THE INFLUENCE OF COW-CALF GRAZING SYSTEMS ON CARBON FLUX

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ABSTRACT

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Grazing management has been identified as the most suitable means to increase forage production and reduce GHG emissions from the cow-calf sector, while potentially increasing carbon sequestration. The grazing management applied to pastures determines factors such as forage growth, residuals accumulation, manure distribution, and soil properties, which create favorable or unfavorable conditions for microbial populations to develop. Methane, N_2O and CO_2 production and consumption in soils are microbial processes. Forage maturity determines forage quality and CH_4 production in the rumen. This study applied a system-based approach to assess net GHG exchange, in terms of C equivalent, and soil organic C accumulation in pastures grazed with cow-calf herds under different stocking rate and densities, and non-grazed pasture sites. Data were collected post-grazing and at a farm-scale and the variability associated to GHG emissions from pasture soils was very large. CO_2 emissions did not differ between systems. Soil and ambient temperature and soil water content had effects on CO_2 emissions. The effect of grazing was not conclusively observed on CH_4 and N_2O emissions. In addition, soil and ambient temperature and soil water content did not conclusively explain CH_4 and N_2O emissions. Other soil properties might be better predictors of CH_4 and N_2O, such as water filled pore space (WFPP) or soil O_2 content. Further research is needed to confirm the effect of WFPS and O_2 content on GHG emissions. We did not observe any clear trade-offs between GHG; generally GHG emissions increased from 2011 to 2013, which was likely associated to weather changes. Our results indicate that grazing management did not affect daily enteric CH_4 emissions from

lactating beef cows. Additionally, CH_4 emissions tended to be lower than reported values for lactating beef cows. The selective grazing allowed cows managed with different grazing strategies to eat forage with similar qualities that met nutritional requirements with reduced CH_4 emissions. Results indicate forage quality might be a better predictor to daily CH_4 emissions than DMI. Grazing systems resulted in higher C equivalent flux than non-grazed pasture sites, which was a result mainly of enteric CH_4 emissions. However, the effect of greater enteric CH_4 contribution from high stocking rate systems was offset by GHG exchange from the soil, and C equivalent flux was not different between grazing systems. High stocking rate, low stocking density system potentially increased total SOC stock, the addition of SOC to deeper layers and SOM. However, low stocking rate, high stocking density systems accrue litter on top of the soil. SOM decomposition rate was slower on the low stocking rate, high stocking density system, which could allow for greater resilience to adverse conditions. Long-term research is needed to confirm SOC sequestration potential of these systems and SOM decomposition rates. Grazing management should be adaptive and farm management decisions are inherent to management. Both grazing systems have opportunities to improve ecosystems services at the farm level, including animal production and food provisioning.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

ADF……Acid detergent fiber

AT……..Ambient temperature

BD……..Bulk density

BW……Body weight

$BW^{0.75}$…….Metabolic body weight

C…….Carbon

Ceq…….Carbon equivalent

Ceq_{flux}…….Net GHG exchange in terms of C equivalent

CH_4…….Methane

CO_2……Carbon dioxide

CP…….....Crude protein

Cr_2O_3……..Chromium oxide

DM…….Dry matter

DMI……....Dry matter intake

DNDF…….Digestible neutral detergent fiber

DNDFI……Digestible neutral detergent fiber intake

ECD……Electron capture detector

$F_{CH4cows}$……...C equivalent flux of enteric CH4 from cows

$F_{CH4soil}$……C equivalent flux of CH4 from pasture soil

F_{CO2}…….C equivalent flux of CO2 from pasture soil

FID……..Flame ionization detector

F_{N2O}…….....C equivalent flux of N2O from pasture soil

GC……..….Gas chromatograph

GE……..….Gross energy

GEI……..…Gross energy intake

GHG……..…..Greenhouse gas

IVTD……..…….*In vitro* true digestibility

LU……..…….Livestock unit

LW……..……Live weight

N…….Nitrogen

N_2…….....Nitrogen gas

N_2O…….Nitrous oxide

NDF……....Neutral detergent fiber

NDFI……...Neutral detergent fiber intake

O_2……Oxygen

SF_6……...Sulfur hexafluoride

SOC…….....Soil organic carbon

SOM……Soil organic matter

ST……Soil temperature

TSN…….Total soil nitrogen

WC……..Water content in the soil

WFPS……..Water filled pore space

CHAPTER 1

LITERATURE REVIEW

1.1. Introduction

The competitive beef market and consumer concern about climate change and global warming have led the beef industry to search for production systems capable of maintaining beef supplies while mitigating environmental impacts. In this evolving context, numerous questions are open for discussion. Research focused on beef production, in particular, has considered what role grazing systems play when discussions turn to global warming: should grazing systems be viewed as mitigation options or as active contributors to climate change?

Beef cattle production contributes to global climate change primarily through enteric methane (CH_4) production. The Environmental Protection Agency affirms that the cow-calf sector of the beef industry is the largest CH_4 emitter within U.S. livestock industries, responsible for 58% of all CH_4 emissions compared with 23% from the dairy sector and 19% from the feedlot and stocker sector (EPA, 2013). The agency suggests that total emissions from the beef cow-calf sector are high for several reasons such as; diets, consisting mainly of forages and of varying quality, are generally poorer than diets fed in the dairy or feedlot sectors; the level of management is typically not as good as in other sectors; and the cow-calf population is historically very large. In addition to enteric CH_4, cow-calf grazing systems are associated with other greenhouse gas (GHG) emissions. The urine nitrogen (N) breakdown in pasture soil results in nitrous oxide (N_2O) emissions (Flechard et al., 2007), and feces decomposition are associated to CH_4 emissions (Holter, 1997). On the other hand, Bodelier and Laanbroek (2004) have observed the potential of grassland soils to sink atmospheric CH_4, and Conant et al. (2001) suggested that improved grazing management potentially reduces atmospheric concentrations of carbon dioxide (CO_2) through carbon (C) sequestration on grazing lands.

Grazing management has been identified as the most suitable alternative to improve animal efficiency and reduce GHG emissions from the cow-calf sector (EPA, 2013) while potentially increasing carbon sequestration. The grazing management applied to pastures determines factors such as forage growth, residuals accumulation, and manure distribution. These factors affect soil properties (e.g. soil moisture, temperature, pH, aeration, and density) which create favorable or unfavorable conditions for microbial populations to develop. Methane, N_2O and CO_2 production and consumption in soils are microbial processes. Furthermore, forage growth determines forage quality and CH_4 production in the rumen.

Previous research have identified pasture soils as potential sink for atmospheric CH_4 (Bodelier and Laanbroek, 2004). Zhou et al. (2008) have suggested that grazing intensities have different impact on the structure of the methanotroph community. Nazaries et al. (2011) studying soil CH_4 fluxes from grassland soils observed an increase in CH_4 consumption following changes in soil management. Their work has postulated the hypothesis that methanotroph communities could be intentionally manipulated with field management to increase their CH_4 oxidation activity.

Ruminants excrete on average 85% of the ingested N, and excess dietary N is excreted mainly in the urine (Castillo et al., 2000). Urine deposition on grazed pasture soil provides optimal conditions for N_2O production. While more research is needed to precisely quantify N_2O emissions from beef cattle production systems and to identify specific options for emissions mitigation, efficiency improvements can reduce N_2O emissions (Eckard et al., 2010). Recycling and maintenance of N within the system reduce the need for inorganic fertilization and increase utilization of urine N. de Klein and Eckard (2008) suggested that if animal urine in grazing systems were spread more evenly across paddocks the effective N application rate from urine

deposition would decrease, potentially reducing N_2O emissions. Rotational grazing management has been associated to more evenly distribution of excreta on paddocks (White et al., 2001), but whether rotational grazing effectively reduces N_2O is unknown.

The effects of grazing management on C cycling and distribution have been evaluated before, however, literature does not yet suggest clear relationships between grazing management and C sequestration. Some studies have reported no effect of grazing on soil organic C (SOC; e.g. Milchunas and Laurenroth, 1993), others reported increases (Wienhold et al., 2001) or a decrease (Derner et al., 1997). The lack of clear relationship between C stocks and grazing management has been associated to climate, inherent soil properties, landscape position, plant community composition, and grazing management practices (Reeder and Schuman, 2002); factors that affect C cycling and sequestration potential on grasslands.

Research outcomes are variable with respect to the potential of grazing management to mitigate GHG emissions. Studies analyzed the following issues without searching for interactions within the system: enteric CH_4 production (e.g. Jones et al., 2011), N_2O emission from pasture soil (e.g. Horvath et al., 2010), and accumulation of SOC (e.g. Schipper and Sparling, 2011). However, a holistic view considers different ecological processes and their relationships, perhaps uncovering new relationships and conclusions.

The primary objective of this work was to apply a system-based approach quantifying GHG flux from 2 grazing management practices and identify the grazing practice that contributes the most to climate change mitigation. The next sections review the latest research conducted in GHG flux, soil properties in grazing systems, pasture management and systems-based approaches for estimating GHG flux in grazing systems.

1.2. Grazing management

The most common grazing management on rangelands is continuous year-round stocking. Previous research suggests that paddocks grazed continuously exhibit uneven herbage consumption, where certain plants are heavily grazed while others are lightly grazed or completely avoided (Witten et al., 2005).

Teague et al. (2004) suggest that the uneven grazing of the paddock initiates a spiral degradation that is accelerated during periods of below average precipitation. Briske et al. (2008) affirms rotational grazing is not superior to continuous grazing in terms of forage and animal productivity and question the efficacy of multi-paddock grazing management for maintaining or improving rangeland conditions. Teague et al. (2009) criticize that Briske et al. (2008) did not take into account plant and animal processes at appropriate spatial and temporal scale and the study was not adaptively managed to achieve desirable soil, vegetation and livestock goals, thus resulting in incorrect interpretations for rangeland management.

Rotational grazing embraces more than paddocks subdivisions and variable rest periods. Rotational grazing is conservation-oriented livestock grazing management with the objective to maintain or improve forage production and forage harvesting efficiency as results of soil chemical, physical and hydrological properties (Teague et al., 2011). Improvement of forage production is related to soil water infiltration rates and water-holding capacity (Snyman, 2003). Conservation-oriented grazing management, when well implemented, is accompanied by several environmental benefits. When the focus goes beyond animal and forage productivity, there is evidence that conservation-oriented grazing management is superior to continuous grazing (Teague et al., 2013). Van der Ploeg et al. (2006) discussed the differences between research conducted at small-scale and focused on a few related parameters (reductionist research) and

systems research involving interaction among elements in whole systems that takes into account many related parameters. They suggested that reductionist research could result in completely different understanding of a subject than whole systems research.

1.3. Grazing management and enteric CH_4 emissions

When focusing on enteric CH_4, grazing animals pose a bigger challenge than feedlot animals because of difficulty to manipulate the diet. Pasture quality becomes a critical factor for achieving reduced CH_4 emissions from grazing animals (Boadi et al., 2004).

Implementing proper grazing management practices contributes to reduced CH_4 emissions from pasture-based systems. Improved grazing management practices that focus on reduction of fiber content of the sward can increase animal productivity and decrease CH_4 per unit of product with less dietary energy lost as CH_4 (Beauchemin et al., 2008). Improving forage quality tends to increase voluntary intake and reduce the retention time in the rumen, promoting more energetically-efficient post-ruminal digestion and reducing the proportion of dietary energy converted to CH_4 (Blaxter and Clappert, 1965).

Forage type affects CH_4 production; legumes often produce lower CH_4 than grasses (McCaughey et al., 1999; Van Dorland et al., 2007). Legumes have lower proportion of structural carbohydrates (lower fiber content), presence of condensed tannins, and faster rate of passage when compared to grasses (Beauchemin et al., 2008). These legumes characteristics have the potential to shift fermentation patterns towards higher propionate production rather than acetate (Johnson and Johnson, 1995).

The grazing management debate between continuous and rotational grazing is included in previous research quantifying enteric CH_4 emissions. McCaughey et al. (1997) observed that CH_4 production was greater from steers continuously grazed at low stocking rates (1.1 steers ha^{-}

^{1}; 306.7 L d^{-1}) than from steers grazed continuously at high stocking rates (2.2 steers ha^{-1}; 242.2 L d^{-1}). They did not observe differences in dry matter intake from steers grazed at different stocking rates. Steers grazing mixed pastures composed of alfalfa and grass emitted 25% less CH_4 than steers grazing grass only pastures (McCaughey et al. 1997).

Methane production tends to increase with forage maturity. Grazing during the beginning of the growing season, when forage is in vegetative stage, produced emissions from steers that were 29% to 45% less compared with steers grazing during mid and late season, when forage is in the reproductive stage (Boadi et al. 2002). Chaves et al. (2006) observed that cattle grazing alfalfa sward in advanced stage of maturity produced more CH_4 as compared with cattle grazing young grass swards. Further research should focus on holistic systems views to establish the relationships among improvements in diet quality, dry matter intake, stocking rates, and net CH_4 production from different production systems (Eckard et al., 2010).

1.4. Grazing management and CH_4 flux from grassland soil

The production and consumption of CH_4 in soils results from biological processes and therefore is directly affected by weather conditions and soil management. Methanogens and methanotrophs have different requirements for growth on the soil. Methanogens are strictly anaerobic *Archaea* that require low redox potential to develop. Methanotrophs are mainly aerobic bacteria with a unique metabolism that uses CH_4 as the only source of C and energy through oxidation into CO_2 (Henneberger et al., 2012). Van den Pol-Van Dasselar et al. (1999) suggests that the production of CH_4 in the soil is mainly controlled by moisture, aeration, type and quantity of soil organic matter (SOM), pH and temperature. The main factors affecting CH_4 consumption are concentrations of CH_4 and oxygen (O_2) in the soil and size of the methanotrophs community. Both methanogens and methanotrophs can be found in various soil

types, but soil properties might support one type of microbial community over the other (Murrell, 2010). The oxidation of CH_4 in aerobic soils represents an important mitigation strategy for atmospheric CH_4 (Lowe, 2006). Saggar et al. (2008) reviewed studies about CH_4 consumption in soils and concluded that forest soils demonstrated the highest potential for CH_4 consumption, followed by pasture soils.

Although grasslands have been identified as a sink for atmospheric CH_4, feces deposited by grazing animals are a CH_4 source. Feces are high in moisture content, microbial communities, and readily available C substrates, which can induce high CH_4 production levels immediately following deposition. Because methanogens are strictly anaerobic, their development in feces is dependent on maintenance of moisture content. Feces deposited on pastures usually dry quickly leading to short term CH_4 emissions. The pattern of CH_4 emissions from feces is quite constant, beginning with an initial peak of emissions and quick declining over time. However, the duration of CH_4 emissions after feces deposition on pasture soils is dependent on weather conditions and previous studies have shown different results. Williams (1993) confirmed that CH_4 emissions from feces in a hot, dry climate cease after 2 d during summer and 3 d during winter. Flessa et al. (1996) found longer emissions lasting approximately 20 d, but decreasing over time as aerobic conditions predominated. More recent studies have shown high levels of CH_4 emissions from feces during the first 2 week following deposition and decreasing over time, but lasting for a period of approximately 35 d (Sherlock et al., 2002). Saggar et al. (2008) point out that after feces decomposition the soil beneath the feces started to oxidize CH_4, becoming a sink for atmospheric CH_4.

1.5. Grazing management and N_2O flux from grassland soil

Intensively managed grasslands are a potential source of N_2O due to soil fertilization and urine deposition by grazing animals (Rafique et al., 2011). Nitrous oxide emission is strongly affected by the rate and timing of organic and inorganic N applications. However, other factors that act as controls for N_2O production are the availability of O_2, N content in the soil, soil moisture and aeration, temperature, pH, and readily available C content. Soil moisture content dictates either nitrification or denitrification as the major process producing N_2O.

Nitrogen fertilization combined with adequate soil moisture can induce N_2O emissions. Eckard et al. (2010) observed that when keeping the N fertilization rate constant, N_2O emissions were dependent on moisture. Luo et al. (2010) observed that the addition of N in small amounts at frequent intervals rather than a small number of large applications could reduce N_2O emissions. Uneven deposition of excreted N by grazing animals can result in "hotspots" of N application equivalent to an application of 400 to 2000 kg N ha^{-1} $year^{-1}$ in the small affected area (Watson et al., 2007). The large amount of N applied in a small area could result in N_2O emissions when combined to appropriate soil conditions to N_2O production (Eckard et al., 2010).

Methods to reduce N_2O emissions from grasslands have been studied. Eckard et al. (2010) affirms that restricting grazing on seasonally wet soils not only reduces N input from urine, but reduces soil compaction, which increases anaerobic conditions on the soil. The combined effect of reduced N fertilization and reduced grazing time on case study farms resulted in 10% reduction in total farm N_2O emissions (Schils et al., 2006). Rotational grazing with appropriate rest periods results in restricted grazing and avoids overgrazing that could result in lower N_2O emissions when compared to continuous grazing.

Improved grazing management provides an overall decrease in N input at farm-scale, by increasing N recycling within the system (Oenema et al., 2005). The introduction of legumes through rotational grazing management can increase soil N, resulting in superior fertility and decreasing the need for fertilization and potentially reducing N_2O emissions (Eckard et al., 2008).

1.6. Grazing management and soil properties

Soil functioning and ecosystem health of rangeland ecosystems rely on plant and litter cover, which are important to provide protection from soil loss and allow soil microbes to function (Bardgett et al., 2009). Plant and litter cover enhance water infiltration, buffer temperatures and decrease soil water evaporation, keeping moisture levels high. These factors enhance soil microbial activity, which promotes soils aggregate stability, sustain plant nutrient status and availability, improve plant growing conditions and result in the incorporation of soil organic matter (SOM). The soil-building factors that affect microbial activity consequently affect GHG production or consumption in the soil, as well as C stock and sequestration.

Grazing management practices affect soil-building factors. For instance, excessive grazing that causes excessive trampling can lead to soil degradation (Herrick and Jones, 2002). Soil degradation is associated with soil compaction and increased bulk density, elevates penetration resistance and reduces aggregate stability (Teague et al., 2011). Management that generates bare ground also causes soil degradation. Bare ground is exposed to the sunlight and temperature is increased, causing decreased microbial activity and accelerated loss of organic matter (Thurow, 1991). Elevated soil temperature and soil loss have a direct negative effect on infiltration rates, soil evaporation, nutrient retention and biological functions that contribute to ecosystem function (Neary et al., 1999). Soil degradation affects GHG production in the soil;

increases loss of C, and decreases potential to sequester C and consume CH_4 in grasslands by decreasing microbial development. Previous research has demonstrated that rotational grazing results in less bare ground, lower soil temperatures and higher soil C than continuous grazing at the same stocking rate (Teague et al., 2010).

The type and amount of vegetation cover influences soil physical parameters and hydrological properties. Plants producing greater amounts of foliage and root biomass increase organic matter aboveground through litter accumulation and belowground through dead root biomass decomposition (Milne and Haynes, 2004). Aboveground litter and plant cover create a more consistent temperature and moisture microenvironment, which in turn favors microbial activity (Devi and Yavada, 2006). These factors enhance formation of stable soil aggregates that increase water infiltration and could improve soil fertility and soil C sequestration potential.

Soil organic C constitutes approximately 60% of SOM and has beneficial effects on the chemical, physical and biological functions of soil (Bardgett et al., 2009). Soil organic C increases the cation-exchange capacity of the soil and water-holding capacity, and contributes to soil structure stability. Organic matter increases adsorption of nutrients, cations and trace elements that are of importance to plant growth. The maintenance of high contents of SOM contributes to overall soil health, as well as for atmospheric C sink (Lal, 2008).

Grassland SOM can be strongly influenced by management. Losses of SOM have occurred due to over grazing and poor pasture management (Conant et al., 2001). However, SOM losses can potentially be reversed and atmospheric C sequestered with good grazing management. Pastures soil C can be increased by eliminating disturbances to the soil and by increasing primary production. Management improvements intended to increase forage productivity usually increase soil C (Conant et al., 2003).

1.7. Carbon balance in grazing systems

The major challenge in whole-system C accounting research is the definition of assumptions and data selection. Often C accounting studies use previous published data and modeling. In that case, there are two important decisions that need attention from grazing beef cattle researchers: the inclusion or not of pastures C sequestration potential, and the choice of enteric CH_4 emissions factors.

The majority of models that estimate GHG balance in agriculture assume that established systems achieve equilibrium conditions in SOC stock and hence do not consider C sequestration potential of soils (e.g. Freibauer et al., 2004). Previous research has suggested that pastures lands may in fact sequester C on an on-going basis. Soussana et al. (2007) challenged the concept of C sink saturation in European grasslands, and Conant et al. (2003) propose that changes in management strategies may also change SOC dynamics. Although some footprint tools consider that pastures provide a greater range of ecosystem services than do agricultural croplands (Pelletier et al., 2001), the exclusion of C sequestration potential may result in a overestimation of GHG emissions.

Pelletier et al. (2010) assumed that pastures were in equilibrium and therefore not sequestering atmospheric C for a C accounting of different beef production systems. However, they performed a sensitivity analysis to include C sequestration potential and verify its impact on results. Phetteplace et al. (2001) estimates C sequestration of 0.12 Mg C ha^{-1} $year^{-1}$ for improved pastures managed with cow-calf herds and 0.40 Mg C ha^{-1} $year^{-1}$ for pastures recently converted to management-intensive grazing. When Pelletier et al. (2010) included the potential of C sequestration proposed by Phetteplace et al. (2001) in their accounting, GHG emissions per mass live weight kg produced would be 8.2 kg less for beef finished on intensively-grazed improved

pastures and hay during the transition phase. Grass-finished beef would produce 15% less GHG than feedlot-finished beef, and their conclusion would have been different. This is just an example of how results could change with different assumptions. The C sequestration rates suggests by Phettepplace et al. (2001) are estimates derived from very specific experimental conditions and other studies have found no C sequestration on pasture soils (Derner et al., 1997). More research is needed to elucidate C sequestration rates in grasslands ecosystems.

Given the importance of enteric CH_4 to overall GHG emissions in beef production, the choice of CH_4 emissions factors can strongly influence modeling outcome. For example, Pelletier et al. (2010) quantified GHG emissions from feedlots using a CH_4 emissions factor of 5.5% instead of the 3% suggested by the Intergovernamental Panel of Climate Change (IPCC), because their modeled diets did not contain 90% concentrates. They performed a sensitivity analysis to calculate the impact of changing the CH_4 emissions factor and found that estimated emissions would be 6% lower using the 3% factor. However, the sensitivity analysis considered the diet in question fed only during the finishing stage, which contributes 30% of total emissions.

Previous studies quantifying whole-system GHG emission from grazing beef cattle production verified that intensive grazing could reduce emissions compared with less intensive grazing beef systems (De Ramus et al., 2003). Best management practices in grazing systems could reduce enteric CH_4 emissions by as much as 22% compared to continuous grazing (De Ramus et al., 2003). The management strategy chosen and entered into the footprint tool influences greatly the output results. Pasture utilization rates may range from 30% to 90%, with the highest utilization rates achieved in well-managed pastures where cattle rotated through paddocks daily (Gerrish et al., 2002). Net emissions from grazing systems management at the high end of this range were lower and the resource efficiencies were improved compared to

grazing systems managed with 60% pasture utilization rates (Pelletier et al, 2010). In addition, most studies do not consider the potential benefits of organic-managed pastures and feed input production strategies. Research in Ireland suggested that organic beef production might lower emissions and improve resource use efficiencies (Casey and Holden, 2006).

Grazing management is also associated with stocking rates. The primary factor that modifies the C flux returned to the soil by excreta is the grazing pressure, which varies with the annual stocking rate. Secondary effects of grazing on the C cycle of a pasture include the role of excreta returns for SOM mineralization and N cycling (especially in nutrient-poor grasslands), and the role of hoof activity and defoliation by animals which reduce the leaf area and canopy photosynthesis (Soussana et al., 2010).

An integrated approach that allows the simultaneous quantification of the 3 most important GHG (CO_2, N_2O and CH_4) is desirable as management choices to reduce emissions involve potential trade-offs. For example, improving the primary production of grasslands by N fertilizer supply may favor belowground C storage but likely leads to increased N_2O and CH_4 emissions (Vuichard et al, 2007). There is a clear need to investigate different grasslands sites to further reduce uncertainties in C flux and GHG balances.

We propose the use of rotational grazing management practices to originate discussion of GHG emissions, in terms of C-equivalent (Ceq), and soil C accumulation in cow-calf grazing systems. A system-based approach is used to assess C accumulation and losses in pastures grazed with cow-calf management differing in stocking rate and density. We understand that the C cycle involves a large number of sources and sinks. However, the purpose of this study was to focus on GHG emissions from pasture soils, CH_4 emissions from cows and soil organic C accumulation in pasture soils. Future studies may add other components to the cycle, such as

microbial development and other gases emissions. The central hypothesis of this study was that rotational grazing with high stocking rate and low stocking density decreases enteric CH_4 emissions, but increases GHG emissions from pasture soil and decrease soil organic C stocks in the soil. The specific objectives of this study were to:

- Quantify CO_2, N_2O and CH_4 emissions from pasture soils grazed with cow-calf pairs managed with different stocking rate and density;
- Quantify enteric CH_4 emissions from grazing cows managed with different stocking rate and density;
- Quantify C and N stocks in pasture soils grazed with cow-calf pairs managed with different stocking rate and density;
- Apply a system-based approach to determine the net GHG emissions and C flux from cow-calf pairs managed with different stocking rate and density.

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CHAPTER 2

PASTURE-DERIVED GREENHOUSE GAS EMISSIONS IN COW-CALF GRAZING SYSTEMS

2.1. Introduction

Grazing management has been identified as the most suitable alternative to improve production efficiency and reduce GHG emissions from cow-calf systems (EPA, 2007). The grazing management applied to pastures determines factors such as forage growth, accumulation of residues, soil compaction, and manure distribution. These factors, in turn, affect soil properties (e.g. soil moisture, temperature, pH and aeration), which create favorable or unfavorable conditions for microbial population development. Given that production and consumption of CH_4, N_2O and CO_2 in soil are microbial processes, the flux of these important GHG from grassland soils are inter-dependent on grazing management.

Assessing the impact of land use and land use change on emissions requires attention to the relationships among all GHG (Robertson et al., 2000). In grasslands ecosystems, CO_2 is exchanged with the soil and vegetation, N_2O is emitted by soils, and CH_4 is emitted by grazing livestock and exchanged with the soil (Soussana et al., 2004). Management choices to reduce emissions involve important trade-offs. For example, preserving grasslands and adapting grasslands management to improve C sequestration may actually increase N_2O and CH_4 emissions at farm scale (Smith et al., 2001). Within grassland ecosystems, N_2O emissions tend to be highly variable in space and time (Oenema et al., 1997), mainly due to heterogeneous distribution of urine and variability of soils properties that control soil moisture (Mosier et al., 1998). Depending on factors that regulate the activity of soil microbes responsible for CH_4 production and consumption, grasslands soils can be small CH_4 sinks (Chan and Parkin, 2001) or sources (Allard et al., 2007).

There is a lack of information regarding the simultaneous evaluation of CO_2, CH_4 and N_2O in pasture soils. The objective of this study was to quantify GHG fluxes from pasture soils

grazed with cow-calf pairs managed with different stocking rate and density. The central hypothesis was that high stocking rate, low stocking density grazing systems resulted in greater GHG emissions from pasture soils, because of shorter rest periods resulting in more often manure deposition.

2.2. Material and Methods

2.2.1. Study site and pasture management

This study was conducted at Michigan State University Lake City AgBioResearch Center (latitude 44°18'N, longitude: 85°11'W; elevation 377 m) located in northwest MI. The region, developed over glaciated soils, was primarily deciduous and coniferous forest before farming entered the area in the 1860's. In the study site, 64% of the area is dominated by the Nester soil series, comprised of well drained sandy loams containing 1 to 6% slopes. The remaining area is dominated by the Kawkawlin soil series which is characterized by heavier soil texture and gentle slope (NRCS, 1999). Ongoing and historic weather data, including precipitation and maximum and minimum air temperature were obtained from an onsite weather station (NOAA, 2013).

Cow-calf pairs were managed with 2 rotational grazing management practices differing in stocking rate and density; an intensive system with high stocking rate and low stocking density, and an extensive system with low stocking rate and high stocking density. The system with low stocking rate and high stocking density (SysA) consisted of 120 cow-calf pairs rotating on a total of 120 ha, divided into 0.7 ha paddocks. Cow-calf pairs were moved to a new paddock 3 times daily (at approximately 0600 h, 1200 h and 1700 h). The equivalent stocking rate was 1 cow ha^{-1} and the stocking density was approximately 100,000 kg live weight (LW) ha^{-1}. The rest period varied from 60 to 90 d during the course of the growing season depending on plant growth. Cow-calf pairs grazed each paddock 2 to 3 times per year. The system with high

stocking rate and low stocking density (SysB) consisted of 4 cow-calf pairs rotating on 1.6 ha pasture, divided into 0.08 ha paddocks. Cow-calf pairs were moved to a new paddock once daily (at approximately 0800 h). The equivalent stocking rate was 2.5 cows ha^{-1} and the stocking density was 28,000 kg LW ha^{-1}. The rest period varied from 18 to 30 d during the course of the growing season depending on plant growth. Cow-calf pairs grazed each paddocks 4 to 5 times per year. The pasture sites in SysB were irrigated as needed, whereas there was no irrigation applied to SysA pasture sites. The only fertilization application was on SysB pasture sites that received urea fertilization (23 kg of actual urea) on June 3rd of 2011 (approximately 30 d before the start of gas sampling, see dates below).

In addition to these 2 systems, grazing-exclusion pasture sites (GE) were monitored in order to account for GHG emissions from non-grazed pastures. The use of a non-grazed pasture site was important to confirm that any differences found between SysA and SysB were attributed to the grazing management practices implemented.

Soil emission sample collection occurred in paddocks most recently occupied by cows. Animal management was dependent on forage growth; consequently different paddocks were assigned as pseudoreplicates for measurements of gas flux during each year and period. The use of pseudoreplicates might not be ideal, but justification for using this approach centers on long-term value of the grazing systems (Liebig et al., 2010) and monitoring at the farm scale. Controlled experiments using genuine replication, with all variables held constant except the treatment, usually deliver most definite results. However, the definition of genuine replications is not practical when addressing landscape ecological impacts and questions at farm scale (Hargrove and Pickering, 1992; Teague et al., 2011).

Cow-calf pairs in SysB were rotating through 0.08 ha paddocks. In order to use similar size paddocks as pseudoreplicates for the 3 treatments, 0.08 ha paddocks were randomly selected in SysA and GE area (3 pseudoreplicates per treatment).

Sample collection started on day 1 post-grazing and continued for 14 d in each paddock. The 14-d sampling period was repeated twice yearly in each of 3 years (2011 to 2013); at the beginning of the grazing season and at the end of the grazing season. The GE area was sampled in 2012 and 2013. During 2011, SysA and SysB paddocks were sampled twice daily; in the morning starting at 0900 h and ending at approximately 1200 h, and in the afternoon starting at 1500 h and ending at approximately 1800 h. Because the time of day did not have significant effect on GHG flux daily data were obtained. Based on results from 2011, sampling was performed once daily in the following years; starting at 0900 h and ending at approximately 1300 h.

The sampling dates were: from July 7th to August 3rd (period 1 - P1) and from August 13th to August 26th (period 2 - P2), 2011; from May 18th to May 31st (P1) and from August 21st to September 3rd (P2), 2012; from May 20th to June 3rd (P1) and August 26th to September 8th (P2), 2013.

2.2.2. Soil emission measurements

In order to characterize soil types in each paddock, ten 30-cm depth soil samples were collected randomly per paddock and composited into 1 sample. Samples were dried at 65°C and analyzed for particle size and pH by the Michigan State University Soil and Plant Nutrient Laboratory. Particle size analysis (clay, silt, and sand) was assessed using sedimentation as described by Bouyoucos (1951). Soil pH was measured potentiometrically in a 1:2.5 soil water suspension, with buffer solutions of pH 4 and 7.

Gas samples were collected using the static chamber methodology (Holter, 1997). Although a well known and largely implemented methodology, studies often modify methods to fit sampling conditions. Our modifications are within method guidelines for use of static chambers (de Klein and Harvey, 2013).

Chambers of 7.6 L (base plus cap) were placed in paddocks to collect gas samples. Within each paddock, a grid was used to evenly distribute 10 static chambers. Within each grid subdivision (n = 10), chambers were placed randomly (Gilbert, 1987) immediately after grazing. Sample collection started 24 h after chamber placement to allow soil microbial populations to stabilize and avoid over-estimation or under-estimation of emissions.

Static chambers were composed of a base (stainless steel ring, 20.3 cm o.d.; 15.2 cm height) and a cap (PVC, 23.5 cm o.d.; 9.4 cm height). Caps contained a rubber strap around them to seal chambers when closed and avoid atmospheric air from entering chamber headspace. Caps contained a vent hole (4 mm o.d.) to avoid pressure perturbations and subsequent mass flow, and a sampling port. The sampling port was sealed with a rubber stopper (Molded Thermogreen, 9.5 mm, Supelco, Bellefonte, PA).

Static chambers remained closed for the 20-min gas collection period and remained open between sample collections throughout the 14-d period. It is generally assumed that molecular diffusion is sufficiently rapid within the chamber headspace such that homogeneous gas concentrations exist when sampling (Livingston and Hutchinson, 1995).

Gas sample collection procedures were as follows: at 0900 h the first chamber was closed (capped) and the first sample of headspace gas (0 min) collected; then samples were collected at 5, 10, and 20 min post-closing to permit calculation of gas fluxes. Eighteen 0-min samples from each treatment were collected (18 out of 30 chambers per treatment). de Klein and Harvey

(2013) propose that 0-min samples are not necessary because this sample is equivalent to atmospheric concentration of the gases of interest.

Gas was collected from the chamber headspace with 20 mL plastic syringes with Luer-Lok Tip (BD, Franklin Lakes, NJ) and Precision Glide needles (0.8 mm x 40 mm, BD, Franklin Lakes, NJ). Syringe contents were transferred to pressurized 20 mL vials with beveled tops and rounded bottoms (23 mm x 75.5 mm x 12.5 mm, Supelco, Bellefonte, PA). Vials were sealed with straight plug stopper, natural red rubber septa (20 mm, Weathon, Millville, NJ) or rubber and silicone septa (20 mm, Leal Technologies, Carrboro, NC). We previously tested the 2 types of septa described and verified that septa type did not increased or decreased the storage time. In addition to the septa, vials were sealed with natural aluminum crimp tops (o.d. 20 mm, Weathon, Millville, NJ). Vials were pressurized at approximately -27.6 kPa (maximum flow: 1.90 cfm; maximum pressure: 413.7 kPa; maximum vacuum: 15000 kPa , GAST, Benton Harbor, MI). Vial pressure was checked with a Media Gauge series digital pressure gauge (ultra low pressure range of ≤ 103.4 kPa, 0.25% full scale accuracy, SSI Technologies, Inc., Janesville, WI). Sample vials were stored at room temperature and transported to a Michigan State University laboratory for analyses by gas chromatograph (GC).

The GC (Shimadzu GC-2014) was equipped with electron capture (ECD) and flame ionization detectors (FID; Shimadzu, Addison, IL). Carrier gas was ultra-purity nitrogen gas, with total flow of 40 mL min^{-1} and purge flow of 0.5 mL min^{-1}. The column oven was maintained at 75°C, FID at 250°C and ECD at 325°C. The GC was equipped with a headspace automatic sampler (COMBI Pal LEAP Technologies, Carrboro, NC). Calibration curves contained at least 5 points and were generated with standard gas of concentrations: 3903 ppm CO_2, 20.42 ppm CH_4 and 4.015 ppm N_2O. Standard gas was diluted with atmospheric air in vials

to 25%, 50% and 75% to generate the curve, which also included a 0 point (atmospheric air) and 100% (non diluted standard gas). The gas chromatograph was calibrated at beginning of analyses and once every 2 weeks.

2.2.3. Soil gas flux calculation and dependent variable measurements

Flux was calculated based on gas concentration determined by chromatography, atmospheric pressure and chamber volume (Equation 2.1). Chamber volume was measured once the chamber ring was placed in the ground to account for field variability.

Equation 2.1. Greenhouse gas flux calculation from static chambers placed on pasture soil.

$$GHG_{flux} = GHG \times P \times V_{ch} / (R \times T)$$

where GHG_{flux} (µg of gas of interest $chamber^{-1}$) is the mass of gas of interest (e.g. CO_2, CH_4 or N_2O) per chamber; GHG (ppm) is the concentration of gas of interest determined by gas chromatography; P (atm) is atmospheric pressure; V_{ch} (cm^3) is the chamber volume; R (atm L mol^{-1} K^{-1}) is the ideal gas constant, and T (K) is the ambient temperature inside the chamber.

The following conditions were recorded daily during the data collection period: atmospheric pressure, soil water content (WC), soil temperature (ST) and ambient temperature (AT). Atmospheric pressure was measured prior to sample collection with a Druck DPI 705 digital pressure indicator (combined non-linearity, hysteresis and repeatability of ± 0.1% full-scale, maximum torque of 2.259 Nm; GE, Fairfield, CT). Soil moisture measurements were taken adjacent to each chamber once daily following gas collection, at a 12 cm depth. Soil moisture was monitored with a soil water content reflectometer (HydroSense system with sensor CS620, display CD620 and two 12 cm-long rods, accuracy: ± 3.0% volumetric water content with electrical conductivity < 2 dS m^{-1}; range: 0% to saturation, Campbell Sicentific, Logan, UT). ST and AT measurements were recorded inside 2 chambers randomly selected per paddock

(3 paddocks per treatment; 6 measurements per treatment) once daily. Soil temperature was monitored at a 5 cm depth with a soil thermometer (Digital thermometer, Taylor, Oak Brook, IL) immediately before gas collection. AT was the air temperature inside the chamber during sample collection time and was measured with the same thermometer used for ST. Following ST measurements, the thermometer was placed inside the chamber from which ST was measured. Chambers were closed and sampling proceeded; when sampling was finished, AT was recorded immediately after chambers were opened.

2.2.4. Statistical analysis

Paddock was the experimental unit (3 pseudoreplicates per treatment). Chambers were considered multiple measurements of each experimental unit. Statistical analyses were conducted using SAS Software (Version 9.2; SAS Institute, 1987). A completely randomized design considered the term year × period (year and period compressed) as repeated measure, and chamber nested within area and treatment as the random term.

The model was as follows:

$$y = \mu + \rho_{i(k*l)} + \tau_l + \gamma_k + \lambda_m + \pi_j + \tau\gamma_{lk} + \tau\lambda_{lm} + e_{jlkmi}$$

where μ is the overall mean, $\rho_{i(k*l)}$ is the random effect of the ith chamber ($\rho_{i(k*l)} \sim N(0, \sigma^2_\rho)$) nested within the kth year and the lth treatment, τ_l is the fixed effect of the lth treatment, γ_k is the fixed effect of the kth year, λ_m is the fixed effect of the mth period, π_j is the fixed effect of the jth day, $\tau\gamma_{lk}$ is the interaction between the lth treatment and the kth year, $\tau\lambda_{lm}$ is the interaction between the lth treatment and the mth period, and e_{jlkmi} is the residual term ($e_{jlkmi} \sim N(0, \sum)$). Statistical analyses were performed using SAS Software (Version 9.2; SAS Institute, 1987). The explanatory variables tested were ST, AT and WC. All tests were performed with 95% confidence ($\alpha = 0.05$). If the main effect of year was significant means were discussed separately

by year. If the main effects of period or treatment were significant the interaction of treatment × period was tested.

2.3. Results and Discussion

2.3.1. Study site and soil properties

Ongoing and historical precipitation and minimum and maximum air temperatures for the studied months are summarized in Table 2.1. The volume of water applied by irrigation on SysB pasture sites during each year is shown in Table 2.1.

Gas sampling was performed in different pastures sites during each year and period depending on the animal rotation schedule. The sampling sites in GE were maintained constant for all sampling occasions. The soil type across grazing systems was predominantly sandy loam. A summary of particle size fractions and soil pH in each pasture is described in Table 2.2. All pH values were below the threshold for alkaline soils (Savadogo et al., 2007).

The first sampling year (2011) was conducted in order to verify and adjust the GHG collection methodology and results were preliminary. For that reason, the GE areas had not been included yet and no data on soil and ambient properties and GHG emissions are available. In addition, WC on SysA pasture sites was not monitored during the 2011 grazing season (Table 2.3).

Soil and ambient temperature and WC varied with treatment, year ($P < 0.01$) and period, for the 3 grazing seasons (Table 2.3). The interaction treatment × period was not significant for WC during the 2012 grazing season. The 2011 grazing season was the only year in which a decrease in ST and AT from P1 to P2 was observed. It was expected that temperatures would increase as the grazing season progressed and daily temperatures became higher. During 2011 the 2 periods were conducted from July 7th to August 26th, with 10 d between periods. During

2012 and 2013 sampling was conducted at the beginning (late May) and at the end of the grazing season (late August, refer to Section 2.2.1 for dates). The effects of weather changes in ST, AT and WC are therefore clearer during the 2012 and 2013 grazing seasons.

When ST and AT increased from P1 to P2, there was a decrease in WC. The SysA pastures sites were irrigated as needed during the 3 years, and higher WC was expected. During the 2012 grazing season, WC was higher for SysB pastures in both periods; GE and SysA were not significantly different. However, during the 2013 grazing season, SysA pasture sites had higher WC during P1, and SysB and GE were not significantly different. During P2, WC was higher in SysB pasture areas (irrigated) than in GE and SysA pasture areas (non-irrigated).

2.3.2. CO_2 emissions

The CO_2 emissions varied with year ($P < 0.01$), treatment, period and treatment × period (Table 2.4). During the 2011 grazing season, the CO_2 emissions pattern changed considerably between periods. Across treatments, CO_2 emissions were higher during P1. During 2011 P1, SysA pasture sites had higher CO_2 emissions than SysB pasture sites. However, during P2, CO_2 emissions from SysA were greatly reduced and SysB pasture sites had higher CO_2 emissions than SysA pasture sites. These differences are interesting mainly because during the 2011 grazing season the time in between periods P1 and P2 was only 10-d. These results indicate the importance of temporal variability and long-term monitoring of CO_2 emissions from pasture soils.

Emissions of CO_2 during 2012 did not vary between treatments ($P = 0.23$). CO_2 emissions increased from P1 to P2 for GE and SysB pasture sites ($P < 0.01$). CO_2 emissions from SysA remained constant throughout the grazing season. During the 2013 grazing season, CO_2 emissions considerably decreased from P1 to P2 (pooled by treatments). During P1, CO_2

emissions were not significantly different among treatments. During P2, CO_2 emissions from SysB pasture sites were greater than from GE and SysA pasture sites.

In this study GHG fluxes were monitored always post-grazing (each period lasted 14 d, starting on day 1 post-grazing). Grazing results in animal trampling, litter accumulation and excreta deposition, which affect soil properties and microbial growth. The variability observed in results might be associated to the changes in soil properties caused by grazing. However, we did not monitored GHG fluxes before grazing to determine the variability of GHG fluxes.

For all treatments, year and periods, negative CO_2 fluxes were observed (data not shown). Nevertheless, the average CO_2 emissions were all positive (Table 2.4), indicating emissions. Although there was some consumption of CO_2 on these pasture soils, on average they were considered CO_2 emitters.

Regarding the year to year variation, generally CO_2 emissions increased from 2011 (129.5 mg CO_2 m^{-2} h^{-1}) to 2013 (242.1 mg CO_2 m^{-2} h^{-1}). The only exception are emissions during P1 that decreased from 2011 to 2012. However, in 2013, emissions during P1 were the highest observed across years and periods. Generally CO_2 emissions decreased from P1 (203 mg CO_2 m^{-2} h^{-1}) to P2 (139 mg CO_2 m^{-2} h^{-1}) across treatments (2011 and 2013).

Short-term studies have shown that grasslands may be a sink for atmospheric CO_2 during peak biomass accumulation periods, but annual data are limited (Frank et al., 2002). Our results suggest that grasslands, directly after grazing, are a source of CO_2. Kim et al. (1992) reported average hourly CO_2 fluxes for grassland sites of 170 mg CO_2 m^{-2} h^{-1} from May to October. Their results are comparable to the CO_2 fluxes observed in the present study (approximately 171 mg CO_2 m^{-2} h^{-1}) across treatments and years from May to September. Soussana et al. (2007) observed CO_2 emissions from grasslands, taking into consideration CO_2 fluxes from soils and

vegetation, and found European grasslands either as sinks of CO_2 or near equilibrium. A few studies have suggested that C budgets of grasslands ecosystems are near equilibrium (e.g. Bruce et al., 1999). Dugas et al. (1999) found an average annual flux of 239 g CO_2 m^{-2} $year^{-1}$ for a tallgrass prairie and interpreted it as near equilibrium, given that an adjacent newly established perennial pasture produced fluxes 10 times greater. In the present study, CO_2 fluxes were greater than the fluxes observed by Dugas et al. (1999) and Bruce et al. (1999), which could be associated to monitoring post-grazing. Previously reported annual estimates include CO_2 fluxes from winter and fall, which are lower when compared to grazing season fluxes, resulting in lower annual average.

Kim et al. (1992) reported that during plant senescence, the net CO_2 flux was in balance with the atmosphere, but during droughts and after plant senescence, fluxes were about -3 g CO_2 m^{-2} d^{-1}. These results contrast the present study because, although negative fluxes were observed in some instances across years, treatments and periods, mean fluxes were always positive (Table 2.4). Similarly, Gilmanov et al. (2005) indicated that a source activity is possible for mixed prairie ecosystems in North America, especially during years with lower than usual precipitation.

For non-grazed mixed-grass prairie, Frank and Dugas (2001) estimated annual flux of 167 g CO_2 m^{-2}. If CO_2 fluxes were monitored during winter and fall in the present study, annual flux might have been lower. It is not accurate to estimate annual fluxes based on monitoring performed only during spring and summer seasons due to grazing and weather conditions that vary by season. Annual CO_2 fluxes must be inferred based either on long-term monitoring of emissions throughout the year, or on modeling, taking into consideration the different grazing managements and weather and soil conditions of each season. Frank (2002) supports that the large vegetation diversity in grasslands and annual climatic variability requires that CO_2 fluxes

be measured across a wide range of grasslands types. Accurate estimates of annual CO_2 flux depend on obtaining precise estimates of dormant season fluxes (Frank, 2002).

Our results did not demonstrate conclusively an increase in CO_2 emissions because of grazing. When differences between treatments were observed, SysB had greater CO_2 emissions; during 2011 (126 mg CO_2 m^{-2} h^{-1} and SysB was 132 mg CO_2 m^{-2} h^{-1}, for SysA and SysB on average, respectively) and during P2 of 2013 (151 mg CO_2 m^{-2} h^{-1} from SysA compared to 220 mg CO_2 m^{-2} h^{-1} from SysB; Table 2.4). The greater CO_2 emissions on SysB could be associated to faster SOM decomposition rate and C cycling. In SysB cow-calf pairs were rotated thru the pasture sites 4 to 5 times per year. In SysA animals were rotated thru paddocks 2 to 3 times per year. We believe that the greater rotation frequency in SysB resulted in (i) greater herbage defoliation, which stimulated plant and root growth; (ii) more frequent excreta deposition and faster decomposition rate of excreta, because of more frequent animal trampling impact; (iii) higher soil water content, because of the need of irrigation to maintain the conditions of this grazing management (Table 2.3). All these factors contribute to microbial growth, which is associated to SOM decomposition and C cycling that could have increased CO_2 emissions. It was observed that grasslands release CO_2 from the soil as a response to management practices (Soussana et al., 2004; Ammann et al., 2007). The induction of CO_2 emissions by grazing was associated to microbial activities (Pucheta et al., 2004), and increased soil temperature and respiration resulting from reduced leaf area and increased penetration of light into the soil surface (Bremer et al., 1998).

Soil temperature, AT and WC affected CO_2 emissions during all years and periods (Table 2.5). This is in agreement with previous papers. Frank (2002) who studied CO_2 fluxes from gazed pasture sites observed that the variation in annual precipitation, short-term droughts

periods and temperature stress effects on CO_2 fluxes are factors that contribute to the inter-annual flux variability.

Soussana et al. (2007) modeled CO_2 emissions from grazed and non-grazed pastures sites and indicated that the between year variability was strong for some of their monitoring sites, but mainly for the ones under grazing management.

2.3.3. CH_4 emissions

There was a year effect on CH_4 emissions ($P < 0.01$). Methane emissions were not significantly different between treatments during P1 of 2011 (Table 2.6). There was a period effect in 2011 ($P < 0.01$) even though the periods were close together in time, when compared to periods in 2012 and 2013. During 2011, period and treatment × period effects were observed, and during P2 emissions from SysB pasture sites were higher than emissions from SysA pastures sites (Table 2.6). From P1 to P2 CH_4 emissions increased across treatments. Both treatments consumed CH_4 during P1, but SysB became a CH_4 source during P2.

During the 2012 grazing season, emissions from SysA and SysB were significantly different during P1 (0.07 and 0.03 mg CH_4 m^{-2} h^{-1}, respectively, $P = 0.02$), but CH_4 emissions from SysB and GE were not significantly different (-0.01 mg CH_4 m^{-2} h^{-1} from GE). During P2, there was no statistical difference among the 3 treatments. There was also no period effect in 2012.

Similar to CO_2 emission, CH_4 emissions in 2013 were generally higher. There was no treatment effect on CH_4 emissions during P1. During P2, GE became a sink for CH_4 and CH_4 emissions increased in SysA and SysB.

Regarding across year variation, 2013 was different ($P < 0.01$), with higher emissions during P1 and P2. Observed fluxes were noticeably low and the SEM significantly large. Several

studies have been conducted to explain the variability associated to CH_4 emissions (e.g. Wachinger et al., 2000; Van den Pol-Van Dasselaar et al., 1999).

In a recent study comparing grazed and non-grazed pasture sites, Wei et al. (2012) observed average CH_4 flux of -0.06 mg CH_4 m^{-2} h^{-1}. These results are comparable to the uptake observed during the 2011 grazing season of the present study. However, pasture sites of the present study did not continue to store CH_4 in 2012 or 2013. Wei et al. (2012) noticed that the lowest uptake occurred during intense rainy days. Factors that increase WC facilitate increased CH_4 production over uptake, because methanogens are predominantly anaerobic whereas methanotrophs are aerobic microorganisms. The highest uptake observed in the present study occurred on GE pasture sites (-0.12 mg CH_4 m^{-2} h^{-1}, Table 2.6), when WC was the lowest (12.5%, Table 2.3). The effect of WC on CH_4 flux was expected because it was confirmed in previous studies (Le Mer and Roger, 2001; Curry, 2007), however in this study the effect of WC on CH_4 emissions was not consistently significant.

Methanogens and metanotrophs can develop in all soil types. It is the balance between the 2 microbial communities and the net CH_4 flux that defines a soil as sink or source of CH_4. Because the present study demonstrated both emissions and consumption of CH_4 we postulate that both microbial communities were present in the soils monitored. Zhou et al. (2008) suggested that grazing intensities have different effects on the structure of the methanotroph communities. Abell et al. (2009) demonstrated that methanotroph type II were not influenced by grazing, while the CH_4 oxidation and the abundance of type I metanotrophs increased during grazing.

CH_4 fluxes varied with treatment on 3 occasions: during P2 of 2011, P1 of 2012 and P2 of 2013. However, the differences among systems were not consistent. During P2 of 2011 SysB

produced greater CH_4 than SysA (0.03 compared to -0.03 mg CH_4 m^{-2} h^{-1}, respectively). During P1 of 2012 SysA produced greater CH_4 than GE, but emissions were not significantly different than SysB; SysB and GE did not differ (-0.01, 0.07 and 0.03 mg CH_4 m^{-2} h^{-1} for GE, SysA and SysB, respectively). During P2 of 2013, grazing systems produced greater CH_4 than GE, but SysA and SysB did not differ (-0.12, 0.14 and 0.14 mg CH_4 m^{-2} h^{-1} for GE, SysA and SysB, respectively). Our results are too variable to allow conclusions on the impact of grazing on CH_4 fluxes. Given that we observed different emissions patterns in each year, we support the importance of long-term studies on CH_4 flux quantification in grazing systems. Additionally, because of the period effect on CH_4 emissions, we suggest that more research needs to be conducted to monitor CH_4 fluxes throughout the year. The significantly effect of period during 2011 ($P < 0.01$) highlights the importance of repeated monitoring of CH_4 emissions throughout the grazing season. Periods were 10 d apart during 2011, and CH_4 emissions significantly decreased from P1 to P2 in both systems.

Despite the fact that CH_4 fluxes were monitored post-grazing, there was no clear increase of CH_4 emissions from grazing systems as compared to GE. Greater CH_4 emissions from pasture soils shortly after grazing were expected, as it was suggested in previous studies that fecal decomposition results in CH4 production (Saggar et al., 2004; Flessa et al., 1996). Although on average systems did not differ, we observed increased CH_4 emissions from grazed pasture sites during the first days of sampling (day 1 to day 4), followed by decreasing fluxes and stabilization (data not shown).

Contrary to CO_2 emissions, the effects of soil characteristics and ambient temperature on CH_4 emissions varied with year. During 2011 and 2013 grazing seasons, both ST and AT had positive effects on CH_4 emissions (Table 2.7). During the 2012 grazing season, none of the

variables had positive effects, and the CH_4 emissions and the differences observed among treatments must have been due to variables that were not accounted for. Methane production is influenced by several factors, such as (i) the aeration of the soil, as methanogenic microorganisms require anoxic conditions to produce CH_4, (ii) the presence of alternative electron acceptors, such as nitrate and sulphate, (iii) type and amount of available organic matter, (iv) the size of the methanogenic population, and (v) type of vegetation (Van del Pol-Van Daselaar et al., 1999)

Methanogens can grow in anaerobic micro pores of aerobic soils, and methanotrophs can grow in aerobic micro pores of soils with high higher WC. For that reason, we believe that monitoring water-filled pore space (WFPS) or O_2 content in the soil could serve as better predictors of CH_4 production or consumption from grasslands soils. More research is needed to verify the relationship between WFPS and O_2 content and CH_4 flux.

Methane production also depends on substrate availability in the soil. Joulian et al. (1996) reported that organic matter quantity and quality influences CH_4 production and that methanogenesis is influenced more by the availability and composition of the substrate than by the density of methanogens. Bergman et al. (1998) stressed that substrate availability, rather than abiotic factors such as temperature and pH, might be a predominant constraint for CH_4 productivity under held conditions. The fact that variability on the landscape scale cannot be explained adequately from easily measurable factors makes it reasonable to ask whether it can be traced back to processes on smaller scales (Wachinger et al., 2000).

2.3.4. N_2O emissions

The effect of year was significant for N_2O emissions ($P < 0.01$). N_2O emissions did not vary between systems during P1 of 2011. During P2 of 2011, SysB had greater N_2O emissions

(59.8 μg N_2O m^{-2} h^{-1}) than SysA (22.5 μg N_2O m^{-2} h^{-1}; Table 2.8). During the 2012 grazing season the differences between treatments were not significant (P = 0.66). There were no effects of period (P = 0.82) nor of the treatment × period interaction (P = 0.11).

The grazing season of 2013 was different compared with 2011 and 2012. Pasture sites grazed with SysA had considerably greater N_2O emissions during P1 (152 μg N_2O m^{-2} h^{-1}) than GE (29 μg N_2O m^{-2} h^{-1}) and SysB (84 μg N_2O m^{-2} h^{-1}) pasture sites. During P2, although GE had negative N_2O fluxes, no significant differences were observed among treatments (Table 2.8).

The N_2O fluxes variability is larger when compared to the other GHG. SEM was greater than means emissions from GE and SysA during P2 of 2012 (Table 2.8). Based on these results we cannot conclude that grazing increased N_2O emissions as has been previously suggested (Liu et al., 2011).

According to Flechard et al. (2007), the average N_2O fluxes from intensively and extensively grazed European grasslands were 1.77 and 0.48 kg N ha^{-1} $year^{-1}$, respectively. The range of N_2O emissions observed in this study was wider, varying from -2.0 to 13.4 kg N ha^{-1} $year^{-1}$ (from GE during P2 and SysA during P1 in 2013 grazing season). However, some of the pasture sites studied by Flechard et al. (2007) received external N inputs, which likely increased N_2O emissions. N_2O fluxes were observed to reach 3 kg N ha^{-1} $year^{-1}$ without grazing or applying fertilizer, while the emission increases up to 20 kg N ha^{-1} $year^{-1}$ over a grazed field with the use of 200 kg N ha^{-1} yr^{-1} of nitrogen fertilizer (Colbourn, 1992).

Although some differences were found among treatments, the pattern was not consistent from year to year. During P1 of 2011, SysB had greater emissions than SysA, but during P1 of 2013, SysB had greater N_2O emissions than SysA and GE. Wei et al. (2012) observed different emissions pattern in different years. Their pasture sites acted as a weak N_2O source during 2009

and as a weak sink during 2010. The only occasion where we verified N_2O sink was by GE in P2 of 2013. However, 2013 was the year when we detected the highest variation in the data collected, and consequently N_2O emissions did not vary among treatments during P2. Studies agree that soils are generally sources for N_2O, and negative N_2O values are usually associated to measurement errors. Chapuis-Lardy et al. (2007) reviewed the potential of agricultural soils to sink N_2O from the atmosphere. The authors suggested that the soil potential to sink N_2O needs further research, mainly regarding elucidation of the microbial communities and processes involved on N_2O sink.

Similarly, the processes involved on N_2O production in soils still need elucidation. The main processes involved in N_2O production are nitrification (autotrophic and heterotrophic) and denitrification (carried out by denitrifiers or nitrifiers). It is difficult to differentiate the proportion of N_2O coming from nitrifier denitrification from the proportion coming from denitrification, because they are basically the same process. Coupled nitrification-denitrification has also been identified as an important source of N_2O in soils. Coupled nitrification denitrification occurs under conditions that are favorable to both processes, occurring mainly in micro sites in the soils. Khdyer and Cho (1983) performed a very interesting experiment to determine the extent that each process (nitrification and denitrification) occurred after the addition of urea uniformly mixed under similar and constant O_2 availability. Nitrification occurs mainly at the surface layers where aerobic conditions predominate. Denitrification dominated in the deeper anaerobic layers. In the intermediate layers, N_2O production was highest, where there was an aerobic-anaerobic interface promoting both nitrification and denitrification to occur simultaneously. This experiment indicated that, as it was postulated for CH_4, N_2O production or

consumption can happen simultaneously in micro pores anaerobic or aerobic, which also increases the variation in measurements.

Nitrous oxide emissions from soil usually occur in "hotspots" associated with urine and fertilizer residue despite diffused nutrient spreading (Flechard et al., 2007). Temporal and spatial variations contribute to uncertainty in N_2O fluxes and the field and annual estimations (Soussana et al., 2007). The possibility that N_2O is formed mainly in hotspots is a contributor to the large variability, mainly in this study because N_2O fluxes were sampled post-grazing.

The effects of ST, AT and WC on N_2O emissions were not consistent across year (Table 2.9). During the 2011 grazing season, both AT and ST were positively correlated with N_2O emissions. During the 2012 grazing season, ST and WC were positively correlated to N_2O emissions, but AT was not. In the 2013 grazing season, none of the variables were positively correlated to N_2O emissions.

Soil temperature influenced N_2O emissions in 2011 and 2012. Denitrification rates and soil microbial activity positively relate to ST (Franzluebbers et al., 2002; Sulzman et al., 2005). N_2O soil flux commonly increases with the increased soil temperature (Ludwig et al., 2001). Moisture content in soils defines either nitrification or denitrification as the major process producing N_2O. However, the presence of anaerobic microsites in dry soils and of aerobic microsites in wet soils possible make WFPS a better indicator of processes prevailing on N_2O production than soil water content alone. Increased moisture content, or decreased WFPS, shifts the importance from nitrification to denitrification in terms of N_2O and nitrogen gas (N_2) production. A generalized relationship between WFPS and emissions of N_2O and N_2 from nitrification and denitrification has been postulated (Granli and Bockman, 1995), and it has been used since then. When soil water content is low, O_2 is plentiful and nitrification proceeds to

nitrate, resulting in little emission of N_2O and no N_2. As soil water content increases, mineralization increases, and nitrification increasingly produces N_2O rather than nitrate. Over the range of moderate soil water content denitrification becomes more important initially with high ratio of N_2O to N_2. When the soil water content is high, nitrification stops and denitrification proceeds increasingly to N_2. This relationship suggests that the maximum production of N_2O occurs when the soil water content is at about field capacity and both nitrification and denitrification are occurring (coupled nitrification-denitrification). It is likely that WFPS could be a better predictor of N_2O emissions than WC.

2.4. Conclusion

This study was conducted post-grazing and at a farm-scale and the variability associated to GHG emissions was large. We observed year and period effects. Within each year, treatment differences were also variable. There was a tendency of greater CO_2 emissions from SysB pasture sites. ST, AT and WC had effects on CO_2 emissions. CH_4 and N_2O emissions were observed from pasture sites of the 3 systems, but the effect of grazing was not conclusively observed on CH_4 and N_2O emissions. In addition, ST, AT and WC did not conclusively explain CH_4 and N_2O emissions. We suggest that other soil properties might be better predictors of CH_4 and N_2O, such as WFPS or O_2 content in the soil. Further research is needed to confirm the effect of WFPS and O_2 content on GHG emissions. We did not observe any clear trade-offs between GHG; generally GHG emissions increased from 2011 to 2013, which was likely associated to weather conditions, such as higher daily temperature and precipitation events.

This study indicates that GHG emissions from pasture soils are still uncertain when monitored at a farm scale. The variability observed on the results of this study raise questions about the current knowledge on GHG fluxes when applied to a farm level. Further research is

needed on GHG fluxes monitoring from different grazing systems, on long-term (during different periods throughout the grazing season, and different years) and before and after grazing, in order to allow conclusions on the impact of grazing systems on GHG fluxes.

Table 2.1. Ongoing and historic precipitation, maximum and minimum air temperature at the study site and total irrigation applied to paddocks.

	Max Temp	Min Temp	Precipitation	Irrigation[1]
	^{0}C		cm	
2011 grazing season				
May	19.5	7.0	5.8	
Jun	24.5	11.0	8.2	
Jul	29.3	15.1	6.1	
Aug	26.4	11.7	9.2	
Sep	21.1	6.7	7.9	11.2
2012 grazing season				
May	21.8	6.9	13.5	
Jun	25.8	11.3	7.7	
Jul	31.3	14.4	18.6	
Aug	25.8	15.4	5.0	
Sep	21.6	5.1	8.8	22.4
2013 grazing season				
May	20.9	5.7	7.7	
Jun	24.6	10.4	4.7	
Jul	27.5	12.7	5.2	
Aug	26.6	10.1	10.5	
Sep	21.4	6.3	4.2	16.8
1981-2010				
May	18.7	4.9	8.2	
Jun	24.1	10.3	8.6	
Jul	26.5	12.6	7.1	
Aug	25.3	11.6	9.4	
Sep	20.8	7.1	9.2	-

[1]Total cm of water applied on each 1.6 ha paddock of SysB during the grazing season (SysB: 2.5 cows ha^{-1} stocking rate and 28,000 kg LW ha^{-1} stocking density).

Table 2.2. Summary of soil characteristics in the study area.

Systems[1]	Size fractions, %			Soil type	pH
	Sand	Silt	Clay		
GE	70.1	16.0	13.8	sandy loam	5.9
SysA	55.0	23.5	21.5	sandy clay loam	6.0
SysB	52.8	26.8	20.4	sandy clay loam	6.2

[1] GE: grazing exclusion; SysA: 1 cow ha^{-1} stocking rate and 100,000 kg LW ha^{-1} stocking density; SysB: 2.5 cows ha^{-1} stocking rate and 28,000 kg LW ha^{-1} stocking density.

Table 2.3. Soil properties of pasture sites grazed with two different management strategies and non-grazed pasture sites.

Systems[1]	2011 grazing season		2012 grazing season		2013 grazing season	
	P1	P2[2]	P1	P2	P1	P2
ST[3], ^{0}C						
GE	-	-	17.4[a]	19.9[a]*	14.9[a]	20.8[a]*
SysA	22.4[a]	19.7[a]*	17.1[a]	20.0[a]*	15.5[b]	21.4[b]*
SysB	21.9[b]	17.8[b]*	15.7[b]	18.6[b]*	14.5[c]	19.2[c]*
SEM	0.07		0.07		0.08	
Source of Variation						
Treatment	<0.01		<0.01		<0.01	
Period	<0.01		<0.01		<0.01	
Treatment x Period	<0.01		<0.01		<0.01	
AT[4], ^{0}C						
GE	-	-	23.3[a]	27.1[a]*	19.1	23.6[a]*
SysA	27.4[a]	23.7[a]*	22.7[a]	25.1[b]*	19.1	25.4[b]*
SysB	25.9[b]	20.9[b]*	21.5[b]	23.9[c]*	19.5	23.8[a]*
SEM	0.18		0.17		0.18	
Source of Variation						
Treatment	<0.01		<0.01		<0.01	
Period	<0.01		<0.01		<0.01	
Treatment x Period	<0.01		<0.01		<0.01	
WC[5], %						
GE	-	-	15.1[a]	13.4[a]*	26.9[a]	12.5[a]*
SysA	-	-	15.0[a]	13.8[a]*	32.1[b]	12.8[a]*
SysB	14.6	14.9*	18.9[b]	16.5[b]*	27.9[a]	17.4[b]*
SEM	0.28		0.43		0.52	
Source of Variation						
Treatment	-		<0.01		<0.01	
Period	<0.01		<0.01		<0.01	
Treatment x Period	-		0.11		<0.01	

[1] GE: grazing exclusion; SysA: 1 cow ha^{-1} stocking rate and 100,000 kg LW ha^{-1} stocking density; SysB: 2.5 cows ha^{-1} stocking rate and 28,000 kg LW ha^{-1} stocking density

[2]Jul 7th – Aug 3rd (P1), Aug 13th – 26th (P2), 2011; May 18th – 31st (P1), Aug 21st – Sep 3rd (P2), 2012; May 20th – Jun 3rd (P1), Aug 26th – Sep 8th (P2), 2013.

[3]ST: soil temperature.

[4]AT: ambient temperature.

[5]WC: soil water content.

Mean differences within columns indicated by letter ($P < 0.05$). Mean differences within rows indicated by symbols ($P < 0.05$).

Table 2.4. CO_2 emissions from of pasture sites grazed with two different management strategies and non-grazed pasture sites.

Systems	Period		Effects		
	P1	P2	Treatment	Period	Treatment x Period
2011 grazing season	mg m^{-2} h^{-1}				
GE	-	-	0.35	<0.01	<0.01
SysA	160.1[a]	93.7[a]*			
SysB	144.8[b]	119.5[b]*			
SEM	4.6				
2012 grazing season					
GE	125.2	140.6*	0.23	<0.01	<0.01
SysA	121.3	126.0			
SysB	111.9	143.0*			
SEM	4.4				
2013 grazing season					
GE	313.7	119.6[a]*	<0.01	<0.01	<0.01
SysA	314.1	151.0[a]*			
SysB	333.7	220.5[b]*			
SEM	9.4				

[1]GE: grazing exclusion; SysA: 1 cow ha^{-1} stocking rate and 100,000 kg LW ha^{-1} stocking density; SysB: 2.5 cows ha^{-1} stocking rate and 28,000 kg LW ha^{-1} stocking density.
[2]Jul 7th – Aug 3rd (P1), Aug 13th – 26th (P2), 2011; May 18th – 31st (P1), Aug 21st – Sep 3rd (P2), 2012; May 20th – Jun 3rd (P1), Aug 26th – Sep 8th (P2), 2013.
Mean differences within columns indicated by letter ($P < 0.05$). Means difference within rows indicated by symbols ($P < 0.05$).

Table 2.5. Effects of soil properties on CO_2 emissions from pasture sites grazed with two different management strategies and non-grazed pasture sites.

Grazing season	Effects[1]		
	ST, ^{0}C	AT, ^{0}C	WC, %
	P-value		
2011	<0.01	<0.01	-
2012	0.02	<0.01	<0.01
2013	<0.01	<0.01	0.02

[1]ST: soil temperature; AT: ambient temperature; WC: soil water content.

Table 2.6. CH_4 emissions from pasture sites grazed with two different management strategies and non-grazed pasture sites.

Systems	Period		Effects		
	P1	P2	Treatment	Period	Treatment x Period
2011 grazing season	mg m^{-2} h^{-1}				
GE	-	-	0.14	<0.01	<0.01
SysA	-0.08	-0.03[a]			
SysB	-0.10	0.03[b]*			
SEM	0.01				
2012 grazing season					
GE	-0.01[a]	0.01	0.02	0.88	0.31
SysA	0.07[b]	0.05			
SysB	0.03[a]	0.04			
SEM	0.02				
2013 grazing season					
GE	0.03	-0.12[a]	<0.01	0.27	<0.01
SysA	0.11	0.14[b]			
SysB	0.15	0.14[b]			
SEM	0.05				

[1] GE: grazing exclusion; SysA: 1 cow ha^{-1} stocking rate and 100,000 kg LW ha^{-1} stocking density; SysB: 2.5 cows ha^{-1} stocking rate and 28,000 kg LW ha^{-1} stocking density.
[2]Jul 7th – Aug 3rd (P1), Aug 13th – 26th (P2), 2011; May 18th – 31st (P1), Aug 21st – Sep 3rd (P2), 2012; May 20th – Jun 3rd (P1), Aug 26th – Sep 8th (P2), 2013.
Mean differences within columns indicated by letter ($P < 0.05$). Means difference within rows indicated by symbols ($P < 0.05$).

Table 2.7. Effects of soil properties on CH_4 emissions from pasture sites grazed with two different management strategies and non-grazed pasture sites.

Grazing season	Effects[1]		
	ST, ^{0}C	AT, ^{0}C	WC, %
		P-value	
2011	0.09	<0.01	-
2012	0.62	0.04	0.78
2013	0.52	0.03	0.55

[1]ST: soil temperature; AT: ambient temperature; WC: soil water content.

Table 2.8. N_2O emissions from pasture sites grazed with two different management strategies and non-grazed pasture sites.

Systems	Period		Effects		
	P1	P2	Treatment	Period	Treatment × Period
2011 grazing season	μg N_2O m^{-2} h^{-1}				
GE	-	-			
SysA	32.3	22.5[a]	0.05	0.67	<0.01
SysB	43.7	59.8[b]			
SEM	9.5				
2012 grazing season					
GE	3.5	5.6	0.66	0.82	0.11
SysA	9.4	3.0			
SysB	6.0	8.6			
SEM	2.8				
2013 grazing season					
GE	30.2[a]	-30.1[a]*	<0.01	<0.01	<0.01
SysA	151.3[b]	13.7[a]*			
SysB	98.9[c]	29.2[ab]*			
SEM	13.4				

[1]GE: grazing exclusion; SysA: 1 cow ha^{-1} stocking rate and 100,000 kg LW ha^{-1} stocking density; SysB: 2.5 cows ha^{-1} stocking rate and 28,000 kg LW ha^{-1} stocking density.
[2]Jul 7th – Aug 3rd (P1), Aug 13th – 26th (P2), 2011; May 18th – 31st (P1), Aug 21st – Sep 3rd (P2), 2012; May 20th – Jun 3rd (P1), Aug 26th – Sep 8th (P2), 2013.
Mean differences within columns indicated by letter ($P < 0.05$). Means difference within rows indicated by symbols ($P < 0.05$).

Table 2.9. Effects of soil properties on N_2O emissions from pasture sites grazed with two different management strategies and non-grazed pasture sites.

Grazing season	Effects[1]		
	ST, ^{0}C	AT, ^{0}C	WC, %
	P-value		
2011	<0.01	<0.01	-
2012	0.01	0.38	<0.01
2013	0.01	0.35	0.46

[1]ST: soil temperature; AT: ambient temperature; WC: soil water content.

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CHAPTER 3

ENTERIC METHANE FROM LACTATING BEEF COWS

3.1. Introduction

The production of CH_4 by cattle has become a subject of scientific debate as the concern over climate change increases. In order to understand the C flux in grazing systems, quantify and understand the impact of management on enteric CH_4 production is warranted. The primary factor affecting enteric CH_4 production are the quantity and quality of the diet. As forage fiber content increases, nutrient digestion and passage rate decelerate, increasing the overall time of ruminal fermentation and subsequent enteric CH_4 production. Therefore, implementing management tools at the farm level that increase pasture quality potentially decreases enteric CH_4 emissions from grazing cows (Beauchemin et al, 2008). Grazing management is the most suitable option to reduce CH_4 emissions from cows. Grazing management is a combination of stocking rate, density and rest periods. These factors define the relationship between herbage demand and supply (Animut et al., 2005), herbage utilization efficiency, animal performance and production per hectare (Pinares-Patino et al., 2007). Despite, the influence of grazing management strategies on enteric CH_4 emissions, especially from beef cows, is not completely clarified.

The majority of studies measuring CH_4 emissions from the beef industry focused on steers or heifers (Ricci et al., 2013). Previous research has measured emissions from lactating beef cows fed mixed alfalfa-concentrate diets (Reynolds and Tyrrel, 2000) or grazing alfalfa-grass and grass-only pastures (McCaughey et al., 1999). The influence of management decisions in the context of systems on CH_4 emissions from beef cows is missing. The objective of this study was to compare CH_4 emissions from lactating beef cows grazed with different combinations of stocking rate and density. Our hypothesis was that low stocking rate and high stocking density grazing management results in lower forage quality, because of longer rest

periods that resulting in lower forage quality increases enteric CH_4 emissions from lactating beef cows.

3.2. Material and Methods

3.2.1. Pasture management

All animal procedures were approved by the Michigan State University Animal Care and Use Committee (protocol no. 04/12-078-00).

Cow-calf pairs were managed with 2 rotational grazing management practices differing in stocking rates and density; an intensive system with high stocking rate and low stocking density, and an extensive system with low stocking rate and high stocking density. The system with low stocking rate and high stocking density (SysA) consisted of 120 cow-calf pairs rotating on a total of 120 ha, divided into 0.7 ha paddocks. Cow-calf pairs were moved to a new paddock 3 times daily (at approximately 0800 h, 1200 h and 1600 h). The equivalent stocking rate was 1 cow ha^{-1} and the stocking density was approximately 100,000 kg LW ha^{-1}. The rest period varied from 60 to 90 d during the course of the growing season depending on plant growth. Cow-calf pairs grazed each paddocks 2 to 3 times per year. The system with high stocking rate and low stocking density (SysB) consisted of 4 cow-calf pairs rotating on 1.6 ha pasture, divided into 0.08 ha paddocks. Cow-calf pairs were moved to a new paddock once daily (at approximately 0800 h). The equivalent stocking rate was 2.5 cows ha^{-1} and the stocking density was 28,000 kg LW ha^{-1}. The rest period varied from 18 to 30 d during the course of the growing season depending on plant growth. Cow-calf pairs grazed each paddock 4 to 5 times per year. The pasture areas in SysB were irrigated as needed, whereas there was no irrigation applied to SysA pasture sites.

The SysA described was simulated in a 1.6 ha pasture closer to the head-gate area, to facilitate transportation of cow-calf pairs to the head-gate area for sample collection.

Enteric CH_4 measurements were collected from respired air of grazed cows, twice during the grazing season using sulfur hexafluoride (SF_6) as a tracer gas technique (Johnson and Johnson, 1995). Sampling occurred during the grazing seasons of 2012 and 2013, in 2 periods at the following dates: from June 4th to June 10th (Period 1 – P1), and from September 4th to September 11th (Period 2 – P2), 2012; from June 5th to June 10th (P1), and from September 11th to September 17th, (P2) 2013. Methane was collected twice daily for 7 d. The first collection started at 0830 h and the second collection started at 1530 h daily. Each collection lasted approximately 1 h. Cows were moved to a chute area for each collection.

Cannulated Angus cows were stratified on weight, age and parity and assigned to each treatment (n = 6). Groups were randomly assigned to treatments (SysA and SysB) for the first periods within each sample year. For the second period in each year, groups assignments were reversed. To achieve the desired grazing density additional cows were added to each herd as needed.

During P1 of 2012, hematomas developed in a subset of cows and after attending veterinarian consultation, we attributed the swelling to head-gate pressure used while collecting samples. During P2 of the 2012 grazing season, 2 cows that still exhibited swelling were eliminated from the sampling. Therefore, P2 was performed with 10 cows (5 replicates per treatment). Cows were cannulated in January 2013. Due to complications post-surgery, 1 cow showed health issues and was euthanized, a replacement cow similar in weight, age and parity was selected for the second year. During 2013, cows did not show any health issues and both periods were performed with 12 cows.

3.2.2. Enteric methane measurements

Enteric CH_4 was quantified with the SF_6 as a tracer gas technique. Pre-calibrated permeation tubes containing SF_6 were placed into the reticulum-rumen of each animal via bolus in 2012 and through the cannula in 2013. Expired gases were collected with a sampling apparatus containing a collection canister (PVC) and modified halter. The permeation tubes, canisters and halters were built following protocol described by Johnson et al. (2007).

To collect enteric CH_4 and SF_6 samples, the canisters were vacuumed to approximately -2.7 kPa with a vacuum pump (2 stages, Robinair, Owatonna, MN). After the collection period, canisters were connected to a dilution system and the final pressure was recorded. Nitrogen was then added slowly until canister pressure reached 117.2 kPa. Pressure readings were recorded to calculate the dilution factor (Johnson et al., 2007). Pressure was measured with a Druck DPI 705 digital pressure indicator (combined non-linearity, hysteresis and repeatability of ± 0.1% full-scale, maximum torque of 2.259 Nm; GE). After pressurization to 117.2 kPa, the contents of the canisters were transferred under positive pressure to evacuated vials, described in Chapter 2, section 3. Methane and SF_6 concentrations were determined by gas chromatography as described in Chapter 2, section 2.2.2.

To adjust for background CH_4, air samples were collected at 2 points upwind of each pasture strip, using the canister method. Canisters were placed on fences adjacent to grazed paddocks. Background canisters were sampled following cow sampling; twice daily for 7-d (first collection at 0930 h and the second collection at 1630 h daily).

Daily CH_4 emissions and the CH_4/SF_6 ratio of concentrations in breath samples were calculated after adjustment for background gas concentrations (Johnson and Johnson, 1995).

Methane emission rate was calculated from collected SF_6 and CH_4 concentrations and from SF_6 permeation tube release rate according to the Equation 3.1.

Equation 3.1. Methane emissions rate from grazing cows.

$$QCH_4 = QSF_6 \times ([CH_4]_{cows} - [CH_4]_{background}]) / [SF_6]$$

where QCH_4 is the CH_4 emission rate (g min^{-1}), QSF_6 is the permeation tube SF_6 release rate (g min^{-1}), $[CH_4]_{cows}$ is the CH_4 concentration quantified from cows (µg m^{-3}), $[CH_4]_{background}$ is the CH_4 concentration quantified from background canisters (µg m^{-3}), and $[SF_6]$ is the SF_6 concentration in samples (µg m^{-3}).

3.2.3. Intake determination and forage analyses

In order to determine intake, chromic oxide (Cr_2O_3) was used as a marker. Fecal output was determined from the passage kinetics of Cr_2O_3 (Fenton and Fenton, 1979). A dosage of 6 g of Cr_2O_3 was administered to the animals twice daily for 7 d, via oral bolus in 2012 and cannula in 2013. Fecal samples were collected during the last 4 d of dosage period. Feces were dried at 65°C until constant weight. Dried feces were ground on a Wiley mill (Carbon steel, 4 adjustable hard tool steel knives, Thomas Scientific, Swedesboro, NJ) and analyzed by atomic absorption spectrophotometry. The atomic absorption spectrophotometer was equipped with 357.9 nm wavelength lamp and air-acetylene flame (Perkin Elmer, Waltham, MA). Fecal Cr_2O_3 concentrations were used to estimate fecal output according to Equation 3.2 and dry matter intake (DMI) was calculated based on Equation 3.3. Indigestibility of DM was determined based on diet apparent digestibility. Cows were weighed at the beginning (d-1) and at the end (d-7) of each sampling period.

Equation 3.2. Fecal output determination based on marker Cr_2O_3.

Fecal output, g d^{-1} = (marker consumed, g d^{-1}) / (marker concentration in feces, g g of DM^{-1})

Equation 3.3. Dry matter intake (DMI) determination based on fecal output.

$$\text{DMI, kg d}^{-1} = \text{fecal output, g d}^{-1} \times 100 / \text{indigestibility of DM (\%)}$$

Pre and post-grazing forage samples were collected twice during the 7-d period; on d 2 and d 6. Within each 1.6 ha pasture, 3 forage sampling sites (approximately 0.5 ha) were designated as forage sampling areas. Each sampling area was equally split into 2 zones (west and east). The day prior to grazing, the pre-grazing pasture biomass was sampled within each zone by clipping 3 randomly placed 0.25 m^2 quadrats to a 5 cm stubble height with Gardena 8803 battery operated harvest shears (Ulm, Germany). Samples were composited by sampling zone, weighed and an average wet weight from each zone was recorded. After mean wet weights were recorded, forage samples from both zones were combined, thoroughly mixed and a 200 g subsample was collected. Subsamples were oven-dried at 60° C for 48 h weighed and grounded in a Wiley mill (Carbon steel, 4 adjustable hard tool steel knives, Thomas Scientific, Swedesboro, NJ). The same sampling process was repeated after cattle grazed the paddock to determine the post-grazing (residual) biomass. The pre-grazing and post-grazing samples were sent to DairyOne (Ithaca, NY) for NIR analysis. The parameters analyzed were: crude protein concentration (CP%), acid detergent fiber (ADF%), neutral detergent fiber (NDF%), lignin concentration (%) and *in vitro* total digestibility (IVTD%). Forage gross energy (GE, MJ kg^{-1}) and digestible NDF (DNDF) were determined based on calculations proposed by the Nutrient requirements of cattle protocol (NRC, 2001). Botanical composition was determined in each paddock pre-grazing. Species composition was estimated using the dry-weight-rank method (Mannetje and Haydock, 1963).

3.2.4. Statistical analysis

For the first period in each year cow-calf pairs were randomly assigned to treatments. For the subsequent periods, a crossover design was implemented in a double repeated measures design, with period and day as repeated measures. The variance-covariance matrix structure defined was unbalanced autoregressive to account for the double repeated measures. The model was as follows:

$$y = \mu + \rho_j + \tau_l + \gamma_k + \lambda_m + \pi_i + \tau\gamma_{lk} + \tau\lambda_{lm} + e_{jlkmi}$$

where μ is the overall mean, ρ_j is the random effect of the jth cow ($\rho_j \sim N(0, \sigma^2_\rho)$), τ_l is the fixed effect of the lth treatment, γ_k is the fixed effect of the kth year, λ_m is the fixed effect of the mth period, π_i is the fixed effect of the ith day, $\tau\gamma_{lk}$ is the interaction between the lth treatment and the kth year, $\tau\lambda_{lm}$ is the interaction between the lth treatment and the mth period, and e_{jlkmi} is the residual term ($e_{jlkmi} \sim N(0, \sum)$). Statistical analyses were performed using SAS Software (Version 9.2; SAS Institute, 1987). All tests were performed with 95% confidence ($\alpha = 0.05$).

3.3. Results and Discussion

3.3.1. Herbage mass, botanical composition and forage nutritional characteristics

There was an effect of year ($P < 0.01$) and means are shown separately for 2012 and 2013. Pre-grazed forage growth was not affected by treatments (Table 3.1). A greater herbage mass pre-grazing was expected on SysA pasture sites, because of longer rest periods (60 to 90 d as compared to 18 to 30 d on SysB pasture sites). Irrigation, as needed, and frequent defoliation on SysB pasture sites might have increased the forage production at these sites, explaining the lack of difference. Cow-calf pairs grazed SysB pasture sites 4 to 5 times per year, whereas SysA pasture sites were grazed 2 to 3 times per year. The greater frequency of grazing applied to SysB pasture sites might have stimulated forage growth. Additionally, SysB had greater amount of

legumes during P2 than SysA (Table 3.2) and likely greater amount of N available to plants, which might also have contributed to forage growth.

Herbage disappearance might not be an accurate predictor of intake, however observed herbage disappearance was in agreement with the DMI estimated using Cr_2O_3 as a marker. Dry matter intake was not different between cows grazed with SysA or SysB (Table 3.4), which is in agreement with the treatments effects found on herbage disappearance ($P > 0.05$). Post-grazing herbage mass was greater for SysA during P2 in both years, 2012 and 2013 (Table 3.1). SysA pasture sites were given longer rest periods, and forage offered to cows were reproductive and mature. We believe that cows grazed selectively, trampling down on a great amount of forage mass, increasing post-grazing herbage mass.

The pastures sites were comprised of mixed grass and legume species. The 3 most predominant species observed in each pasture site are depicted in Table 3.2. Grasses were predominant and represented from 67 to 96% of the pastures across periods and treatments. However, legumes were always found, contributing from 3 to 21% of the pastures across periods and treatments (Table 3.2). Frequently more than 1 species of legume was present (data not shown).

The same species were present throughout the year but in different proportions. In SysA pasture sites orchard grass (*Dactylis glomerata*) and bromegrass (*Bromus inermis*) were the predominant grasses and birdsfoot trefoil (*Lotus corniculatus*) was the predominant legume, but red (*Trifolium pratense*) and white clover (*Trifolium repens*) were also observed (Table 3.2). In SysB pasture sites, Kentucky (*Poa pratensis*) and orchard grass were the predominant grasses over the 2 years of study (Table 3.2). White clover was the most predominant legume during P1 and red clover was predominant during P2. The grass-legume ratio of pasture sites varied from

96:4 (SysA pasture sites during P2 of 2012) to 69:4 (SysB pasture sites during P2 of 2013). Our results are in agreement with previous research that found few species responsible for large proportion of the DM production in mixed grass and legumes pastures (Sanderson et al., 2005; Skinner et al., 2006).

Previous studies have shown that grazing intensity did not affect species composition (Ren et al., 2012; Dumont et al., 2008; Kruess and Tscharntke, 2002). The proportion of legumes tended to be higher in SysB pasture sites during from P1 to P2. The SysA pasture sites were provided longer rest periods, consequently at the end of the grazing season (P2) the plants were tall and reproductive. The SysA grass height during P2 may have shaded and impaired the development of legumes, decreasing their proportion. Shorter grazing returns results in frequent defoliation, which maintains grasses at shorter heights. Shorter grasses have less shading effect, allowing the development of plants with different growing habits, such as legumes. Alfalfa (*Medicago sativa*) has an upright growth habit, red clover and birdsfoot trefoil have upright to decumbent, and white clover have prostrate growth habit (Hannaway, 2004). Indeed, we observed the predominance of red and white clover on SysB pasture sites, that with more frequent defoliation had shorted grasses, allowing the development of prostrate growth habit legumes (Table 3.2). Another factor that might have contributed to the decreased abundance of legumes during P2 in SysA pasture sites was dry weather during the summer (end of the season - P2). The SysB pastures areas were irrigated as needed, which might have allowed the growth of legumes throughout the year.

Botanical shift affects forage quality, complicating the estimation of the nutritional value of a pasture (Skinner et al., 2004; Belesky et al., 1999). Dumont et al. (2009) suggested that the distribution evenness of plant species is greater under high stocking rates. In order to decrease

the error associated with botanical composition on estimation of forage quality, we collected 3 replicates of composited random samples per treatment. However, animals graze selectively and the forage quality results may not accurately represent the DM consumed by cow-calf pairs. Grazing animals select their feed and prefer living to dead material, younger to older material, leaf to stem and legume to grass leaves (Hodgson et al., 1990; Popp et al., 1997).

The nutritional characteristics of the pastures differed by year ($P < 0.01$), therefore means are presented separately for 2012 and 2013 grazing seasons (Table 3.3). Period and treatment effects were observed for forage nutritional characteristics. During P1 forage characteristics were quite similar between treatment pasture sites. As the grazing season proceeded the impact of grazing management increased, mostly due to longer rest periods given to SysA pasture sites.

Crude protein content increased in SysA pasture sites from P1 to P2 of 2012, and remained constant from P1 to P2 during 2013. The change in NDF content was the same in both years; increased from P1 to P2 in SysA pasture sites but remained constant in SysB pasture sites. Based on NDF, lignin and CP content forage quality decreased in SysA pasture sites during P2 (Table 3.3).

The larger differences observed (between SysA and SysB pasture sites) during P2 was expected. At the beginning of the grazing season (P1), forage growth is independent of the grazing system. Four months later (P2), SysB pasture sites were comprised of mature forages in the reproductive stage. The SysB pastures sites, managed with shorter rest periods, maintained young, vegetative forage throughout the season. Irrigation and frequent defoliation might have contributed to higher forage quality of SysB pasture sites during P2 allowing the development of legumes during P2 (Table 3.2). The lower grass-legume ratio in SysB pasture sites is reflected in increased or maintained CP and NDF composition.

3.3.2. Animal performance

Cows were maintained in 1 single herd for most of the year, being divided into SysA or SysB herd approximately 14 d before the sampling for diet adaptation. Therefore, treatment effects on body weight (BW) cannot be determined. Body weights increased from 2012 to 2013 ($P < 0.01$) and from P1 to P2 (Table 3.4).

There were no treatment effects on DMI, although SysA cows had lower DMI than SysB cows during P1. There was a period effect on DMI ($P < 0.01$), likely associated with decreased intake of SysA cows during P1 (12.8 kg DMI d^{-1}) compared to SysB cows during P1 (15.2 kg DMI d^{-1}). DMI of SysA and SysB cows increased from P1 to P2 (4 and 1.1 kg DMI d^{-1} increase, respectively). In this study, cows ingested on average 2.6% (SysA) and 2.8% (SysB) of their BW. These values are in agreement with previous studies. Marston et al. (1998) suggested that DMI of lactating beef cows varies from 2.3 to 2.7% of BW for cows grazing average or high quality forages, respectively. Hatfield et al. (1989) observed DMI from 14.8 to 16.1 kg DM d^{-1} (on average 2.8% of BW) for lactating beef cows with different milk production levels.

Forage or animal management-related factors do not explain the lower intake of SysA cows during P1. It is possible that the stress associated to sampling, such as the use of canister and halter, and moving to the head-gate area twice daily, could have decreased DMI (cows were not halter broken prior to the sampling periods). Another hypothesis is that the decreased DMI observed could be error associated with the use of Cr_2O_3 as a marker. Previous research (Smith and Reid, 1955) reported that the excretion of chromium might not be constant during the day. Feces were sampled twice daily during the present study in order to decrease the variation of marker excretion, but in some occasions, fecal collection was not possible because the cows had defecated on their way to the head-gate area.

Although forage quality generally decreased in SysA pasture sites during P2, DMI did not change between systems (during P2). We believe that, despite the poorer forage quality observed in SysA, cows grazed selectively. The botanical composition analysis indicated the presence of legumes during P2 in both systems. Herbage mass analysis indicated no treatment differences. In addition, in SysA cow-calf pairs were moved to a new paddock 3 times daily and once daily in SysB. We believed that these factors altogether - presence of legumes, high herbage mass and rotational schedule - provided the opportunity for cow-calf pairs to select what to eat and match nutritional requirements.

3.3.3. CH_4 emissions from beef cows

Emissions are described as daily emissions per head (g CH_4 d^{-1}), daily emissions mass expressed per unit of intake (GE, NDF or DNDF intake; GEI, NDFI and DNDFI respectively), or unit of metabolic body weight (g CH_4 kg $BW^{-0.75}$).

Treatment effects were observed for CH_4 emissions as a percent of GEI and per unit DNDFI (Table 3.5). The significant difference on CH_4 as a percent of GEI is probably a result of the lower DMI found for SysA cows during P1. The highest daily emissions were observed from SysA cows during P1, which might have led to greater loss of CH_4 as a percent of GEI and reduced DMI.

Enteric CH_4 emissions in this study are within the range reported by others using the SF_6 tracer method from yearling heifers, first-calf heifers and mature cows (120 to 255 g CH_4 d^{-1}, De Ramus et al., 2003), and cows and steers (150 to 240 g CH_4 d^{-1}, Pavao-Zuckerman et al., 1999). McCaughey et al. (1999) reported CH_4 emissions varying from 267 to 294 g CH_4 d^{-1} from first-calf, early lactation heifers grazing grass-only and alfalfa pastures. Our results varied from 195 to 249 g CH_4 d^{-1} and were lower than the values found by McCaughey et al. (1999). The authors

also observed higher emission rates per unit $BW^{0.75}$ (2.6 g CH_4 kg $BW^{-0.75}$) and higher loss of CH_4 as a percent of GEI, varying from 7 to 9.5%. Pinares-Patino et al. (2007) conducted an experiment to compare CH_4 emissions from grazing heifers managed under high (2.2 livestock units; LU) and low (1.1 LU) stocking rates. They observed similar daily emissions (on average 216.6 g CH_4 d^{-1}), but higher CH_4 emission rate (7% of GEI) compared to the present study.

Johnson et al. (1994) suggested CH_4 losses as a percent of GEI varying from 2 to 12% for cattle fed diets with different composition. Published values for grazing cattle approximate 6% and represents studies including steers (Kennedy and Charmley, 2012) and heifers (Chaves et al., 2006; Boadi and Wittenberg., 2002). Cattle eating high forage diets typically release a greater percentage of their dietary energy as CH_4 than cattle eating grains (Freetly and Bown-Brandl, 2013). Enteric CH_4 emissions range from 3% for feedlot cattle to 6% of GEI lost as CH_4 for grazing cattle (IPCC, 2006). Kurihara et al. (1999) observed CH_4 emissions rate as high as 11% of GEI. Our values varied from 3.8 to 6.4% of GEI, which could be considered low for grazing lactating beef cows. The highest value (6.4% for SysA cows during P1) was a result of high daily emission and low DMI.

Emissions rate is a function of DMI; higher DMI explains lower emission rate as a percent of GEI. Cows in the present study had comparatively higher DMI than animals from previous studies focusing on enteric CH_4 emissions. McCaughey et al. (1999) observed heifers with 10 kg d^{-1} DMI on average. Pinares-Patino et al. (2007) reported heifers consuming on average 9 kg DM d^{-1}, whereas our cows consumed, on average, 15 kg DM d^{-1}. Daily CH_4 production increases with DMI (Boadi et al., 2002). Ricci et al. (2013) studied the correlation between daily CH_4 emissions and several variables such as $BW^{0.75}$, DMI, GEI, CP, NDF and lignin. Stronger correlations were observed for DMI and GEI (on average r = 0.83). A weaker

correlation was with $BW^{0.75}$ (r = 0.64). Crude protein and NDF were correlated but not as strongly (r = 0.04 and 0.20, respectively). Lignin was negatively correlated with CH_4 emissions. These results suggest that intake related variables individually explained a substantial proportion of the variation in observed CH_4. Therefore, we expected to observe higher daily CH_4 emissions due to higher DMI than previous research, and increased from P1 to P2 given that intake increased and overall forage quality decreased during P2.

Bannink et al. (2010) performed a study using simulations to verify the impact of fertilization and grass stage of maturity on cattle. They concluded that the quality of the forage had more effect on CH_4 emission than DMI. This concept is applicable to our results. The rotational grazing management implemented, provided new paddocks every 8 to 12 hours (SysA) or each day (SysB). The rotational grazing practice allowed cows to select for higher quality forage at every feeding event. Consequently, despite the higher DMI compared to previous research, our cows were selecting high quality forage and produced comparatively lower CH_4 emissions.

It has been suggested that continuous set stocking allows maximum selective grazing, which results in higher response per animal than from rotational grazing (Matches and Burns, 1995). De Ramus et al. (2003) have suggested that when forage quality is low, low stocking density and continuous stocking allow the animals to select "portions of the forage plant" that are higher in quality. We are in agreement with De Ramus et al. (2003), mainly because continuous grazing reduces forage diversity, which requires animals to seek portions of the plants with higher quality, given that they do not have a wide range of plant diversity to choose from. Selective grazing is the cause of uneven usage of pasture (Teague et al., 2004), which leads to overgrazing and diversity reduction under continuous grazing practices (Teague et al., 2013). In

our pasture sites, forage diversity was wide enough that cows were able to select higher quality plants, instead of higher quality portions of the same plants.

3.4. Conclusion

Botanical composition analysis showed that both grazing systems implemented in this study allowed the development of grasses and legumes throughout the grazing season. Grazing system with shorter rest periods (SysB) had greater proportion of legumes and overall greater forage quality at the end of the season, than grazing system with longer rest periods (SysA). However, decreased forage quality at the end of the grazing season did not decrease DMI of cows in SysA. We believe that both grazing systems implemented in this study provided opportunities for selective grazing of different plant types, even though overall forage quality decreased at the end of the grazing season in pastures grazed with low stocking rate and high stocking density system.

Our results indicate that grazing management did not affect daily CH_4 emissions from lactating beef cows. Additionally, CH_4 emissions tended to be lower than reported values for lactating beef cows. The selective grazing resulted from the management systems implemented in this study allowed cows managed with different grazing strategies to eat forage with similar qualities that met nutritional requirements with reduced CH_4 emissions. The results in this study suggest that forage quality might be a better predictor to daily CH_4 emissions than DMI. Further research is needed to confirm that hypothesis.

Table 3.1. Forage mass pre and post-grazing and forage disappearance for pastures grazed with different grazing management strategies.

Systems[1]	Periods[2]			Effects		
	P1	P2	SEM	Treatment	Period	Treatment × Period
2012 grazing season						
Pre-grazing , kg ha^{-1}					P-value	
SysA	2278	3824*	127	0.74	<0.01	0.06
SysB	2670	3508*				
Post-grazing, kg ha^{-1}						
SysA	1375	2580^{a}*	121	0.15	0.10	<0.01
SysB	1978	1454^{b}				
Disappearance, %						
SysA	38	26^{a}	6	0.09	0.30	0.23
SysB	32	52^{b}				
2013 grazing season						
Pre-grazing , kg ha^{-1}						
SysA	3851	3735	157	0.83	0.05	0.14
SysB	4240	3443				
Post-grazing, kg ha^{-1}						
SysA	2776	2167^{a}*	82	0.06	<0.01	<0.01
SysB	2963	1524^{b}*				
Disappearance, %						
SysA	26	42*	5	0.28	0.03	0.76
SysB	30	55				

[1] SysA: 1 cow ha^{-1} stocking rate and 100,000 kg LW ha^{-1} stocking density; SysB: 2.5 cows ha^{-1} stocking rate and 28,000 kg LW ha^{-1} stocking density.
[2]Jun 4th - Jun 10th (P1), Sep 4th - Sep 11th (P2), 2012; Jun 5th - Jun 10th (P1), Sep 11th - Sep 17th (P2), 2013.
Mean differences within columns indicated by letters ($P \leq 0.05$). Mean differences within rows indicated by symbols ($P \leq 0.05$).

Table 3.2. Botanical composition of pastures grazed with different grazing management strategies.

Systems[1]	Periods[2] P1		P2	
2012 grazing season		%		%
SysA	Orchard (*Dactylis glomerata*)	70	Bromegrass (*Bromus inermis*)	85
	Birdsfoot trefoil (*Lotus corniculatus*)	17	Orchard (*Dactylis glomerata*)	11
	Dandelion (*Taraxacum officinale*)	13	Red clover (*Trifolium pratense*)	4
SysB	Kentucky (*Poa pratensis*)	34	Timothy (*Phleum pratense*)	70
	Orchard (*Dactylis glomerata*)	54	Red clover (*Trifolium pratense*)	15
	White clover (*Trifolium repens*)	7	Bromegrass (*Bromus inermis*)	11
2013 grazing season				
SysA	Kentucky (*Poa pratensis*)	50	Bromegrass (*Bromus inermis*)	51
	Orchard (*Dactylis glomerata*)	17	Orchard (*Dactylis glomerata*)	26
	Red/white clover (*T. pratense*/*repens*)	7	Birdsfoot trefoil (*Lotus corniculatus*)	9
SysB	Kentucky (*Poa pratensis*)	54	Orchard (*Dactylis glomerata*)	55
	Orchard (*Dactylis glomerata*)	30	Kentucky (*Poa pratensis*)	14
	Red/white clover (*T. pratense*/*repens*)	3	Red clover (*Trifolium pratense*)	21

[1]SysA: 1 cow ha^{-1} stocking rate and 100,000 kg LW ha^{-1} stocking density; SysB: 2.5 cows ha^{-1} stocking rate and 28,000 kg LW ha^{-1} stocking density.
[2]Jun 4th - Jun 10th (P1), Sep 4th - Sep 11th (P2), 2012; Jun 5th - Jun 10th (P1), Sep 11th - Sep 17th, (P2), 2013.

Table 3.3. Nutritional characteristics of pastures grazed with different grazing management strategies.

Systems[1]	Periods[2]		Effects		
	P1	P2	Treatment	Period	Treatment × Period
2012 grazing season					
CP[3], %					
SysA	11.7	11.4[a]	<0.01	0.08	<0.01
SysB	12.3	15.5[b]*			
NDF[4], %					
SysA	65.6	73.9[a]*	<0.01	0.32	<0.01
SysB	65.1	64.1[b]			
Lignin, %					
SysA	3.9	6.1[a]*	<0.01	<0.01	<0.01
SysB	3.1	4.8[b]*			
IVTD[5], %					
SysA	78.1	66.7[a]*	<0.01	<0.01	<0.01
SysB	80.3	78.9[b]			
GE[6], MJ kg^{-1}					
SysA	18.8	17.6	0.64	0.02	0.44
SysB	19.5	17.6			
2013 grazing season					
CP, %					
SysA	13.2	8.1[a]*	<0.01	<0.01	<0.01
SysB	13.2	11.4[b]			
NDF, %					
SysA	62.5	66.8[a]*	<0.01	0.04	0.25
SysB	60.2	62.1[b]			
Lignin, %					
SysA	3.3	6.6*	0.27	<0.01	0.83
SysB	3.1	6.4*			
IVTD, %					
SysA	82.5	58.4[a]*	<0.01	<0.01	<0.01
SysB	83.9	65.1[b]*			

Table 3.3. (cont'd)

Systems[1]	Periods[2]		Effects		
	P1	P2	Treatment	Period	Treatment × Period
GE, MJ kg^{-1}					
SysA	17.6	17.4	0.35	<0.01	0.05
SysB	17.6	17.3			

[1]SysA: 1 cow ha^{-1} stocking rate and 100,000 kg LW ha^{-1} stocking density; SysB: 2.5 cows ha^{-1} stocking rate and 28,000 kg LW ha^{-1} stocking density.
[2]Jun 4th - Jun 10th (P1), Sep 4th - Sep 11th (P2), 2012; Jun 5th - Jun 10th (P1), Sep 11th - Sep 17th, (P2), 2013.
[3]CP: Crude protein.
[4]NDF: Neutral detergent fiber.
[5]In vitro total digestibility.
[6]Gross energy.
Mean differences within columns indicated by letters ($P \leq 0.05$). Mean differences within rows indicated by symbols ($P \leq 0.05$).

Table 3.4. Body weight, dry matter intake, gross energy intake, NDF intake and digestible NDF intake of cows grazed with different grazing management strategies.

Systems[1]	Periods[2]		Effects		
	P1	P2	Treatment	Period	Treatment × Period
BW[3], kg head^{-1}					
SysA	545.1	571.3*	0.8	<0.01	0.29
SysB	544.5	561.4*			
SEM	14.4				
DMI[4], kg d^{-1}					
SysA	12.8^{a}	16.8*	0.16	<0.01	<0.01
SysB	15.2^{b}	16.3*			
SEM	0.42				
GEI[5], MJ d^{-1}					
SysA	226.3^{a}	296.7*	0.16	<0.01	<0.01
SysB	268.7^{b}	286.6*			
SEM	7.5				
NDFI[6], g kg^{-1}					
SysA	8.0^{a}	11.6^{a}*	0.58	<0.01	<0.01
SysB	9.4^{b}	9.8^{b}			
SEM	0.28				
DNDFI[7], g kg^{-1}					
SysA	5.6^{a}	5.4	0.02	<0.01	<0.01
SysB	6.8^{b}	5.5*			
SEM	0.17				

[1]SysA: 1 cow ha^{-1} stocking rate and 100,000 kg LW ha^{-1} stocking density; SysB: 2.5 cows ha^{-1} stocking rate and 28,000 kg LW ha^{-1} stocking density.
[2]Jun 4th - Jun 10th (P1), Sep 4th - Sep 11th (P2), 2012; Jun 5th - Jun 10th (P1), Sep 11th - Sep 17th, (P2), 2013.
[3]BW: Body weight.
[4]DMI: Dry matter intake.
[5]GEI: Gross energy intake.
[6]NDFI: Neutral detergent fiber intake.
[7]DNDFI: Digestible neutral detergent fiber intake.
Mean differences within columns indicated by letters ($P \leq 0.05$). Mean differences within rows indicated by symbols ($P \leq 0.05$).

Table 3.5. Methane emissions from cows grazed with different grazing management strategies.

Systems[1]	Periods[2]		Effects		
	P1	P2	Treatment	Period	Treatment × Period
g CH_4 d^{-1}					
SysA	249.3	235.9	0.13	0.96	0.66
SysB	195.3	206.0			
SEM	20.0				
g CH_4 kg [3]DMI^{-1}					
SysA	21.0[a]	12.1*	0.17	0.08	<0.01
SysB	11.3[b]	14.1			
SEM	1.80				
CH_4 % of [4]GEI					
SysA	6.4[a]	4.1*	0.05	0.09	0.02
SysB	3.8[b]	4.2			
SEM	0.46				
g CH_4 kg [5]$BW^{-0.75}$					
SysA	2.2	2.0	0.29	0.81	0.68
SysB	1.8	1.8			
SEM	0.20				
g CH_4 kg [6]$NDFI^{-1}$					
SysA	32.6[a]	19.2*	0.06	0.03	0.01
SysB	19.1[b]	21.0			
SEM	2.22				
g CH_4 kg [7]$DNDFI^{-1}$					
SysA	46.5[a]	43.0	0.02	0.41	0.1
SysB	26.7[b]	37.8			
SEM	3.77				

[1]SysA: 1 cow ha^{-1} stocking rate and 100,000 kg LW ha^{-1} stocking density; SysB: 2.5 cows ha^{-1} stocking rate and 28,000 kg LW ha^{-1} stocking density.
[2]Jun 4th - Jun 10th (P1), Sep 4th - Sep 11th (P2), 2012; Jun 5th - Jun 10th (P1), Sep 11th - Sep 17th, (P2), 2013.
[3]DMI: Dry matter intake.
[4]GEI: Gross energy intake.
[3]$BW^{-0.75}$:Metabolic body weight.
[6]NDFI: Neutral detergent fiber intake.
[7]DNDFI: Digestible neutral detergent fiber intake.
Mean differences within columns indicated by letters ($P \leq 0.05$). Mean differences within rows indicated by symbols ($P \leq 0.05$).

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CHAPTER 4

CARBON FLUX ASSESSEMENT IN COW-CALF GRAZING SYSTEMS

4.1. Introduction

GHG fluxes from grasslands ecosystems are intimately linked to grazing management. In grasslands, CO_2 is exchanged with the soil and vegetation, N_2O is emitted by soils and CH_4 is emitted by animals and exchanged with the soil. When CO_2 exchange with vegetation is included on net GHG exchange calculation, these ecosystems are usually considered GHG sinks (Soussana et al, 2007; Allard et al., 2007). Similarly, the inclusion of SOC change in net GHG exchange accounting might result in grasslands with GHG sink potentials (Liebig et al., 2010).

Grasslands management choices to reduce GHG budget may involve important trade-offs. Allard et al. (2007) and Soussana et al. (2007) studied net GHG exchange from grasslands including CO_2 exchange with the vegetation, and observed net CO_2 equivalent sink activity, but with different trade-offs. Allard et al. (2007) observed that enteric CH_4 emissions expressed as CO_2 equivalent strongly affected GHG budget in intensive and extensive managed grasslands (average 70% offset of total CO_2 sink activity). Soussana et al., (2007) observed that addition of enteric CH_4 and N_2O emissions from pasture soils to CO_2 sink activity of grasslands resulted in relatively small offset of total CO_2 sink activity (19% average). The small trade-off observed by Soussana et al. (2007) was not enough to affect the CO_2 equivalent sink potential of the sites studied.

Management of grasslands modifies SOC storage (Conant et al., 2001; Schuman et al., 2002), potentially increasing C sequestration (Follet et al., 2001). Grasslands management primarily affects SOC storage by modifying C inputs to the soil, including root turnover and C allocation between roots and shoots (Ogle et al., 2004). Liebig et al. (2010) suggested that the factors contributing to net GHG exchange decreased in relative impact in the order of SOC change, soil-atmosphere N_2O flux, enteric CH_4 emissions, CO_2 emissions associated with N

fertilizer production and application, and soil-atmosphere CH_4 flux. Similarly, Roberston et al. (2000) observed that SOC change and N_2O flux control net GHG exchange in agroecosystems.

In this study we assessed the net GHG exchange (in terms of Ceq flux) of 2 grazing systems differing in stocking rate and density. We hypothesized that low stocking rate, high stocking density systems have lower C flux resulting from less animals per area, and higher accumulation of SOC because of longer rest periods.

4.2. Material and Methods

4.2.1. Pasture management and GHG collection

Cow-calf pairs were managed with 2 rotational grazing management practices differing in stocking rates and density; an intensive system with high stocking rate and low stocking density, and an extensive system with low stocking rate and high stocking density. The system with low stocking rate and high stocking density (SysA) consisted of 120 cow-calf pairs rotating on a total of 120 ha, divided into 0.7 ha paddocks. Cow-calf pairs were moved to a new paddock 3 times daily (at approximately 0600 h, 1200 h and 1800 h). The equivalent stocking rate was 1 cow ha^{-1} and the stocking density was approximately 100,000 kg LW ha^{-1}. The rest period varied from 60 to 90 d during the course of the growing season depending on plant growth. Cow-calf pairs grazed each paddocks 2 to 3 times per year. The system with high stocking rate and low stocking density (SysB) consisted of 4 cow-calf pairs rotating on 1.6 ha pasture, divided into 0.08 ha paddocks. Cow-calf pairs were moved to a new paddock once daily (at approximately 0800 h). The equivalent stocking rate was 2.5 cows ha^{-1} and the stocking density was 28,000 kg LW ha^{-1}. The rest period varied from 18 to 30 d during the course of the growing season depending on plant growth. Cow-calf pairs grazed each paddocks 4 to 5 times per year. The pasture sites in SysB were irrigated as needed, whereas there was no irrigation applied to SysA

pasture sites. The only fertilization application was on SysB pasture sites that received urea fertilization (23 kg of actual urea) on June 3rd of 2011 (approximately 30 d before the start of gas sampling, see dates below). In addition to these 2 systems, grazing-exclusion pasture sites (GE) were monitored in order to account for GHG emissions from non-grazed pastures. The use of a non-grazed pasture site was important to confirm that any differences found between SysA and SysB were attributed to the grazing management practices implemented. The soil type across treatments pasture sites was predominantly sandy loam.

SysA and SysB areas were sampled during 3 years (2011 to 2013). Sampling for all treatments was repeated in 2 periods; at the beginning of the grazing season (period 1 – P1) and at the end of the grazing season (period 2 – P2). The first year was considered a preliminary year, for the purpose of adjusting the methodology for GHG from soils collection. For that reason, GE pasture sites were not sampled, dates of periods monitored were closer together in time as compared to 2012 and 2013, soil bulk density (BD) was not monitored, soil was sampled to 10 cm depth, and enteric CH_4 emissions were not monitored. For details on dates of each period and methodologies used on GHG emissions from soils and enteric CH_4 emissions refer to Chapter 2, section 2.2 and Chapter 3, section 3.2. Soil texture and pH in each treatment are described in Table 2.1, Chapter 2.

Soil sample collection occurred in paddocks most recently occupied by cows. Soil samples were collected from 0.08 ha paddocks (3 pseudoreplicates per treatment). Soil sampling occurred approximately 20 days post-grazing. The sampling dates were: August 1st, and August 28th, 2011; June 3rd and September 15th, 2012; June 30th and September 28th, 2013.

4.2.2. Soil bulk density determination

Soil BD samples were collected with a 7.6 cm diameter and 7.5 cm height brass ring, avoiding disturbance of soil structure. Samples were weighed, dried at 105°C to constant weight, and re-weighed. Bulk density was calculated by dividing the dry weight by the soil core volume (Blake and Hartge, 1986). Soil BD was not assessed during 2011. Soil BD was monitored in different depths to allow SOC stock calculation (described below). However the distinction of BD at the 0 to 5 cm and 5 to 10 cm depths was not possible because of the ring height (7.5 cm). For that reason, BD in the top soil was assessed from 0 to 7.5 cm and it was used to calculate SOC stock at 0 to 5 cm and 5 to 10 cm depths. SOC stock at 10 to 20 cm was calculated with BD of 10 to 17.5 cm depth, and SOC stock at 20 to 30 cm was calculated with 20 to 27.5 cm BD.

4.2.3. Soil organic matter and C and N stocks determination

During 2012 and 2013, the soil pool was assessed at different depths: 0 to 5 cm, 5 to 10 cm, 10 to 20 cm, and 20 to 30 cm. SOC and TSN stocks were not monitored during 2011. A 0 to 30 cm depth is often used to report C stocks in soils (Schipper and Sparling, 2011). Previous studies suggest that changes in soil C and N can extend throughout the soil profile rather than just in the topsoil (Schipper et al., 2007; Franzluebbers and Stuedemann, 2009). Therefore, sampling occurred at different depths to illustrate changes along the profile and address the concern that changes in the surface soil may not represent storage in deeper horizons (Blanco-Canqui and Lal, 2008). For each replicate (0.08 ha paddock) 10 soil samples were randomly collected at each depth and composited per paddock. Soil samples were dried at 65°C separated in 2 sub samples. One sub sample was sent to the Michigan State University Soil and Plant Nutrient Laboratory for SOM determination. SOM was determined by wet digestion and colorimetry (Schulte and Hopkins, 1996). The second sub sample was ground manually with a

pestle and mortar and sent to Michigan State University Great Lakes Bioenergy Research Center Laboratory for analysis of C and N.

Soil OC and total soil N (TSN) from soil samples were determined by an Elemental Combustion System (ECS 4010 CHNSO Analyzer, Costech, Valencia, CA). The ECS uses combustion and gas chromatography with thermal conductivity detector and helium as carrier gas to determine N_2 and CO_2. We tested for the presence of inorganic C in the soils of the study area and concluded that no inorganic forms were present, thus total C represents SOC. Carbon: nitrogen ratio was calculated for 0 to 30 cm depths.

Soil OC and TSN stocks were calculated based on soil layers of fixed depth (Equation 4.1). However, given that we observed high variability on BD between years and among treatments, we corrected SOC and TSN values for a fixed mass of soil, as suggested by Ellert et al. (2002; Equation 4.2 to 4.4 use SOC as example of calculations). This approach includes the selection of a reference soil mass (M_{ref}), which is the lowest soil mass to the prescribed depth from all sampling sites. The M_{ref} is then used to determine the soil mass to be subtracted from the deepest core segment (excess mass of soil: M_{ex}) so that mass of soil is equivalent to all sampling sites

Equation 4.1. Soil organic carbon and nitrogen stock calculated based on soil layers of fixed volume.

$$SOC_{FD} = \Sigma\, C_i \times BD_i \times L_i \times 0.1$$

where SOC_{FD} is SOC stock to fixed depth (Mg ha^{-1}), C_i is organic carbon concentration in depth *i* (mg C g^{-1} dry soil), BD_i is the bulk density of soil in depth *i* (g m^{-3}), and L_i is the length of the depth *i* (cm).

Equation 4.2. Determination of soil mass in each depth.

$$M_{soil} = \Sigma\, BD_i \times L_i \times 100$$

where M_{soil} is mass of soil to a fixed depth (Mg ha^{-1}), BD_i is bulk density of soil in depth *i* (g/m^3), and L_i is the length of the depth *i* (cm).

Equation 4.3. Determination of mass of excess soil in each depth.

$$M_{ex} = M_{soil} - M_{ref}$$

where M_{ex} is mass of excess soil (Mg ha^{-1}), M_{soil} is the mass of soil to a fixed depth (Mg ha^{-1}), and M_{ref} is the lowest soil mass selected from all sampling sites and depths (Mg ha^{-1}).

Equation 4.4. Determination of SOC stock to fixed mass of soil.

$$SOC_{FM} = SOC_{FD} - M_{ex} \times C_{dl}/1000$$

where SOC_{FM} is the SOC stock for a fixed mass of M_{ref}, M_{ex} is mass of excess soil (Mg ha^{-1}), and C_{dl} is organic carbon concentration in the deepest depth (mg C g^{-1} dry soil).

4.2.4. C flux calculations

In this study, fluxes from the ecosystem to the atmosphere are considered a contribution to the atmosphere budget. Therefore, positive GHG emissions indicate emissions to the atmosphere and negative GHG emissions indicate sink activity. According to Chapin et al. (2002) and adapted later by Soussana et al., (2007) the net GHG exchange (NGHGE) of a managed grassland ecosystem is calculated as:

$$NGHGE = NEE + F_{CH4} + F_{N2O}$$

where NEE is the net ecosystem exchange of CO_2 that includes emissions from soil and plant respiration, F_{CH4} is the CH_4 flux from soil and F_{N2O} is N_2O flux from the soil. We adapted the calculation to obtain the net GHG exchange in terms of C equivalent (Ceq_{flux}). The Ceq_{flux} for each site was calculated by adding CH_4 and N_2O emissions to CO_2 emissions using the global warming potential of each of these gases at the 100-year time horizon (IPCC, 2007; GWP_{N_2O} = 298 and GWP_{CH4} = 25), as follows

$$Ceq_{flux} = F_{CO2} + F_{CH4soil} + F_{N2O} + F_{CH4cows}$$

where F_{CO2} is the C equivalent flux of CO_2 from the soil, $F_{CH4soil}$ is the C equivalent flux of CH_4 from the soil, $F_{CH4cows}$ is the C equivalent flux of enteric CH_4 from the cows, and F_{N2O} is the C equivalent flux of N_2O from the soil. In contrast to Soussana et al. (2007) our F_{CO2} does not include CO_2 lost by plant and animal respiration. The largest part of organic C ingested during grazing is highly digestible and is respired shortly after intake (Soussana et al., 2007). Additional C loss (5% of digestible C) occurs through enteric CH_4 emissions, which was accounted for by the term $F_{CH4cows}$. We did not account for enteric CH_4 from the calves. The non-digestible C (from 25 to 40% of the intake depending on herbage digestibility) is returned to the pasture mainly as feces (Soussana et al., 2007). We did not differentiate between manure-derived emissions and soil-derived emissions. Soil emissions sampling was post-grazing and hence we assume that any emissions from feces or urine decomposition is accounted for in the soil term.

Soussana et al. (2007) and Chapin et al. (2002) included the C lost from the system through plant biomass export. Because our calculations are limited to the grazing season we assumed no C loss via herbage cutting and removal from the sampled sites. C loss from herbage decomposition on top of the soil is assumed to be included in CO_2 and CH_4 emissions from the soil, SOM and SOC content. There was no addition of C into our systems by organic fertilization and hence it is not included on the calculations. We did not account for C leaching from pasture soils.

In order to allow summation of GHG fluxes from soil and cows and determination of Ceq_{flux}, $F_{CH4cows}$ (originally in g CH_4 cow day^{-1}) was converted to an area basis (g CH_4 ha d^{-1}), using stocking rates of each system: SysA = 1 cow ha^{-1}, and SysB = 2.5 cows ha^{-1}. We monitored

only the grazing season and the Ceq_{flux} is shown as daily average flux, because extrapolation to annual flux would be inaccurate.

SOC stock change was not included in the Ceq_{flux} determination because SOC content was monitored for a period of 2 years, which is not considered long enough to detect accurate SOC changes (Schuman et al., 2002). However, we consider SOC stock in our discussion of Ceq_{flux} because the main objective of this study was to show the importance of looking at different pools when assessing GHG emissions from grazing systems. SOC stock is an important pool to consider in any C flux accounting.

4.2.5. Statistical analysis

SOC and TSN stocks data were analyzed as a completely randomized design. Statistical analyses were performed using SAS Software (Version 9.2; SAS Institute, 1987). Paddocks were considered experimental units and were treated as the random term, and the compressed term year × period was considered a repeated measure. We associated the effects of year and period to the variability of the data, and hence means are shown pooled my year and period. The main reason for showing pooled means was that the length of this study was not long enough to allow assessment of SOC change in time, and showing means by year could lead to inaccurate conclusions. All tests were performed with 95% confidence ($\alpha = 0.05$). Soil and animal GHG emissions data were analyzed as described in Chapter 2, Section 2.3.3 and Chapter 3, Section 3.2.3, respectively.

Ceq_{flux} data were analyzed as a completely randomized design. Paddocks were considered experimental units and were treated as the random term, and the compressed term year × period was considered a repeated measure. When the main effect of year was significant differences were discussed separately by year. When the main effects of treatment or period were

significant the interaction treatment × period was evaluated and pre-planned comparisons within treatment and period were performed. All tests were performed with 95% confidence ($\alpha = 0.05$).

4.3. Results and Discussion

4.3.1. Soil characteristics

Soil sampling was performed in different pasture sites during each year and period sampled, depending on animal management. The sampling sites in GE were maintained constant for all sampling occasions. A summary of particle size fractions in each pasture size is described in Table 2.1, Chapter 2.

Soil BD values were different from 2012 to 2013 ($P < 0.01$), but did not change from P1 to P2 ($P = 0.19$). Therefore means are poled by period. Soil BD increased with soil depth but no treatment effects were observed (Table 4.1). The accumulation of litter over time is a result of rotational grazing, with adequate rest periods for regrowth. The presence of organic litter dissipates the animal trampling impact, resulting in less compaction and lower soil BD of the soil (Sanjari et al., 2008). The accumulation of litter protected grazed soils from compaction, resulting in no BD differences between grazing systems and GE. Savadogo et al. (2007) and Franzluebbers and Stuedemann (2009) reported BD values similar to this study.

Soil BD has been found to increase because of grazing in soils with large quantities of fine soil particles (clay + silt) that are more sensitive to animal traffic and compaction (Vanhaveren, 1983; Abdelmagid et al., 1987). Our pasture sites were predominantly comprised of sand particles, and mostly sandy loam.

4.3.2. SOC and TSN stock and SOM content

We observed year and period effects on SOC stocks ($P < 0.01$ and $P = 0.05$, respectively), which are likely associated to spatial and temporal variability. Soil C stocks

display high spatial variability, especially in grasslands. Cannell et al. (1999) found a coefficient of variation of 50% when evaluating spatial variability of C stocks in grasslands as compared to 15% in arable lands. Previous research have associated the variability to sampling at different depths (Bird et al., 2002), climate (Conant et al., 2001), texture (mainly clay content; Parton et al., 1987), and lack of evaluation of C distribution within the grazing system (Schumann et al., 1999). The ability to detect change in SOC stocks depends on the time since the original sampling, spatial homogeneity of the soil and intensity of sampling (Schipper et al., 2010). In this study, sampled paddocks (pseudoreplicates) were different at each year and period (see Section 4.2.), which did not allow spatial homogeneity between soil samples. In addition, Conant et al. (2001) suggested that periods of 5 to 10 years for a field scale study would be adequate to detect changes in SOC stock. Therefore, the change observed from 2012 to 2013 cannot be associated to SOC stock change (i.e. accumulation or loss). However, because the studied grazing systems were implemented at the study site for 5 years prior to 2012, the relative change between treatments may be considered.

Table 4.2 illustrates SOC stock means by treatment pooled by year and period. On average, SOC stock was higher for SysB pasture sites, and the difference between GE and SysA was not significant (63, 42 and 47.4 Mg C ha^{-1} for SysB, GE and SysA respectively, $P < 0.01$). In SOM, N and C are predominantly covalently bonded (Schipper et al., 2010) and thus the pattern of TSN accumulation in pasture sites was highly correlated to SOC accumulation (Table 4.2). SysB pasture sites had higher TSN stocks compared to GE and SysA (4.85, 3.44 and 3.95 Mg N ha^{-1}, for SysB, GE and SysA respectively, $P < 0.01$). A similar relationship between C and N reported by Pineiro et al. (2009).

The effects of grazing management on C cycling and distribution has been evaluated before, however, literature does not yet suggest a clear relationship between grazing management and C sequestration. Some studies have reported no effect of grazing on SOC stock (e.g. Milchunas and Laurenroth, 1993), others reported increases (Weinhold et al., 2001) or a decrease (Derner et al., 1997). Differences in findings between SOC stocks and grazing management has been associated with factors that affect C cycling and sequestration potential on grasslands, such as: climate, inherent soil properties, landscape position, plant community composition, and grazing management practices (Reeder and Schuman, 2002). The management applied to the land affects soil's ability to retain organic C. Practices that increase plant productivity and C inputs to the soil, and decrease soil exposure to sunlight and erosion allow greater C accumulation (Parton et al., 1987).

Reeder and Schuman (2002) studied the impact of heavy or light grazing on SOC stocks, compared to non-grazed areas. In their evaluation of the 0 to 30 cm layer, they observed significantly higher SOC stock in grazed pastures (67 Mg C ha^{-1}) compared to non-grazed pastures (58 Mg C ha^{-1}). The range of SOC stock observed was from 55 Mg C ha^{-1} to 100 Mg C ha^{-1}. We observed wider range of SOC stock values among all treatments (from 25 to 113 Mg C ha^{-1}; data not shown). The greater variability observed in this study might be associated to the sampling in different pasture sites at each year and period. Sanjari et al. (2008) observed lower SOC stock values for rotational grazing, continuous grazing and non-grazed pasture sites in 5 years of monitoring (on average 25 Mg C ha^{-1}). However, increased SOC content in rotational grazing pasture sites compared to continuous grazing or non-grazed pasture sites was observed by Sanjari et al. (2008) and associated to greater grass growth and rest periods. Southorn (2002) attributed the greater SOC accumulation in rotational grazing systems to the larger proportion of

plant material being incorporated into the soil. In addition, adequate rest periods is a key driver in the recovery of grazed species and increase in aboveground organic material, followed by its subsequent incorporation into the soil, resulting in increased SOC (Gillen et al., 1991).

In this study, SysA pasture sites were given longer rest periods (60 to 90 d) than SysB pasture sites (18 to 30 d). Nevertheless, the increased SOC stock of SysB pasture sites suggested that grazing management of SysB is increasing SOC stocks at a faster rate than SysA or GE ($P < 0.01$; Table 4.2). Naeth et al. (1991) suggested that grazing, such as that in SysB, reduces litter mass accumulation because animal traffic enhances physical breakdown and incorporation of litter into the soil. It is likely that more frequent grazing in SysB reduced litter accumulation, and enhanced physical breakdown increasing litter decomposition and incorporation into the soil. Frequent grazing also could have stimulated forage and roots development, increased soil water content and microbial development, enhancing the rate of decomposition of litter and transfer of C into deeper layers of the soil (Sharif et al., 1994). Root decay, although not measured in this study, was identified as another reason for increased SOC under rotational grazing systems. Intensive defoliation under a single grazing event results in cessation of plant respiration, leading to death of roots within a few hours after grazing, in order to equalize biomass (Sanjari et al., 2008). In SysB defoliation was intensive and more frequent than in SysA.

In SysA, forage offered to cow-calf pairs was mature and in reproductive stage, which resulted in selective grazing by cows for higher quality plants (see discussion on Chapter 3, Section 3.3.3). Forage that was not ingested was trampled down, resulting in greater litter accumulation on soil surface (Table 3.1, Chapter 3). The significantly lower SOC stock in SysA and GE compared to SysB might be the result of immobilization of C in excessive aboveground plant litter, due to longer rest periods (SysA) or non-grazing (GE).

Soil organic C constitutes approximately 60% of SOM (Bardgett et al., 2009). Consequently, the differences in SOM content between treatments were similar to the differences observed for SOC stocks. SysB had higher SOM content to 30 cm than SysA or GE that did not differ (4.07%, 3.33% and 3.22%, for SysB, SysA and GE, respectively, $P < 0.01$). SOM decreased throughout the soil profile in all treatments (Figure 4.1).

In SysA pasture sites, animal trampling was more intense at each grazing occasion (due to higher stocking density), but it was less frequent (longer rest periods). The higher stocking density might have contributed to the formation of litter on soil surface, but without frequent animal trampling, it is likely that litter decomposition happened at a slow rate. Because of higher stocking density, cow-calf pairs grazed each paddock of SysA for a short period of time (8 to 12 h). The short time of grazing was likely not prolonged enough to accelerate litter decomposition and incorporation into the soil. Reeder and Schuman (2002) suggests that a build-up of litter on the soil surface affects soil temperature and soil water content, which will, in turn, affect plant residue and SOM decomposition rates.

When observing the SOC distribution along the soil profile, SysB contained higher SOC content in the 20 to 30 cm layers compared with SysA ($P = 0.02$) and GE ($P = 0.03$; Figure 4.2). It was interesting to find that SysB pasture sites had accumulated C mainly in deeper layers. We expected that, because of the long rest period and lack of irrigation on SysA, deep-rooted plant species would develop and significantly contribute to SOC accumulation in deeper layers, as it was observed before (Fisher et al., 1994). However, botanical composition did not support that hypothesis (Table 3.2, Chapter 3). Legumes were found to be present on both SysA and SysB pasture sites, and the same grasses species were found on both systems (although on different proportions).

The surface depth (0 to 10 cm) generally contains the highest levels of labile C, indicative of rapid turnover. This labile C is important mainly to ecosystem function and microbial development. It represents the C participating in C cycling within the ecosystem and is not representative of sequestered C. Carbon sequestered in deeper layers, indicates favorable conditions for root penetration and high levels of microbial activity. Deeply sequestered C enhances ecosystem hydrology and nutrient recycling. Additionally sequestration of C on deeper layers provide long-term benefits, because C is less susceptible to loss from surface-soil disturbances (Franzluebbers and Stuedemann, 2009). Our data supports earlier findings that change in soil C can extend throughout the soil profile (Schipper et al., 2010; Schipper et al., 2007). Schipper et al. (2010) observed that despite the apparent long residence time of soil C in deep horizons, SOC moves through 1 m-deep horizons more rapidly than previously thought. The frequent trampling effect caused by the cow-calf pairs in SysB resulted in disruption of surface soil crust and soil aggregates, increasing SOM decomposition and SOC incorporation in deeper depths (Liu et al., 2004; Neff et al., 2005). Intensive grazing has been associated to high rate of SOM decomposition (Sanjari et al., 2008).

TSN concentration was also highly stratified with depth and followed SOC accumulation (Figure 4.3). Conant et al. (2005) and Franzluebbers and Stuedemann (2009) find that changes in SOC stock were closely related to changes in TSN stock. There are potential benefits as a result of coupling between soil C and N changes. For example, the sequestration or loss of 1 Mg C is associated with approximately 100 kg of N gained or lost (Schipper et al., 2010). There was no treatment effect on C:N ratio (Table 4.2). The relatively high C:N ratio observed in this study suggest that C and N immobilization is the dominant processes over mineralization (Du Preez and Snyman, 1993).

4.3.3. Total C equivalent flux

Means are shown separately by year and period for F_{CO2}, $F_{CH4soil}$, F_{N2O}, $F_{CH4cows}$ and Ceq_{flux} (year effect $P < 0.01$; Table 4.3). Daily means are presented in order to allow discussion on the overall Ceq_{flux} between grazing systems and non-grazed pasture sites (Table 4.4).

Grazing systems versus non-grazed pasture sites - Generally, grazing systems had higher Ceq_{flux} than GE pasture sites, except during P2 of 2012, when the difference between SysA and GE was not significant (Table 4.3). The increased Ceq_{flux} from grazing systems was expected because $F_{CH4cows}$ was considered zero for GE. However, the difference between grazing systems and GE was substantially small.

The initial hypothesis was that Ceq_{flux} would be increased in grazing systems not only due to enteric CH_4, but also because of manure decomposition in pasture soils. However, during 2012 the difference between grazing systems and GE was approximately 3 kg C ha d^{-1}, which approximates $F_{CH4cows}$. This suggests that during 2012, grazing did not increase GHG flux from the soil. The Ceq_{flux} pooled by treatment during 2012 (average 10.3 kg C ha d^{-1}) was greater when compared to 2011 (9.6 kg C ha d^{-1}) and 2013 (19.8 kg C ha d^{-1}). The year of 2012 was relatively dry, with precipitation concentrated in a few days during the grazing season (Table 2.1, Chapter 2). The low soil moisture content could have decreased GHG flux from the soil in all pasture sites. The year of 2011 does not include $F_{CH4cows}$.

During 2013, the difference in Ceq_{flux} between grazing systems and GE was greater (approximately 8 kg C ha d^{-1} during P1, and 11 kg C ha d^{-1} during P2) than the contribution of $F_{CH4cows}$ (on average 3.3 kg C ha d^{-1}). Generally, during 2013 GE pasture soils had decreased F_{CO2}, $F_{CH4soil}$, and F_{N2O} compared to grazing systems. GE pasture sites were the only ones with

observed N_2O and CH_4 sink activities, during the 2013 grazing season. The higher levels of moisture in the soil (compared to 2012) likely increased microbial activity, resulting in increased GHG exchange from pasture soils. During P2 of 2013, SysB had greater Ceq_{flux} than SysA and GE. It was the only occasion when the difference between grazing systems was observed.

SysA versus SysB

During 2011, $F_{CH4cows}$ was not monitored and Ceq_{flux} represents the addition of F_{CO2}, $F_{CH4soil}$ and F_{N2O} (Table 4.3). F_{N2O} and $F_{CH4soil}$ were not different between treatments in neither period. During P2, SysB had greater F_{CO2} than SysA (7.64 and 6.07 kg C ha^{-1} d^{-1}, respectively), which resulted in greater Ceq_{flux} from SysB pasture sites than SysA during P2. Pooled by treatment, Ceq_{flux} decreased considerably from P1 to P2 (11.2 and 8.2 kg C ha^{-1} $d^{-}1$, for P1 and P2, respectively; $P < 0.01$). Because there were no consistent differences in F_{N2O} and $F_{CH4soil}$ from P1 to P2, the decrease in Ceq_{flux} is due only to the decrease in F_{CO2}. These results suggest that, when $F_{CH4cows}$ is not taken into account, F_{CO2} seems to be the driver of Ceq_{flux} in grazed pastures.

During 2012, $F_{CH4cows}$ is included in Ceq_{flux}. The differences between systems observed in F_{CO2}, $F_{CH4soil}$, F_{N2O}, or $F_{CH4cows}$ were not significant, and consequently the difference between systems in Ceq_{flux} was likewise not significant (Table 4.3). Despite the greater stocking rate of SysB (2.5 cows ha^{-1}) compared to SysA (1 cow ha^{-1}), $F_{CH4cows}$ were not significantly different between grazing systems during P2. We expected greater $F_{CH4cows}$ from SysB because of the greater number of cows per hectare. However, the results suggest that SysA cows had relatively high enteric CH_4 emissions, during 2012 (Table 4.3)

During 2013, SysB had higher Ceq_{flux} when compared to SysA during P2 (22.49 versus 13.40 kg C ha^{-1} d^{-1}, respectively; $P < 0.01$). The increased Ceq_{flux} from SysB was a result of greater $F_{CH4cows}$ compared to SysA during P2 (6.22 versus 1.61 kg C ha^{-1} d^{-1}, respectively; $P = 0.02$), because SysB did not have increased GHG emissions from soils compared to SysA (Table 4.3). During P1, again SysB had greater $F_{CH4cows}$ compared to SysA (3.26 versus 1.93 kg C ha^{-1} d^{-1}, respectively $P = 0.03$). However, Ceq_{flux} was not different between grazing systems (24.11 and 23.35 for SysA and SysB, respectively, $P = 0.13$). The decreased $F_{CH4cows}$ in SysA, was offset by the numerical increased F_{N2O}, which increased Ceq_{flux} of SysA. These results suggest that the contribution of enteric CH_4 to Ceq_{flux} may be not always be the driver of higher GHG emissions. Robertson et al. (2000) showed that half of the total net CO_2 equivalent emissions from arable sites was contributed by N_2O production. Our results indicate that under specific circumstances this concept might apply to grasslands. Results from Soussana et al. (2007) indicate that despite the large error in enteric CH_4 measuring, the CH_4 emission rate would not lead to a large change in the net GHG exchange of the studied grasslands.

Daily Ceq_{flux} pooled by year and period

In order to allow the comparison between treatments across years and periods, we pooled daily means (Table 4.4). It is important to keep in mind that we sampled only during the grazing season. By not monitoring Ceq_{flux} during the winter, early spring or late fall, the pooled daily means cannot be extrapolated to annual means.

Daily Ceq_{flux} from grazing systems was higher than non-grazed pasture sites by approximately 5.8 kg C ha^{-1} d^{-1} ($P < 0.01$). The largest contributor for the greater Ceq_{flux} from grazing systems compared to GE was $F_{CH4cows}$. However, pooled across years grazing systems

also had higher F_{N2O} and $F_{CH4soil}$ than GE. Between grazing systems the difference in Ceq_{flux} ($P = 0.60$) was not significant. The only flux that was different between grazing system was $F_{CH4cows;}$ SysB had greater $F_{CH4cows}$ than SysA (4.91 versus 2.09 kg C ha^{-1} d^{-1}, respectively; $P < 0.01$). The increased $F_{CH4cows}$ from SysB was a consequence of higher stocking rate, because daily enteric CH_4 emissions were not difference between systems across years (Table 3.5, Chapter 3). The contribution of $F_{CH4cows}$ in SysB was not large enough to increase Ceq_{flux}.

Typical N_2O emissions from grasslands soils converted into C equivalent range between 0.3 and 3 kg C ha^{-1} d^{-1} (Machefert et al., 2002). Freibauer et al. (2004) observed N_2O fluxes of 0.7 kg C ha^{-1} d^{-1} from grasslands. On the other hand, Soussana et al. (2007) studied grasslands GHG flux throughout the year and found N_2O emissions varying from -0.08 to 2.4 kg C ha^{-1} d^{-1}. In the present study, we observed F_{N2O} from 0.06 to 1.35 kg C ha^{-1} d^{-1}.

Regarding $F_{CH4soil}$, we observed sink activity ($F_{CH4soil}$ range was from -0.16 to 0.14 kg C ha^{-1} d^{-1}, whilst Soussana et al. (2007) when monitoring CH_4 fluxes throughout the year obtained higher emissions (0.2 to 1.3 kg C ha^{-1} d^{-1}). They associated the lower sink activity observed to the presence of grazers, suggesting that grazing reduces the on-site sink activity for CH_4. In fact, the negative mean of $F_{CH4soil}$ in the present study was from GE pasture sites (Table 4.3). Deposition of excreta by animals is expected to produce CH_4 emissions at a very low level (as compared to application of organic fertilizers; Jarvis et al., 2001), but may increase N_2O emissions (Smith et al., 2001).

In the present study, very low $F_{CH4soil}$ was observed and when differences between treatments were observed they were due to F_{CO2}, F_{N2O} or $F_{CH4cows}$ (Table 4.3). Liebig et al. (2010) suggested that factors contributing to net GHG exchange in grasslands were decreased in relative

impact order of SOC change, soil-atmosphere N_2O flux, enteric CH_4 emissions and soil-atmosphere CH_4 flux.

We did not include SOC change in Ceq_{flux} determination, and the differences in N_2O fluxes were not significant between grazing treatments, which resulted Ceq_{flux} differences that were not significant between grazing systems. Liebig et al. (2010) including SOC change in the GHG exchange determination, observed negative net GHG from heavily and moderately grazed grasslands. Allard et al. (2007) and Soussana et al. (2007) also observed negative GHG exchange from grasslands, because CO_2 exchange with the vegetation was included on the determination of net GHG exchange. The annual mean Ceq_{flux} from SysB was lower than the annual mean Ceq_{flux} from SysA (Table 4.4), although means were not statistically different. However, if SOC change was included on Ceq_{flux} these results and conclusions could change. SOC stock results suggested that potentially SysB is accumulating higher SOC than SysA (Table 4.2), but long-term monitoring of SOC stock in the study is needed to allow incorporation of SOC change in Ceq_{flux} determination.

Generally, the higher stocking rate in SysB increased $F_{CH4cows}$, but did not affect $F_{CH4soil}$ and F_{N2O}. We believe that the lower stocking density in SysB and irrigation allowed shorter rest periods, frequent herbage defoliation, faster return of nutrients to soils from excreta deposition, increased plant growth and roots development. These factors, in addition to greater TSN content in SysB, might have contributed to microbial development and faster nutrient cycling, decreasing GHG emissions from soils. It was demonstrated in Section 4.3.2 that SysB is potentially increasing SOC stocks at a faster rate than SysA or GE. Similarly, SOM content was higher in SysB compared to SysA and GE, which suggests faster litter decomposition. SOC accumulation on deeper layers (20 to 30 cm) was greater in SysB, which also suggests potential

of C sequestration. In addition, SysB gives the producer more flexibility in terms of animal production. Because of shorter rest periods and frequent defoliation forage quality remained high and constant throughout the grazing season (Table 3.3, Chapter 3). The maintenance of forage quality permits the production of different types of animals, such as finishing steers for instance, which permits the producers to aggregate value to their final product according to market changes.

In SysA there was a decrease in forage quality from P1 to P2 (Table 3.3, Chapter 3) but $F_{CH4cows}$ was not increased, which was associated to selective grazing. We observed the development of legumes in both systems, indicating that the grazing management is not depleting the development of specific plant species, and selective grazing is allowed in both systems. SysA does not need irrigation and longer rest periods results in litter accumulation on the top soil, with slow decomposition rate. It is possible that the SOM slower decomposition rate of SOM in SysA could provide greater resilience to SysA compared to SysB.

It is important to remember that we monitored GHG exchange during the grazing season only. We did not account for emissions in other periods other than post-grazing, and hence annual emissions may not be accurate. Similarly, we are assuming that the grazing seasons of both systems were of the same duration. If one system allowed prolonged or shortened grazing season, Ceq_{flux} would change.

4.4. Conclusion

Grazing systems had greater Ceq_{flux} than non-grazed pasture sites. The largest contributor to increased Ceq_{flux} from grazing systems was enteric CH_4 emissions. However, on an annual basis, grazing systems also had increased N_2O and CH_4 emissions from pasture soils, compared to non-grazed pasture sites. Non-grazed pasture sites were the only sites with CH_4 sink activity.

The effect of greater enteric CH_4 contribution from SysB, due to higher stocking rate than SysA, was offset by GHG exchange from the soil. Hence, our results indicate no clear difference in C equivalent flux between the grazing systems studied, when SOC change is not incorporated. SysB potentially increased total SOC stock, the addition of SOC to deeper into the soil horizon and SOM content to 30 cm. SysA, with longer rest periods, allowed litter accumulation on the top soil, resulting in slower SOM decomposition rate, which can result in greater resilience in the long-term.

Grazing management should be adaptive and farm decisions are inherent to grazing management. Both SysA and SysB have opportunities to improve ecosystems services at the farm level, including animal production and food provisioning. Long-term research is needed to confirm SOC stock and SOM decomposition rates of these systems. The incorporation of C sequestration into the determination of Ceq_{flux} could change results and possibly differentiate the grazing systems studied.

Table 4.1. Soil bulk density in pasture soils grazed under two management strategies and non-grazed.

Soil depth, cm	Systems[1]		
	GE	SysA	SysB
2012 grazing season		g cm^{-3}	
0 to 5	1.27	1.20	1.25
5 to 10	1.27	1.20	1.25
10 to 20	1.57	1.25	1.35
20 to 30	1.43	1.47	1.44
SEM		0.05	
Source of Variation			
Treatment	0.11		
Depth	<0.01		
Treatment x Depth	0.11		
2013 grazing season			
0 to 5	1.46	1.57	1.39
5 to 10	1.46	1.57	1.39
10 to 20	1.65	1.58	1.62
20 to 30	1.65	1.59	1.57
SEM		0.04	
Source of variation			
Treatment	0.14		
Depth	<0.01		
Treatment x Depth	0.36		

[1] GE: grazing exclusion; SysA: 1 cow ha^{-1} stocking rate and 100,000 kg LW ha^{-1} stocking density; SysB: 2.5 cows ha^{-1} stocking rate and 28,000 kg LW ha^{-1} stocking density.

Table 4.2. Soil organic carbon and total soil nitrogen stocks in pasture soils grazed under two management strategies and non-grazed.

Systems[1]	Stocks		
	SOC[2]	TSN[3]	C:N
	Mg ha^{-1}		
GE	42.0[a]	3.44[a]	21.0
SysA	47.4[a]	3.95[a]	18.7
SysB	63.0[b]	4.85[b]	19.4
SEM	3.8	0.2	
Source of Variation			
Treatment	<0.01	<0.01	0.06

[1]GE: grazing exclusion; SysA: 1 cow ha^{-1} stocking rate and 100,000 kg LW ha^{-1} stocking density; SysB: 2.5 cows ha^{-1} stocking rate and 28,000 kg LW ha^{-1} stocking density.
[2]SOC: soil organic carbon.
[3]TSN: total soil nitrogen
Means differences within columns indicated by letters ($P < 0.05$).

Table 4.3. GHG exchange from pasture soils and animal and total C equivalent flux from pasture sites managed under two different management strategies and non-grazed pasture sites.

Systems[1]	Soil emissions F_{CO2}[2]		F_{N2O}[3]		$F_{CH4soil}$[4]		Animal Emissions $F_{CH4cows}$[5]		Total emissions Ceq_{flux}[6]	
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
2011 grazing system	kg C ha^{-1} d^{-1}									
GE	-	-	-	-	-	-	-	-		
SysA	10.54	6.07[a]*	1.16	0.80	-0.18	-0.07	-	-	11.35	6.77[a]*
SysB	9.74	7.64[b]*	1.19	1.59	-0.21	0.06*	-	-	10.69	9.57[b]
SEM	0.41		0.32		0.04				0.64	
Source of Variation										
Treatment	0.28		0.07		0.25				0.03	
Period	<0.01		0.96		0.02				<0.01	
Treatment × Period	<0.01		0.08		0.04				<0.01	
2012 grazing season										
GE	8.24	9.13	0.11	0.05	0.01[a]	0.003	0	0	8.38[a]	9.18[a]
SysA	8.04	8.31	0.44	0.08	0.14[b]	0.08	3.28	2.26	12.06[b]	10.75[ab]
SysB	7.11	9.26*	0.31	0.19	0.08[a]	0.07	4.89	3.43	12.17[b]	12.73[b]
SEM	0.50		0.11		0.04		0.63		0.57	
Source of Variation										
Treatment	0.43		0.19		<0.01		0.12		<0.01	
Period	0.15		0.09		0.38		0.03		0.97	
Treatment × Period	0.07		0.33		0.51		0.68		0.06	

Table 4.3. (cont'd)

Systems[1]	Soil emissions F_{CO2}[2]		F_{N2O}[3]		$F_{CH4soil}$[4]		Animal Emissions $F_{CH4cows}$[5]		Total emissions Ceq_{flux}[6]	
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
2013 grazing season	kg C ha^{-1} d^{-1}									
GE	19.96	8.57^{a}*	0.96^{a}	-0.88	0.20	-0.17			20.77^{a}	7.71^{a}*
SysA	19.72	10.75^{ab}*	4.75^{b}	0.35*	0.23	0.33^{b}	1.93^{a}	1.61^{a}	26.13^{ab}	13.40^{b}*
SysB	21.49	14.97^{b}*	3.23^{b}	0.82	0.26	0.35^{b}	3.26^{b}	6.22^{b}	28.13^{b}	22.49^{c}
SEM	1.36		0.70		0.18		0.84		1.96	
Source of Variation										
Treatment	<0.01		<0.01		<0.01		0.02		<0.01	
Period	<0.01		<0.01		0.78		0.11		<0.01	
Treatment × Period	0.04		0.03		0.02		0.05		<0.01	

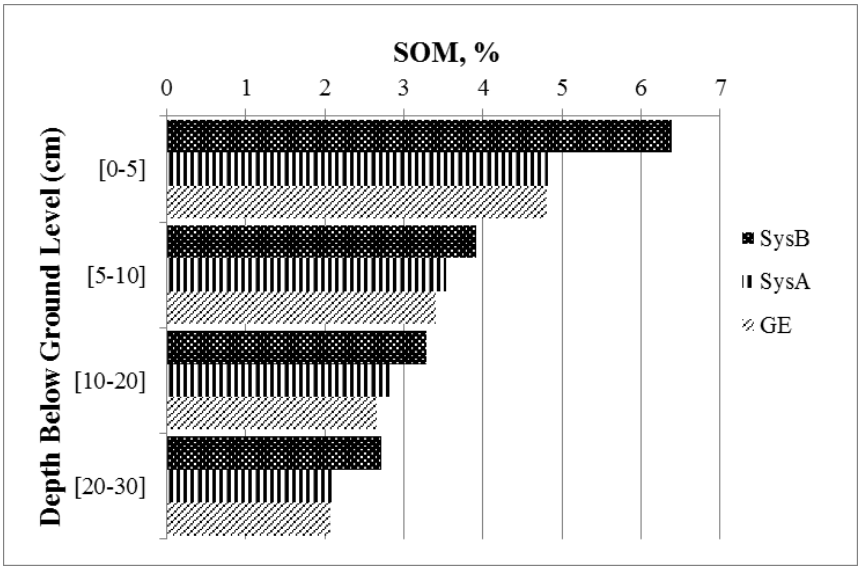
[1]GE: grazing exclusion; SysA: 1 cow ha^{-1} stocking rate and 100,000 kg LW ha^{-1} stocking density; SysB: 2.5 cows ha^{-1} stocking rate and 28,000 kg LW ha^{-1} stocking density.
[2]F_{CO2}: C equivalent flux of CO_2 from the soil.
[3]F_{N2O}: C equivalent flux of N_2O from the soil.
[4]$F_{CH4soil}$: C equivalent flux of CH_4 from the soil.
[5]$F_{CH4cows}$: C equivalent flux of enteric CH_4 from the cows.
[6]Ceq_{flux}: net GHG exchange in terms of C equivalent.
Means differences within columns indicated by letters ($P < 0.05$). Means differences within rows indicated by symbols ($P < 0.05$).

Table 4.4. Daily GHG emissions from soil and animal managed under two different grazing strategies and non-grazed pasture sites.

Systems[1]	Soil emissions			Animal Emissions	Total emissions
	F_{CO2}[2]	F_{N2O}[3]	$F_{CH4soil}$[4]	$F_{CH4cows}$[5]	Ceq_{flux}[6]
	kg C ha^{-1} d^{-1}				
GE	9.87[a]	0.25[a]	-0.09[a]	0	8.88[a]
SysA	10.03[a]	1.56[b]	0.13[b]	2.09[a]	13.96[b]
SysB	11.47[b]	1.17[b]	0.10[b]	4.91[b]	15.34[b]
SEM	0.66	0.32	0.08	1.09	0.74
Source of Variation					
Treatment	0.17	<0.01	<0.01	0.02	<0.01

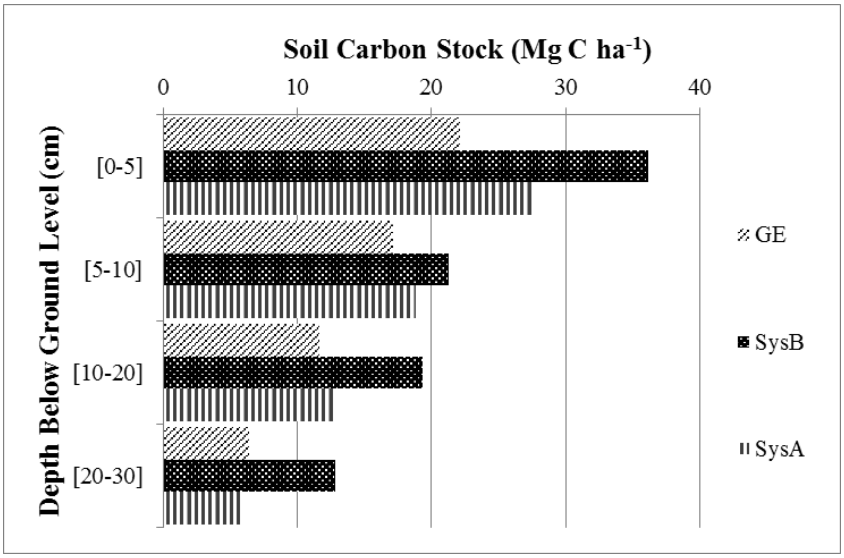
[1]GE: grazing exclusion; SysA: 1 cow ha^{-1} stocking rate and 100,000 kg LW ha^{-1} stocking density; SysB: 2.5 cows ha^{-1} stocking rate and 28,000 kg LW ha^{-1} stocking density.
[2]F_{CO2}: C equivalent flux of CO_2 from the soil.
[3]F_{N2O}: C equivalent flux of N_2O from the soil.
[4]$F_{CH4soil}$: C equivalent flux of CH_4 from the soil.
[5]$F_{CH4cows}$: C equivalent flux of enteric CH_4 from the cows.
[6]Ceq_{flux}: net GHG exchange in terms of C equivalent.
Means differences within columns indicated by letters ($P < 0.05$).

Figure 4.1. Soil organic matter in pasture soils grazed with two different grazing management strategies and non-grazed pastures sites.



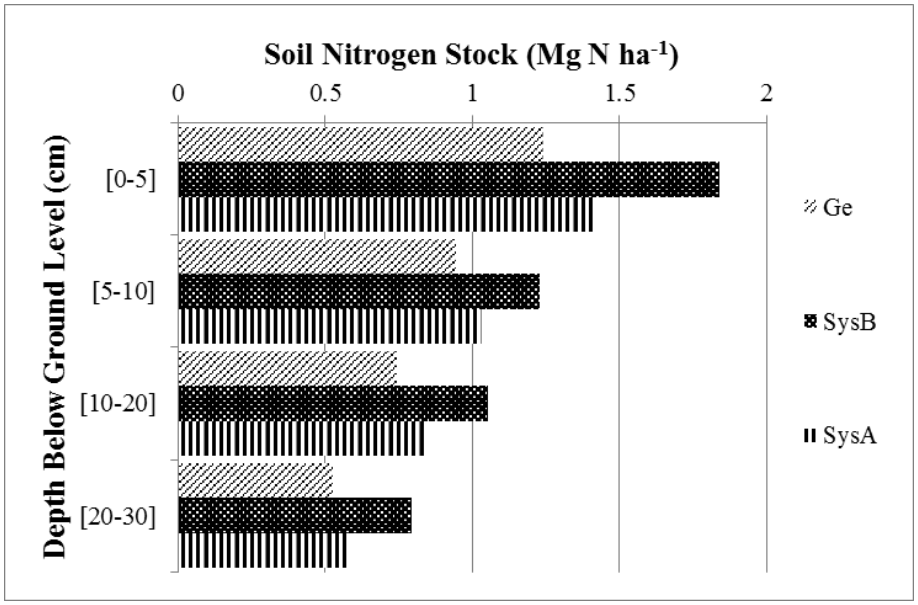
GE: grazing exclusion; SysA: 1 cow ha^{-1} stocking rate and 100,000 kg LW ha^{-1} stocking density; SysB: 2.5 cows ha^{-1} stocking rate and 28,000 kg LW ha^{-1} stocking density.

Figure 4.2. Soil carbon stock in pasture soils grazed with two different grazing management strategies and non-grazed pastures sites.



GE: grazing exclusion; SysA: 1 cow ha^{-1} stocking rate and 100,000 kg LW ha^{-1} stocking density; SysB: 2.5 cows ha^{-1} stocking rate and 28,000 kg LW ha^{-1} stocking density.

Figure 4.3. Total soil nitrogen stock along the soil profile in pasture soils grazed with two different grazing management strategies and non-grazed pastures sites.



GE: grazing exclusion; SysA: 1 cow ha^{-1} stocking rate and 100,000 kg LW ha^{-1} stocking density; SysB: 2.5 cows ha^{-1} stocking rate and 28,000 kg LW ha^{-1} stocking density.

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CHAPTER 5

SUMMARY

The main objective of this study was to apply a system-based approach to determine the influence of grazing systems on C flux. We monitored CO_2, CH_4 and N_2O emissions from pasture soils, enteric CH_4 emissions from cows and SOC and TSN stocks in pasture soils. We understand that the C cycle involves a large number of sources and sinks. Future research may add other components to the C flux determination initiated in this study, such as C leaching, microbial development, other gaseous emissions, CO_2 exchange with the vegetation and from cows respiration, and CH_4 emissions from calves.

This study was conducted at a farm-scale, following animal rotation schedule, which did not allow the use of true replication. The variability associated to CO_2, CH_4 and N_2O emissions from pasture soils was considerably high. We observed different results in each of the years and periods studied. GHG emissions from pasture soils are associated to manure decomposition in the field. In this study, GHG emissions were monitored always post-grazing, which might also have contributed to the variability of results. There was a tendency that SysB increased CO_2 emissions from pasture soils. Grazing systems did not conclusively influence CH_4 and N_2O emissions from pasture soils, and none clear trade-offs were observed between GHG emissions. Generally, GHG emissions increased from 2011 to 2013, which could be associated to different weather conditions in each year. On average, pooled by year, grazing resulted in greater emissions of N_2O and CH_4 when compared to non-grazed pasture sites. Methane sink activity was observed only on non-grazed pasture soils.

The soil and ambient properties monitored were soil temperature, ambient temperature and soil water content. These properties affected CO_2 emissions, but did not conclusively explain CH_4 and N_2O emissions. The soil and ambient properties monitored were chosen based on previous studies that found relationships between these properties and GHG emissions. The

results of this study did not support previous findings, and suggest that at a farm-scale, CO_2, CH_4 and N_2O fluxes could be mainly influenced by other soil properties. We suggest WFPS or O_2 content in the soil as possible better predictors of GHG from pasture sites at farm scale. Further research is needed to confirm the effect of WFPS and O_2 content on GHG emissions. This study indicates that GHG emissions from pasture soils are still uncertain when monitored at a farm scale.

The DMI of cows observed was relatively high when compared to previous studies. However, enteric CH_4 emissions observed tended to be lower than reported values. Previous research suggested that enteric CH_4 production increased with increased DMI. We hypothesized that the grazing management implemented in this study provided opportunities for selective grazing of different plant types, allowing cows to ingest high quality diets, while producing low amounts of enteric CH_4. These results suggest that diet quality might be a better predictor to daily CH_4 emissions than DMI, mainly due to methodological limitations to accurately determine DMI from grazing animals. Additionally, the differences observed in daily CH_4 emissions between grazing systems were not significant at 5%. Possibly, the selective grazing resulted from the management systems implemented in this study allowed cows managed with different grazing strategies to eat forage with similar qualities that met nutritional requirements with reduced CH_4 emissions. However, further research is suggested to determine the effects of selective grazing on enteric CH_4 emissions from grazing cows.

When combining GHG emissions from pasture soils and enteric CH_4 emissions from cows to determine the net C equivalent flux, our results showed that grazing systems had greater C equivalent flux than non-grazed pasture sites, and the differences observed between grazing systems were not significant. It is possible that the effect of greater enteric CH_4 contribution

from higher stocking rate systems was offset by GHG exchange from the soil, resulting in non-significant difference between grazing systems. Intensive grazing (high stocking rate, low stocking density) potentially increased total SOC stock, the addition of SOC to deeper into the soil horizon and SOM content to 30 cm when compared to extensive grazing (low stocking rate, high stocking density).

Although SysB allowed for more animals per hectare, we did not observed a significant difference between grazing systems in daily C-equivalent flux, when expressed as kg of C equivalent per hectare. However, these results are limited to the assumptions and limitations of this work. The implementation of SysA requires more land than SysB, hence if the results were expressed in a land basis, conclusions could change. Additionally, an important assumption of this work was that both grazing systems would allow for the same duration of grazing season. If one system allowed for longer grazing season, and C equivalent flux was expressed in terms of total length of grazing season (instead of daily values), conclusions could change. The inclusion of other components of the C cycle on the calculation of C equivalent flux could potentially change conclusions. For instance, CO_2 exchange with the vegetation and C sequestration potential. SOC stock was greater in SysB; if the potential of SysB to increase C sequestration in the soil is confirmed, it could result in a significant difference of C equivalent flux between systems. Results obtained in this work are only valid within the assumptions and limitations of the study and further research is encouraged. Additional topics that still deserve future research include the relationships between GHG fluxes from soils and microbial communities, the role of nitrogen in the C equivalent flux, processes involved on CH_4 and N_2O sink activity and the sink potential of grasslands soils.

Both systems studied have benefits and could be implemented under specific conditions and objectives of the farmer. SysA could be implemented in marginal lands, where irrigation is not possible and crop production is difficult. Additionally, SysA represents a low input opportunity to reverse land degradation in the farm. High stocking densities grazing systems (such as SysA) were associated to attenuation or reversal of land degradation in arid ecosystems. SysB, with shorter rest periods and frequent defoliation of the forage, allowed the development of greater forage quality throughout the grazing season. The maintenance of high quality forage provides flexibility of operations to the farmer, according to market prices. For instance, SysB allows the production of finishing steers, as well as cow-calf operations. This flexibility of operation aggregates value to the final product of the farm.

Concerning the total C equivalent flux from grazing systems versus non-grazed pasture sites, indeed the former had greater C equivalent flux. The greater C equivalent flux from the grazing systems was associated to greater GHG emissions from pasture soils and enteric CH_4 emissions. However, the difference between grazed and non-grazed pasture sites observed in this study was considerably small (approximately 6 kg C ha^{-1} d^{-1}). The benefits of grazing systems, such as animal production and food provisioning, overcome the small increase in C equivalent flux when compared to non-grazed pasture sites.

This study is unique because it included monitoring at the field of CO_2, CH_4 and N_2O from pasture soils and enteric CH_4 emissions from cows simultaneously. Other similar studies did not monitor the 4 emissions sources and used reported values to determine C equivalent flux. Observed results raise questions about the current knowledge on C equivalent flux when applied to a farm level. Small-scale, reductionist research do not focus on interactions among all elements of a system and could result in completely different results than a system-based

research. Nevertheless, system-based C flux accounting of grazing systems are very limited and needed.