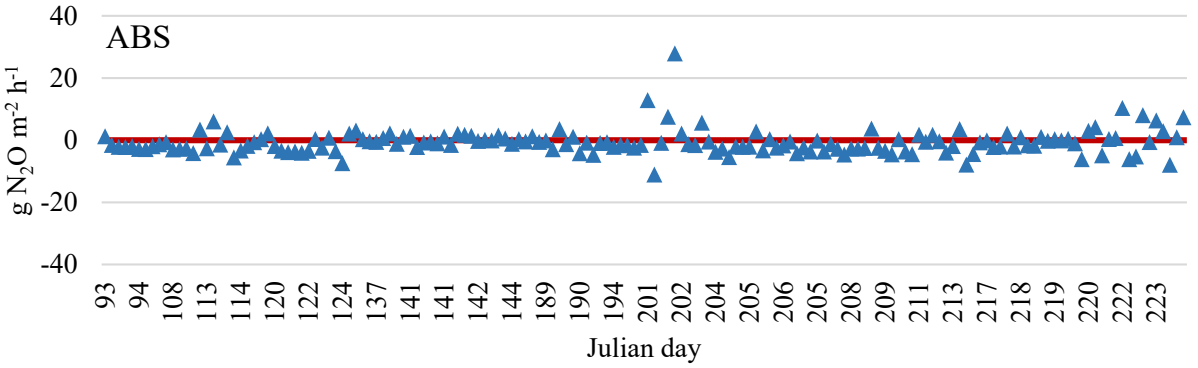

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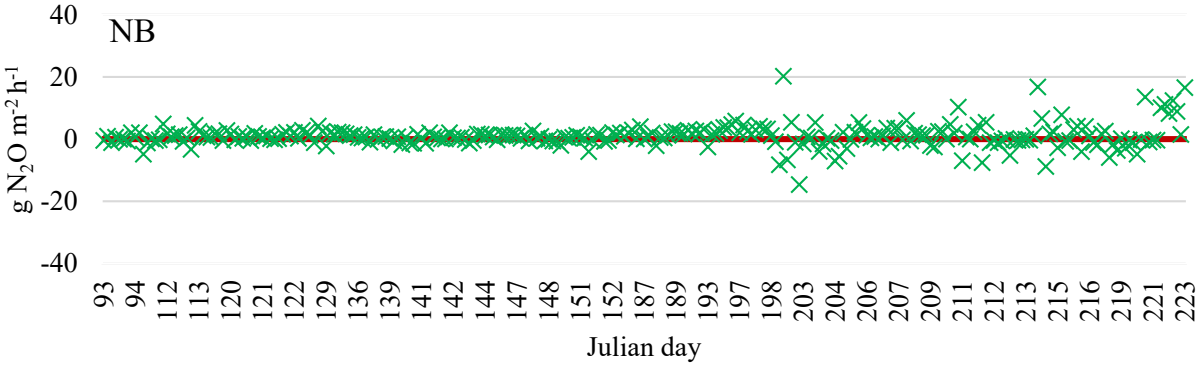

Figure 6-1. Nitrous oxide (N_2O) fluxes from the automated greenhouse gas system in a temperate grass under three different burning regimes: (1) annually burned summit, (2) annually burned in a terrain slope (ABS), and (3) unburned summit (NB).

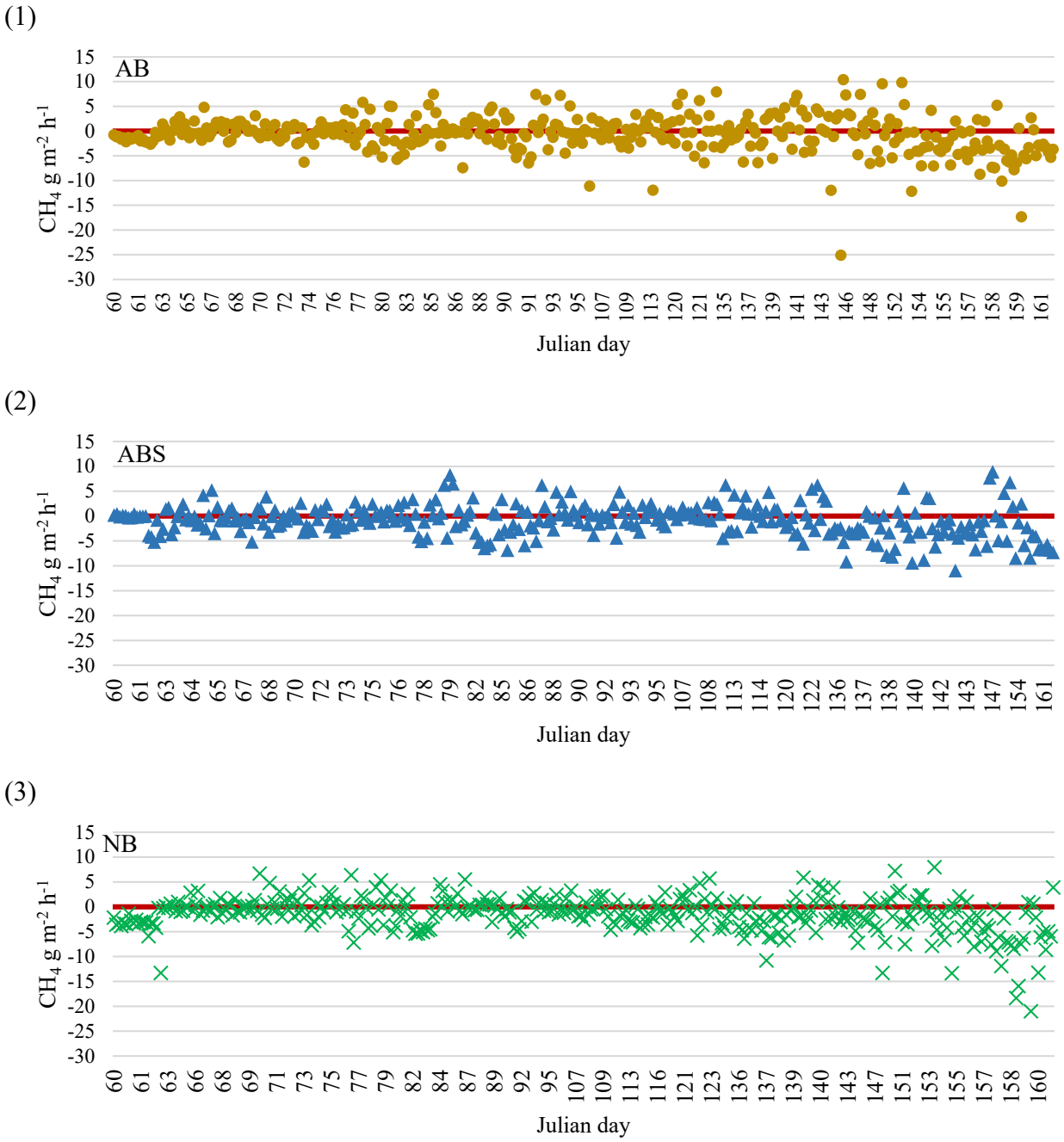

Figure 6-2. Methane (CH_4) fluxes from the automated greenhouse gas system in a temperate grass under three different burning regimes: (1) annually burned summit, (2) annually burned in a terrain slope (ABS), and (3) unburned summit (NB).

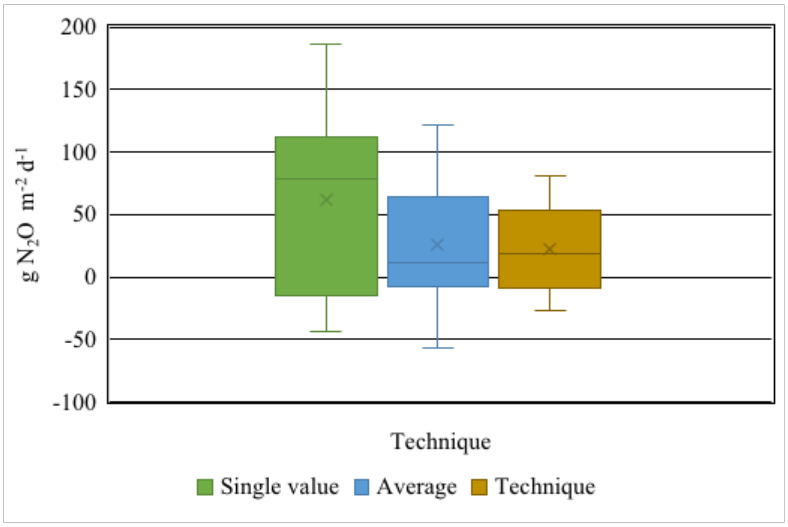

Figure 6-3. Daily N_2O value technique considering a single value during the day, average of daily fluxes, and the cumulative of daily fluxes using linear interpolation. Wiskers represents the standard deviation of the mean ($p<0.05$).

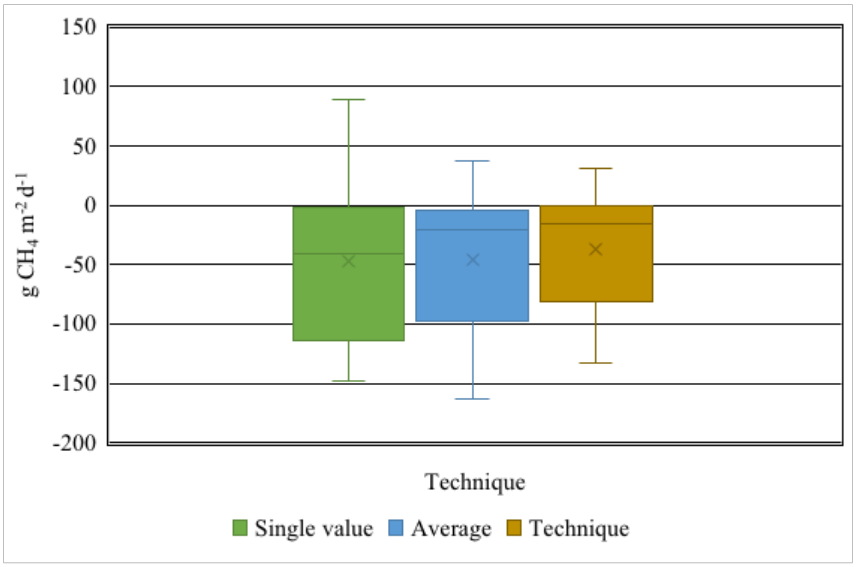

Figure 6-4. Daily CH_4 value technique considering a single value during the day, average of daily fluxes, and the cumulative of daily fluxes using linear interpolation. Whiskers represents the standard deviation of the mean.

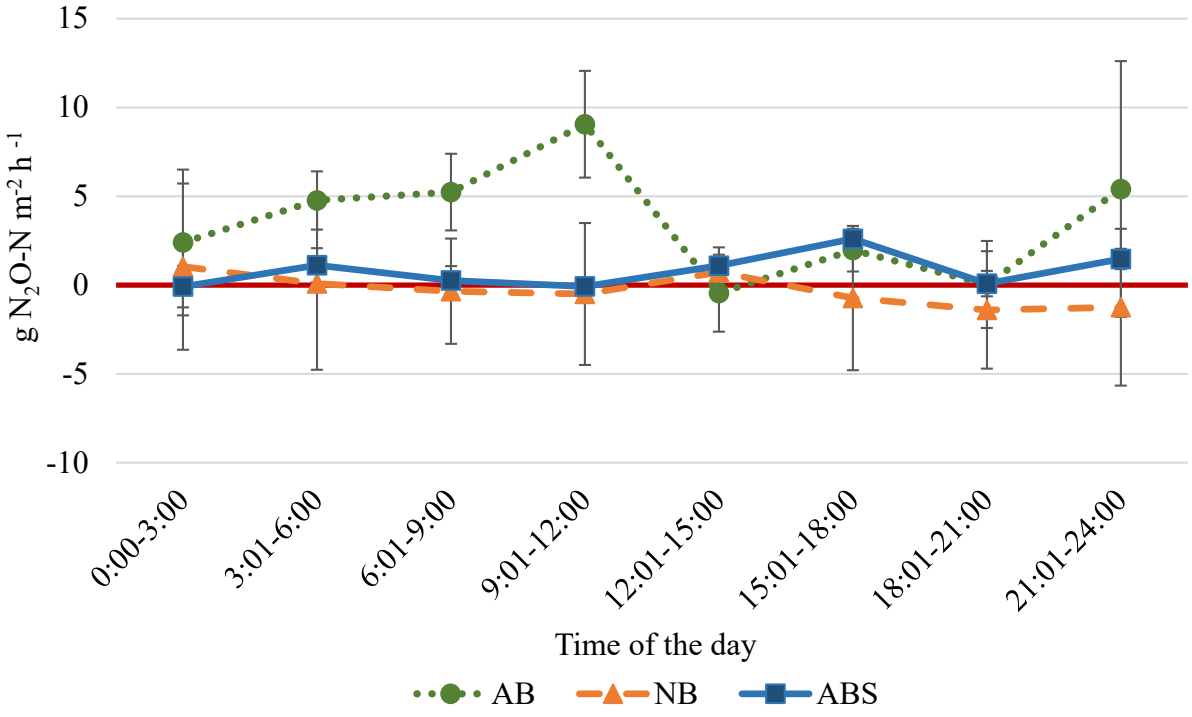

Figure 6-5. Diurnal cycle of N_2O dynamics during a 24 h d period under three different burning regimes: annually burned in a flat surface (AB), no burned in a flat surface (NB), and annually burned in a slope (ABS). Sampling times during the day were distributed as 1, 2, 3, 4, 5, 6, 7 and 8 for 00:00 to 03:00, 03:01 to 06:00, 06:01 to 9:00, 9:01 to 12:00, 12:01 to 15:00, 15:01 to 18:00, 18:01 to 21:00, and 21:01 to 24:00, respectively. Error bar represents the standard deviation of the mean.

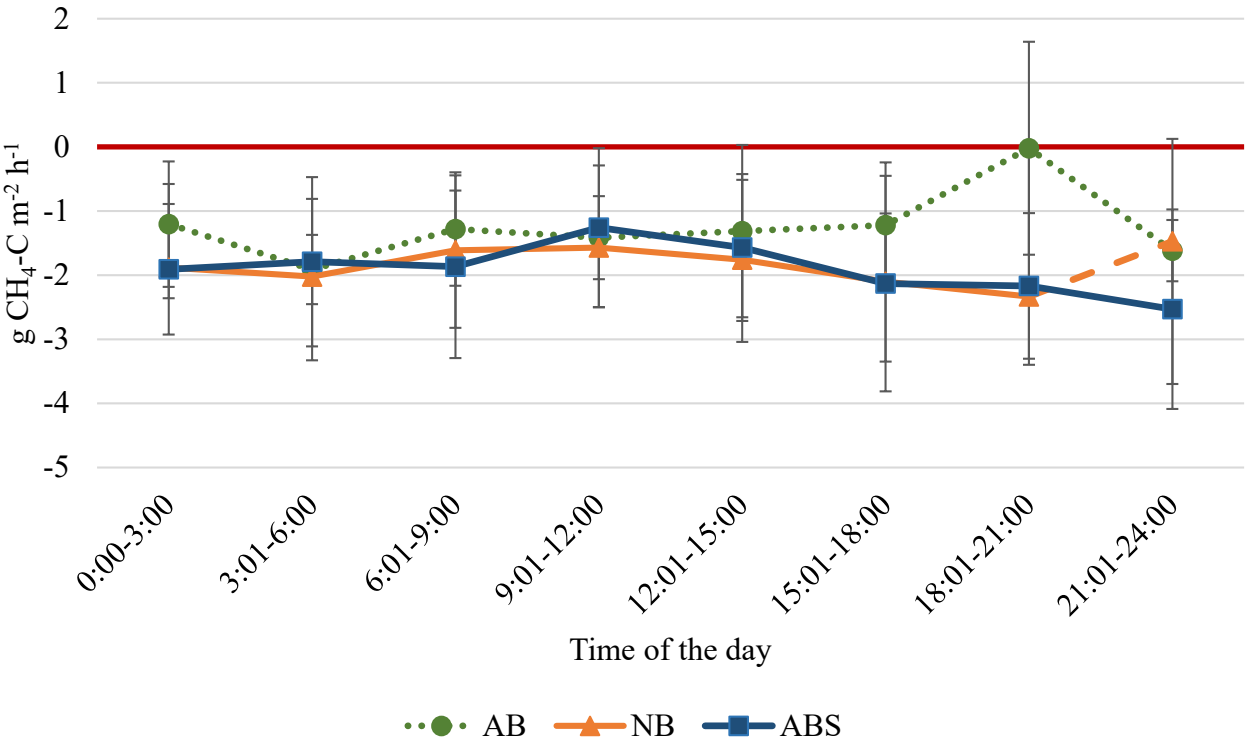

Figure 6-6. Diurnal cycle of CH_4 dynamics during a 24 h d period under three different burning regimes: annually burned in a flat surface (AB), no burned in a flat surface (NB), and annually burned in a slope (ABS). Sampling times during the day were distributed as 1, 2, 3, 4, 5, 6, 7 and 8 for 00:00 to 03:00, 03:01 to 06:00, 06:01 to 9:00, 9:01 to 12:00, 12:01 to 15:00, 15:01 to 18:00, 18:01 to 21:00, and 21:01 to 24:00, respectively. Error bar represents the standard deviation of the mean.

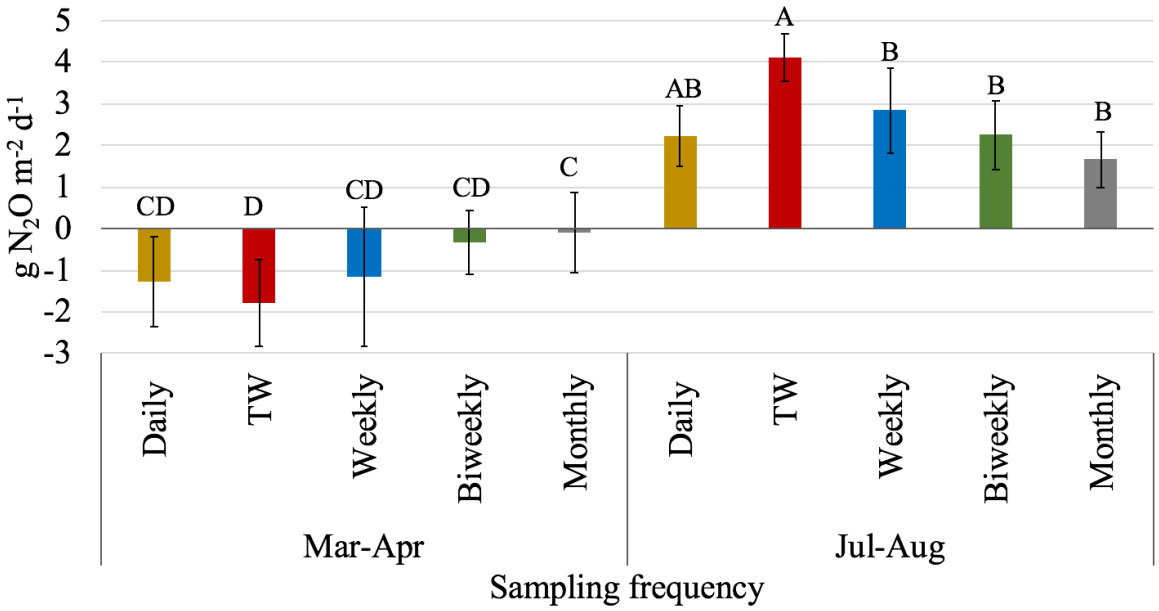

Figure 6-7. Budget estimation of N_2O fluxes using a mimic of the static chamber system for the season of spring (April and May) and summer (July and August). Sampling frequency studied was base in a single daily value, twice a week sampling (TW), weekly, biweekly, and monthly sampling. Different letters indicate significant differences by PROC GLM $p<0.005$.

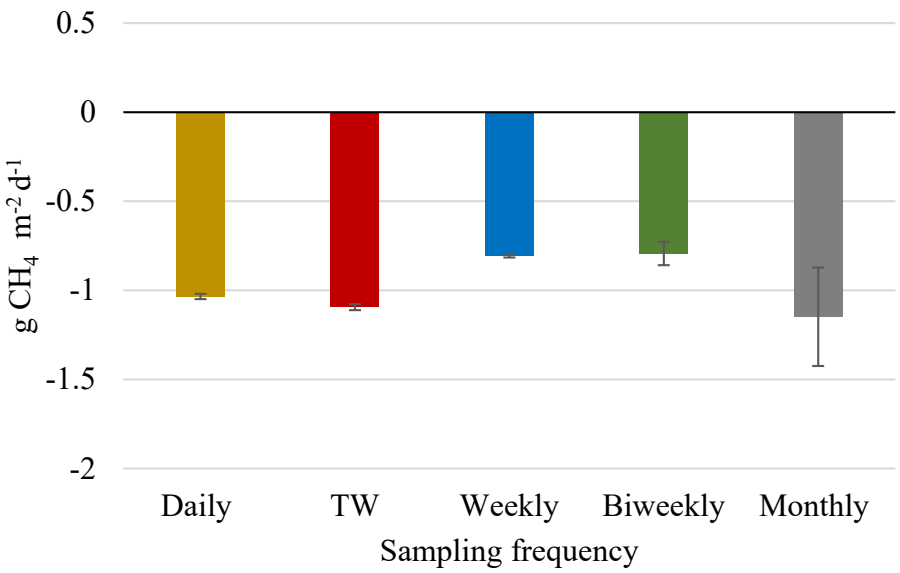

Figure 6-8. Budget estimation of CH_4 fluxes using daily values from a mimic of a static chamber system for the spring season. Sampling frequency studied was base in a single daily value, twice a week sampling (TW), weekly, biweekly, and monthly sampling. Different letters indicate significant differences by Proc GLM $p<0.005$

Table 6-1. Soil nutrient availability from the studied site, before the experiment establishment, under undisturbed annually burned pastures in Lazy N Ranch in Pottawatomie County, Kansas.

pH[1]	NH_4^+-N[2]	NO_3^--N[2]	Ca	Cu[3]	Fe[3]	K[4]	Mg	Mn[5]	Na	Zn[3]	P-Melich[5]
	----------	----------	----------	mg/kg	----------	----------	----------	----------	----------	----------	----------
6.5	0.41	0.17	3,178	1	102	374	460	21	59	4	4

[1] Soil pH was determined using a 1:1 soil:water method.
[2] KCl extraction for inorganic nitrogen (N), NH_4^+ and NO_3^-.
[2] DTPA extraction for Cu, Fe, Mn, and Zn both analyzed by a Inductively Coupled Plasma Spectrometer.
[3] Ammonium Acetate extraction for K.
[4] Samples were analyzed by Mehlich 3 Phosphorus for P (Lachat Quickchem 8000, Loveland, CO, USA).

Table 6-2. p-values for the greenhouse gases of nitrous oxide (N_2O) and methane (CH_4) fluxes in a temperate grassland under annually burned in flat surface and slope, and no burning in flat surface.

Factor	N_2O	CH_4
Time (T)	0.0104	0.0931
Julian day (JD)	<.0001	<.0001
Burning regime (BR)	0.0061	<.0001
T*JD	<.0001	<.0001
T*BR	0.0276	<.0001
JD*BR	<.0001	<.0001
T*JD*BR	0.0002	<.0001

Table 6-3. p values for nitrous oxide (N_2O) and methane (CH_4) daily flux by one daily value, and the three different techniques: sum of the fluxes, an average of the fluxes, and cumulative of the fluxes during 1 d period. Also, for diurnal cycle of N_2O and CH_4 dynamics during a 24 h d period under three different burning regimes: annually burned in a flat surface (AB), no burned in a flat surface (NB), and annually burned in a slope (ABS).

Method	**Daily flux**
N_2O	0.0482
CH_4	0.8195

Table 6-4. p-values for nitrous oxide (N_2O) and methane (CH_4) daily for the diurnal cycle of N_2O and CH_4 dynamics during a 24 h d period under three different burning regimes: annually burned in a flat surface (AB), no burned in a flat surface (NB), and annually burned in a slope (ABS).

Factor	**N_2O**	**CH_4**
Time (T)	0.2573	0.9700
Site (S)	<.0001	0.0783
T x S	0.0416	0.8915

Table 6-5. p-values of nitrous oxide (N_2O) and methane (CH_4) fluxes within sampling frequency for the greenhouse gases

Factor	N_2O	CH_4
Frequency (F)	0.7940	0.8474
Months (M)	<.0001	-
F x M	0.0164	-

Chapter 7 - Summary

Grazing systems have been identified as one of the main causes of current increases in atmospheric greenhouse gases (GHG) and global climate change. Understanding the effects of beef production on the environmental systems provide better tools to the agricultural, scientific, and the governmental community regarding the future management practices of the cattle management to tackle its effect on GHG and increasing cattle systems resilience to climate variability (Campbell et al., 2016). Current environmental assessments for restore grazed pastures, increase cattle resilience and decrease its environmental footprint includes biotechnology application in animal and pastures traits, and efforts to integrate soil and water conservation practices (Peters et al., 2013; Steiner et al., 2014; Rao et al. 2015, Rojas-Downing et al., 2017).

This project aimed to provide a framework on N dynamics in grazed systems by quantifying soil nitrous oxide (N_2O) and methane (CH_4) fluxes, inorganic N dynamics, and the soil microbial community interaction in two temperate grazed prairies in Kansas, USA and a tropical pasture in Cali, Colombia. The general objective was to quantify soil N_2O and CH_4 fluxes, inorganic nitrogen (N), and soil microbial dynamics in temperate grazed systems. Also, our research attempts to provide a framework of soil N_2O and CH_4 flux dynamics, differences in the diurnal cycle fluxes, and accuracy in GHG cumulative estimations considering sampling frequency.

From the research at Konza Prairie Biological Station, from July 2014 to December 2017, we conclude that N_2O fluxes were relatively low and varied as a source or a sink of N_2O; and the grassland was mostly a CH_4 sink. Similarly to Allard et al. (2007), we conclude the role of

grazing systems on unfertilized grasslands outweigh their N_2O and CH_4 emissions footprint under annually burned, and further CH_4 uptake capacity is 3-yr patch burning. Also, higher CH_4 sink capacity from 3 yr patch burning supports Fuhlendorf and Smeins (1999) findings who suggested patch burning provide habitat heterogeneity resulting in an increase on N_2O and CH_4 uptake, bolstering of N dynamics, and providing a more diverse ecological niche.

Results from the summer field study, during 2016 and 2017, focused on urine and manure patches under different water regimes (high precipitation, ambient conditions, and drought) registered significant changes in soil N dynamics, and N_2O and CH_4 fluxes. Additionally, results identified soil microbial communities in this temperate grassland were able to recover within 7 d to high precipitation conditions and N additions from urine and manure patches. Moreover, this research indicates N_2O and CH_4 fluxes cover about 0.22% and 0.19% of the total grazed area. Therefore, and agreeing with Ramirez et al. (2012) results, our conclusion implies that N depositions from urine and manure patches, from cattle grazing under low animal density, are not sufficient for causing significant detrimental environmental effects on temperate grazed pastures. As mentioned, conclusions were made under the studied conditions and we recommend considering spatial variability from grazed systems, animal density, and animal behavior to estimate urine and manure patches as an additional factor on grazed grassland GHG budget estimations.

The research at the International Center for Tropical Agriculture (CIAT) at Cali, Colombia aimed to provide information on N dynamics as a result of the plant-soil-microbial interactions considering the biological nitrification inhibitor capacity of two *Brachiaria* pastures. We conclude *Brachiaria* humidicola 16888 trait decreased soil nitrification rates, increased AMF and actinomycetes, suppress AOB during high nitrification rates, and produce more biomass.

Our conclusions recognize the efficiency of the biological nitrification inhibitor (BNI) from *Brachiaria* grasses to reducing N losses and controlling soil microbial communities.

Finally, by studying the temporal dynamics of N_2O and CH_4 fluxes in a temperate grazed grassland we determine N_2O fluxes are highly dynamics within a day and over the seasons, considering months during spring and summer, and as a result of burning regimes. We suggest that GHG sampling should be planned on a seasonal basis considering expected weather conditions. Estimating cumulative N_2O and CH_4 fluxes should consider strategies to reduce the influence of additional biotic factors therefore reducing estimation errors (Parkin and Kaspar, 2006; Mishurov and Kiely, 2011).

Further research should address the understanding of long-term resistance of grazing systems and how to optimize the synergy of agricultural practices and technology for nutrient cycling, water use and quality, soil carbon (C) storage, GHG mitigation, and other ecological services. Economic viability of integrating sustainable practices on extensive land areas and small stakeholders should also be developed. Some specifics projects to consider for future research could include the study of differences between GHG and N dynamics in different vegetation to have a better understanding of the effect of grass species, the aboveground biomass role on N_2O and CH_4 dynamics for sustain biodiversity, and its role in soil C sequestration capacity. These efforts could provide insight on the grasslands terrestrial sink capacity to offset emissions from grazed lands in temperate and tropical regions.

Our conclusions indicate temperate grasslands are a minimal source of N_2O and sink of CH_4. By controlling animal density, and soil cover with specific plant traits, such as *Brachiaria sp*., ranchers could increase nutrient efficiency, and reduce GHG losses from manure and urine patches and decrease the environmental footprint of grazed pastures.

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Appendix A - Supplemental data for Chapter 3

C1A

C3B

C3A

Figure A- 1. Experimental sites at Konza Prairie Biological Station. The watershed C1A was annually burned, C3B burned every 3 yr, burned in 2014 and 2017, and C3A also burned every 3 yr burned in 2013 and 2016. Benfield soils series was predominant in the three watersheds. This series was characterized on upland zones in grasslands covered by tallgrass prairie grasses.

Retrieved from: https://soilseries.sc.egov.usda.gov/OSD_Docs/B/BENFIELD.htm

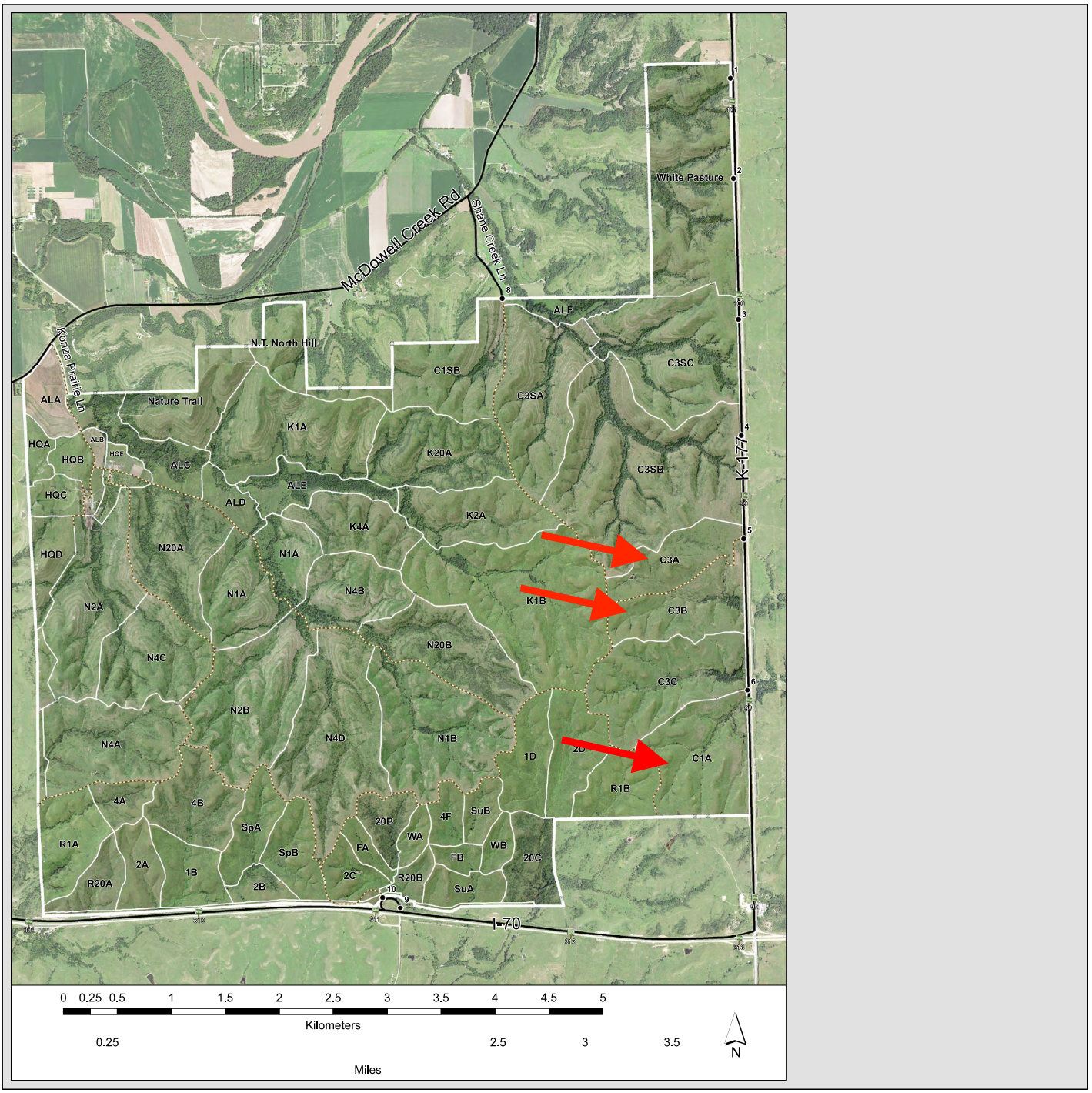

Figure A- 2. Konza Prairie Biological Station watershed map.

Retreived from: https://kpbs.konza.k-state.edu/treatments.html

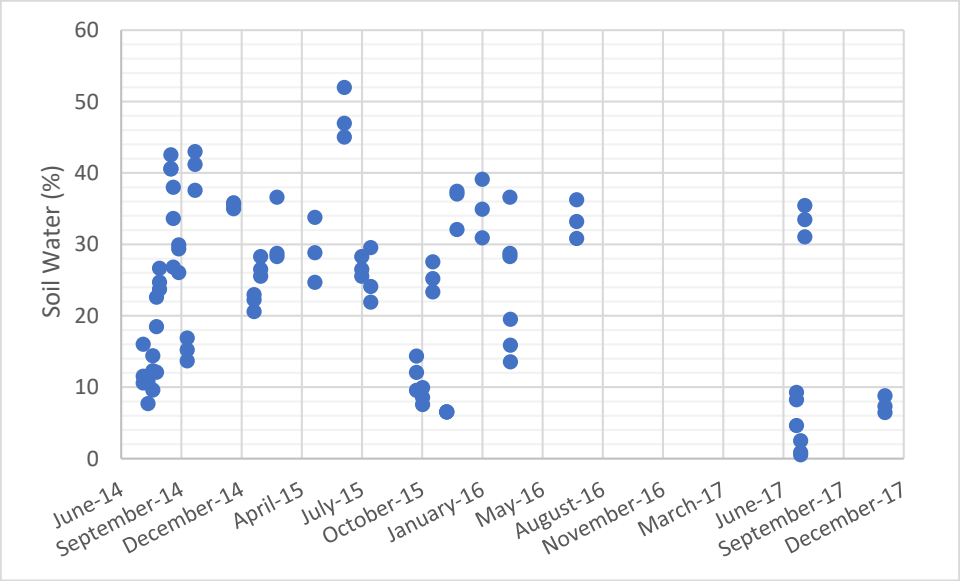

Figure A- 3. Soil water content dynamics in the watersheds C1A (annually burned), and C3A and C3B (3yr patch burned) at Konza prairie from June 2014 to December 2017.

Table A-1. Total precipitation (PCPN) (mm) during the GHG emissions. Total precipitation from June to December of 2014, January to December of 2015, 2016, and 2017. Data gathered from Konza Prairie Biological Station LTER.

Year	PCPN (mm)
2014	483
2015	1,000
2016	993
2017	726

Table A-2. Monthly precipitation (PCPN) (mm) during the GHG emissions data study. Summation of daily precipitation episodes by month from June to December of 2014, January to December of 2015, 2016, and 2017. Data gathered from data Konza Prairie Experimental Station LTER.

Month	2014	2015	2016	2017
	----------PCPN (mm)----------			
Jan	-	2.1	15.8	35.445
Feb	-	37.0	12.2	13.7
March	-	8.0	15.1	49.5
April	-	72.3	107.7	122.6
May	-	200.1	234.5	103.1
June	179.9	134.4	40.2	33.5
July	23.4	148.1	121.7	98.3
August	87.0	80.2	192.2	159
September	23.7	105.7	157.3	17.7
October	108.4	3.1	62.5	84.6
November	6.4	41.1	7.9	3.6
December	54.3	163.3	26.0	4.8

Table A-3. Average mean air temperature (ºC) during the GHG emissions data study. Average of monthly air temperature calculated from daily values from June to December of 2014, January to December of 2015, 2016, and 2017. Data gathered from data Konza Prairie Experimental Station LTER.

Month	2014	2015	2016	2017
	----------Mean Air temp (ºC) ----------			
Jan	-	1.9	3.2	3.6
Feb	-	2.9	3.8	5.1
March	-	4.2	8.1	7.9
April	-	10.7	11.4	11.8
May	-	14.5	14.7	14.6
June	20.7	20.1	20.6	20.2
July	22.1	23.2	23.7	23.2
August	22.9	23.0	23.5	21.8
September	19.8	21.7	21.9	20.7
October	15.7	17.4	17.0	16.5
November	8.4	12.5	13.7	10.4
December	5.8	7.2	6.0	6.9

Table A-4. Average mean soil temperature (ºC) during the GHG emissions data study. Average of monthly soil temperature calculated from daily values from June to December of 2014, January to December of 2015, and 2016, and January to July of 2017. Data gathered from data Konza Prairie Experimental Station LTER.

Month	2014	2015	2016	2017
	----------Mean Soil temp (ºC) ----------			
Jan	-	2.3	2.8	-2.0
Feb	-	2.4	4.3	-0.8
March	-	5.2	8.7	6.1
April	-	11.4	12.2	13.5
May	-	15.3	15.2	16.4
June	20.7	21.0	21.5	23.5
July	22.1	23.5	23.8	25.7
August	22.4	23.3	23.8	25.1
September	21.2	21.5	21.6	23.5
October	15.7	16.4	16.6	16.7
November	8.4	11.6	12.7	10.8
December	5.8	6.6	5.0	4.0

Equation A-1. Equation used for gas concentration by Ideal Gas Law:

Mol trace gas L^{-1}=

(V trace gas mol L^{-1} * $1L^{-1}$* P atm)/(0.08206 L atm mol^{-1} $^oK^{-1}$)*(273+T^{o}C)^{o}K

Where:

P=pressure V=chamber volume n= moles of gas

R= gas law constant T= temperature

Flux calculation:

flux (nL L^{-1} h^{-1}) = $(C_{A1,2}-C_0)^2/[t_{A1,2}x(2xC_{A1,2}-C_3-C_0)]x \ln[(C_{A1,2}-C_0)/(C_3-C_{A1,2})]$

Where:

C_0=headspace concentration at time 0

$C_{A1,2}$= average of the headspace concentrations at time 1 and 2

C_3= average headspace concentrations at time 3

$t_{A1,2}$= is the time interval corresponding to average of time 1 and 2

Table A-5. Net balance of the grassland (a), and the grazed grassland (b) from June to December 2014.

(a)

Watershed	CH_4	N_2O	Grassland balance
	kg CO_2-eq ha^{-1} $year^{-1}$		
C1A	-19	3	-16
C3A	-24	17	-7
C3B	-22	16	-6

(b)

Watershed	CH_4-C	Grazed grassland balance
	kg CO_2-eq ha^{-1} $year^{-1}$	CO_2-eq kg cow/calf land unit $year^{-1}$
C1A	-5.9	1.8
C3A	-7.5	0.1
C3B	-6.8	0.9

Table A-6. Significance of F values for the variables CH_4 and N_2O fluxes for the effects of date, burning regimes (watershed) and it interaction from July 2014 to December 2017.

Factors	**CH_4**	**N_2O**
Date (D)	<.0001	0.5231
Watershed (W)	0.4190	0.3757
D*W	0.2784	0.5957

Appendix B - Supplemental data for Chapter 4

Additional information on animal management at Lazy N Ranch

The Lazy N Ranch has 300 ha land use for cattle grazing from April to August (5 months). The cattle (*Bos taurus*) population is characterized as having 3 ha per cattle. The average cattle weight is 363 kg, and animals have a straight grass diet with an estimated 11 kg grass and 22.7 L of water consumed. Daily grass consumption was estimated by an approximate eating rate of 3% of it body weight per day. Water consumption was estimated to 1.9 to 3.8 L of water per each 45 kg of body weight; for this experiment, we used the value of 2.8 L of water per each 45 kg of body weight.

Table B-1. Laboratory analysis was run by SDK Laboratories (Hutchinson, KS).

Urine	
Ammonia	**Nitrate**
mg/kg	**mg/kg**
1950	208

Manure				
Moisture	**Dry matter**	**TKN- Total Kjeldah Nitrogen**	**P_2O_5-P**	**Potash-K_2O**
%	**%**	**mg/kg**	**mg/kg**	**mg/kg**
93.32	16.68	2990	1030	1020

Table B-2. F values for the factors precipitation patterns, urine and manure patches, and it interaction on N_2O and CH_4 flux accumulation over the incubation time.

Factor	N_2O	CH_4	N_2O	CH_4
	--------2016--------		--------2017--------	
Precipitation patterns (P)	0.147	0.7287	0.1157	0.4718
Cattle manure and urine (CMU)	0.1086	0.5297	0.4612	0.9879
P*CMU	0.7024	0.431	0.9461	0.7985

Table B-3. Nitrous oxide (N_2O), and methane (CH_4) flux accumulation during the 28 d incubation from ambient (AM), drought (DR) and high precipitation (HP) conditions, and control (CO), manure (MN), and urine (UR) patches.

	----------N_2O (g ha^{-1}) ----------			**----------CH_4 (g ha^{-1}) ----------**		
	AM	**DR**	**HP**	**AM**	**DR**	**HP**
	----------2016----------					
CO	-34.4	1.1	1.5	33.9	105.8	17.9
MN	-71.6	12.5	12.5	-44.7	153.8	28.0
UR	-24.4	64.2	31.6	51.0	44.8	74.8
	----------2017----------					
CO	7.2	67.1	108.8	18.9	-1.5	-131.5
MN	4.7	32.2	73.7	30.8	-121.1	-10.4
UR	22.6	72.8	149.9	-86.8	136.1	-28.9

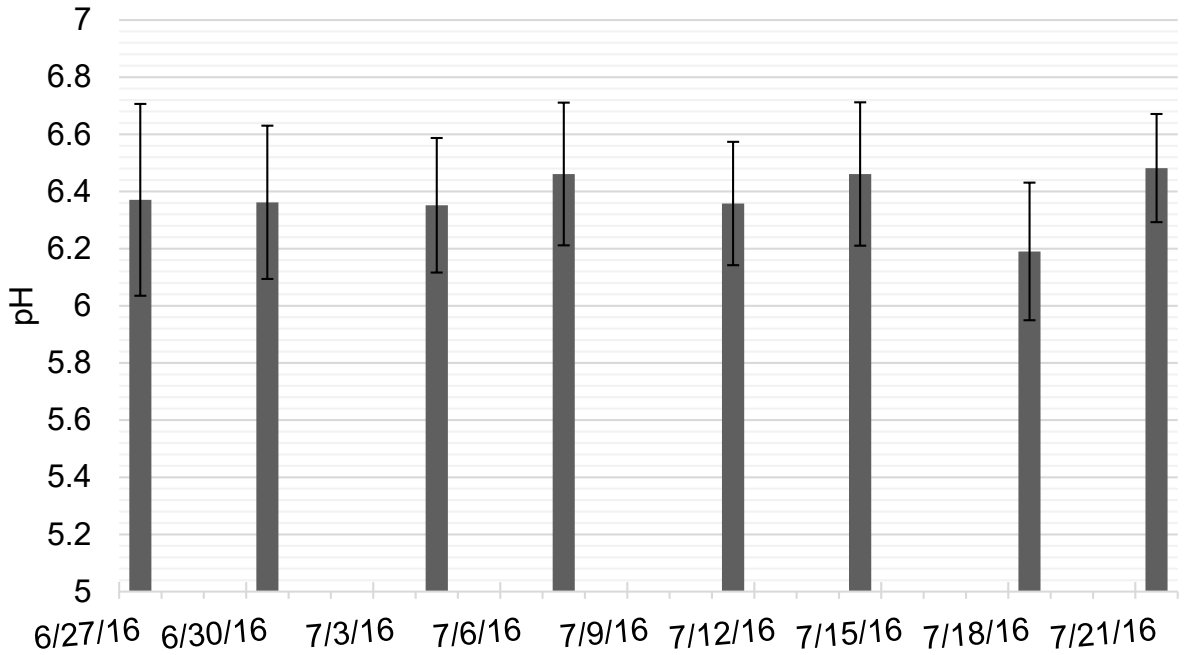

Figure B-1. Soil pH in the 28 d of incubation as a result of the interaction of the treatments under the cattle urine (UR), and manure (MA) patches and the control (CO) treatment under the precipitation conditions: high precipitation (HP), drought (DR), and control (CO). Error bars represent standard error of the mean.

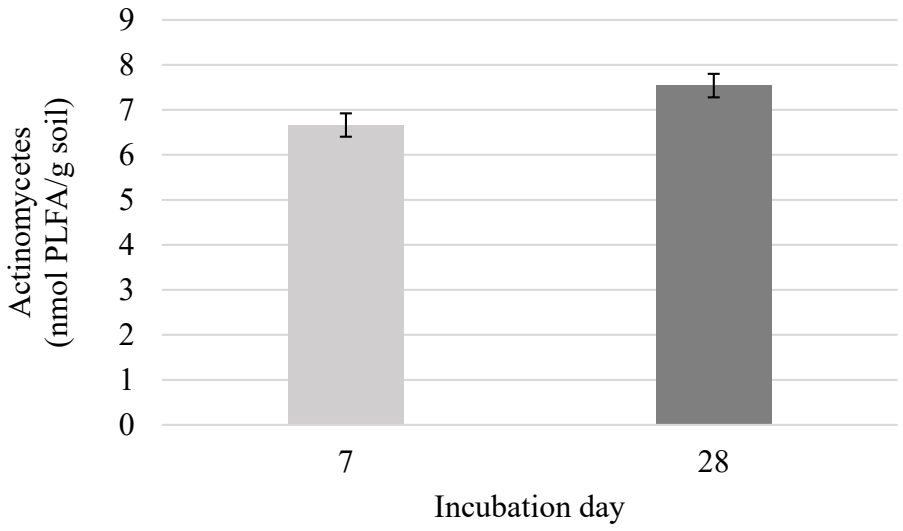

Figure B-2. Mean (n=4) changes in the actinomycetes group abundance from the 7 and 28 d of incubation. Overlapping standard error bars are not significantly different (LSD protected, $p<0.05$).

Appendix C - Supplemental data for Chapter 5

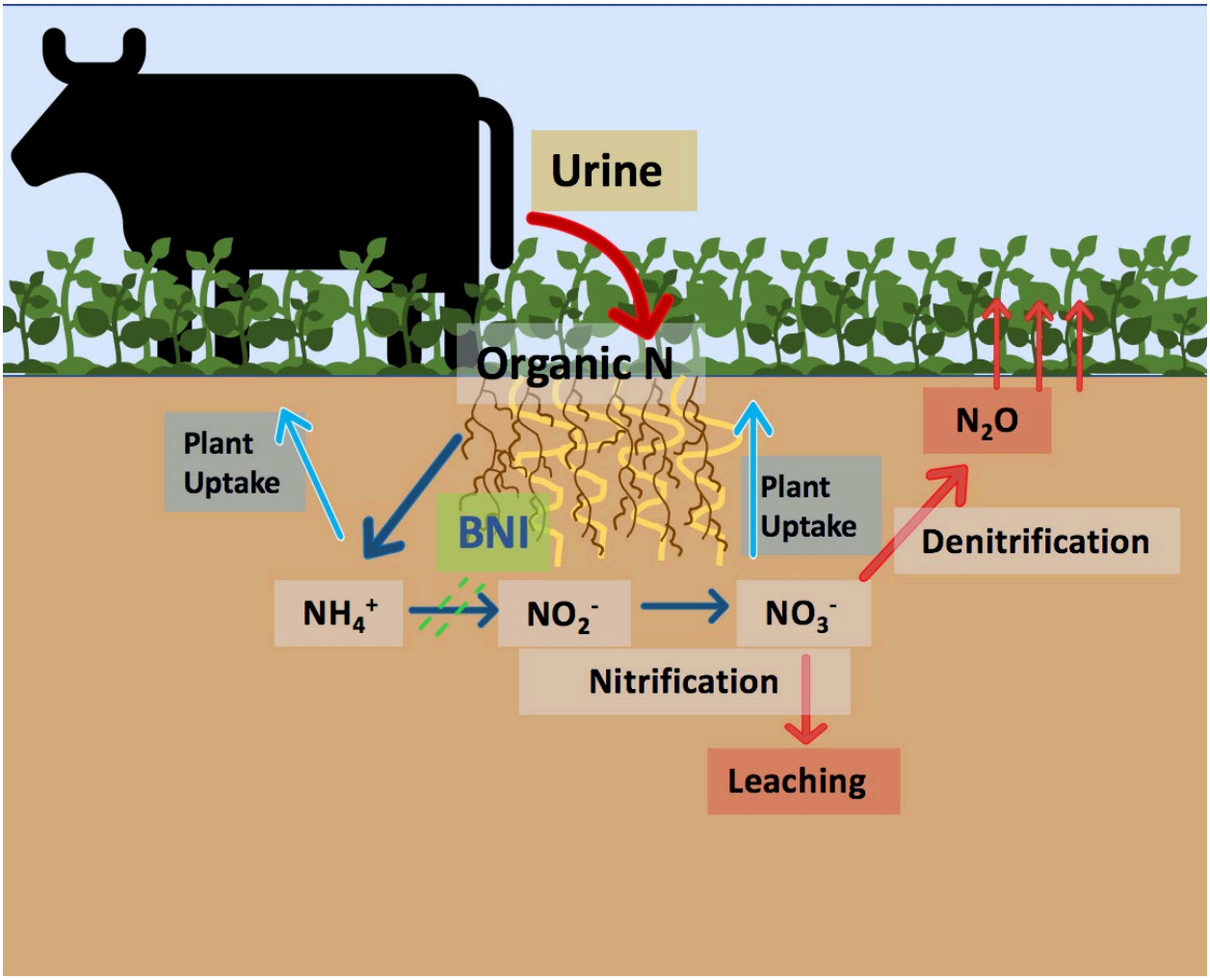

Figure C-1. Graphical abstract for the project of characterization of soil nitrogen (N) dynamics, as ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-), nitrous oxide (N_2O) fluxes, and soil microbial dynamics as influenced by biological nitrification inhibition (BNI) from *Brachiaria* grasses.

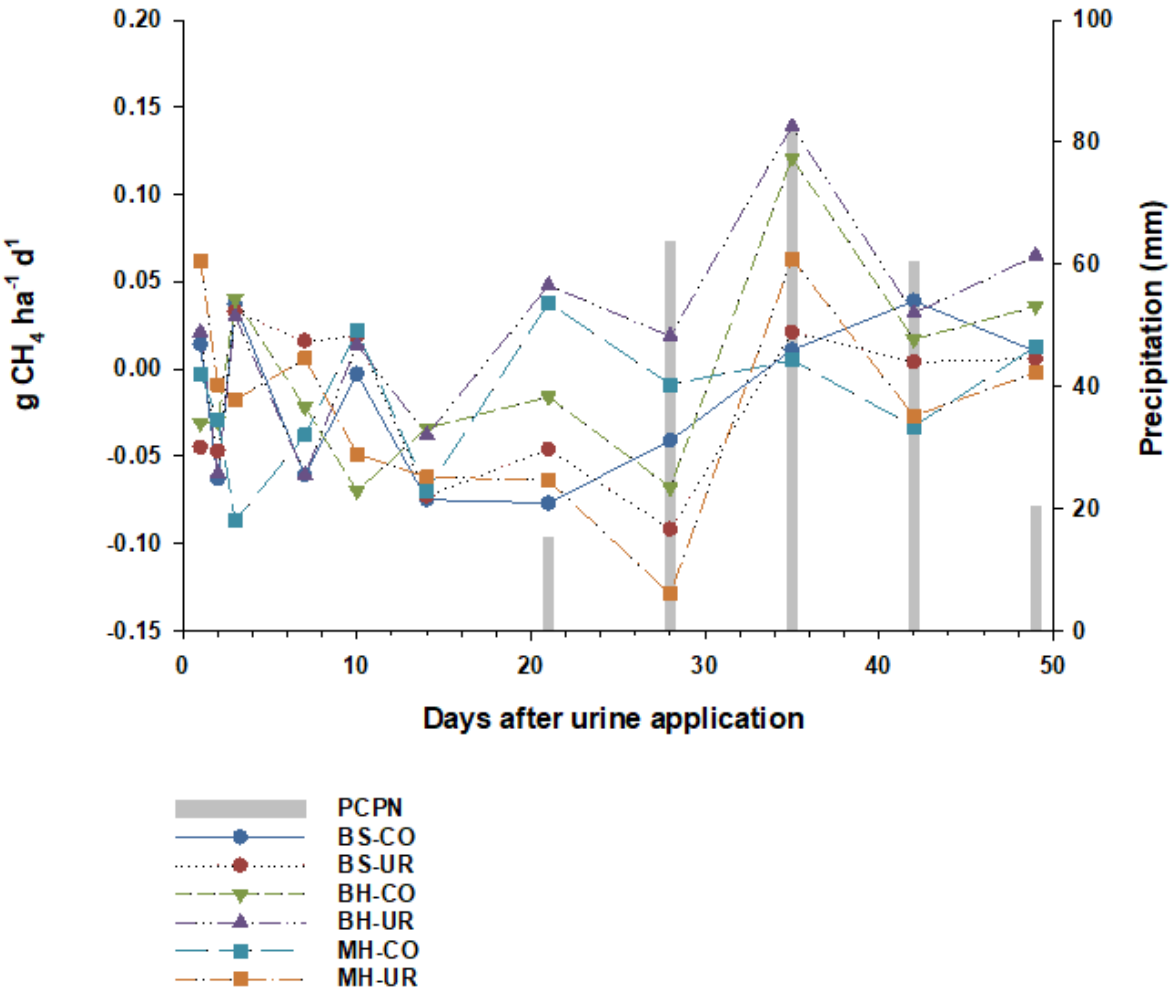

Figure C-2. Methane (CH_4) emissions over the 49 d. Six treatments were studied: bare soil (BS), Brachiara humidicola 16888 (BH), and *Brachiaria mulato* hybrid 1 (MH) each soil cover had a secondary treatment which was the application of bovine urine (UR), and the control (no nitrogen application).

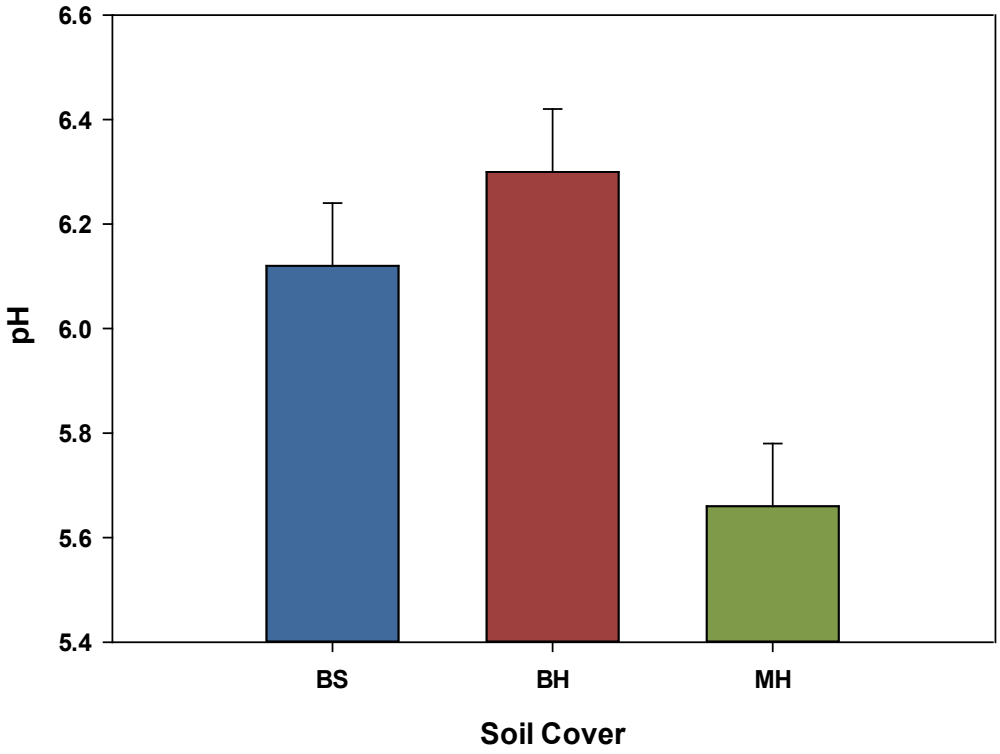

Figure C-3. Soil pH from the studied treatments of bare soil (BS), *Brachiara humidicola* 16888 (BH), and *Brachiaria mulato* hybrid 1 (MH) during the 48-d experiment. Significance differences were calculated by Proc Mixed $p>0.05$; bars represent the mean value and error bar indicate the standard deviation.

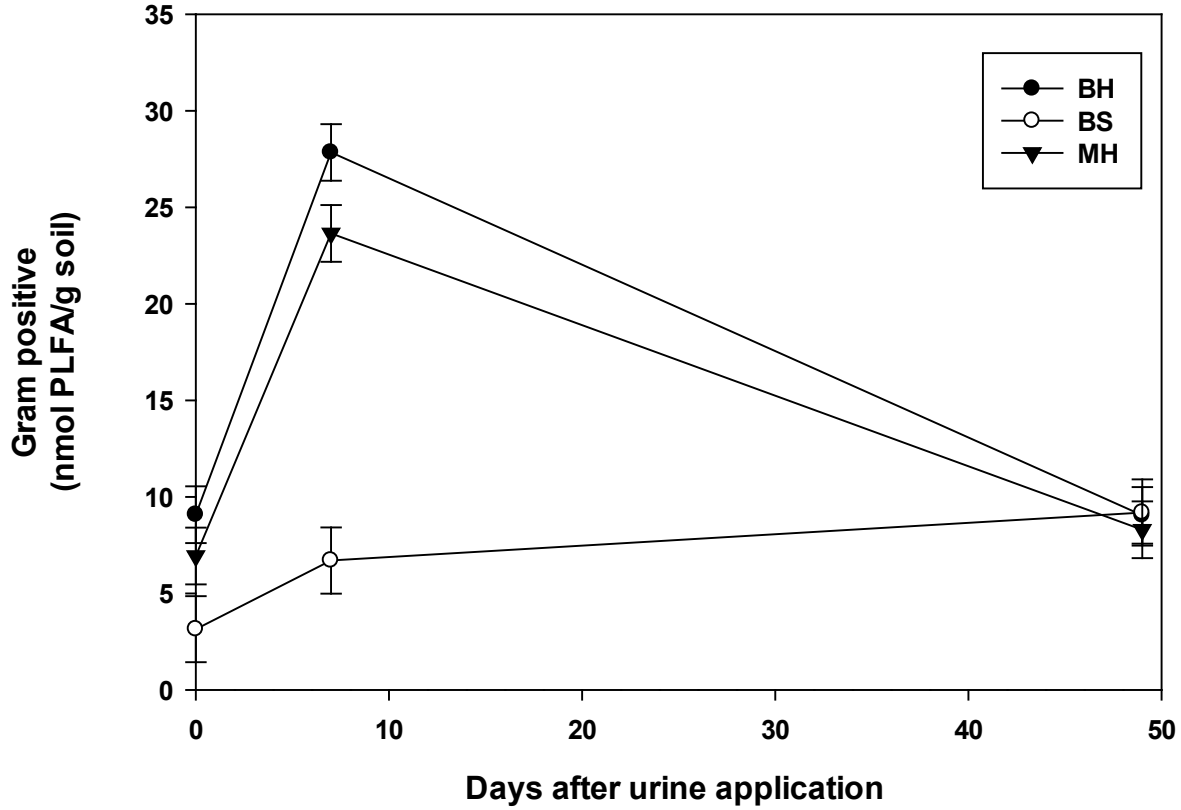

Figure C-4. Gram positive bacteria dynamics from bare soil (BS), *Brachiaria humidicola* 16888 (BH), and *Brachiaria mulato* hybrid 1 (MH) over time 2 h, 7 and 49 d after urine application. Significance differences were calculated by Proc Mixed $p>0.05$, errors bars indicate standard deviation.

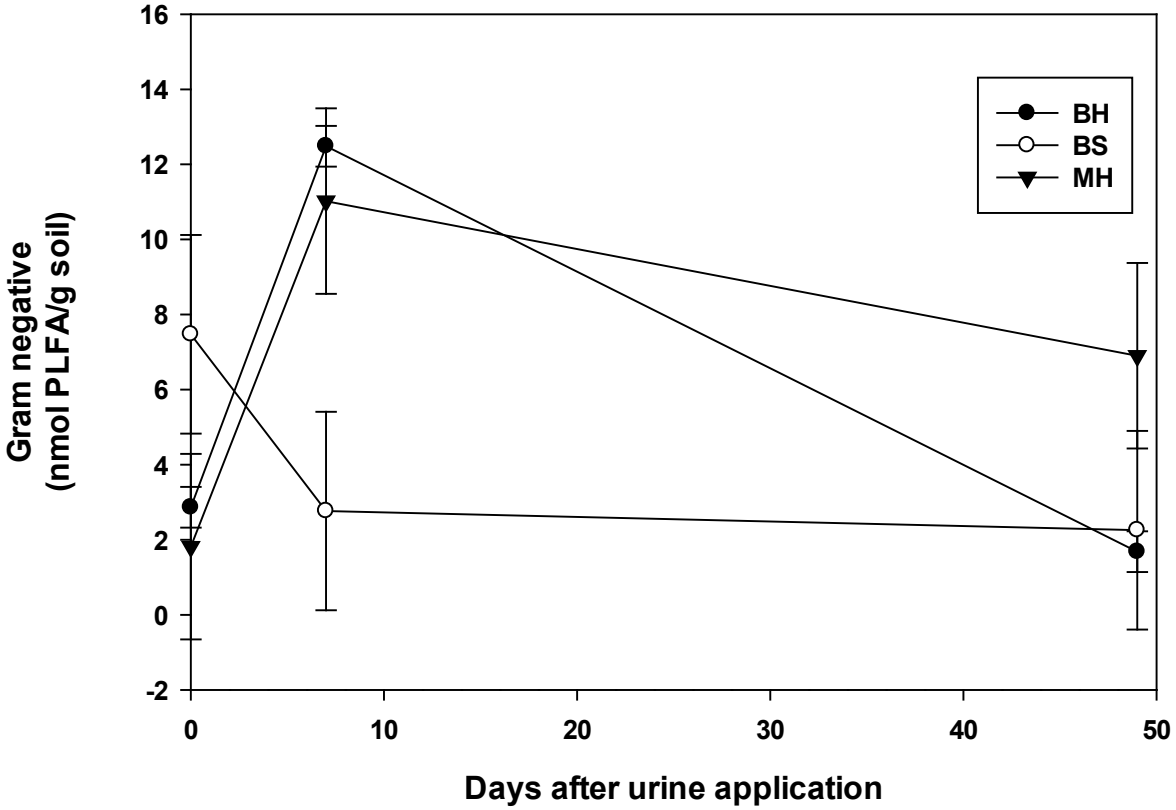

Figure C-5. Gram negative bacteria dynamics from bare soil (BS), *Brachiaria humidicola* 16888 (BH), and *Brachiaria mulato* hybrid 1 (MH) over time 2 h, 7 and 49 d after urine application. Significance differences were calculated by Proc Mixed $p>0.05$, errors bars indicate standard deviation.

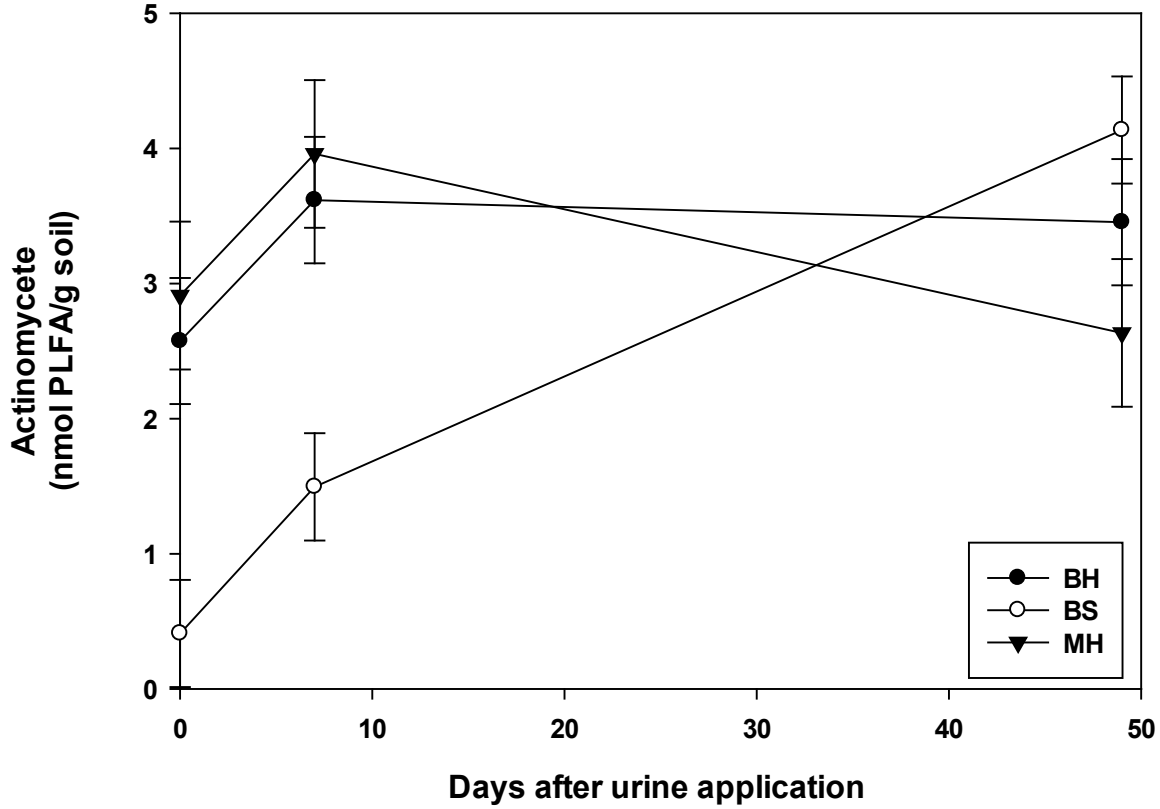

Figure C-6. Arbuscular mycorrhizal fungi (AMF) dynamics on bare soil (BS), *Brachiaria humidicola* 16888 (BH), and *Brachiaria mulato* hybrid 1 (MH) over time 2 h, 7 and 49 d after urine application. Significance differences were calculated by Proc Mixed $p>0.05$, errors bars indicate standard deviation.

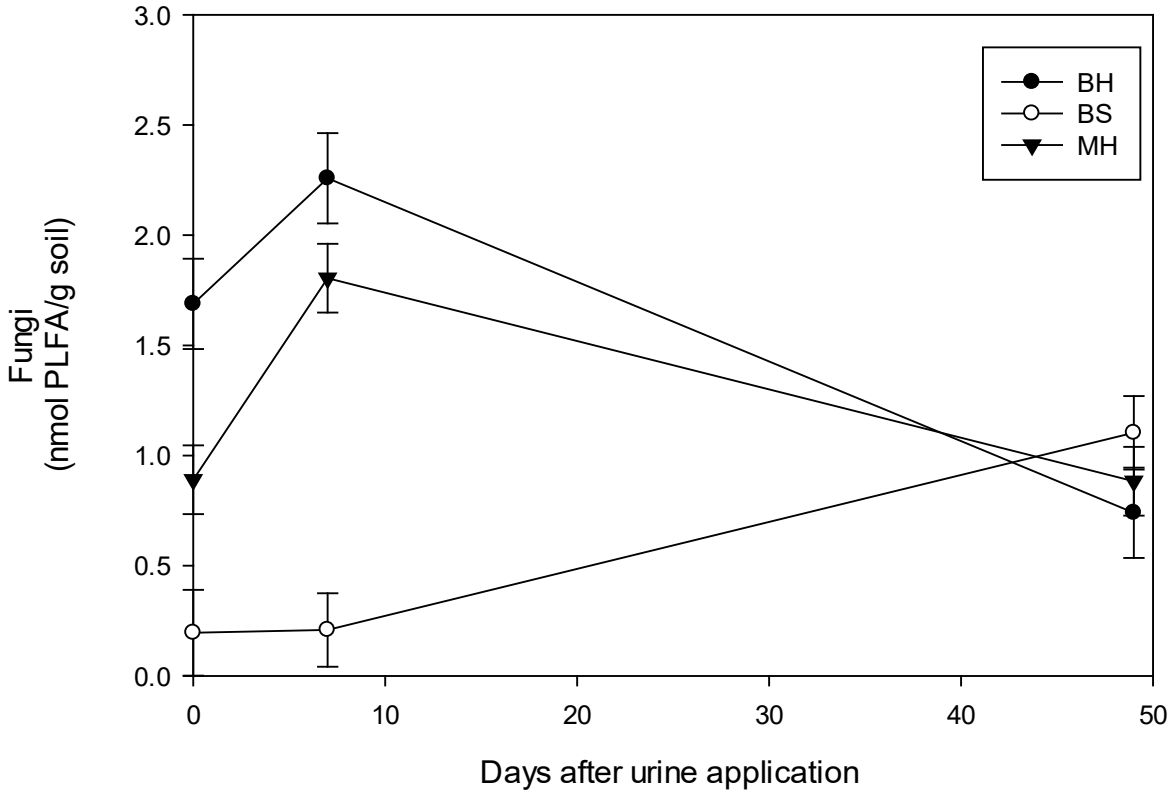

Figure C-7. Fungi dynamics on bare soil (BS), *Brachiaria humidicola* 16888 (BH), and *Brachiaria mulato* hybrid 1 (MH) over time 2 h, 7 and 49 d after urine application. Significance differences were calculated by Proc Mixed $p>0.05$, errors bars indicate standard deviation.

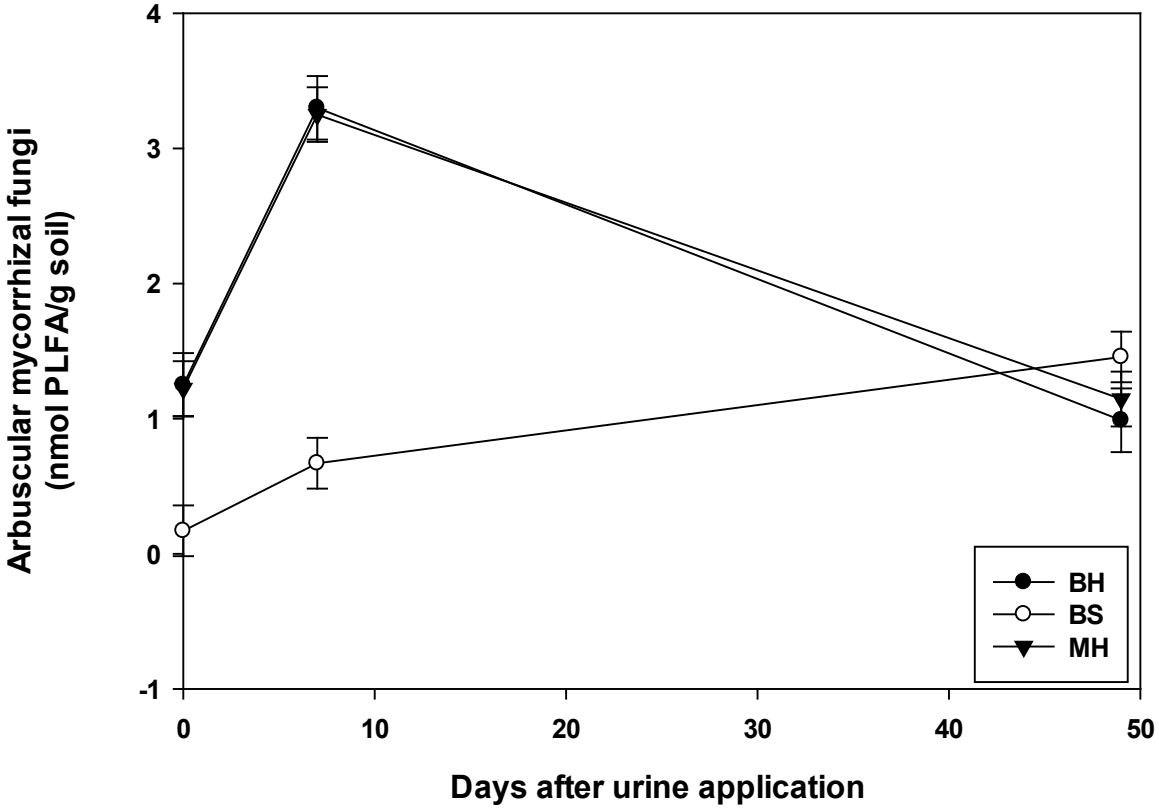

Figure C-8. Arbuscular mychorrizal fungi (AMF) dynamics on bare soil (BS), *Brachiaria humidicola* 16888 (BH), and *Brachiaria mulato* hybrid 1 (MH) over time 2 h, 7 and 49 d after urine application. Significance differences were calculated by Proc Mixed $p>0.05$, errors bars indicate standard deviation.

Appendix D - Supplemental data for Chapter 6

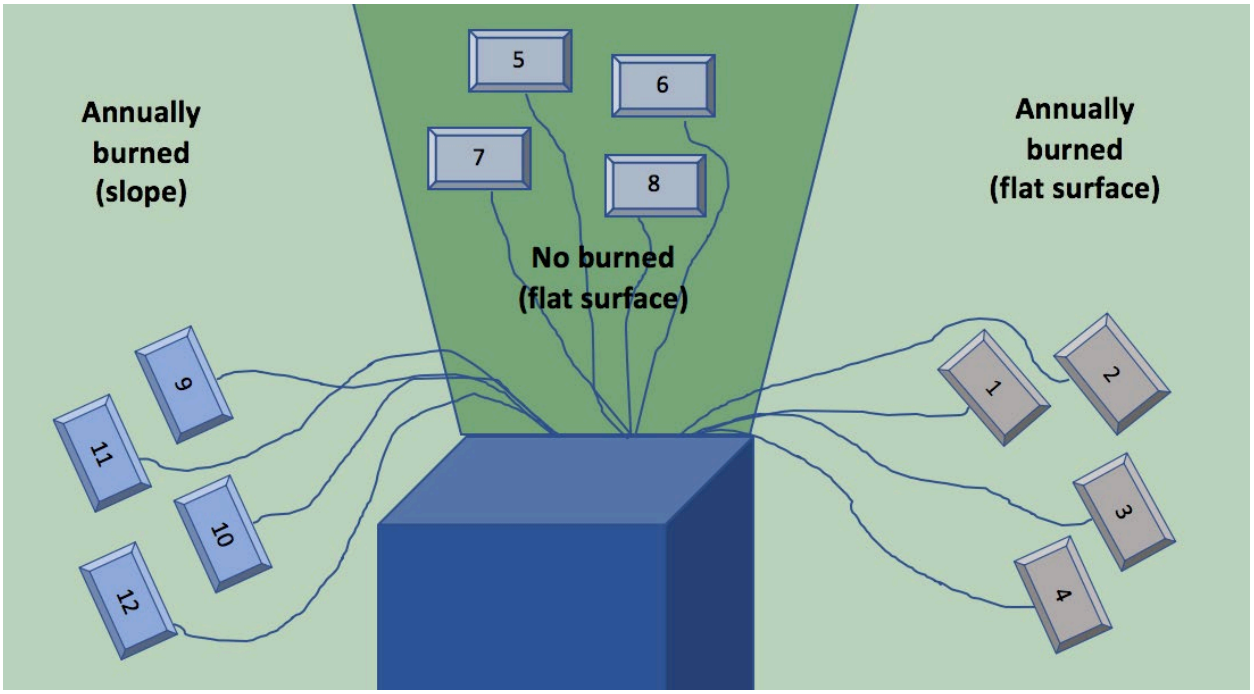

Figure D-1. Schematic design of experimental site at Lazy N Ranch with the three studied areas of annually burned in a flat surface, no burned in a flat surface, and annually burned in a slope.

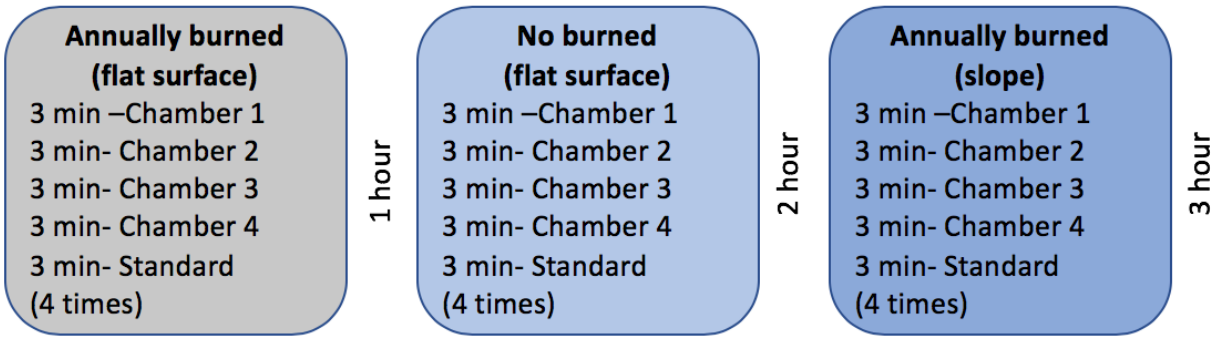

Figure D-2. The twelve chamber sampling sequence divided in the three plots.

Equation D- 1. Considering the ideal gas law, the mol volume needs to be corrected for air pressure and temperature as described by Butterbach et al. (2011):

$$\frac{Chamber\ vol\ (m^3) mol\ weight\ (g\ mol^{-1}) slope (ppmv\ min^{-1})}{chamber\ area\ (m^2) mol\ vol\ of\ CH_4 or\ N_2O\ (m^3\ mol^{-1})} 60x10^6$$

Appendix E - Supplemental data for Quality Control and Quality Assurance: Greenhouse gas sampling and analysis

Before each sampling, each vial of 20 mL (22x75 mm) glass vial (Wheaton, New Jersey, USA) was closed with a 20 mm gray butyl stopper (Labco Limited, Wales, UK) and sealed with a 20 mm unlined seal open top aluminum (Labco Limited, Wales, UK). Each vial was vacuum evacuated the four following the next steps:

1. 3 min vacuum evacuated
2. 30 sec with flow of Helium
3. 3 min vacuum evacuated
4. Using an empty syringe, each vial was checked if it was completely evacuated.

Once vials were ready for sampling, each vial was labeled with numbers corresponding to the plot or watershed the chamber and time of sampling. An identification list with the vials numeral code was taken to each field sampling; in this same list, we also gathered information from each sampling such as air temperature, abnormal conditions in the area, and soil water data in case of malfunction of the phone application for the Stevens Water Monitoring Systems (Stevens Water Monitoring Systems, Inc., Portland, Oregon, USA). Additionally, two extra vials were taken to the field in case of a previously labeled vial went missing or broken.

Gas samples were collected by placing a closed vented chamber cap over the buried anchor, sealing the division between the buried anchor and the vented chamber cap with a 3.5 to 7 cm rubber band. Gas samples inside the chamber were taken by using a 25 mL syringe with a 0.6 mm x 25 mm needle (BD PrecisionGlideTM Needle, Franklin Lakes, NJ, USA). The sampling began by placing a chamber over the PVC core and sealing it with a rubber strap. Once the chamber was sealed, the first sample (0 min) was taken and then successive samples

were taken 15, 30, and 45 min after sealing; each gas samples was transferred to the previously clean, evacuated vial, and labeled vial.

Gas samples were analyzed for CO_2, CH_4 and N_2O using a Bruker Scion 456 Gas Chromatography (Scion Instruments©, Austin, TX). The GC was calibrated daily using analytical-grade standards containing 0.2, 0.512, 3.5, and 15.3 mg kg^{-1} N_2O, 4.0 mg kg^{-1} CH_4, and 495, 800, and 993 mg kg^{-1} CO_2. Each daily sampling set (45 samples for Konza project (Chapter 3), and 81 samples for the summer 2016, and 2017 study (Chapter 4)) was analyzed under the same calibration. The samples were analyzed in batches of 30 samples; each batch had a standard vial with the concentrations of 0.2 mg kg^{-1} N_2O, 4.0 mg kg^{-1} CH_4, and 495 mg kg^{-1} CO_2 in position 1, 16, and 30 to assure reading quality of the GC during the samples analysis.

After the GC analysis, the standards samples were verified to determine reading accuracy. Also, for assuring the chamber was sealed an R^2 of the CO_2 concentrations were calculated for each chamber expecting an R^2 higher than 0.85; in case of a $R^2<0.85$ all data set was not considered for the flux analysis. Also, samples with values higher than the standard deviation of the mean were not considered in the flux estimations. During the gas concentration and flux calculation analysis, when missing temperature data we considered temperature data gathered from the Konza LTER Experimental Station Headquarters (Chapter 3) (https://climhy.lternet.edu) and the National Weather Service Forecast Office (Chapter 4) (http://w2.weather.gov/climate/index.php?wfo=top). For the study in Chapter 3, from summer 2014 to December 2017, about 18% of the total sample days were discarded. In the case of Chapter 4, about 20% of the samples were discarded.

In the case of Chapter 6, the FluxNet 3.3 Software (Baldochi et al., 2001) considered the CH_4 and N_2O concentration files, the temperature file, and the status file in which every

troubleshooting was registered therefore identifying inaccurate data files or "broken files," and removing them from the flux calculation. Also, the FluxNet output file was reviewed, and data with the R^2 >0.85 was considered for the statistical analysis.

References:

Baldocchi, D., Falge, E., Gu, L.H., Olson, R., Hollinger, D., Running, S., Anthoni, P., Bernhofer, C., Davis, K., Evans, R., Fuentes, J., Goldstein, A., Katul, G., Law, B., Lee, X.H., Malhi, Y., Meyers, T., Munger, W., Oechel, W., Paw U, K.T., Pilegaard, K., Schmid, H.P., Valentini, R., Verma, S., Vesala, T., Wilson, K., Wofsy, S., 2001. FLUXNET: a new tool to study the temporal and spatial variability of ecosystem-scale carbon dioxide, water vapor, and energy flux densities. Bulletin of the American Meteorological Society 82 11, 2415-2434.