

HOW DO MANAGEMENT DECISIONS IMPACT THE SUSTAINABILITY OF UPPER
MIDWEST BEEF CATTLE GRAZING?

By

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ABSTRACT

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The conversion of grass to beef is less efficient than the process of converting feed to human-edible protein in nonruminants, although ruminal fermentation does allow for the utilization of complex carbohydrates. This has resulted in the cattle industry receiving increased attention due to its perceived high carbon (C) footprint. While ruminants have evolved to fill the niche of converting human inedible carbohydrates into usable end-products for growth, this process results in considerable greenhouse gas (GHG) emissions as byproducts of the reticulo-rumen fermentation process. Life Cycle Assessments have shown that the grazing sector of the cattle industry, cow-calf and stocker cattle, may be responsible for 70 to 80% of the industry's total C footprint. Therefore, research is needed to explore the soil-plant-animal interrelationships and generate data to provide management recommendations to producers to improve the C footprint of their operation. As it relates to agriculture, sustainability has three legs that must be met addressed to improve its sustainability: 1) social- the strategy must maintain social license to operate, 2) environmental- the strategy must reduce negative environmental consequences, and 3) economic- producers must remain economically viable. This dissertation examines how management decisions impact grazing beef cattle through two of the three legs of sustainability: environmental and economic sustainability. The literature review focuses on the role on enteric methane on the C footprint of the beef industry, as this topic "keeps the lights on" in many laboratories due to increased consumer concern about the industry's C footprint. However, key

nutritional abatement strategies that goes beyond enteric methane are management decisions that improve the efficiency of ruminal fermentation.

Matching cows to the production environment is a critical management decision, as selecting cows too small or too large would result in an inefficient utilization of the forage base. This idea is explored chapter 2, modeling the biological and economic output of a Michigan beef herd. We conducted a backwards looking enterprise budget analysis and forward-looking net present value analysis on the Lake City AgBioResearch Center Red Angus herd to determine what size cow best fits the present production environment. We found that as cow size increased, within the range of cow body weights of 430.83 to 634.92 kg, calculated net returns per unit of land decreased by \$10.27/ha.

The last two chapters compare environmental tradeoffs between two different forage mixtures grazed by beef cattle: a biologically diverse, mixed-specie pasture (**COMP**), and a simple binary mixture of alfalfa:orchardgrass (**SIMP**). The hypothesis was that the COMP mixture would have reduced enteric methane production, increased forage productivity due to the diversity of the mixture, and greater soil C accumulation. Both treatments performed similarly in animal performance, soil GHG fluxes, and soil organic C and total N stocks. Animals grazing COMP mixtures tended to have lower enteric CH₄ emissions compared to SIMP, but there was no difference in emission intensity across three grazing seasons. This project serves to supplement the dearth of literature comparing a simple and diverse forage mixture. More research on the long-term performance of the diverse and simple forage mixtures and the utilization of new technologies such as eddy covariance flux towers will help provide a more robust examination on the environmental tradeoffs between forage mixtures than the methodologies utilized in this experiment.

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KEY TO ABBREVIATIONS

ADF.....	Acid detergent fiber
ADG	Average daily gain
aNDF.....	neutral detergent fiber
BCS	Body condition score
BW	Body weight
C	Carbon
CH ₄	Methane
CO ₂	Carbon dioxide
COMP.....	Complex forage treatment
CP	Crude protein
CT	Condensed tannins
CW	Carcass weight
CWP	205-d adjusted weaning weight
DBW	Dam body weight adjusted to body condition score 5
DM	Dry matter
DMI.....	Dry matter intake
DMP	Daily methane production
EI	Emission Intensity
GEI.....	Gross energy intake
GEM.....	GreenFeed™ emission measurement system
GHG.....	Greenhouse gas

GTP	Global temperature potential
GWP	Global warming potential
GWP ₁₀₀	Global warming potential over 100 year timeframe
iADF	Indigestible acid detergent fiber
IPCC	International panel on climate change
IVTDM48	In vitro true digestibility of dry matter over 48 hrs
LCA	Life cycle assessment
LWG	Live weight gain
N	Nitrogen
N ₂ O	Nitrous oxide
NDFI	Neutral detergent fiber intake
NPV	Net present value
OM	Organic Matter
PAC	Portable accumulation chamber
RFI	Residual feed intake
SF ₆	Sulfur hexafluoride
SIMP	Simple forage treatment
SOC	Soil organic carbon
TSN	Total soil nitrogen
WEOC	Water extractable organic carbon
WEON	Water extractable organic nitrogen
WW	Weaning weight
Y _m	Methane yield per unit of intake

YW Yearling weight

CHAPTER 1
LITERATURE REVIEW

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1.1 Introduction

The environmental impact of the beef industry has received increased public attention due to its perceived impact on climate change. A recent International Panel on Climate Change (IPCC; IPCC, 2019) report estimated that 23% of global anthropogenic greenhouse gas (GHG) emissions was from agriculture, forestry, and other land uses. These sources contribute an estimated 44% of all methane (CH₄) emissions ($4.5 \pm 1.4 \text{ GtCO}_2\text{e y}^{-1}$), with enteric CH₄ from ruminants responsible for 46%, of the $4.5 \pm 1.4 \text{ GtCO}_2\text{e y}^{-1}$ or $2.1 \text{ GtCO}_2\text{e y}^{-1}$ (IPCC, 2019; IPCC, 2013). Anthropogenic GHG have been rising since the start of the industrial revolution and will continue to rise with increased fossil fuel (e.g., coal, gas and oil) combustion (Place and Mitloehner, 2010).

In the United States, the agriculture sector contributes about 9% of total GHG emissions, whereas transportation, industry and electricity sectors are the majority emitters (about 79% of total) (EPA, 2019). The Paris Climate Accord funded a special IPCC climate report in 2018 that recommended developed countries should reach zero emission targets in an “as soon as possible” window and reduce global emissions by 45% by 2030. Thus, while the United States agriculture emission footprint is considerably less compared with other U.S. sectors and the world, the zero-emission target implies mitigation from all sectors barring marked improvement in carbon (C) sequestration technologies (Rogeli et al., 2018). Of the 9.1% attributed to U.S. agriculture production, about 60% is due to animal agriculture, and, about 60% of that is attributed to biogenic enteric CH₄ emissions from all domesticated ruminants (3.2% of total U.S. emissions; EPA, 2019). Methane is a potent GHG with a global warming potential (GWP) 28 times that of CO₂ (IPCC, 2014). Agriculture contributes about 37.8% of domestic CH₄ emissions from the combined sources of enteric, manure management rice cultivation and field residue burning, with

enteric CH₄ emissions being the largest agricultural source at 27% of U.S. CH₄ emissions (EPA, 2019). Because of methane's contribution to the overall agriculture emission footprint, addressing mitigation opportunities is essential to reach reduced emission targets.

Recent Life-Cycle Assessments (LCA) have highlighted the need to focus on the grazing sectors of the U.S. beef industry, with the cow-calf and stocker cattle components contributing approximately 70 to 80% of total GHG from the U.S. beef sector (Rotz et al., 2019; Rotz et al., 2015; Alemu et al., 2017). The reason for this is two-fold. First, cattle consuming a high forage diet have increased CH₄ emissions; and, secondly breeding stock live on the land continuously and produce one calf per year (Rotz et al., 2015). To date there have been several reviews detailing the impact of nutrition on enteric CH₄ production and mitigation options (Johnson and Johnson, 1995; Boadi et al., 2004; Beauchemin et al., 2008; Hristov et al., 2013). Considering the contribution of the grazing sector to enteric CH₄ emissions the primary objective of this review is to focus on mitigation strategies from a grazing perspective and to explore how soil-plant-animal interrelationships can be manipulated and enhanced to reduce CH₄ emissions and improve ecosystem functioning and overall system sustainability.

1.2 Sources of Methane Emissions

1.2.1 Enteric Methanogenesis

Enteric CH₄ is a natural by-product of the anaerobic fermentation process in the reticulo-rumen and hindgut in ruminants (Patra, 2012; **Figure 1.1**). The rumen is an anaerobic environment where large numbers of symbiotic bacteria, protozoa, and fungi derive their energy from consumed feedstuffs. The digestion end-products of their digestion are primarily microbial cell protein and VFA (primarily acetate, butyrate, and propionate) that the host animal utilizes to meet its own metabolic needs (Krehbiel, 2014). This symbiotic relationship allowed ruminants to

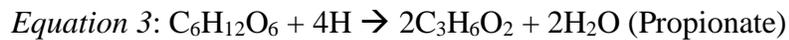
evolve across a multitude of biomes to fill an ecological niche utilizing complex carbohydrates, chiefly cellulose, that most mammalian species cannot digest (Knapp et al., 2014). This evolution led to the rise of a complex microbial community that include methanogenic species that differ from methanogens in other populations because it lacks cytochrome proteins responsible for electron transfer (Muñoz-Tamayo et al., 2019; Knapp et al., 2014). In most ecosystems this would be an energetic disadvantage, but instead it allows the methanogens to survive in the reticulo-ruminal environment (Muñoz-Tamayo et al., 2019). In addition to the VFAs and protein produced during the fermentation process, gaseous CO₂ and H₂ are produced. These serve as the primary substrates for methanogenic archaea to produce CH₄, typically through the hydrogenotrophic pathway:



This process of cellular respiration by methanogens utilizes the H₂ to produce CH₄ and H₂O thereby preventing metabolic hydrogen from accumulating in the reticulo-rumen. Hydrogen removal is crucial for healthy ruminal fermentation as accumulation limits the ability of microbial populations to oxidize the cofactors responsible for electron transfer, thereby reducing carbohydrate degradation, microbial growth rate, and synthesis of microbial cell protein (Beauchemin et al., 2009; Wolin, 1974; McAllister and Newbold, 2008).

Enteric CH₄ production is driven primarily by level of feed intake and dietary fiber concentrations (Johnson and Johnson, 1995; Hristov et al., 2013). Methane production increases with greater intake due to impacts on ruminal passage rate and carbohydrate fermentation (Johnson and Johnson, 1995). The form of carbohydrate also influences CH₄ production (Johnson and Johnson, 1995; Hristov et al., 2013). Cattle consuming a high concentrate diet compared with a diet high in cell wall fiber results in less dietary energy lost as CH₄ through

impacts on ruminal pH, shifting microbial populations, and a decrease in the acetate:propionate ratio (Johnson and Johnson, 1995). The production of acetate is greatest in cattle fed high fiber diets and its production increases CH₄ production by increasing the amount of metabolic H₂, whereas propionate acts as a hydrogen sink:



Numerous other factors including forage processing and quality, lipid content, forage secondary compounds, and dietary additives also alter CH₄ production. Due to the myriad of factors influencing enteric CH₄ production, energy losses in the form of CH₄ can range from 2 to 12% of GE intake (Johnson and Johnson, 1995). Therefore when including its impact on global climate change, taking strides to mitigate CH₄ production is both economically and environmentally beneficial. Figure 1 displays the role of enteric CH₄ in the biogenic C cycle. Carbon dioxide is fixed in plants via photosynthesis, and this C is then converted into CH₄ after consumption of plant material by ruminants and expelled out of the mouth of the animal. This CH₄ has a residence time of about 9 to 12 yrs in the atmosphere before being broken back down into CO₂.

1.2.2 Regional Emissions

An inherent complexity when discussing global emission mitigation is the regional specificity of emissions and the societal cost-benefit relationship at the more localized level. Therefore, it is important to compare regional emission rates to determine where improvement is needed and why some regions favor lower CH₄ intensity (CH₄ per unit of product, typically kg carcass weight in beef cattle) compared to others. Globally, 2.8 gigatonnes of enteric CH₄ GHG are annually produced, with cattle being responsible for 77% of the total (FAO, 2013).

However, the emission rate is not equal across countries with developing countries contributing as much as 75% of total global GHG emissions from ruminants (Herrero et al., 2013). In terms of CH₄ emission intensity (kg CO₂ eq./kg carcass weight; **CW**), Sub-Saharan Africa and Southern Asia have the greatest with 41 and 50 kg CO₂ eq/kg CW (Gerber et al., 2013). The developed regions of Western and Eastern Europe range from 5 to 7 kg CO₂ eq./kg CW (Gerber et al., 2013). North America and Oceania are estimated to have an emission intensity of 11, whereas the emission intensity of Latin America is about 24 (Gerber et al., 2013). In developing countries, greater CH₄ emission intensities are driven by poor feed digestibility, low slaughter weights, greater age at slaughter and poor animal husbandry (Gerber et al., 2013; Herrero et al., 2013). In developed countries, emission rates are low due to improved grazing management leading to greater diet digestibility, more intensive feeding practices, and temperate conditions (Gerber et al., 2013; Herrero et al., 2013). All of these result in improved dietary quality throughout the lifespan of the animal, thereby reducing days on feed and emitting less enteric CH₄ emission per unit of feed consumed (Gerber et al., 2013; Herrero et al., 2013). In addition, European countries mitigate the footprint of their beef sector by producing 80% of their beef from dairy animals (Gerber et al., 2013). Overcoming the economic, political, and educational complexities of developing countries are essential for these regions to reduce their enteric CH₄ emissions and improve their C footprint. Beef production in these countries is not focused primarily on food production and nutrient security as in developed countries (Tedeschi et al., 2017). In developing countries livestock provide wealth, draft power, fuel, and religious significance that are not as important in developed countries (Smith et al., 2013). It is estimated that livestock production plays a major role in the livelihoods of more than 1 billion people in Africa and Asia, and that two-thirds of the livestock managers are women (Smith et al., 2013).

Mitigation strategies for these countries must balance the improvements in efficiency with the underlying complexities of the local populations and their cultures, and not be detrimental to human health, and environmental sustainability.

1.3 Quantification Methods

The “gold standard” for measuring enteric CH₄ production is the respiration chamber, and similarly the head-box method that quantifies enteric CH₄ production by multiplying airflow through the chamber by the difference in CH₄ concentration in and out measured by a gas analyzer (Hill et al., 2016). These methods are based on indirect calorimetry and provide very precise and accurate estimates of gas production (Hill et al., 2016). However, restricted movement of animals in the respiration chambers and head boxes creates an artificial environment that does not reflect a normal production environment and could limit feed intake (Storm et al., 2012). Additionally, an issue in estimating farm-scale emissions is that grazing cattle are selective grazers, forming food preferences over time which play a critical role in meeting their nutritional needs (Provenza, 1995). This can result in animals selecting a higher quality diet than the average of the available forage base; this adds uncertainty to applying confinement emission quantification methods to grazing animals. Additionally, the artificial environment created within the chamber can result in lower DMI (Huhtanen et al., 2019). The respiration chamber or head-box was used in the vast majority of studies used to develop emission equations and C accounting. Therefore, there are potential flaws in that these methodologies may not adequately capture farm-scale emissions when comparing changes in management and environment. In an examination of chamber data, Huhtanen et al. (2019) found that using these methods underestimated DMI compared with that found in grazing studies and gave lower total enteric CH₄ emission estimates. Current methods to quantify enteric CH₄

production in grazing environments are the sulfur hexafluoride tracer technique (SF_6), the GreenFeed™ gas quantification system (**GEM**; C-Lock Inc., Rapid City, SD), various field-level emission quantification methods, and animal models (Gunter and Beck, 2018; Hill et al., 2016).

1.3.1 Sulfur Hexafluoride Tracer Technique

The first method to demonstrate repeatable accuracy in quantifying emissions in an open-air environment, such as grazing, and allow for natural forage selection was the SF_6 technique (Gunter and Beck, 2018; Hill et al., 2016; Zimmerman, 1993). This method utilizes SF_6 as a tracer gas based on the assumption that the standard SF_6 emission rate is equal to the CH_4 emission rate (Johnson et al., 1994). Prior to the experiment beginning, the release rate of the SF_6 bolus is estimated in vitro before the bolus is placed into the rumen (Zimmerman, 1993). A halter with a stainless-steel collection vessel and a capillary tube attached to a collection canister is then placed around the animals' head to collect respired air from the animal (Zimmerman, 1993; Johnson et al., 1994). Enteric CH_4 emission rate can then be determined by gas chromatography using the ratio of $\text{CH}_4:\text{SF}_6$ multiplied by the standard SF_6 release rate, and corrected for background SF_6 concentration (Johnson et al., 1994). This method initially was revolutionary because it allowed researchers to quantify emissions from free-grazing animals (Johnson and Johnson, 1995). However, later research literature showed that the difference between SF_6 and respiration chambers may be > 10% at times (Storm et al., 2012). The limitations of the SF_6 method may explain these, at times unacceptable, error rates including the curvilinear release rate of the standard SF_6 gas from the intraruminal bolus over time, labor, and the inability to collect emissions from the hindgut (Storm et al., 2012; Vlaming, 2007). The method relies on a presumably continuous and constant release rate of SF_6 from the permeation tubes. Yet measured pre- and post-experiment release rates of SF_6 from permeation tubes displayed a curvilinear

release rate in the laboratory (Storm et al., 2012; Vlaming, 2007). This could result in a decrease in release rate of 6 to 11% while in the rumen (Vlaming, 2007). Therefore, studies that use permeation tubes of differing release rates within experiment may result in inaccurate CH₄ emission estimates (Vlaming, 2007; Pinares-Patino et al., 2008). Additionally, hindgut CH₄ emissions that are not absorbed into the blood stream and respired out of the lungs may account for 1 to 11% of CH₄ emissions (McGinn et al., 2006). Lastly, the use of SF₆ is paradoxical as it is a potent GHG with a GWP of 22,800 (Vlaming, 2007).

1.3.2 GreenFeed™ Emission Measurement System

Based on the previously outlined limitations of the SF₆ technique, the GEM system was developed by using spot measurements to estimate enteric CH₄ production (Hristov et al., 2015; Gunter and Beck, 2018). Similar to SF₆, this method is able to estimate CH₄ emissions from free grazing animals, but is less intrusive and relies on spot measurements to estimate daily production (Hristov et al., 2015). The GEM uses a portable head-box either in a free-stall or on a trailer that utilizes bait feed to entice animals to place their head in the head-box (Hristov et al., 2015). When an animal places its head in the hood, the volume of air that is drawn from around the animal's head and shoulders is measured and a subsample is analyzed by non-dispersive near infrared gas analyzers for CO₂ and CH₄ concentrations (Hristov et al., 2015). These results are then compared with background gas concentrations prior to the animal entering the hood to determine gas emission rates. Spot estimates are then averaged over the course of sampling period to estimate each animal's daily gas production (Gunter and Beck, 2018). Previous research indicates the GEM can result in highly variable data sets due to greater within-day and within-animal variability that is more difficult to capture with a spot measurement system (Hammond et al., 2016). However, the system proved to be sufficiently accurate with adequate

number of visits over an extended period of time (Cottle et al., 2015). In a power analysis of cattle visiting a GEM, Gunter and Bradford (2017) found that accurate daily estimates can be obtained if animals visit the unit an average of 2.4 times per day for 4.8 to 6.3 d. Hammond et al. (2015) reported that GEM in a grazing experiment was not able to capture treatment differences that were evident in respiration chambers using 4-d sampling periods with an average of 1.6 visits per d. However, recent studies on the duration of sampling and number of adequate visits may explain these differences as more visits may be required in order to detect treatment differences during short sampling periods (Hammond et al., 2015; Gunter and Beck, 2018). Additionally, these studies focused on meal-fed cattle which have a larger diurnal variation than grazing animals which emphasizes the importance of the timing of visits to the head box (Gunter and Beck, 2018). Lastly, because this system is less expensive than chamber methods and requires less labor than the SF₆ method, larger samples sizes (more experimental replicates) are possible compared with other sampling techniques and aids in efforts to minimizing experimental and sampling errors (Gunter and Bradford, 2015).

1.3.3 Portable Accumulation Chambers

Another method that relies on spot sampling is the portable accumulation chamber (**PAC**) has been used to estimate CH₄ production (Goopy et al., 2011). This method has typically been used with grazing sheep and has showed moderately high correlations with the respiration chamber method ($r = 0.71$), as well as identifying relative emission changes without scaling up to daily CH₄ production (**DMP**) estimates (Goopy et al., 2011; Hegarty, 2013). These chambers are boxes that are open at the bottom, clear-sided and sealed at the bottom with high density foam rubber. The chambers have three sampling ports located on the superior, posterior and lateral walls and the chamber is placed on industrial grade rubber (Goopy et al., 2011; 2016). Animals

are positioned on top of the rubber bottom and the box is lowered down, sealed, and sampling durations last from 1- to 2-h (Hammond et al., 2016). Studies comparing PAC to respiration chambers have found moderate correlations at both a 1- and 2-h sampling durations (Goopy et al., 2011; Goopy et al., 2016; Robinson et al., 2015; Robinson et al., 2016). Goopy et al. (2011) in a study comparing respiration chambers with PAC using sheep, found $R^2 = 0.42$ to 0.48 for 2 h sampling periods and 0.39 to 0.43 in 1-hr sampling periods. Measuring ewes for 40 to 60 min, Robinson et al. (2016) determined that these estimates had a repeatability of about 0.47 once adjusted for BW and ADG, but did not alter the animals intake like respiration chambers. Researchers using the PAC method have noted that repeatability can be impacted by time of sampling and feeding schedules which can cause issues with repeatability by altering the diurnal pattern of DMP (Hammond et al., 2016). This causes issues when scaling up to estimate DMP, which is less of an issue when using the GEM because spot samples are obtained throughout the day if the animals utilize the head-box (Hammond et al., 2016; Gunter and Beck, 2018).

1.3.4 Micrometeorological Techniques

On the field level, micrometeorological techniques including flux-gradient, eddy covariance and inverse dispersion models are being developed to provide herd scale emission estimates (Hill et al., 2016; Storm et al., 2012; McGinn, 2013; Harper et al., 2011). These methods measure atmospheric CH_4 concentrations and meteorological variables to estimate herd-level, animal group, or farm-level emission rates. Measuring emissions on a farm scale allows researchers to have a better understanding of how mitigation strategies are impacting the operation and to test strategies on a large number of animals (McGinn et al., 2019; Harper et al., 2011). These methodologies have an advantage over individual animal sampling techniques because they do not require animal handling or bait feeding that may alter animals behavior and

diet selection and allow for long-term comparison between management strategies (Harper et al., 2011; Coates et al., 2017). However, spatial variability and low intensity of emissions from grazing animals have limited the adoption of these methods beyond feedlot and confinement operations and may require animals to be grazed at high densities in order to estimate emissions (Flesch et al., 2018; Dengal et al., 2011). There are numerous different micrometeorological methodologies each having its own set of advantages and disadvantages and proper field configurations, which are outside the scope of this review but have been reviewed in depth by McGinn (2013), Flesch et al. (2018) and Harper et al. (2011).

1.4 Mitigation of Methane in Grazing Environments

Methane production in grazing environments is a combination of many different factors interacting to influence individual animal intake, performance, and emission rates. Dietary quality (e.g., digestibility) plays an important role in the production of CH₄ (Hristov et al., 2013; Knapp et al., 2014; Johnson and Johnson 1995). Feeding highly fermentable carbohydrates, such as the starch in high concentrate diets, results in lower enteric CH₄ production per unit of feed DM consumed by ultimately shifting microbial populations to favor propionate production and increased ruminal rate of passage (Moe and Tyrrell, 1979; Johnson and Johnson, 1995). However, feeding all cattle only diets high in digestible carbohydrates would reduce the advantage to convert complex carbohydrates with high fiber content and use of untillable land into useable end-products, chiefly milk or meat (Carvalho et al., 2018). Additionally, this would lessen the benefits of grazing animals on grassland productivity and health. Therefore, the question posed to scientists, and ultimately producers, should be how to leverage soil-plant-animal interrelationships to meet productivity goals of the present and future, without compromising social and ecological outcomes (Tilman et al., 2011).

1.4.1 Managing the Forage Base

1.4.1.1 Forage Quality

Proper management of the forage base, the type of forage being grazed, and the stage of forage maturity can impact CH₄ emissions and productivity of cattle and can serve to improve or degrade the land base (Beauchemin et al., 2008; Teague et al., 2004). Improving pasture quality can improve dietary digestibility and result in decreased enteric CH₄ emissions (Archimède et al., 2011; Beauchemin et al., 2008; Hristov et al., 2013). Archimède et al. (2011) conducted a meta-analysis examining CH₄ production of C3 and C4, cold and warm season species respectively, grasses and cold and warm season legumes (all legumes utilize the C3 photosynthetic pathway). Grasses utilizing the C3 photosynthetic pathway are typically considered cool or temperate grasses, and those utilizing the C4 pathway are considered warm or tropical grasses (Archimède et al., 2011). The authors indicated that cattle fed C4 grasses had greater CH₄ production than cattle fed C3 grasses and both warm and cold legumes. Cattle fed warm-season legumes produced the least CH₄ per kg DMI and per kg OM intake compared with the other diets (Archimède et al., 2011). The results are similar to those of Margan et al. (1998) who compared two C4 species and a C3 grass and found that C4 grasses produced 23% more CH₄ than a C3 grass grown under the same subtropical conditions. These results also align with established literature about CH₄ production from forages. Grasses that utilize the C3 photosynthetic pathway are normally considered higher “quality” than C4 grasses because they are typically lower in fiber including decreased lignin production, and greater in protein content (Barbehenn et al., 2004). This results in lower CH₄ production values based on the correlation between fiber content of the diet and CH₄ production (Blaxter and Wainman, 1964; Moe and Tyrrell, 1979). Similarly, McCaughey et al. (1999) found that first calf heifers grazing a mixture

of alfalfa-meadow bromegrass (*Medicago sativa* L. and *Bromus biebersteinii*) treatment lost less energy as CH₄ compared with heifers grazing a 100% meadow bromegrass pasture at 7.1 vs. 9.5% of gross energy intake (**GEI**) lost as methane, respectively. Waghorn et al. (2002) examined the CH₄ production of young ram lambs fed 10 different forage rations: a mixture of ryegrass-white clover pasture, Lucerne, sulla, chicory, red clover, lotus, and mixtures of sulla and Lucerne, sulla and chicory, and chicory with red clover. They found that lambs consuming the ryegrass-white clover pasture produced the most CH₄ (g/kg DMI basis) as compared with the other 10 treatments and reported a 10-fold range in overall CH₄ emissions. These results clearly highlight the wide range of emissions that occur even in high quality forages. However, these studies were all conducted with monoculture or a simple forage mixture. Studies using diverse forage bases resulted in reduced enteric CH₄ production as it allows animals to select plants and plant parts and build their own diet when grazed at lower stocking densities (Chiavegato et al., 2015; DeRamus et al., 2003; MacAdam and Villalba, 2015; Provenza, 1995; Provenza and Villalba, 2006).

The quality of a forage diet is not solely determined by the type of forage being offered. Forage maturity impacts enteric CH₄ production by altering the nutrient density and digestibility of the forage, thereby lowering its quality. As forages mature, fiber content increases and lignin deposition in the cell wall increases as the plants shift from primary cell wall growth to secondary cell wall thickening resulting in decreased digestibility and intake (Jung and Allen, 1995). Boadi and Wittenberg (2002) examined three different forage diets classified as high-quality (legume/grass mixed hay, 61.5% IVOMD), medium quality (grass hay, 50.7% IVOMD), or low-quality (grass hay, 38.5% IVOMD) and found that DMI, digestible organic matter digestibility, and GEI was lowest for low-quality hay. However, CH₄ emissions as a percent of

GEI were not affected by stage of maturity, but the reductions in DMI and digestibility resulted in CH₄ emissions per unit of digestible OM consumed being highest on the low-quality diet. Similarly, Pinares-Patiño et al. (2003) measured CH₄ production of Charolais cows grazing timothy grass monocultures at four differing stages of maturity: early vegetative, heading, flowering, and senescence. Organic matter intake was greatest at heading compared with other stages of maturity, but the GEI lost as CH₄ was not impacted by maturity. The production of CH₄ did decrease, however, with increasing fiber digestibility. Muñoz et al. (2016) reported that when emissions were reported per unit of milk yield in dairy cows grazing pastures of differing forage quality, the less mature, higher quality pasture resulted in a reduction in CH₄ emissions. Similarly, in a study comparing low and high stocking rates in France with Holstein-Friesian heifers, CH₄ emissions per unit of digestible feed intake was decreased with increasing digestibility and heifers consuming the higher quality forage in the high stocking rate treatment were heavier at the end of the grazing season (Pinares-Patiño et al., 2007). These studies highlight that when considering the impact of forage quality and maturity on enteric CH₄ emissions, production may best be expressed on an intake or animal production basis rather than GEI lost (Beauchemin et al., 2008).

1.4.1.2 Grazing Management

Along with improving pasture quality, grazing management strategies have the potential to decrease CH₄ production (Savian et al., 2018; DeRamus et al., 2003; Hristov et al., 2013). However, the literature is inconsistent on the short- and long-term impacts of different grazing management strategies. Ruminants selectively graze and form food preferences over time which play a critical role in meeting their nutritional needs (Provenza, 1995). Matches and Burns (1995) and DeRamus et al. (2003) agreed that continuous set stocking allows for maximum diet

selection, which results the capture of short-term gains in animal performance. However, long-term impacts on enteric CH₄ production between continuous and rotational management is an area of recurrent inconsistency. Savian et al. (2014), in a comparison of continuous vs. rotational grazing in Brazil indicated that continuous grazing resulted in lower emission intensity (CH₄/kg ADG) compared with rotational grazing. Yet by refining the rotational management, Savian et al. (2018) later showed that rotating cattle among paddocks at a target residual forage height of 11 cm resulted in decreased CH₄ production compared with that of a “traditional” rotational grazing, which had targeted pre- and post-grazing sward heights of 25 cm and 5 cm, respectively.

DeRamus et al. (2003) determined that when incorporating rotational grazing consisting of best management practices, with periodical fertilization and animals being rotated frequently, annual enteric CH₄ production was reduced by 22% compared with continuous stocking. Conversely, McCaughey et al. (1997) compared enteric CH₄ production and voluntary intake of steers grazing in one of two grazing strategies: continuous or 10-paddock rotational grazing with animals moved based on forage availability, at two stocking rates: 1.1 or 2.2 steer/ha. They found that neither voluntary intake or CH₄ production were impacted by management strategy.

Considering that sustainability is a multi-pronged goal, perhaps the benefits of a grazing management strategy occur beyond short-term animal performance. One hypothesis suggests that the variable results in enteric CH₄ production from different grazing management is that animals in the studies are able to select high quality diets within the treatments and perform well.

However, some literature argues that the impacts of management are more crucial to the long-term performance of the supporting environment. One suggestion is that the use of continuous stocking results in imbalanced patch grazing pressure which can result in overgrazing of preferred forages and ecosystem process impairment (short- or long-term damage to normal

ecosystem function). This leads to a reduction in plant diversity, increases the proportion of undesirable or low-quality forages, and ultimately leads to soil erosion (Teague et al., 2004; Teague et al., 2016). Additionally, the loss of high-quality forages from over-grazing will result in a lower amount of solar energy captured and thus reduce the amount of solar energy converted to forage and then to useable end-products (e.g., meat and milk).

1.4.1.3 Forage Secondary Compounds

Grazing ecosystems are complex and impact emissions through other mechanisms beyond intake and forage quality. In grazing lands containing a forage base of differing forages at different stages of growth, cattle may capture synergies due to primary and secondary compounds present in the plants resulting in improved performance (Provenza et al., 2003; Villalba et al., 2010). Plant secondary compounds or metabolites consist of a broad spectrum of compounds including flavonoids, tannins, pectins, glycosides, terpenoids, and sesquiterpene lactones, just to name a few. With a wide range of compounds comes a wide range of ecological functions performed by the compounds, but the compounds of interest from a grazing perspective are those that act as a defense against herbivory (the consumption of plants by animals; Iason, 2005). Some of these defense compounds can be toxic to the animal or act as a deterrent through taste, and animals learn either by experience or animal-to-animal learning which plants to consume and how to minimize the impacts of toxic compounds (Provenza, 1995). However, some of these compounds have shown promise to improve animal productivity, health and mitigate enteric CH₄ production. Compounds of interest are saponins, condensed tannins, and essential oils (Hristov et al., 2013). Lagrange et al. (2017) found that with cattle grazing diverse pastures containing different legumes have divergent impacts on CH₄ production, potentially due to differences in secondary compounds. They noted that cattle grazing a diverse

pasture with saponin containing alfalfa had greater CH₄ emissions per unit of BW gain than cattle on pastures with tannin-containing sainfoin or tannin-containing birdsfoot trefoil, as well as improved animal performance. In a study feeding alfalfa hay or sainfoin hay, beef heifers produced less CH₄ on an OM basis when fed the tannin-containing sainfoin hay (Chung et al., 2013). Similarly, Grainger et al. (2009) found that dairy cows grazing rye grass supplemented with 163 g/d or 326 g/d of condensed tannins (CT) had reduced enteric CH₄ emissions, although the CT also caused a corresponding decrease in milk yield and intake. Tannins are able to reduce enteric CH₄ because of its affinity to bind protein and carbohydrates in the rumen which decreases ruminal fiber digestion. However, its inclusion at high dietary concentrations can decrease voluntary feed intake. Min et al. (2003) suggested that tannins impact rumen protein degradation when consumed at 20 to 45 g CT/kg DM, and at >55 g/kg DM can inhibit voluntary feed intake and forage digestibility. Tannins also may serve to improve animal performance through bloat control and providing some antiparasitic properties (Hristov et al., 2013). However, tannin research is difficult and inconsistent due to difficulties in isolating tannins and because different forages may contain different structural forms of tannins.

1.4.2 Mitigation Strategies Directed Toward Animals

Mitigating enteric CH₄ emissions by acting directly on the animal and not the forage base is problematic in grazing environments partially due to the difficulty in estimating DM intake of grazing animals (level of intake is a major driver of enteric CH₄ production), the infrequency of supplementation and(or) variable level of individual animal supplement intake (Buddle et al., 2011; Hristov et al., 2013). Common mitigation strategies are supplementation to increase animal performance (thereby decreasing emission intensity, although not always done specifically to decrease emissions, e.g., protein supplementation), supplements that directly alter

ruminal fiber digestion or methanogens, breeding for more efficient animals, and vaccinating against methanogens (Hristov et al., 2013; Phetteplace et al., 2001; Grainger and Beauchemin, 2011). Other strategies, such as nitrate supplementation do not seem practical because of issues in getting the inhibitor to the animal in useful quantities and(or) risks of toxicity (Hristov et al., 2013; Llonch et al., 2017).

1.4.2.1 Supplementation

DeRamus et al. (2003) and Shibata and Terada (2010) hypothesized that improving the efficiency of feed energy use has the greatest potential for mitigating enteric CH₄ production. Outside of improving the forage base, providing supplementation to meet the nutritional needs of different classes of grazing animals is a mitigation option. Producers have long supplemented grazing ruminants to improve performance during times of nutritional deficiencies in the forage crop, such as the late summer slump or winter dormancy that affects many forage bases across the U.S. (Caton and Dhuyvetter, 1997). Similar to estimating emissions of grazing animals, accurate and precise supplementation are difficult because energy requirements for grazing animals are not the same as for confinement-fed cattle (Caton and Dhuyvetter, 1997). The most common forms of beef cow supplementation are energy supplementation, when intake of C skeletons are the limiting nutrient for ruminal microbial growth, and protein supplementation when low quality forages (i.e., CP < 7 %) are the primary diet. Energy supplementation can come in many forms, such as corn grain or fat supplementation, depending on the goals of the producers. For example, when cattle are grazing wheat pasture that is high in water and protein contents, but low in fiber, corn supplementation can improve animal performance (Hogan, 1982). Although not specifically done to alter CH₄ emissions, by improving animal performance this strategy indirectly improves emission intensity (Thompson et al., 2019). Similarly, during times

of forage dormancy, protein is supplemented to improve performance to meet microbial N requirements (McCollum and Horn, 1990; DeRamus et al., 2003). Protein supplementation does not directly impact rumen methanogens, unlike energy supplementation strategies, and can come in a multitude of forms including pelleted feed, limit grazing cool season forage and lick blocks, among others (DeRamus et al., 2003; Leng, 1993). McCollum and Gaylean (1985) in a cross-over design utilizing ruminally cannulated steers reported that cottonseed meal (37.9% CP) fed at 800 g/animal per d improved forage utilization by improving IVDMD and animal performance. Unfortunately, there is limited literature on the impact of supplemental protein on enteric CH₄ emissions. Decades ago, Leng (1993) and Moss (1994) suggested that this strategy could be particularly beneficial in developing countries where the animal nutrition is potentially poor. They also suggested that using targeted supplementation in these countries could reduce enteric CH₄ emissions by improving the nutritional balance in the rumen. DeRamus et al. (2003) using the SF₆ technique reported that heifers grazing ryegrass ad libitum had one-tenth of the CH₄ emission intensity compared with animals limit grazing ryegrass for 1 h during the spring, but that limit-grazing for 4 h resulted in similar enteric CH₄ emissions. Boadi et al. (2002) compared grazing steers supplemented with rolled barley grain at 2, 4, and 4 kg/head per d during the early, mid, and late grazing season, respectively, with steers grazing alfalfa and meadow grass pastures without supplementation. They reported that supplementation reduced forage intake, increased total OM consumed, but did not impact overall enteric CH₄ emissions. It was hypothesized from the results that the greater forage quality and quantity were the major factors influencing animal responses. However, considering the high forage quality available to the steers, one should not apply these results to cattle grazing lower quality forage where supplementation might improve the nutritional status of the rumen. Considering the heavy reliance on forage quality by

governing bodies when estimating CH₄ emissions (IPCC, 2006), it would be beneficial to do more long-term monitoring due to the variation among different production environments, changing forage quality throughout the year, and supplementation strategies that exist to improve animal performance. Considering the advancements in modern emission quantification techniques, along with the lowered cost of monitoring, long-term studies should be conducted.

Lipid supplementation is a plausible approach to directly reduce enteric CH₄ emissions (Beauchemin et al., 2009). Nutritionally, lipids are categorized by their impacts on ruminal activity and fiber digestion (Jenkins, 1997). There is a wealth of literature exploring the impact of fats on ruminal CH₄ production. Lipid supplementation apparently alter emissions through reductions in ruminal fiber digestion, although there is some evidence for a suppressive effect on bacteria and protozoa activity (Hristov et al., 2013). An additional mode of action is unsaturated fatty acids acting as a H₂ sink during the process of biohydrogenation in the rumen. This reduces the H₂ pool available to methanogenic archaea; however, this process appears to have minimal impact and was suggested to only account for 1 to 2% of metabolic H₂ (Johnson and Johnson, 1995; Jenkins et al., 2008). A group of lipids, including animal-based tallow and grease, oils from plants (eg., soybean oil and cottonseeds), and high fat byproducts (e.g., distillers grains) can alter ruminal CH₄ production (Hristov et al., 2013). The most likely mode of action for these supplements is coating fiber particles to protect them from ruminal microbes. When supplemented at appropriate dietary concentrations, these lipid sources can reduce CH₄ production without altering DM digestibility or forage intake (Patra, 2012; Grainger and Beauchemin, 2011). There has been extensive research done with dietary lipids but unfortunately the majority was conducted with dairy or beef cattle fed total mixed rations with significant proportions of concentrate feeds (Beck et al., 2019). In two separate experiments using the GEM,

Beck et al. (2018, 2019) examined the impact of whole cottonseed supplementation and rumen bypass fat or soybean oil on enteric CH₄ emissions of steers grazing old world bluestem pastures. In both experiments CH₄ production was reduced when steers were offered whole cottonseed or soybean oil. Rumen bypass fat did not alter CH₄ production (g/d) but did when expressed as g/kg of BW gain and lowered CH₄ yield (Y_m) (9.7% vs. 8.0% for control vs. bypass fat-supplemented cattle). Carvalho et al. (2016) examined Nellore steers grazing C4 grasses offered no supplement, palm oil, linseed oil, rumen bypass fat, or whole soybeans supplemented at 1% of BW. They found that the linseed oil treatment reduced CH₄ emissions per kg BW but none of the lipid supplements improved animal performance or DM intake. The authors hypothesized that a reduction in ruminal fiber digestibility may have been responsible for the lack of improvement in performance. These studies show that different lipid sources can potentially be beneficial both environmentally and economically through impacts on performance, but overfeeding lipids can be detrimental although results are not consistent across different lipid sources as some studies have shown reductions in DMI and productivity (Grainger and Beauchemin, 2011; Hristov et al., 2013). Grainger and Beauchemin (2011) conducted a meta-analysis and showed that, while some supplemental lipids do reduce CH₄ production, there were significant linear and curvilinear responses in CH₄ production from diets containing up to 13% lipids, and dietary fat content should not be greater than 6 to 7%, DM basis (Patra, 2012). The persistence of CH₄ reduction was inconsistent in the literature (Grainger and Beauchemin, 2011). Studies such as those conducted by Martin et al. (2011) and Grainger et al. (2009) have resulted in long-term emission mitigation that may be due to animal type or diet variability. More long-term experiments should be conducted with grazing beef cows to determine how impactful lipid supplementation could be at the herd emissions level, as well as to explore economical methods of supplementation.

Ionophores are common feed additives provided to both grazing and confinement fed cattle because of their impact on animal health and efficiency (Byers and Schelling, 1984; Callaway et al., 2003). The most frequently used ionophore, monensin, improves energy and N utilization by selective inhibition of gram-positive ruminal bacteria which favors propionate production (Hristov et al., 2013; Beauchemin et al., 2008). Ionophores effectively inhibit gram-positive bacteria by facilitating the transport of ions across the cell membrane which leads to the disruption of the chemi-osmotic gradient (Bergen and Bates, 1984). A greater proportion of gram-negative bacteria shifts the acetate:propionate ratio, favoring production of the more energetically efficient propionate that acts as a H sink thereby potentially reducing CH₄ production (Place et al., 2011; Appuhamy et al., 2013). However, the impact of ionophores on CH₄ production was inconsistent and sometimes not detected in studies in grazing environments (Appuhamy et al., 2013; Thompson et al., 2019). In a meta-analysis of 22 studies, Appuhamy et al. (2013) reported that including monensin at 32 mg/kg DM reduced CH₄ yield (% GE lost as CH₄) by $0.33 \pm 0.16\%$ for steers consuming total mixed rations. In an experiment with dairy cows grazing a predominantly ryegrass sward with monensin supplemented at 471 mg/d, no differences were detected compared with cows not receiving monensin (Grainger et al., 2010). A respiration chamber experiment was conducted concurrently with the grazing experiment. In cows receiving the same monensin dose and offered fresh-cut ryegrass no difference on CH₄ production was detected from monensin supplementation. Similarly, Grainger et al. (2008) dosed monensin using a controlled release capsule and reported that enteric CH₄ yield of dairy cows grazing ryegrass pasture was not impacted by monensin supplementation. In a respiration chamber experiment with steers consuming fresh chopped winter wheat at increasing intake levels and offered a monensin containing supplement (158 g/head per d), CH₄ production tended

to be reduced for supplemented animals compared with non-supplemented animals at 115 L/d vs. 130 L/d, respectively ($P = 0.06$; Shreck et al., 2017). There was no difference in GEI lost as CH_4 when animals were fed at 1.5x maintenance. However, in an experiment with beef steers and heifers grazing winter wheat offered and energy supplement containing monensin (34 mg/kg DM), Thompson et al. (2019) found that increasing level of supplement intake reduced CH_4 emission intensity (g of CH_4 /kg of BW gain). Whereas cattle fed TMR have displayed long-term reductions in CH_4 production, potential explanations for the inconsistency in grazing trials could be variable dietary quality of different forage bases, the rate of intake, and the lack of studies conducted using beef cattle in grazing environments (Odongo et al., 2007; Appuhamy et al., 2013; Hristov et al., 2013; Grainger et al., 2008). Even though monensin may result in reductions of CH_4 emissions and has proven to improve feed efficiency and reduce bloat, it was banned in the European Union since 2006 and its classification as an antibiotic could result in increased scrutiny in future years in other regions.

Although not a new feed additive, recent research showed the potential anti-methanogenic properties of *Asparagopsis* species (seaweed) inclusion in the diet, both *in vitro* and *in vivo* (Li et al., 2018; Roque et al., 2019a; Roque et al., 2019b; Machado et al., 2015). *Asparagopsis* microalgae produces bioactive compounds including bromoform and dibromochloromethane giving it the same potential mode of action as the chemical additive bromochloromethane that decreased enteric CH_4 emissions (Tomkins et al., 2009). In an *in-vitro* dose-response experiment, Machado et al. (2015) tested 10 doses of *Asparagopsis* inclusion from 0 to 16.7% OM incubated with a grass hay substrate. Methane production was reduced by 99% at 2% OM inclusion level. When included at 0.5% or 1% OM basis in a TMR, Roque et al. (2019b) reported that CH_4 emissions from dairy cows sampled with a GEM were reduced by 43% at the

1% inclusion level after adjusting to account differences in DMI. Methane reduction of this magnitude would make seaweed supplementation with *Asparagopsis* inclusion one of the most noteworthy mitigation strategies tested *in vivo*. Additionally, when offered to sheep fed a high-fiber pelleted diet Li et al. (2018) reported that ruminal propionate concentrations increased with *Asparagopsis* supplementation and CH₄ production decreased over a 72-d period.

Supplementation of *Asparagopsis* in grazing environments at different intakes and frequencies of supplementation is needed to establish its efficacy in high forage diets, beyond high concentrate confinement feeding.

Another possible avenue for enteric CH₄ mitigation was the discovery that CH₄ emissions might be heritable, suggesting that genetic selection of animals might be a viable mitigation possibility (Pinares-Patino et al., 2013; Roehe et al., 2016). It is hypothesized that this is possible because the host animal asserts some form of influence over its own microbiota (Roehe et al., 2016). Some researchers suggested that the mechanisms controlling the microbiome may be in the interactions with receptors in the rumen wall or salivary antibodies (Tapio et al., 2017). Goopy et al. (2016) hypothesized that the physical structure of the rumen could explain the differences between genetic lines, but their results were not consistent. Additionally, their results implied, although indirectly, sire environmental interactions indicating that selecting cattle for reduced CH₄ emissions in one environment may not improve performance as the animal moves from a grazing environment to the feedlot. In a study analyzing about 3,400 records, along with the records reported by Pinares-Patiño et al. (2013), Jonker et al. (2018) reported heritability estimates of 0.23 and 0.13 for CH₄ g/d and Y_m, respectively, in New Zealand sheep. Estimates in Angus cattle was similar as reported by Hayes et al. (2016). They reported heritability values of 0.27 and 0.29 for CH₄ production and Y_m from 737 Angus cattle in Australia. Additionally, new

research found significant differences between breeds. In a study of Nelore and Angus cattle grazing during the growing period and finishing in a feedlot, Maciel et al. (2019) determined that Nelore cattle produced significantly less CH₄ (g/d) than Angus cattle regardless of diet.

However, in the finishing period the Angus steers reached finishing weight faster and therefore produced the same amount of CH₄ during the finishing phase as Nelore steers (Maciel et al., 2019). More work to refine the selection technique and characterization of beef herds in other countries could potentially allow for reductions in the carbon footprint of the beef production.

Methane production is partially dependent on the quantity of feed consumed which led researchers to explore selecting animals based on their residual feed intake (**RFI**; Hegarty et al., 2007). Residual feed intake is defined as the difference between actual feed intake and the expected rate of intake needed for that animal to meet its maintenance requirements plus the desired production level (e.g., milk, meat, wool; Arthur et al., 2001). Research on this strategy was inconsistent and variable across environments (Hegarty et al., 2007; Velazco et al., 2016; McDonnell et al., 2016). In a study with low- and high-RFI Limousin x Fresian heifers enteric CH₄ production, DMI, and performance were measured in both grazing and confinement environments (McDonnell et al., 2016). The study indicated that animals with low-RFI actually had greater CH₄ emissions on the basis DMI and GEI than high-RFI animals. These results were similar to those of Velazco et al. (2016) and Flay et al. (2019), who found that cattle with heavier BW and low-RFI had greater DMP compared with high-RFI cattle. Other studies, such as those conducted by Hegarty et al. (2007) and Lawrence et al. (2011), reported low-RFI cattle had lower enteric CH₄ emissions. These inconsistent results demonstrate that RFI classifications are not consistent across diets or animals, particularly growing animals, currently limiting RFI's application as a mitigation tool.

1.4.2.2 Vaccination Against Methanogens

Vaccination against ruminal methanogens is another potential strategy which thus far has produced inconsistent results. This approach is based on the generation of an antibody response that is delivered to the rumen through salivary secretions to neutralize methanogens (Buddle et al., 2011). Such a vaccination potentially would be easy for many producers to implement because they already use some type of annual vaccination protocol; thus vaccination might be cost-effective (Buddle et al., 2011). Cook et al. (2008) demonstrated that concentrations of avian anti-methanogen antibodies can reduce CH₄ production *in vitro*. In a study with mature wether sheep, Wright et al. (2004) isolated methanogens and provided sheep with a 3-methanogen mixture, a 7-methanogen mixture followed by the 3-methanogen mixture, or adjuvant only. Primary and secondary immunizations were given subcutaneously 153 d apart. Testing CH₄ emissions with both respiration chambers and the SF₆ technique, they found a significant 7.7% reduction in enteric CH₄ production 4 wk post-secondary immunization in sheep provided the 3-methanogen mixture. Wedlock et al. (2010), although not measuring enteric CH₄ emission, did find that antisera selected from methanogen fractions produced a strong antibody response in sheep, with both IgG and IgA responses detected in the saliva. However, in a study using a vaccine attempting to account for 52% of the methanogens present in the rumen, Williams et al. (2009) found that CH₄ production actually increased by 18% in sheep after 3 vaccinations, opposite of the effect expected. Minimal research has examined the efficacy of methanogen vaccination in cattle. Subharat et al. (2015), using 5-mo old male Holstein-Friesian calves, provided subcutaneous anti-methanogen vaccinations isolated from *Methanobrevibacter ruminantium* M1. After vaccination, the authors detected a strong IgG response and a moderate IgA response in the serum and saliva of inoculation animals, as well as rumen antibodies.

In vivo vaccination trials showed promise but may be limited by current knowledge of the rumen methanogen population (Boadi et al., 2004). In the study by Williams et al. (2009), the 5-methanogen species vaccination resulted in increased CH₄ production, even with the desired immune response being observed. The authors hypothesized that this was because the vaccine was not targeting the species responsible for most of the CH₄ production or that some unknown conditions are necessary to see an abatement response recorded in previous studies (Williams et al., 2009). It also was noted by authors that any vaccine formulation is diet and environment-specific, with a broad-spectrum out of the reach of current research (Williams et al., 2009; Hook et al., 2010). Whitford et al. (2001) found that most rumen methanogens are difficult to culture and that the majority of species, at that time, had yet to be isolated. For the vaccination approach to show promise in the future it will be critical to successfully culture highly productive methanogen species and to do so across production environments as methanogen populations can vary widely by region and diet (Hook et al., 2010).

1.5 Soil Methanotrophy

Outside of CH₄ oxidation by OH⁻ radicals in the atmosphere, the only major sink of atmospheric CH₄ is oxidation by soil microbes (Saggar et al., 2008; Hartmann et al., 2011). Soils can be either a source or sink of CH₄, with mostly obligate aerobic methanotrophs located in the upper levels of upland soils responsible for oxidizing atmospheric CH₄ to CO₂, but the ecological controls involved are still poorly understood creating large uncertainties (Saggar et al., 2008; IPCC, 2013). It is estimated that methanotrophic activity in soil may remove about 9 to 47 Tg(CH₄)/yr and this mostly occurs in aerobic soils (IPCC, 2013). Activity of soil methanotrophs is dependent on the diffusion rate of CH₄ and biological activity of soil (Mosier et al., 1998). After a rainfall, CH₄ is immediately emitted from the soil, with this emission rate gradually

decreasing as soil dries and more CH₄ is oxidized as the diffusion becomes less suppressed as less water fills the pore networks (Mosier et al., 1998; Hartmann et al., 2011). Forest soils with well-developed soil-structure typically displays the greatest CH₄ oxidation rate, followed by immature forests, then native range land, and lastly intensively managed agriculture lands have the lowest CH₄ sink potential due to increased and frequent disturbance and fertilizer application (Mosier et al., 1998). Temperate forest soils have CH₄ uptake rates ranging from 12.8 to 25.6 kg (CH₄)/ha per yr, compared with agriculture soils having maximum oxidation rates of 1.6 to 3.2 kg (CH₄)/ha per yr (Smith et al., 2000). Only soils with high water tables were reported to be sources of CH₄ (Smith et al., 2000). Nitrogen fertilizer application has a negative relationship with methanotroph activity because ammonium may have an inhibitory effect on the enzymes responsible for CH₄ oxidation (Dunfield and Knowles, 1995). However, these results are inconsistent in the literature. It was hypothesized by Hütsch et al. (1993) that it may take at least 7 yr of fertilizer application before inhibitory effects are seen. Uptake rates also display significant temporal variation between seasons (Mosier et al., 1998). Methane uptake rates are greatest in summer months when drier soil conditions are present and less in cold wetter months when methanotroph activity is suppressed and methanogen activity is greater (Mosier et al., 1998). Even with considerably lower oxidation rates, some agricultural soils may still be able to offset CH₄ emissions from animal excreta and part of enteric CH₄ emissions but few studies have been conducted in this area and results have been inconsistent (Saggar et al., 2004; 2007). Mosier et al. (1991) compared CH₄ uptake rates in a native pasture, an annually fertilized pasture, and a non-irrigated wheat field and found that N fertilization of the native grassland reduced CH₄ uptake rate by 35% compared with the native pasture. The non-irrigated wheat field resulted in a further decrease of 15% from the fertilized native pasture (Mosier et al., 1991). These results are

similar to those of Willison et al. (1995) who reported that long-term application of ammonium-N fertilizer resulted in reduced CH₄ uptake by soils. However, in a study on the impact of plant diversity and fertilizer application on CH₄ and N₂O fluxes in Germany, it was found that CH₄ uptake decreased with increasing plant diversity regardless of fertilization (Niklaus et al., 2016). It was hypothesized that this was due to increased soil moisture which lowered the diffusion rate of CH₄. However, other potential explanations for the inconsistency is that N concentrations were not high enough to be inhibitory, fertilizer may not have been applied to fields prior to experiment onset, or water content of the soils was the limiting factor before enzyme inhibition. In a grazing study on three different types of steppe in Inner Mongolia, China (meadow steppe, typical steppe, and desert steppe) and three different grazing rates (light, moderate, and heavy grazing) it was reported that light grazing did not change CH₄ uptake rates, but moderate and heavy grazing reduced uptake by 6.8 to 37.9% (Tang et al., 2013). However, in a synthesis of 43 studies conducted in China, including the previous study, only heavy grazing rates consistently decreased CH₄ uptake (Tang et al., 2019). While the synthesis by Tang et al. (2019) reviews a depth of literature on uptake rate in grazing ecosystems, there is a lack of research incorporating enteric CH₄ production rates and the potential of different mixtures to offset parts of the enteric CH₄ budget.

1.6 Carbon Accounting

The Paris Climate Accord set an aggressive goal of keeping global temperature rise below 1.5°C compared with pre-industrial levels by the year 2100 (Rogeli et al., 2018). To reach this goal all industries must reach net zero emissions, particularly in developed countries, as soon as possible (Rogeli et al., 2018). Methane production is a large component of the beef industry's C footprint because it is a potent GHG with a global warming potential (**GWP**) over a 100 year-

time frame (**GWP₁₀₀**) 28 times that of CO₂ according to IPCC AR5 (IPCC, 2014). With a supporting population of approximately 32 million head of beef cows in the US, and higher CH₄ emission rates with high forage diets compared with higher concentrate diets, this results in a C footprint that heavily favors intensive production environments while the cow-calf production sector contributes 70 to 80% of the total CO₂ equivalents budget (Rotz et al., 2019; Alemu et al., 2017; Heflin et al., 2019). Therefore, the greatest emission reduction potential lies in reducing enteric CH₄ emissions, especially from the cow herd (Rotz et al., 2019). The importance of enteric CH₄ from grazing ruminants in the biogenic C cycle, however, provides an additional layer of complexity in the discussion of enteric CH₄ mitigation that should be mentioned. In the U.S., CH₄ from ruminants has always been a large component of the C cycle during the transition from wild to farmed ruminants (Kelliher and Clark, 2009). Two studies examining pre-European enteric CH₄ emissions from the large bison herds and other wild ruminants compared with modern day farmed ruminants found that emission rates pre-European settlement were similar to modern emission rates (Hristov, 2011; Kelliher and Clark, 2009).

The metrics being used when conducting current life cycle assessments and monitoring progress of mitigation are in continual discussion and the benefits of reductions of short-lived pollutants, such as CH₄, may be overstated because of this (Allen et al., 2018; Pierrehumbert, 2014). A carbon footprint represents the totality of emissions from a system, with different GHGs contribution converted to a CO₂ equivalents basis; this may be misrepresenting the burden of enteric CH₄ (Allen et al., 2018). Greenhouse gases are typically converted into CO₂ equivalents using a global warming potential metric over varying time scales, and the time scale that is selected to calculate the equivalent metrics can have significant impact on the final computed C footprint (IPCC, 2013). For example, if GWP₂₀ (meaning over a 20-yr timeframe) is

used CH₄ is considered 84 times more potent than CO₂, but when this is expressed over 100-yr timeframe then the GWP falls to 28 (IPCC, 2013). The high heat trapping potential increases with shorter timeframes because CH₄ is a short-lived pollutant with an atmospheric half-life of about 9 to 12 yr before being oxidized by hydroxy radicals, compared with CO₂ which can remain in the atmosphere for thousands of years (IPCC, 2013; **Figure 1.1**). This has led some to question the use of GWP₁₀₀ and to suggest other metrics including global temperature potential (**GTP**) and GWP*, although both are relatively new and lack policy influence (Shine et al., 2005; Allen et al., 2018). Using GTP₁₀₀ metric CH₄ is equal to just 4 times CO₂, down from 28 using GWP₁₀₀, but N₂O only changes from 265 to 234 using GTP₁₀₀ (IPCC, 2013). The metric GWP* takes a slightly different approach to determine the impact of short-lived pollutants by equating a yearly one tonne increase in the rate of CH₄ emission with a one-off pulse release of 100 x GWP₁₀₀ tonne of CO₂ (pulse release to calculate temperature impact of methane over 100 yrs; Allen et al., 2018). This allows CH₄ to be considered on a decadal time-scale, one that is more appropriate given its atmospheric lifetime. This may allow future models to more appropriately consider the behavior of CH₄ in the atmosphere by allowing its warming potential to reach zero which is not possible with CO₂ due to its long atmospheric lifespan, unless sequestration is considered (Allen et al., 2018). The implication, therefore, is that if cattle numbers remain static, there should be no increase in radiative forcing and likewise any decrease in cattle numbers or emission rate should actually have a cooling effect on a 100-yr timeframe versus consistent radiative forcing during the same time.

Livestock Environmental Assessment and Performance Partnership (FAO, 2016) which standardizes LCA modeling, requires IPCC Tier II or III calculations to compute enteric CH₄ production (FAO, 2016). Studies that utilize Tier II methodologies use a standard conversion

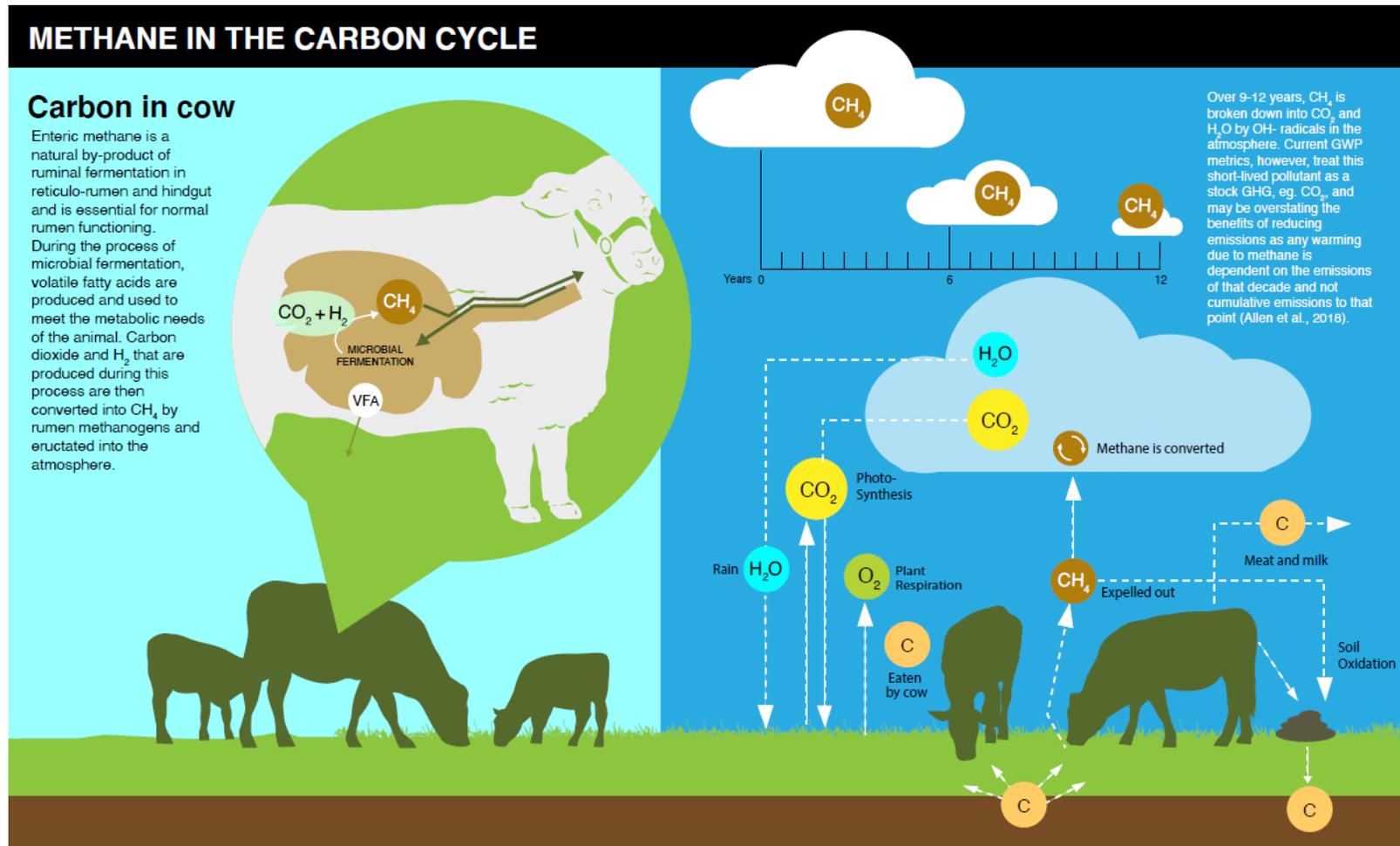
factor for enteric CH₄ production, 6.5% of GEI is lost as CH₄ for grazing cattle and 3.5% of GEI for cattle on feedlot diets, which arise from studies that are not associated with normal grazing patterns. This can result in non-representative DMI based on the CH₄ measurement techniques that were used. Research using new technologies, including the SF₆ tracer technique and GEM, indicate that the conversion factor for grazing cattle may overestimate emissions in some environments (Stanley et al., 2018; Chiavegato et al., 2015; Stackhouse-Lawson et al., 2012). Life cycle assessments conducted by Stackhouse-Lawson et al. (2012) and Stanley et al. (2018) using data collected for production environments in California and the upper Midwest, respectively, both indicated that the IPCC Tier II methodologies were overestimating emissions by up to 15%. With GHG monitoring technologies evolving rapidly to better sample the complex grazing environment where respiration chamber methodologies have limitations, more long-term monitoring studies and updated LCA calculations are needed to improve the accuracy of the industries C footprint for grazing cattle.

1.7 Applications: Current and Future

Producer decisions impacting the soil-plant-animal interrelationships show promise in reducing the CH₄ emission rates from cattle. Improving the quality of the forage base by incorporating high quality, readily digestible forages and grazing strategies that improve the quality of the forage base, while potentially sacrificing short-term gains in animal performance, can result in the reduction of CH₄ production. Additionally, mitigation tools such as lipid supplementation, supplementing *Asparagopsis* (seaweed), incorporating forages with beneficial secondary compounds, and genetic selection for reduced enteric CH₄ production may be viable tools for beef producers to lower their C footprint. Improvements in grazing emission estimates and micrometeorological techniques also will give researchers better insights in future years on

how animal management and grazing strategies impact whole herd emissions and landscapes. In particular, as financial markets for C and ecosystem services develop over the coming decades these landscape monitoring systems may serve an important role in the future of agriculture and allow producers to benefit on other ecosystem services they provide rather than just provisioning services. However, these systems must be cost effective, accurate, and precise for these services to be fairly compensated. Lastly, changing from the GWP₁₀₀ metric to GWP* may allow researchers to better understand the impacts of short-lived pollutants on climate change and be an improved representation of how enteric CH₄ production operates as part of the natural C cycle.

FIGURE 1.1: Methane in the carbon cycle¹



¹Adapted from sacredcow.info

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CHAPTER 2

COW EFFICIENCY: MODELING THE BIOLOGICAL AND ECONOMIC OUTPUT OF A MICHIGAN BEEF HERD

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Abstract

In recent decades, beef cattle producers have selected cattle for biological traits (i.e., improved growth) to maximize revenue, leading to an increase in average cow body size. However, matching cow size to the production environment would allow producers to maximize productivity and economic returns per unit of land. This may help meet the goals of sustainable intensification, but environmental complexity and varying cow-calf production strategies dictates a regional approach. The objective of this experiment was to examine the biological efficiency and economic returns of a Northern Michigan cow-calf operation. We hypothesized that biological efficiency and economic returns would decrease with increasing cow body size. Data were collected from a Red Angus cow herd located at the Lake City AgBio Research Center in Lake City, MI from 2011 to 2018 on cow age, weight, and body condition score at weaning, and subsequent 205 d adjusted calf weaning weight (WW), sex, and yearling weight. Biological efficiency was defined as WW as a percentage of cow body weight (DBW). Enterprise budgeting techniques were used to calculate expected net returns from 2011 to 2018 after classifying cows into 11 BW tiers at 22.67 kg intervals beginning at 430.83 kg. Forward-looking net present value (NPV) was calculated using the same tier structure, for a 10-yr production cycle with the baseline being a 200 d grazing season. Weaning weight increased with increasing DBW ($P < 0.01$), but the percentage of cow body weight weaned was reduced by $-38.58 \times \ln(\text{DBW})$ ($P < 0.01$). This led to cows weaning 26.38 kg/ha more with every 100 kg drop in DBW. Expected net returns from 2011 to 2018 did not differ by DBW tier on a per cow basis but did on a per ha basis with a decrease in \$10.27/ha with each increase in DBW tier ($P < 0.01$). Net present value was maximized in the baseline scenario at 453.51 kg DBW and decreased in value

as DBW increased. These results suggest that for a Northern Midwestern cow-calf herd, comparatively lighter cows provide a higher economic value on a land basis.

2.1 Introduction

Matching cow size to the specific environment plays a key role in the long-term sustainability of the operation but selection for biological performance (i.e. average daily gain, kg/d) indicators may be disrupting this balance. This can be seen by the increase in cow size in recent decades (Johnson et al., 2010; Scasta et al., 2015). Producers choose growth traits in an effort to maximize revenue which has led to increases in mature cow size. Additionally, producers have been incentivized by packers to produce bigger, heavier carcasses (Johnson et al., 2010). However, this may be hampering the long-term environmental and economical sustainability of cow-calf operations if producers have to adjust stocking rates due to selecting for traits which provide short-term benefits (Doye and Lalman, 2011; Miller et al., 2001). In conjunction with these economic incentives, beef producers have a tendency to subconsciously favor larger animals because of the perceived benefits in growth, but they do not always consider the feed requirements of the herd are increasing and fail to adjust for herd size (Reuter, 2017; Doye and Lalman, 2011). Therefore, recommendations are required for producers to make informed decisions on suitable cow sizes for their specific environments. The objective of this study was to model the relationship between cow body size and age on calf weaning weight, yearling performance, and economic returns of a Northern Michigan cow herd. We hypothesized that weaning weight efficiency and economic returns would decrease with increasing cow body size.

Biological variables such as cow body weight and weaning weight play an important role in production efficiency. Dickerson (1970) defined efficiency as the ratio of total cost to total

animal product and highlighted the need to maximize production per female relative to their metabolic body weight. Previous literature, including classical work by Urick et al. (1971) and Dinkel and Brown (1978), showed that as cow body weight increases producers may realize moderate improvements in calf weaning weights. Recent studies including Kuhlert et al. (2013), Mourer et al. (2010) and Dobbs et al. (2011) showed that for each additional 100 kg of cow BW, calf weaning weight may increase from 2.3 to 10 kg depending on stocking rate and environmental conditions. However, the ability of heavier cows to wean heavier calves may not come efficiently (Doye and Lalman, 2012; Scasta et al., 2015). Lalman and Beck (2019) reported that mature cow size has been increasing and requires increased income to offset the increase in mature cow size. Whitworth et al. (2006) examined the biological efficiency of the University of Arkansas beef herd and found the efficiency was lowest with heavier cows. This would imply that as cow size has increased their ability to wean more kg has been compromised.

Biological efficiency ratios are incomplete metrics for genetic selection criteria and are more useful when paired with an economic analysis. An important factor that influences biological efficiency and cattle performance is the maintenance overhead (energy intake required for maintenance) and this will vary by production environment (Arango and Van Vleck, 2002). Doye and Lalman (2011) performed an economic analysis of two cow-herds in the Southern Plains of the U.S. of differing sizes (498.86 kg vs. 634.92 kg) and two pasture types (native and improved pasture). They reported that larger cows weaned more weight and therefore generated more revenue from the sale of the calves. Bir et al. (2018) calculated the net present value (**NPV**) of increasingly heavier cows and found that 430.84 kg cows provided the largest return across native vs improved pasture and beef breeds tested. The type of cow that optimizes a production environment in one region may not in another, investigations into beef cow efficiency in

Michigan and the Northern Midwest has yet to be done. Understanding the production efficiency of Michigan beef cows will provide insights for improving the region's sustainability as it relates to sustainable intensification. The concept of sustainable intensification implies the maximization of production per unit of land in a manner that meets all three pillars of sustainability- planet, people, and profit (Makkar, 2013). If lighter cows require less land to produce a similar amount of product and be as or more profitable, this would address two of the three pillars. However, sustainable intensification does not specify how to meet these goals and regional complexity means that different strategies will be needed depending upon local climate, production environment, and management.

2.2 Materials and Methods

To examine both the biological and economic efficiency of cows of differing weights a multi-step approach was conducted. Collected data were subject to statistical analysis to develop biological output models. Additionally, economic costs and prices were collected to conduct a backward-looking enterprise budget analysis of the cow herd. These biological output models, costs, and prices were then used in a forward-looking net present value analysis of modeled cow herds of increasing weight classes.

2.2.1 Animals and Forage

Data for this experiment were obtained from the Lake City AgBioResearch Station cow herd from 2011 to 2018 in northwest Michigan (Lake City, MI). Average annual precipitation and temperature are detailed in **Table 2.1** (usclimatedata.com).

The cow-herd from 2011 to 2018 was Red Angus breeding cows and resulted in a total of 1,038 cow-calf records, an average of 130 annual records. Only cows that produced a calf each year were recorded in the data set and reasons for culling were not recorded. Cows were

managed in an adaptive multi-paddock grazing management with improved forages including: orchardgrass (*Dactylis glomerata* L.), alfalfa (*Medicago Sativa* L.), timothy grass (*Phleum pretense*), red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), birdsfoot trefoil (*Lotus corniculatus* L.), smooth brome grass (*Bromus inermis* L.), and Kentucky bluegrass (*Poa pratensis*). Grazing onset began when forage availability was determined to be adequate based on visual appraisal, with turn out averaging May 15th and grazing terminated due to snow cover by mid-November. From 2011 to 2018 the farm averaged approximately 200 grazing days annually. Cows were grazed in high stock densities (~150,000 kg cow body weight ha⁻¹) until 2016 when management was shifted to an adaptive rotational grazing with larger paddocks and longer grazing durations with a target grazing density of ~80,000 kg cow body weight ha⁻¹. Winter management consisted of high quality (9-11% CP) grass hay fed *ab-libitum*. Hay was fed by unrolling hay bales across fields and winter-feeding locations were rotated across the farm depending on where animal impact was desired. Cows received *ad-libitum* water and offered free-choice mineral.

Bulls were introduced, after soundness exams, on approximately July 1 each year into heifer groups and at the end of July into mature cow groups, with calving occurring from late March to May. Calves were weaned in October and November (~6 months of age) of each year. Each year at weaning cow body weights were recorded, and cow body condition score (**BCS**) was recorded on a 1 to 9 scale by 2 qualified technicians (Wagner et al., 1988), with the exception of 2018 when BCS was not recorded. Calf weaning weights were taken and adjusted to a 205-d weaning weight with no age of dam adjustment included (**WW**). Weaned calves went into a grass-finishing program described by Stanley et al. (2018) and yearling weights (**YW**) were recorded the spring after weaning. All mature cows were managed the same over the 8-year

period except for a small subset used for a grazing experiment which were removed from the analysis. Retained heifers were managed separately from mature cows and joined the mature cow herd after first calving. Cow body weights were then normalized to a body condition score of 5 using equations described by Fox et al. (1988; **DBW**). For 2018, actual cow body weights were used due to lack of BCS records that year. Biological efficiency was calculated for both 205-d adjusted weaning weight (**CWP**) and yearling weights as a percentage of **DBW**.

2.2.2 Forage Intake and Land Use

Calves were assumed to enter the finishing enterprise at weaning and were not included in forage intake or economic analyses. Forage intake for the cow-calf enterprise was calculated using **NEM** values of the forage base and fed hay as recommended by **NASEM** (2016; see Equation 1). Forage samples from 2012 and 2013 were analyzed by Dairy One (Ithaca, NY) with sampling procedures described by Chiavegato et al. (2015) and hay core samples were taken from 2016 to 2018 and analyzed by DairyLand (Battle Creek, MI). Dry matter intake was calculated as:

$$\text{Equation 1: DMI} = [\text{DBW}^{0.75} \times (0.04997 \times \text{NEM}^2 + 0.04631)] / \text{NEM}$$

Intake of nonpregnant cows included an intake adjustment of 0.0384 (**NASEM**, 2016). During lactation, cows were assumed to have a peak lactation of 8 kg/d at maturity and intake was increased by adding 0.2*daily milk yield adjusted for age of cow (**NASEM**, 2016). Net energy maintenance values were 1.43 Mcal/kg DM for pasture and 1.20 Mcal/kg DM for hay. Yearly forage consumption per cow was estimated using a 200-d grazing season (Lake City Research Center Beef Report, 2014) and 164 d of hay consumption. During the hay feeding period, 115 d were modeled to include environmental stress (i.e. cold stress) with a 5% increase in **DMI** per d using the dry pregnant cow calculation (**NRC**, 2000; usclimatedata.com). Since the

grazing season aligns with the farm calving season, DMI was calculated using the lactating intake. Stocking rate was then calculated using on farm estimates of forage utilization rates from 2014 to 2018, based on cow days per ha and assuming an average forage utilization of 50%. For years falling outside of this range, the average (5506.18 kg/ha) was used.

2.2.3 Economic Analysis

For the 8-year collection period, backward-looking enterprise budgeting techniques were used for the cow-calf operation to calculate expected annual net returns per cow (AAEA, 2000). Cows were placed into tiers depending on their body weight each year, using 22.67 kg increments beginning at <430.84 kg and the last tier being >634.92 kg for analysis (similar to Bir et al., 2018) resulting in 11 enterprise budgets per year. All prices and costs described below are only those belonging to the cow-calf enterprise on the farm.

Expected annual return per cow is calculated as:

Equation 2: Expected Net Return (\$/cow) = Expected Revenue – (Fixed Costs + Variable Costs)

Revenue was calculated for three sources: weaned calf, cull cow sales, and cull bull sales:

Equation 3: Expected Revenue (\$/cow) = WeanedSteerRev. + (WeanedHeiferRev. * 0.6793) * Prob(Cull|Age_t) + CullCowRev. * Prob(Cull|Age_t) + CullBullRev. *(1/125)

Where Price_t is the cull cow price in year t and Prob(Cull|Age_t) is the probability of the cow being culled at age *t*.

Prices for steers and heifers were obtained from Livestock Marketing Information Center using Iowa market data for medium and large frame #1-2 (LMIC, 2019b). Heifer sales were adjusted using a 32.07% retention rate that was recorded on farm over the study period. This high retention rate was due to the management program for first calf heifers to breeding during a

short window (~4 wks). Cull cow and bull prices were based on Lancaster, Pennsylvania sale prices (LMIC, 2019c). Following Azzam et al. (1990), cull cow revenue was calculated as:

$$\text{Equation 4: Cull Cow Revenue} = \text{Cow Weight} * \text{Price}_t * \text{Prob}(\text{Cull}|\text{Age}_t)$$

Variable costs included feed cost, marketing costs, mineral, veterinary costs, equipment/facility repairs, labor, interest on operating capital and other variable costs. Cattle only received supplement in the form of hay during times of limiting forage growth and winter, feed cost was calculated as:

$$\text{Equation 5: Feed cost} = (\text{Land} (\$/\text{ha}) \times \text{Stocking rate} (\text{ha}/\text{cow})) + (\text{Annual Hay Intake per cow} (\text{kg hay}/\text{cow}) \times \text{Hay price} (\$/\text{kg hay}))$$

Forage and hay consumption were calculated as described in the previous section. Annual land cost varied by year, based on USDA NASS published land rental prices (USDA-NASS, 2019). Hay cost was represented by August hay prices in U.S. from 2011 to 2018 sourced from the Livestock Marketing Information Center (LMIC, 2019a). Marketing cost was calculated to be 5% of annual revenue, and mineral cost was estimated to be \$36 per cow in 2019 based on farm consumption rates. This cost was then adjusted back to 2011 prices using index for prices paid published by the USDA NASS (USDA-NASS, 2019). Yearly veterinary costs and equipment/facility repairs were calculated similarly, with 2019 prices estimated to be \$25 per cow and \$7.87 per ha, respectively. Labor was calculated using hourly wage rates from 2011 to 2018 published by the USDA NASS for 500 hours of required labor (USDA-NASS, 2019). Fuel and other costs were calculated to be \$17.20 per ha on-farm in 2014 and was adjusted using the prices paid index (USDA-NASS, 2019). Interest on operating capital was assumed to be 5% of costs annually.

Fixed costs included pasture care, taxes at 3.3% of expenditures, machinery and livestock costs, and miscellaneous cost (10% of overhead cost). Annual machinery depreciation costs for: one 130-hp tractor, one 2011 John Deere Gator, 4 hay rings, a bale unroller, \$15,000 barn, chutes/pens, and headgate were calculated using straight-line depreciation. Useful life for machinery was assumed to be 10 yr with the exception of the barn and chute/pens which had estimated useful years of 30 and 20 years, respectively. Pasture care was comprised of fence, water, seeding (over 30 yr) and lime application every 10 years and cost \$26.04 annually. Straight-line depreciation was used to calculate fixed costs of bulls and cows. Bull weight was estimated by dividing cow weight by 0.70, to service 25 cows and have a 5-year service period, with an initial purchase price of \$3,060 and average price of cull bulls was used to calculate annual depreciation (Bir et al., 2018). Cow depreciation cost was calculated with an initial purchase price of \$1,200 and salvage value of \$782.65 after 10 years, using the average weight of the cow herd as the average cull weight for the herd.

Expenses are detailed for each year in **Table 2.2**.

2.2.4 Statistical Analysis

Biological efficiency data for the entire herd were analyzed using a linear mixed effects model in R (R Core Team, 2019, v. 3.6.1; $N = 1038$), with dependent variables of WW, YW and CWP. Fixed effects were cow body weights adjusted to BCS of 5 as a continuous variable, cow age and sex of the offspring. Year and cow were included as random terms in the final model. Model fit was tested with random terms included as nested or crossed effects using log-likelihood and final models included both year and cow as crossed random terms (Bates, 2010). Dependent variables of interest were adjusted 205-d calf weaning weight as a percent of BCS adjusted DBW, adjusted 205-d weaning weight and yearling weight. Quadratic terms for age and

the natural log of cow body weight were tested for significance and dropped until model fit was not improved based on log-likelihood.

Output per ha (weaning weight/ha) was analyzed via linear regression using adjusted cow body weight and year as explanatory variables. Expected net returns per cow and ha from 2011 to 2018 was analyzed by separating cows into eleven 22.67 kg weight classes, with class 1 being < 430.84 kg and class 11 being > 634.92 kg and used as an explanatory variable with year. Significant differences were declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

2.2.5 Net Present Value

Net present value (**NPV**), a measurement of the value of future cash flows over the lifecycle of the cow, was calculated with increasing cow weights to determine which cow size provided maximum present value over their productive lifetime (Moss et al., 2018). Using the enterprise budgets described above, and the biological efficiency and weaning weight models developed in this research, NPV was forecasted over 10-yr production period similar to Bir et al. (2018) as:

$$\text{Equation 6: NPV} = \text{NPV}_i | \text{CowWght}_{it} - \sum^{10} [\text{Expected NetRet}_i | \text{CowWght}_{it} / ((1 + 0.05)^t \times \text{ha per cow})]$$

where i is the i th cow in yr t and a 5% discount rate. Expenses for 2019-2027 are detailed in **Table 2.3** and the size of the operation was assumed to be 40.5 ha with herd sizes determined by the forage intake and land use developed in this research. Historical prices from the 2004 to 2014 cattle cycle were used to forecast cattle prices and hay prices from 2020 to 2027 (LMIC, 2019b). Future prices were then calculated by the percentage change between years with actual 2019 prices used for the first year of the forecasting model (Bir et al., 2018). Land rental prices changed little for this region over time and were assumed to stay static at \$69.13 /ha. Hay prices

were forecasted using percentage change between years of historical prices from 2004 to 2014. A 1% annual inflation was applied to mineral, veterinary, equipment/facility repairs, and other variable costs (USDA-NASS, 2019). Labor rates were forecasted using percentage change from 2011 through 2018 rates. Assumption regarding marketing costs and interest on operating capital remained the same as the 2011-2018 enterprise budgets. Fixed costs were calculated the same as described above but with prices adjusted to reflect 2019 tax rates, equipment costs, and livestock prices. Pasture care costs stayed static at \$24.49 /ha and machinery/livestock depreciation ranged from \$93.56 to \$95.00 / cow, varying by cull cow weight and herd size for each simulated weight class. The tax rate was a constant 2.8% and miscellaneous cost remained 10% of overhead costs.

Net present values were then calculated for cows weighing 430.84 kg, then increasing in increments of 22.68 kg up to a final weight of 634.92 kg. Cows were projected to reach 85% of their mature weight by age 2, and increase in weight annually by 4% of their mature weight until reaching maximum weight at age 6 (Selk, 2005). The baseline scenario was assumed to have 200 grazing d and 164 d of hay intake, with hay intake being a combination of 49 d without cold stress and 115 d with cold stress (usclimatedata.com). Next, a sensitivity analysis was then performed by altering grazing d and hay intake, without cold stress, in 5 d increments above and below the 200 d baseline. The tested range was 175 to 225 grazing d and 139 to 189 hay intake d. When grazing d increased, hay intake d decreased and vice versa. When hay feeding days were increased, daily consumption was calculated using the pregnant lactation intake equation.

2.3 Results and Discussion

2.3.1 Herd Metrics

Data used for the development of the biological efficiency models and analysis of economic returns were collected from the Lake City AgBioResearch center cow herd from 2011

to 2018. Mean cow age over the course of the recorded period was 4.57 ± 2.11 yr with an averaged non-adjusted BW of 546.11 ± 69.25 kg. Body condition score adjusted cow BW averaged 538.74 ± 65.37 kg (**Table 2.4**), with an average BCS of 5.37 ± 0.44 at weaning over the 8-year period. As cows aged, their body weight increased in a sigmoidal curve with the inflection point of the curve at 534.70 kg which occurred at approximately 4 years of age.

There were a total of 1,035 calves with recorded WW, with an average of 242.65 ± 31.77 kg (**Table 2.4**). Females averaged 14.7 kg less than the male counterparts (234.65 vs. 249.35 kg, females and males, respectively) with 472 recorded female weights and 563 recorded male offspring. These WW were similar to nationwide survey data collected by NAHMS (2009) which reported average WW to be 240.36 kg. Yearling weights were recorded for 868 offspring with an average weight of 327.05 ± 46.17 kg (**Table 2.4**). Similar to WW, female offspring weighed less than males at one year of age, with females weighing 49.66 kg less (299.64 vs. 349.83 kg, females and males, respectively) and consisted of a total of 394 female and 474 male records.

2.3.2 Biological Efficiency Models and Cow Longevity

Calf weaning weight as a percent of cow body weight averaged 45.53% over the study period. The best fit model for CWP included log transformed DBW, calf birth weight, sex, age of cow, and age of cow squared. As DBW increased, the percentage of their body weight weaned decreased significantly (**Table 2.5**; $P < 0.001$). For every 1% increase in body weight, CWP was reduced by 0.38% ($-38.58 \times \ln(\text{DBW}) \pm 1.87$). Sex had a significant relationship with CWP ($P < 0.01$) with female calves having a lower weaning percentage than steers. Age of the cow had a tendency for a quadratic relationship with CWP, where older cows weaned more than younger

cows, but at a diminishing rate ($P = 0.06$). This quadratic relationship was similar to the weaning-weight model developed by Bir et al. (2018).

These results were similar to those of Scasta et al. (2015), who separated cows into 4 BW tiers by 45.5 kg increments: 453 kg, 544 kg, 589 kg, and 634 kg. They found that as cow size increased CWP decreased and reported similar mean efficiencies to those found in the current study. However, their study was conducted in a lower rainfall area where lighter cows were expected to have an energetic advantage. In a study in southwest Arkansas, Beck et al. (2016) showed that although WW increased as cow BW increased, weaning efficiency decreased linearly by 6.7 kg per 100 kg of cow BW. In this study, for every 1% increase in DBW, weaning weight increased 0.36 kg but was offset by the decreased weaning efficiency of 0.38% DBW (**Table 2.6; Table 2.5**; $P < 0.01$). Williams et al. (2018) reported that for cows classified as having high weaning weight ratios, lighter weight cows had higher CWP than heavier cows. However, cows classified as high CWP did consume more feed on a g/kg BW basis.

Age of cow had a quadratic relationship with 205 d adjusted WW, similar to CWP (**Table 2.5**; $P < 0.01$). Age of cow may influence WW and CWP due to a higher maintenance energy requirement of younger cows. Wiseman et al. (2019) reported maintenance energy requirement of 107 kcal ME/kg BW^{0.75} for primiparous Angus cows in Oklahoma, although the relationship between age and maintenance requirements is inconsistent in the literature (NASEM, 2016). Additionally, the effect of age on milking performance could play a role on WW. Andersen et al. (2020) reported a significant difference in milking output between young and mature Hereford x Angus crosses and Angus cows, with young cows averaging 6.6 kg/d and mature cows producing 8.2 kg/d, although the impact of milk productivity on calf weaning weight is inconsistent (Mulliniks et al., 2020).

For yearling weights, the best fit model included Ln(DBW), calf birth weight, sex, age of cow, and age squared (**Table 2.7**). Log transformed DBW did not have a relationship with yearling weight ($P = 0.26$), unlike weaning weights. Age of dam did have a quadratic relationship with yearling weight ($P < 0.01$). Recent literature has not examined the impact of age on yearling weight, but Koch and Clark (1955) reported that dam age impacted yearling weight and was similar to weaning weight, as reported here, but was likely not practically important. When expressed as a percentage of DBW, there was a significant relationship and resulted in a depression in yearling weight efficiency by 0.58% for each additional 1% increase in body weight ($P < 0.001$), similar to the results with weaning efficiency. This is similar to Morris and Wilton (1976) who reported that both weaning and yearling weight efficiencies were superior for smaller cows. It should be mentioned, however, that the animals in the current study were in a grass finishing program and results may be different from grain finishing.

The results of Mulliniks et al. (2018) highlight the flaws of using biological efficiency alone as a selection metric. Reproduction performance was reported to decrease with decreasing cow body size with a 435.83 kg cow having a pregnancy rate of just 86% compared to 97% with a 538.32 kg cow (Mulliniks et al., 2018). Mulliniks et al. (2018) hypothesized that the reduction in pregnancy rate may be due to an imbalance between genetic potential for milk production and forage intake with smaller cows unable to consume enough forage to meet their nutritional demand for lactation. This goes against Stewart and Martin (1981), who reported that lifetime performance of Angus and Milking Shorthorn influenced cattle decreased with increasing mature body weight by -0.007 ± 0.003 calves/kg. However, we did not have records on culling decisions and therefore could not accurately analyze the impact of cow size on longevity. This could alter the results observed in this experiment. Additional research in this area is needed as breed

differences have been reported in previous literature (Núñez-Dominquez et al., 1991; Tanida et al., 1988; Szabó and Dákay, 2009).

2.3.3 Forage Intake and Land Use

As cow size increased, forage consumption increased 1.69 kg of DM/d for each additional 100 kg of body weight. This resulted in 450 kg BW cows consuming approximately 10.84 kg of DM/d and 750 kg BW cows consuming approximately 15.91 kg of DM/d during the grazing period. Over a 200 d grazing season, this resulted in 750 kg BW cows consuming 1,014.3 kg of DM more than 450 kg BW cows (3,181.85 kg of DM vs. 2,167.55 kg of DM, respectively). Over the record period forage productivity ranged from 5,000 kg of DM/ha to 6,587 kg of DM/ha and had an average of 5,662 kg of DM/ha from 2014 to 2018. Forage productivity for 2019 was included in calculating the average forage productivity applied for the years of 2011-2013 which was 5,506 kg of DM/ha. The long-term estimated utilization was 50% and resulted in stocking rates of 0.77 ha/cow for 450 kg BW cows up to 1.15 ha/cow for a 750 kg BW cow. Winter feeding rates followed similar trends. Daily hay intake increased across body weights at a rate of 1.59 kg per 100 kg increase in body weight and resulted in total feeding rates of 1,636.52 kg of DM and 2,418.03 kg of DM for 450 and 750 kg BW cows, respectively. Stokes et al. (1986) simulating the changes in stocking rate with increasing cow size in central Texas on improved forages with a 50% utilization rate and reported a stocking rate of 0.76 ha/cow for a moderate milking 550 kg cow vs. 0.66 ha/cow for a moderate milking 450 kg cow. Lindquist (2014) reported that for Michigan cow-calf operations stocking rates ranged from 1.01 to 1.83 ha/cow, similar to the range calculated here. The stocking rates changed by year according to forage productivity and each year's rates were utilized for the economic analysis. These results led to lighter cows weaning more kg/ha than heavier weight cows, with a decrease in 26.38 kg/ha

with every 100 kg increase in body weight with year included in the model (**Fig. 2.1**; $P < 0.01$; $R^2 = 0.41$). Bir et al. (2018) reported a similar advantage for lighter weight cows weaning more weight per ha. This would indicate that in the Upper Midwest, producer adoption of lighter cows may be an option for meeting the goals of sustainable intensification by maximizing productivity per unit land (Makker, 2013; Tedeschi et al., 2015).

2.3.4 Lake City Expected Net Return 2011-2018

The expected net returns of the Lake City cow herd from 2011-2018 was analyzed with cow body weight classification (1 to 11 in 22.67 kg increments) and year used as explanatory variables and individual cow as a random term. Overall, mean returns were \$338.97 from 2011 to 2018 per cow/yr. Net returns had a significant relationship with year, with highest returns over the trial period in 2014 and 2015 ($P < 0.01$). Both years corresponded with the highest calf prices over this time and average years of forage productivity (5,398.16 and 5,740.53 kg/ha for 2014 and 2015, respectively). Lowest returns occurred in 2016 with a decrease in \$88.21 on the intercept of the regression line, due to a depressed weaned calf price that fall.

Cow body weight classification did not have a significant relationship with expected net returns per cow ($P = 0.19$). These results are different than those reported by Doye and Lalman (2011), who reported that as cows increased in size their expected net returns decreased. The returns in this study reflect that the forage base on the farm was not limiting economic returns of heavier cows on a per cow basis at 200 grazing d, and that cows over this period were generally well matched to the forage base and management with lighter cows offsetting their increased variable costs and heavier cows offsetting their increased feed costs equally on a per cow basis. On a per ha basis, cow body weight classification did have a significant relationship with expected net returns ($P < 0.01$; **Fig. 2.2**). Expected net returns per ha decreased \$10.27 with each

additional body weight classification. Similarly, Beck et al. (2016) reported that cow BW did not impact net returns, but increased stocking rate increased net returns by \$438/ha. The results are reflective of the increased stocking rate potential and output per hectare with lighter cows over the study period.

2.3.5 Net Present Value

Net present value over 10-years was calculated for cows beginning at 430.84 kg and 22.67 kg increments to a maximum weight of 634.9 kg (**Table 2.7; Table 2.8**). Weaning weights were calculated using the equation reported in Table 5. The baseline scenario assumed 200 grazing d and 164 hay feeding d, and the maximum NPV was determined. At the baseline, cows weighing 453.51 kg had the maximum NPV at (\$496.63), followed by the 476.19 kg classification, the 430.84 kg classification and then decreasing in value with each additional 22.67 kg (**Table 2.8**). Ultimately, the 634.92 kg classification had approximately 74% lower NPV compared with the 453.51 kg classification at 200 d grazing. These results were similar to the NPV result of Bir et al. (2018) who found that lighter cows had higher NPV than their heavier counterparts. Nasca et al. (2015), in an economic and environmental analysis of beef production in Argentina, reported that economic efficiency declined with increasing cow weights because of high supplementary feed costs, similar to this study.

The ideal cow weight in this study changed when a sensitivity analysis on the number of grazing days was performed (**Table 2.8; Table 2.9**). When grazing d was increased 5 d, the 430.84 kg classification had the highest returns, and this held true for the rest of the grazing d scenarios. Cows weighing 566.89 kg and higher never provided higher returns than the 453.51 kg classification at 200 d grazing, even at 225 grazing d. Interestingly, when grazing d were reduced the 430.84 kg classification had higher returns compared to their baseline, before

ultimately dropping below the baseline value at 180 d grazing. This shows that lighter cows can withstand a drop in grazing d because hay costs are not as high compared to heavier cows, which may provide producers some protection against adverse weather conditions. Scasta et al. (2015) reported similar results when comparing drought performance of small and large cows. They found that large cows did not maximize their genetic potential during years of drought whereas smaller cows had an advantage because of their lower maintenance energy requirements. Therefore, at 200 d grazing, heavier cows (>476.19 kg) provided increasingly less NPV than the 3 lightest cow weigh classes with 453.51 kg classification providing the highest return. Additionally, the lightest cows (430.84 kg) increased their NPV through 180 grazing d. This indicates that for the Upper Midwest, light cows are more functional for producers as they provide protection against adverse climatic events, which may reduce the number of days on pasture.

2.4 Conclusions

Cow-calf production environments are highly variable and balancing cow-size with both the management and grazing environment may help improve producer profitability (Nasca et al., 2015). These results indicate that in the Upper Midwest utilization of lighter weight cows increases the weaning weight ratio of the herd, may require less land and hay per cow, and potentially increases expected net returns on a per ha basis. The net present value of light weight cows increased as the number of grazing days decreased, as they require less hay compared to their heavier counterparts. This may provide protection for producers against adverse weather events and climatic variability that is predicted to increase in frequency in Michigan (Melillo et al., 2014). Additionally, the increased weaned weight per ha captured by the lighter cows may also meet the goals of sustainable intensification by maximizing production per unit of land

(Makkar, 2013; Tedeschi et al., 2015). This would require a paradigm shift for producers, who often believe that heavier cows maximize profitability and require improved estimates of cow size from producers, as most do not weigh their animals consistently (Reuter, 2017; Doye and Lalman, 2011). Research in other regions, such as that done by Nasca et al. (2015) and Bir et al. (2018), should be done in other regions to provide region specific recommendations for producers on how best to maximize productivity. This study did not include actual animal intakes and milk production, factors that have been reported to impact calf weaning weight and differ for cows with high CWP (Williams et al., 2018). These results do not, however, equate to what is profitable for packer and feedlot operators in the region, as what is profitable for cow-calf producers may not be as profitable for other market segments. There may be potential for collaboration between feedlot and cow-calf sectors, as research has shown that in some regions offspring of small and moderate frame cows were more profitable and had higher BW in the feedlot (Mulliniks et al., 2018). To our knowledge this is the first work to show the relationship between biological efficiency, economic returns, and beef cow body weight in the Upper Midwest region of the United States. The biological efficiency advantage reported here in light weight cows needs continued research by measuring on farm intakes, milk production, and examining how selection for lighter weight cows alters economic returns further up the beef supply chain.

Table 2:1: Average annual climate data Lake City, MI 2011 to 2018

Month ¹	High Temperature, °C	Low Temperature, °C	Precipitation., cm
January	-2.69	-12.19	4.82
February	-1.40	-12.24	3.78
March	4.64	-8.13	4.96
April	10.79	-1.76	11.09
May	20.20	5.98	8.69
June	24.56	10.64	7.32
July	27.63	13.04	8.68
Aug	26.11	12.17	7.55
Sep	22.52	7.76	7.32
Oct	14.46	2.39	10.81
Nov	6.44	-2.77	6.89
Dec	0.24	-7.30	5.00
Total			86.90

¹usclimatedata.com

Table 2:2: Annual expenses and budget assumptions for the model beef cow herd, 2011 though 2018

Expenses	2011	2012	2013	2014	2015	2016	2017	2018
<i>Variable Costs</i>								
Marketing, 5% of Revenue	5%	5%	5%	5%	5%	5%	5%	5%
Feed, \$/ha	31.72	61.72	64.19	74.07	74.07	74.07	69.14	74.07
Hay, \$/ton	196.00	203.00	199.00	207.00	161.00	137.00	147.00	177.00
Mineral, \$/cow	32.72	34.16	34.79	36.65	36.23	34.65	34.88	36.00
Veterinary, medicine, and identification, \$/cow	22.73	23.72	24.16	25.45	25.16	24.07	24.22	25.00
Equipment, and facility repairs, \$/ha	7.22	7.53	7.67	8.20	7.99	7.64	7.69	7.87
Labor, \$/cow	30.75	31.91	32.97	33.53	34.83	36.06	37.00	39.36
Other variable costs (fuel etc.), \$/ha	14.96	15.62	15.90	17.00	16.56	15.84	15.95	16.32
Interest on operating capital ¹ , 5%	5%	5%	5%	5%	5%	5%	5%	5%
<i>Fixed Costs</i>								
Taxes ²	3.30%	3.30%	3.30%	3.30%	3.30%	3.30%	3.30%	3.30%
Pasture care, \$/cow	26.04	26.04	26.04	26.04	26.04	26.04	26.04	26.04
Machinery, Livestock, Interest, \$/cow	28.06	28.06	28.06	28.06	28.06	28.06	28.06	28.06
Miscellaneous, 10% of Overhead	10%	10%	10%	10%	10%	10%	10%	10%

¹Interest on operating capital is 5% of variable costs

²Taxes represent 3.3% of total expenditures

Table 2:3: Annual expenses and budget projections for the model beef cow herd, 2019 through 2027

<i>Expenses</i>	2019	2020	2021	2022	2023	2024	2025	2026	2027
<i>Variable Costs</i>									
Marketing, 5% of Revenue	5%	5%	5%	5%	5%	5%	5%	5%	5%
Feed, \$/ha	69.13	69.13	69.13	69.13	69.13	69.13	69.13	69.13	69.13
Hay, \$/ton	192.51	194.29	240.63	319.06	194.29	210.33	349.37	361.84	354.71
Mineral, \$/cow	36.00	36.36	36.72	37.09	37.46	37.84	38.21	38.60	38.98
Veterinary, medicine, and identification, \$/cow	25.38	25.63	25.89	26.15	26.41	26.67	26.94	27.21	27.48
Equipment, and facility repairs, \$/ha	7.77	7.84	7.92	8.00	8.08	8.16	8.24	8.33	8.41
Labor, Wage, \$/hr	14.61	15.05	15.49	15.93	16.37	16.81	17.25	17.69	18.16
Other variable costs (fuel etc.), \$/ha	16.48	16.65	16.81	16.98	17.15	17.32	17.50	17.67	17.85
Interest on operating capital ¹ , 5%	5%	5%	5%	5%	5%	5%	5%	5%	5%
<i>Fixed Costs</i>									
Taxes ² , \$/cow	2.80%	2.80%	2.80%	2.80%	2.80%	2.80%	2.80%	2.80%	2.80%
Pasture care, \$/ha	24.49	24.49	24.49	24.49	24.49	24.49	24.49	24.49	24.29
	93.56-								
Machinery, Livestock, Interest ³ , \$/cow	95.00
Miscellaneous, 10% of overhead	10%	10%	10%	10%	10%	10%	10%	10%	10%

¹Interest on operating capital is 5% of variable costs

²Taxes represent 2.8% of total expenditures

Table 2.3 cont.

³Varies by cow body weight and herd size

Table 2:4: Cow-calf herd summary statistics, 2011-2018

Total Records	1038	Min	Max
Average Cow Weight, kg	538.74 ± 65.37	367.35	780.05
Average BCS	5.37 ± 0.44	4	7
Recorded Weaning			
Weights	1035		
Overall Average Weight, kg	242.65 ± 31.77	108.84	312.93
<i>Female Records</i>	472		
Average Weight, kg	234.65 ± 29.02	108.84	287.98
<i>Male Records</i>	563		
Average Weight, kg	249.35 ± 32.45	122.49	312.93
Recorded Yearling			
Weights	868		
Overall Average Weight, kg	327.04 ± 46.17	185.94	512.47
<i>Females Records</i>	394		
Average Weight, kg	299.63 ± 33.39	185.94	394.55
<i>Males Records</i>	474		
Average Weight, kg	349.83 ± 42.64	231.29	512.47

Table 2:5: Regression coefficients for predicting calf weaning weight as percent of cow body weight

<i>Predictors</i>	<i>Estimates 95% CI</i>		<i>P</i>
Intercept	277.52	253.78 – 301.26	<0.001
Ln(DBW), kg ¹	-38.58	-42.25 – -34.91	<0.001
Calf Birth Weight, kg ²	0.07	0.02 – 0.13	0.009
Heifers	2.93	-6.38 – 12.25	0.537
Steers	5.95	-3.36 – 15.27	0.211
Age, yr	0.98	0.29 – 1.67	0.005
Age squared, yr	-0.06	-0.12 – -0.01	0.019
Observations	1013		
Marginal R ² / Conditional R ²	0.380 / 0.615		

¹Natural log of normalized cow body weight

²205 d adjusted weaning weight

Table 2.6: Regression coefficients for predicting 205 d adjusted weaning weight

<i>Predictors</i>	<i>Estimates</i>	<i>95% CI</i>	<i>P</i>
Intercept	-47.07	-171.74-77.61	0.460
Ln(DBW), kg ¹	36.92	17.61 – 56.22	<0.001
Calf Birth Weight, kg ²	0.37	0.10 – 0.65	0.008
Heifers	17.73	-30.96 – 66.42	0.476
Steers	33.79	-14.89 – 82.47	0.174
Age, yr	5.80	2.19 – 9.41	0.002
Age squared, yr	-0.41	-0.69 – -0.13	0.005
Observations	1013		
Marginal R ² / Conditional R ²	0.119 / 0.463		

¹Natural log of normalized cow body weight

²205 d adjusted weaning weight

Table 2:7: Regression coefficients for predicting calf yearling weight

<i>Predictors</i>	<i>Estimates</i>	<i>95% CI</i>	<i>P</i>
Intercept	155.72	-38.82 – 350.26	0.117
Ln(DBW), kg ¹	17.46	-12.99 – 47.91	0.262
Calf Birth Weight, kg ²	0.37	-0.08 – 0.81	0.11
Heifers	-5.23	-77.37 – 66.91	0.887
Steers	45.00	-27.12– 117.12	0.222
Age, yr	9.19	3.55 – 14.82	0.001
Age squared, yr	-0.65	-1.09 – .020	0.004
Observations	847		
Marginal R ² / Conditional R ²	0.314 / 0.427		

¹Natural log of normalized cow body weight

²205 d adjusted weaning weight

Figure 2.1: Weaned kg/ha plotted against adjusted cow body weight, by year for model herd

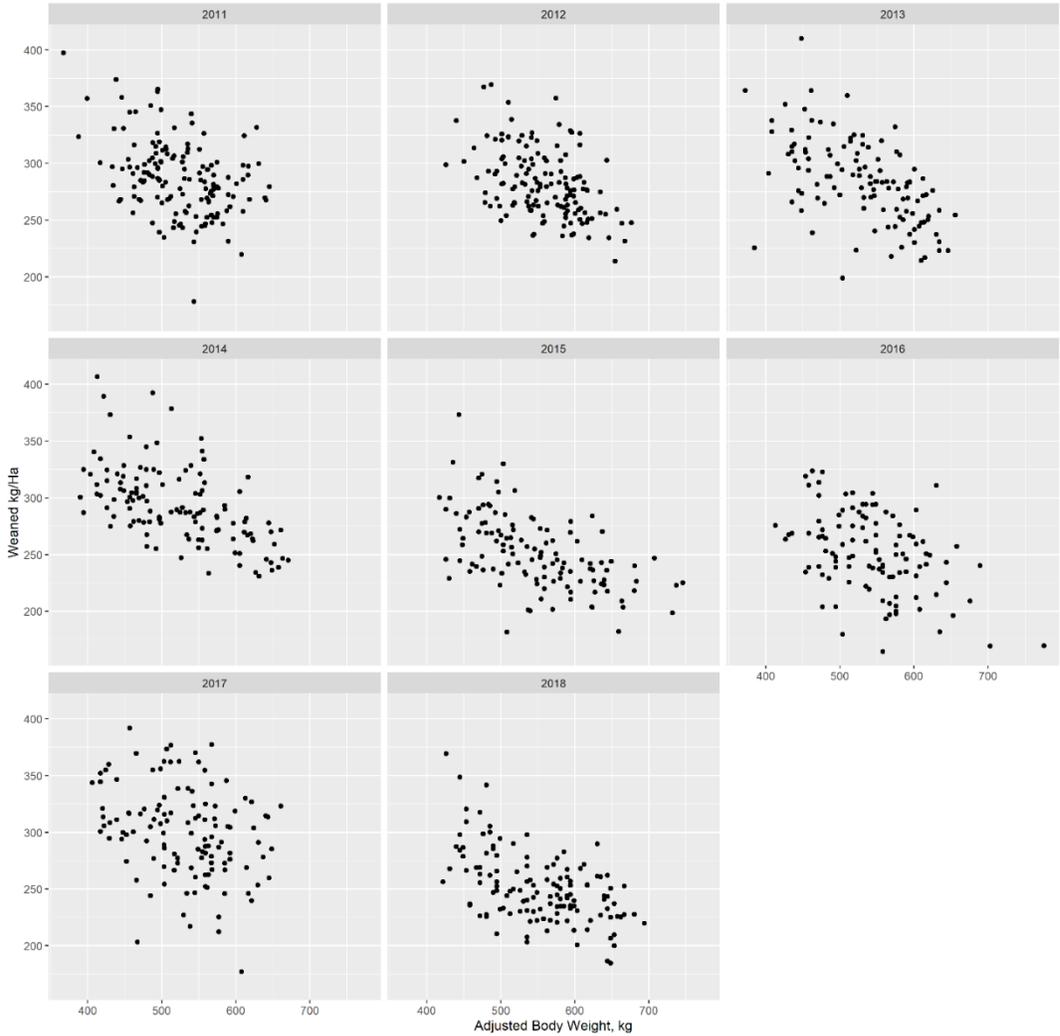


Figure 2.2: Expected Net Return/ha plotted by 11 cow body weight tiers (22.67 kg intervals) beginning at 430.84 kg

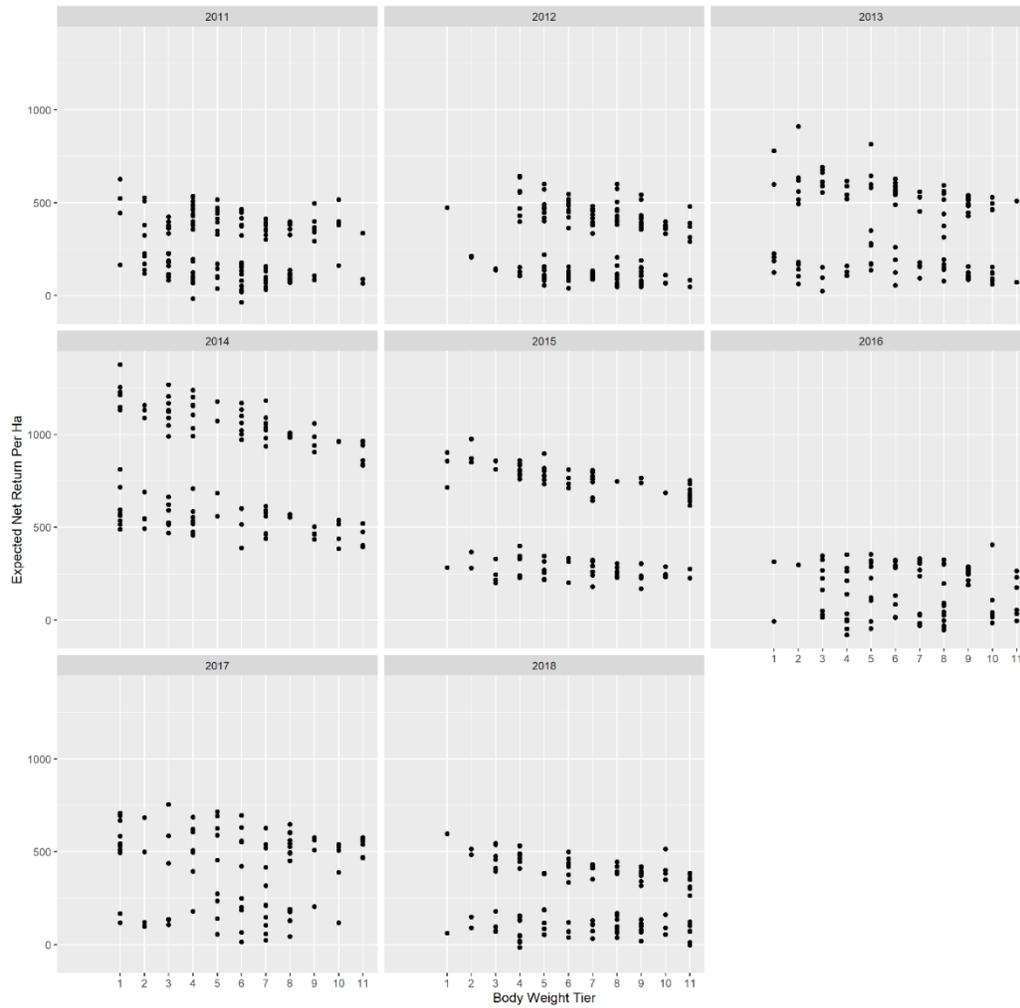


Table 2:8: Net present value at increasing cow body weight and variable grazing d

Cow (kg)	Grazing d ¹										
	175	180	185	190	195	Baseline	205	210	215	220	225
430.84	-\$585.56	-\$561.44	-\$537.32	-\$513.20	-\$489.08	-\$537.44	-\$443.04	-\$421.11	-\$399.18	-\$377.26	-\$355.33
453.51	-\$618.96	-\$594.85	-\$570.75	-\$546.64	-\$522.54	-\$496.63	-\$476.45	-\$454.46	-\$432.47	-\$410.48	-\$388.49
476.19	-\$654.92	-\$630.83	-\$606.74	-\$582.65	-\$558.56	-\$534.46	-\$512.42	-\$490.37	-\$468.33	-\$446.28	-\$424.23
498.87	-\$670.98	-\$646.90	-\$622.82	-\$598.74	-\$574.67	-\$550.59	-\$528.49	-\$506.39	-\$484.29	-\$462.19	-\$440.09
521.54	-\$687.17	-\$663.10	-\$639.03	-\$614.97	-\$590.90	-\$566.84	-\$544.69	-\$522.54	-\$500.39	-\$478.25	-\$456.10
544.22	-\$722.34	-\$698.29	-\$674.23	-\$650.18	-\$626.12	-\$602.07	-\$579.87	-\$557.68	-\$535.49	-\$513.29	-\$491.10
566.89	-\$739.43	-\$715.39	-\$691.34	-\$667.30	-\$643.25	-\$619.21	-\$596.97	-\$574.73	-\$552.50	-\$530.26	-\$508.03
589.57	-\$756.81	-\$732.78	-\$708.74	-\$684.71	-\$660.67	-\$636.64	-\$614.36	-\$592.08	-\$569.81	-\$547.53	-\$525.26
612.24	-\$774.59	-\$750.56	-\$726.53	-\$702.51	-\$678.48	-\$654.45	-\$632.14	-\$609.83	-\$587.51	-\$565.20	-\$542.89
634.92	-\$792.81	-\$768.80	-\$744.78	-\$720.76	-\$696.74	-\$672.72	-\$650.37	-\$628.03	-\$605.68	-\$583.33	-\$560.98

¹Baseline= 200 d grazing

Table 2.9: Relative change (%) in net present value compared to a 430.84 kg cow at 200 grazing d

Cow BW (kg)	Grazing d ^{1,2}										
	175	180	185	190	195	Baseline	205	210	215	220	225
430.84	-8.95%	-4.47%	0.02%	4.51%	9.00%	0.00%	17.56%	21.64%	25.72%	29.80%	33.88%
453.51	-15.17%	-10.68%	-6.20%	-1.71%	2.77%	7.59%	11.35%	15.44%	19.53%	23.62%	27.71%
476.19	-21.86%	-17.38%	-12.89%	-8.41%	-3.93%	0.55%	4.66%	8.76%	12.86%	16.96%	21.06%
498.87	-24.85%	-20.37%	-15.89%	-11.41%	-6.93%	-2.45%	1.66%	5.78%	9.89%	14.00%	18.11%
521.54	-27.86%	-23.38%	-18.90%	-14.43%	-9.95%	-5.47%	-1.35%	2.77%	6.89%	11.01%	15.13%
544.22	-34.41%	-29.93%	-25.45%	-20.98%	-16.50%	-12.03%	-7.90%	-3.77%	0.36%	4.49%	8.62%
566.89	-37.58%	-33.11%	-28.64%	-24.16%	-19.69%	-15.21%	-11.08%	-6.94%	-2.80%	1.33%	5.47%
589.57	-40.82%	-36.35%	-31.87%	-27.40%	-22.93%	-18.46%	-14.31%	-10.17%	-6.02%	-1.88%	2.27%
612.24	-44.13%	-39.66%	-35.18%	-30.71%	-26.24%	-21.77%	-17.62%	-13.47%	-9.32%	-5.17%	-1.01%
634.92	-47.52%	-43.05%	-38.58%	-34.11%	-29.64%	-25.17%	-21.01%	-16.86%	-12.70%	-8.54%	-4.38%

¹Baseline= 200 d grazing

²Positive number reflects positive change in net present value compared to a 430.84 kg cow at the baseline

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LITERATURE CITED

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CHAPTER 3

IMPACT OF FORAGE DIVERSITY ON FORAGE PRODUCTIVITY, QUALITY, BEEF CATTLE PERFORMANCE AND ENTERIC METHANE EMISSIONS

Abstract

Greenhouse gas emissions (**GHG**) from the beef industry is largely attributed to the grazing sector, specifically from beef cattle enteric methane emissions. Therefore, the study objective was to examine how forage diversity impacts forage productivity, quality, animal performance and enteric methane emissions. This study occurred over 3 consecutive grazing seasons (2018 to 2020) and compared two common Midwest grazing mixtures: 1) A botanically diverse, cool-season mixed species mixture (**COMP**), and 2) A simple, 50:50 alfalfa:orchardgrass mixture (**SIMP**). Fifty-six steers and heifers were adapted to a GreenFeed Emission Measurement System each year (**GEM**; C-Lock Inc., Rapid City, SD) and stratified into treatment groups based on acclimation visitation. Each treatment consisted of 4 paddocks, three 3.2 ha and one 1.6 ha paddock, with 8 and 4 animals each, respectively. Forage production was measured bi-weekly in pre-and post-grazed sub-paddocks, and forage quality was analyzed using near-infrared spectroscopy. Shrunken body weights were taken monthly to determine animal performance. Forage productivity did not differ between treatments ($P = 0.69$) but tended lower in 2018 ($P = 0.06$; 2,403 kg DM/ha) than 2019 (2,919 kg DM/ha) and 2020 ($P = 0.10$; 2,808 kg DM/ha). Crude protein was significantly lower for COMP in 2018 compared to SIMP. Forage ADF content was significantly lower for the COMP mixture ($P = 0.02$; 33.38% vs. 34.73% for COMP and SIMP, respectively). The COMP treatment resulted higher IVDMD48 in 2018 and 2019 compared to the SIMP treatment ($P < 0.01$). Animal performance not differ between treatments ($P > 0.50$). There was a tendency for the COMP treatment to have lower enteric CH₄ production on a g/d basis ($P = 0.06$), but no difference was observed on an emission intensity basis (g CH₄/kg gain; $P = 0.56$). These results would indicate that adoption of the complex forage mixture would not result in improved forage productivity, animal performance, or reduced emission intensity compared to the simple forage mixture.

3.1 Introduction

The beef industry has come under scrutiny in recent decades due to its perceived contribution to global warming, with enteric methane (**CH₄**) being the main driver of beef's carbon footprint (Rotz et al., 2019). This has increased research to address mitigation options for both beef and dairy cattle to improve efficiency of production and reduce the carbon footprint (Boadi et al., 2004; Hristov et al., 2013; Thompson and Rowntree, 2020). Recent beef industry life-cycle assessments (**LCA**) indicate that approximately 70 to 80% of the industry's carbon emissions are from the grazing sectors, and predominately from cow-calf production, making mitigation in grazing environments crucial for overall enteric CH₄ mitigation (Rotz et al., 2019; Alemu et al., 2017). Animals in grazing environments consume lower quality diets that are higher in fiber content than those in confinement operations and this high fiber diet drives greater enteric CH₄ per unit of dry matter intake (**DMI**) and with concurrently reduced animal performance (Thompson and Rowntree, 2020). These combine to result in greater total emissions and increased emissions per unit product (e.g., kg carcass weight; Stanley et al., 2018). Tested mitigation strategies include dietary lipid supplementation, vaccination against methanogens, utilizing forages with beneficial secondary compounds, improving forage quality, health interventions, and utilizing other best management practices, all of which have been reviewed extensively (Beauchemin et al., 2008; Hristov et al., 2013; Thompson and Rowntree, 2020; Zubieta et al., 2021). Mitigation strategies that have low barrier for entry and high potential adoption likelihood (i.e., inexpensive and easy to adopt) should be prioritized (Hristov et al., 2013). One such strategy would be the examination of different forage bases to identify differences in animal performance, as well as monitor animal and soil GHG emissions. Proper grazing management and high-quality pastures can result in

reduced enteric CH₄ production by improving the digestibility of the diet (Beauchemin et al., 2008; Archimède et al., 2011).

A common forage mixture in the Midwestern U. S. is a binary alfalfa:orchardgrass mixture which has relatively high nutritive quality. In a meta-analysis of enteric CH₄ production of different forages Archimède et al. (2011) indicated that cool-season forages (eg. those that grow in the Midwest U. S.) typically result in lower enteric CH₄ emissions compared with warm-season grasses per unit of DMI. Temperate grasses utilize the C3 photosynthetic pathway and typically having lower fiber content, decreased lignification and greater protein content (Barbehenn et al., 2004) than C4 tropical grasses. Although there is some inconsistency in research results, the inclusion of legumes is typically thought to reduce enteric CH₄ production through increased DMI and ruminal passage rate, reduced fiber content, improved performance and the presence of condensed tannins in some species, such as birdsfoot trefoil (Beauchemin et al., 2008; Hristov et al., 2013). However, the literature directly comparing enteric methane emissions and animal performance from a simple forage mixture to diverse forage mixtures is lacking (Alemu et al., 2019). Therefore, the objective of this experiment was to examine the forage productivity, forage quality, animal performance and enteric CH₄ emissions of two common Midwest grazing mixtures: a simple (**SIMP**) alfalfa:orchardgrass mixture, and a complex (**COMP**) forage mixture. The hypothesis was that the COMP forage mixture would result in increased forage productivity, improved forage quality, and a reduction in enteric methane emissions.

3.2 Materials and Methods

The use of animals and procedures were approved by the Michigan State Animal Care and Use Committee (Protocol #02-18-019-00).

3.2.1 Experimental Design and Pastures

Experimental research pastures were located at the Michigan State University Lake City AgBioResearch Center (latitude: 44°18'N, longitude: 85°11'W; elevation 377 m; Appendix A) and the experiment consisted of three consecutive grazing seasons from 2018 through 2020. Onsite weather data were collected from a national oceanic and atmospheric association weather station and are reported in Figures 3.1 and 3.2 (NOAA, 2020). Each treatment consisted of 4 experimental units: three 3.2-ha pastures and one 1.6-ha pasture. Pastures were established in the fall of 2017 after tilling research pastures to terminate a previous trial. Two pasture treatments were established: 1) a simple (**SIMP**) mixture, and 2) a complex (**COMP**) forage mixture. The SIMP pastures were seeded with a target forage base of 75% alfalfa (*Medicago sativa* L.) and 25% orchardgrass (*Dactylis glomerata* L.). The COMP mixture was seeded at a rate of 9% alfalfa, 10% orchardgrass, 12% red clover (*Trifolium pratense* L.), 7% white clover (*Trifolium repens* L.), 29% birdsfoot trefoil (*Lotus corniculatus* L.), 12% forage chicory (*Chicorium intybus*), 14% meadow fescue (*Festuca pratensis*), and 7% timothy (*Phleum pratense*). Fertilizer was not applied to any of the treatment plots.

In each 3.2 ha pasture, 8 animals were assigned each grazing season. Animals were allocated 0.2 ha sub-paddocks and managed in utilizing rotational grazing management. In each 1.6 ha pasture, 4 animals were assigned each season. Animals were allocated 0.1 ha sub-paddocks. In 2018, cattle in both treatments were rotated every 2 d for the duration of the grazing season. Two pastures within the COMP treatment were mowed to a 25-cm stubble height on June 17, 2018 to curb growth of reproductive forage chicory. In 2019, cattle assigned to COMP treatments were rotated daily for the first 32 d (two full rotations) then slowed to 2 d moves with the SIMP treatment for the remainder of the year. This was done to increase grazing pressure on

forage chicory in COMP treatment pasture. In 2020, the cattle in COMP pastures were rotated daily for the first 24 d, then all pastures were slowed to 3 d moves for the duration of the grazing season in anticipation of a summer drought.

3.2.2 Animals

Each year, Red Angus steers and heifers ($n = 56$) were selected from the larger acclimation group and stratified into grazing groups based on acclimation visitation, to ensure that each replicate contained animals that would visit the GreenFeedTM emission measurement system (**GEM**; GreenFeedTM, C-Lock Inc, Rapid City S.D) and randomly assigned to a pasture. Animals were placed in treatment periods at d -4 to allow animals to adapt to their paddocks prior to beginning of sampling with the GEM. Animals were offered ad-libitum access to drinking water and commercially available free-choice mineral-vitamin mix (Hubbard Feeds, Mankato, MN).

Year 1 consisted of 56 steers (13 months old; $BW = 317.91 \pm 37.55$ kg) and the grazing season started on June 1, 2018. Grazing duration was a total of 104 d in the experimental pastures. For 28 d (August 17 to September 14), animals were removed due to low forage quantity (<600 kg/ha post-graze residuals) and managed together until grazing could continue. These 28 d were not included in animal performance or enteric methane (**CH₄**) analysis. Prior to grazing (d -7) shrunk body weights were obtained from each animal after a 24 hr shrink. Animals were then reweighed monthly after 12 hr shrinks and average daily gain (**ADG**) was determined via linear regression. When animals were returned to experimental pastures in September, 12 h shrunk weights were taken every two weeks to improve precision of ADG estimates during the short duration the animals returned to treatment pastures. Grazing was terminated on October 10, 2018.

Year 2 consisted of 48 Red Angus heifers and 8 steers (13 months old; BW = 282.59 ± 36.51 kg) selected from the larger acclimation group. Four steers were assigned to each of the 1.6-ha paddocks based on acclimation visitation rate. Heifers were assigned experimental paddocks based on breeding groups as dictated by the breeding goals of the research farm and randomly assigned to a pasture. Bulls were turned out in heifer pastures from July 1 to July 29th for breeding, with one bull per pasture. Shrunken body weights (12 h shrink) were taken prior to grazing onset (d -4) and again monthly for the duration of the grazing season which began May 25, 2019 and ended on October 1, 2019.

Year 3 consisted of 48 Red Angus heifers and 8 steers (13 months old; BW = 267.08 ± 27.88) selected from the larger AHCS acclimation group. Again, 4 steers were stratified to each of the 1.6-ha paddocks based on AHCS acclimation visitation. Heifers were selected based on AHCS acclimation then assigned to breeding groups dictated by farm breeding goals and randomly assigned to a pasture. Bulls were turned out from June 26th to July 24th for breeding. Shrunken body weights (12 h shrink) were taken prior to grazing onset (d -7) and again monthly for the duration of the grazing season which began on May 30, 2020 and ended on September 19, 2020.

3.2.3 Gas Production

Enteric CH₄ and carbon dioxide (CO₂) were estimated using an GEM. Due to only one system being available, it was randomly assigned to one pasture, then rotated between treatment and paddocks every 2 weeks for the duration of the grazing season so that each experimental replicate was monitored in each grazing season. The GEM rotation pattern was randomized each year so that replicates were not analyzed at the same time each year. Animals were allowed a maximum of 4 visits each day, with 6 drops per visit, and a drop dispense interval of 30 sec

between each drop (Thompson et al., 2019). This was done to ensure that animals remained in the partially enclosed chamber for a minimum of 3 min recommended by Velazco et al. (2016). Any visits less than 3 min were removed from the analysis. A minimum of 4 h was required between visits to encourage animals to space visits across the day to capture the diurnal variation in enteric CH₄ production, although for grazing animals this variation may be small (Gunter and Bradford, 2015). The pelleted bait feed used contained 75% alfalfa meal, 22.85% soybean hulls, 2% liquid molasses, and 0.15% Herd Request (Cares Solutions Co-op, White Cloud, MI). Each drop had an average weight of 35 g. Herd Request (Prinova Flavors, LLC, Carol Stream, IL) is a flavoring agent used to encourage animals to visit the AHCS on the high-quality pasture. Pellets were sampled monthly each year and analyzed for nutritive value by a commercial laboratory (DairyLand Laboratories Inc., Arcadia, WI; **Table 3.1**). Visits for each individual animal that met the criteria of a good visit were averaged together across the 2-week sample period to determine emissions.

3.2.4 Forage Intake Measurements

Forage intake was determined using the dual-marker method described by Kartchner (1981) using one experimental unit from each treatment (n = 16). The external marker was titanium dioxide (TiO₂) and the internal marker was indigestible ADF (**iADF**). In 2018, intake was estimated during the last fortnight of the grazing season (Sept. 14 to Sept. 27, 2018). For 2019 and 2020, forage intake was estimated for a fortnight at the beginning and end of each grazing season (May 27 to June 9, 2019 and Sept. 5 to Sept. 18, 2019; June 1 to June 14, 2020 and August 31 to September 13, 2020, respectively). For the first 9 d animals were bolused daily with 10 g TiO₂ (Highwater Clays, Ashville, N. C.) at 0900 h, and during the last 5 d animals were bolused and fecal samples were collected via rectal grab at 0900 and 1500 h. During the

fecal collection period, a grazed forage sample was collected from each sampled pasture using the hand-plucking method by a trained observer to mimic the forage selected by the animals grazing in each paddock on d 3 (Gregorini et al., 2006) during the morning grazing bout (0700 to 1000). Diet samples were dried at 60°C for 48 h to determine DM, then ground through a Wiley mill (Thomas A. Wiley Laboratory Mill, Model 4, Swedesboro, NJ) to pass through a 1-mm screen and transported to East Lansing, MI for analysis.

Fecal samples were immediately placed in a forced-air oven and dried at 60°C for a minimum 3 d and samples were then checked daily until dried to a constant weight. Dried samples were then ground to pass through a 1-mm screen, then composited across d within animal with a target of 3 g per collected sample (Thompson et al., 2019). A subsample of forage and fecal samples were then analyzed for iADF using the procedure described by Bohnert et al. (2002). In duplicate, 0.5 g of each forage and fecal sample were weighed into filter bags (F57; Ankom Technology, Macedon, NY). Forage samples were incubated at 39°C for 16 h in a solution containing 0.1% pepsin (Catalog #9001-75-6, Fisher Scientific, Hampton, NH) and 10% 1 N HCl using a Daisy^{II} incubator (2 L per incubation vessel; Ankom Technology). Samples were then rinsed with warm (39°C) tap water and placed in a mesh bag with the fecal samples. All samples were then placed into the rumen of a cannulated dairy cow located at the Michigan State University Dairy Teaching and Research Center (East Lansing, MI) for 96 h. Upon removal, samples were rinsed with warm (39°C) tap water until the rinse became clear and dried at 50°C. Samples were then analyzed for iADF content to determine digestibility. Titanium content was determined using mass spectroscopy in triplicate using a modified protocol described by Myers et al. (2004). Composite diet samples were analyzed for CP, NDF, ADF,

NE_m, NE_g and TDN via a commercial laboratory (DairyLand Labs Inc., Arcadia, WI) and are presented in **Table 3.2**.

3.2.5 Forage

Forage samples were taken every 2 wk for the duration of each grazing season. In 2018, forage sampling was originally conducted using a rising plate meter (Jenquip, Fielding, New Zealand) with calibrated equations based on clip samples but that method was replaced by the quadrat method after 2 sampling periods due to difficulty in obtaining representative forage heights of bolting chicory. Each sampling week, prior to rotating animals pre-graze samples were collected by randomly placing four 0.25-m² quadrats and clipping to a 5-cm stubble height in each experimental paddock. Samples were then dried at 60°C for 48 h to determine DM and forage productivity. The same sub-paddock was then resampled after animals were rotated out using the same sampling procedure to determine DM and post-graze forage residual. Whole plant samples were then ground to pass through a 2-mm screen (Wiley Mill) and composited by size for pasture and sample type (pre or post-grazed) for each week into a 20-g composite sample and transported to Michigan State University Agronomy Farm (East Lansing, MI) where samples were ground to pass through a 1-mm screen (Udy cyclone mill; Model 3010-030, Udy Corporation, Fort Collins, CO) prior to nutritive analysis. Forage quality was determined using NIRS for crude protein (**CP**), neutral detergent fiber (**aNDF**), acid detergent fiber (**ADF**), in vitro true dry matter digestibility over 48 hrs (**IVTDMD48**), lignin, and ash using the grass hay and mixed hay equations for the COMP and SIMP treatments, respectively, sourced from the NIRS Forage and Feed Consortium (Hillsboro, WI). All samples had scanned spectra with a global H value less than 3.0 and neighborhood H values less than 1.5, indicating the scanned samples were similar to those of the developed equations. To further validate equations, a subset

of 18 samples from each treatment using samples from all years were selected to represent the range of scanned samples and sent to a commercial laboratory for chemical analysis (DairyLand Laboratories Inc., Arcadia, WI). The laboratory used was the same one that developed the NIRS prediction equations to minimize intra-laboratory error. These samples were then used to validate the NIRS equations for CP, aNDF and ADF. For the SIMP treatment, the NIRS equations had an $R^2 > 0.95$ and standard error of prediction less than or equal to 1.6. For the COMP treatment, the NIRS equation had an $R^2 \geq 0.86$ and standard error of prediction less than or equal to 2.2.

Botanical composition in each experimental unit was determined monthly each grazing season using the dry-rank-weight method described by Mannelje and Haydock (1963) by two trained observers. In each experimental unit, 24 locations were randomly sampled by placing a 0.13 m² quadrat and ranking species by observed DM content as: 1 (70% of DM), 2 (21% of DM), or 3 (9% of DM). Observers rotated experimental units each sampling period to minimize observer bias.

3.2.6 Statistical Analysis

All data were analyzed using the MIXED procedure in SAS (SAS Institute Inc., Cary, NC, v 9.4). Pasture was considered the experimental unit and included as a random term. Enteric CH₄, emission intensity (g CH₄/kg gain; **EI**), and CO₂ were analyzed using the fixed effect of treatment, year, the interaction between treatment and year, and the fixed effect of sex. Pasture was considered a random effect along with pasture by year, individual animal nested with year and pasture, and time of sampling nested within year. Emission intensity was calculated using the two shrunk body weights nearest the sampling period to minimize impacts of gains associated with differing points of the grazing season. Forage characteristics were analyzed in a completely randomized design used the MIXED procedure in SAS. Pasture nested in treatment

and year by pasture interaction were included as random terms and wk was included as a repeated measure. Fixed effects were the treatment, year and the year by treatment interaction. Means were separated using the LSMEANS statement with a tukey adjustment. Forage intake was analyzed by year due to unequal sampling periods between years. Statistical significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

3.3 Results

3.3.1 Forage Quality, Quantity, and Botanical Composition

Both pre-graze and post-graze forage mass were significantly impacted by yr ($P < 0.05$; **Table 3.3**) but neither was impacted by treatment or by a treatment x yr interaction ($P \geq 0.48$). Forage mass in 2019 tended to be greater than 2018 ($P = 0.06$; $2,919 \pm 137$ vs. $2,403 \pm 144$ kg DM/ha, respectively). In 2020 pre-grazed forage mass tended to be greater than in 2018 ($P = 0.10$). There was no difference in forage mass between 2020 and 2019. Differences observed between post-grazed forage mass were similar to pre-grazed forage mass with a significant impact by yr ($P < 0.01$). Post-grazed forage mass was lower in 2018, $1,528 \pm 116$ kg DM/ha, compared with 2019 and 2020, $2,074 \pm 111$ kg DM/ha and $2,107 \pm 124$ kg DM/ha respectively ($P < 0.01$), but no differences were observed for 2019 and 2020. Within yr, there were no observed differences in post-graze forage mass between the treatments ($P > 0.10$).

Botanical composition for each treatment is detailed in **Figure 3.3** and **Figure 3.4**. Both treatments had considerable yr to yr variation as expected. In 2018 the SIMP forage mixture was dominated by alfalfa at $61.8 \pm 2.9\%$ and most of the remaining being $34.7 \pm 3.6\%$ orchardgrass. However, by the following yr the percentage of alfalfa was lower at $43.8 \pm 2.9\%$ ($P < 0.01$) and tended to drop again in 2020 to $34.9 \pm 3.2\%$ ($P = 0.06$). This corresponded with yearly increases

in the orchardgrass component to $53.7 \pm 3.6\%$ in 2019 and $60.0 \pm 3.9\%$ in 2020, both greater than in 2018 ($P < 0.01$). For the COMP forage mixture, forage chicory was the dominate species in 2018 at $51.7 \pm 2.4\%$ and alfalfa was the second most present specie at $12.3 \pm 1.5\%$. All combined, orchardgrass, meadow fescue, and timothy 17.3% of the mixture. In 2019 the COMP botanical composition shifted with red clover representing $25.2 \pm 1.8\%$ and white clover being $15.5 \pm 1.5\%$. Forage chicory dropped to $19.4 \pm 2.4\%$ ($P < 0.01$) with the grass species accounting for 28.4% of the mixture. Similarly, the following yr was predominantly a clover-grass mixture with red and white clover accounting for $16.5 \pm 2.0\%$ and $15.2 \pm 1.7\%$, respectively, and the grass species accounting for 38.31% of the pasture composition. Chicory was again lower than the yr prior at $4.6 \pm 2.7\%$ ($P < 0.01$) in 2020. Alfalfa concentration stayed relatively consistent from 2018 to 2019, being $12.3 \pm 1.5\%$ and $9.1 \pm 1.5\%$, respectively, but lower in 2020 at $7.5 \pm 1.6\%$ compared to 2018 ($P < 0.01$).

Pre-graze forage quality results for each yr is presented in **Table 3.4**. There was a yr by treatment interaction present for each nutrient ($P \leq 0.05$), except ADF and there was a tendency for an interaction for aNDF content ($P = 0.07$). Crude protein was 2.2 percentage points less in 2018 for COMP compared with SIMP ($P < 0.05$). However, there was no observed treatment difference in CP for 2019 or in 2020. Crude protein was less in 2020 compared with previous years ($P < 0.01$) for both treatments.

Neutral detergent fiber content was impacted by yr ($P < 0.01$) and had a tendency for a treatment x yr interaction ($P = 0.07$). Each yr aNDF content of each treatment increased ($P < 0.05$). Upon mean separation the COMP treatment did not differ from SIMP in aNDF concentrations across all yrs. Acid detergent fiber content ranged from 32.59% to 35.30% across the three yrs and was impacted by treatment and yr ($P \leq 0.02$) but there was no treatment x yr

interaction ($P = 0.82$). From 2018 to 2019, average ADF content of the treatments increased from 2018 to 2019 by 1.7 percentage points ($P < 0.01$), but no change was observed from 2019 to 2020. Over the three yrs, COMP had 1.35 percentage points less ADF content compared to SIMP. There was a treatment x yr interaction for IVTDMD48 ($P < 0.01$). The SIMP treatment had less IVTDMD48 in 2018 ($P < 0.01$) compared to the COMP, and in 2019 ($P < 0.01$). In the final year treatments did not differ ($P > 0.10$).

Ash content was also impacted by a treatment by yr interaction ($P < 0.01$). The SIMP treatment did not differ in ash content in 2018 compared to 2019 but was lower in 2020 ($P < 0.05$). The COMP treatment was lower ash content each year of the study ($P < 0.05$). Lignin content had a treatment by yr interaction ($P < 0.01$). In 2018, the treatments did not differ in lignin content. The COMP treatment had similar lignin content in 2019 compared to 2018 and was higher than SIMP that yr ($P < 0.05$). The SIMP treatment had less lignin content in 2019 than 2018 ($P < 0.05$) but similar levels in 2020. The COMP treatment had less lignin content in 2020 than previous yrs ($P < 0.05$). Treatments had similar content of lignin in 2020.

3.3.2 Forage Intake

Forage intake ranged from 5.73 kg DM/head per d to 10.93 kg DM/d over the three grazing seasons. Due to differences in sampling procedure among yrs, forage intake results were analyzed by yr. In 2018, there was no impact of treatment on forage intake during the sampling period ($P = 0.76$; **Table 3.5**). In 2019, there was no treatment by sampling period interaction ($P = 0.28$), but there was a period and treatment effect ($P < 0.01$). Cattle in the SIMP treatment consumed less DM (6.83 ± 0.52 kg DM/head per d) compared with those in COMP (9.02 ± 0.54 kg DM/head per d). Dry matter intake was greater during the summer sampling period in 2019 (8.93 ± 0.44 kg DM/head per d) compared with the fall sampling period (6.92 ± 0.43 kg

DM/head per d). In 2020, there was a treatment by sampling period interaction ($P < 0.01$). In the summer sampling period, cattle grazing the COMP treatment consumed more DM compared with those in SIMP, at 9.71 ± 0.72 kg DM/head per d vs. 6.01 ± 0.72 kg DM/head per d, respectively ($P = 0.01$). The cattle on the COMP treatment had a decrease in DMI between the summer and fall sampling periods, with animals consuming 5.73 ± 0.72 kg DM/head per d in the fall period ($P < 0.01$). The intake during the fall sampling period was not statistically different from the animals grazing the SIMP treatment (7.17 ± 0.72 kg DM/head per d; $P = 0.52$).

Gross energy intake ranged from 114.83 MJ/d to 215.49 MJ/d over the three years and results mirrored those of DMI (**Table 3.6**). In 2018, animals from the COMP and SIMP treatments consumed similar levels of GE ($P = 0.55$). In 2019, there was no treatment by sampling period interaction ($P = 0.27$) but there were observed treatment and sampling period effects ($P < 0.01$). Animals grazing the SIMP treatment consumed less GE compared to animals grazing the COMP. Again, GEI was significantly less in the fall sampling period for both treatments compared with the summer sampling period. In 2020, there was a treatment by sampling period interaction ($P = 0.05$). In the summer sampling period, animals grazing COMP consumed more GE than those on SIMP ($P = 0.03$). In the fall sampling period, cattle in both treatments consumed similar amounts of GE.

Neutral detergent fiber intake tended to be different between the treatments in 2018 ($P = 0.06$; **Table 3.7**). In 2019, there was no treatment by time interaction, treatment effect, or effect of sampling period ($P \geq 0.13$). However, in 2020 there was a significant treatment by sampling period interaction ($P < 0.01$). During the summer sampling period, animals on the SIMP treatment consumed less NDF ($P = 0.02$) compared to those on the COMP. The animals grazing the COMP treatment then had a significant ($P < 0.01$) reduction in NDFI between the summer

and fall sampling periods. The SIMP and COMP treatments were not different during the fall sampling period that yr.

3.3.3 Animal Performance and Emissions

Animal performance results are displayed in **Table 3.8**. Animal liveweight gain had a treatment by yr interaction ($P = 0.04$). However, upon mean separation no difference between treatments within yrs was observed. In 2018 live weight gain (**LWG**) was similar between treatments, 96.77 ± 2.10 vs. 101.79 ± 2.11 kg LWG for SIMP and COMP, respectively, and was similar to the results observed in 2020, 104.40 ± 1.93 vs. 98.85 ± 1.92 kg LWG for SIMP and COMP respectively. Performance during 2018 and 2020 production yrs were less than the LWG observed in 2019 where the SIMP treatment had a LWG of 128.90 ± 1.92 kg and the COMP had a LWG of 128.79 ± 1.92 kg. There was a sex effect on LWG with steers gaining more weight than heifers ($P < 0.01$; 120.74 ± 1.62 vs. 99.10 ± 1.30 kg LWG, respectively). Similarly, when considered on a daily basis, animal performance was impacted by yr ($P < 0.01$; **Table 3.8**). In 2020 animals gained significantly less than prior yrs at 0.84 ± 0.03 kg/d compared to 1.02 ± 0.03 and 0.96 ± 0.03 kg/d for 2018 and 2019, respectively. The improved LWG in 2019 was a result of increased grazing duration compared to other yrs but was not due to improved animal performance compared to the 2018 grazing season. There was a significant impact of sex on ADG ($P = 0.04$) with heifers gaining 0.90 ± 0.02 kg/d compared to 0.98 ± 0.03 kg/d for steers.

Enteric methane emissions were not impacted by the treatment by yr interaction ($P = 0.33$) but tended to be impacted by treatment ($P = 0.06$; **Table 3.9**). Neither yr nor sex impacted enteric CH₄ emission rate ($P \geq 0.44$). Cattle grazing COMP had lower enteric CH₄, 193.37 ± 5.52 g CH₄/d, than SIMP, 211.09 ± 5.44 . Emission intensity (**EI**; g CH₄/ kg gain; **Table 3.9**) was

not impacted by any variable ($P \geq 0.36$). Similarly, CO₂ emissions were not affected impacted by treatment, year, their interaction, or sex ($P \geq 0.43$).

3.4 Discussion

The hypothesis that the COMP forage mixture would result in greater forage productivity was rejected in this experiment. Forage productivity was similar between treatments across all 3 yrs and previous literature is inconsistent on the differences in forage productivity at increasing levels of forage diversity (Sanderson et al., 2007; Deak et al., 2007). Small plot studies have indicated that there is no benefit to planting forage mixtures containing more than 4-species (Tracy and Sanderson, 2004). However, in a grazing experiment examining forage mixtures with increasing levels of species inclusion, Deak et al. (2004) found that greater yields were associated with the inclusion of red clover. Similarly, Sanderson et al. (2016) observed a positive relationship between the number of species planted and annual DM yield and that red clover contributed significantly to DM productivity in the first two yrs of the study. High concentrations of forage chicory have also been shown to increase forage biomass (Sanderson et al., 2007), but that was not observed in this study when forage chicory accounted for 52% of pasture DM in the first yr. In 2018, forage quantity was higher for the COMP treatment early in the grazing season as forage chicory encountered favorable environmental conditions (data not shown), however when bolted chicory pastures were mechanically topped by clipping to a 25 cm stubble height (Li and Kemp, 2005). Rainfall in June of 2018 was lower than other yrs of the study and the mechanical topping that occurred that month may explain why chicory did not increase pasture productivity that year. In subsequent yrs early season forage growth was managed more aggressively in the COMP mixture by rotating cattle daily to reduce the likelihood of chicory bolting. Some research has suggested that increased forage diversity may result in improved

forage productivity during dry yrs (Sanderson et al., 2005; Sanderson et al., 2006; Isbell, 2015). However, Deak et al. (2007) observed that complexity of forage mixtures does not always translate to improve forage productivity and that individual species in the mixtures is more important. The results of this study would indicate that no advantage is gained through the adoption of either forage mixture, even with shifting botanical composition.

The shift in botanical composition of both treatments towards greater grass species was expected (Tracy et al., 2018). These results are similar to those reported by Sanderson et al. (2005) in the Northeastern U.S. The authors examined different forage mixtures containing two, three, six, and nine species of grass, forbs and legumes grazed by lactating dairy cows. They reported that, after two yrs of grazing, orchardgrass dominated the pastures at the start of the third yr after stand establishment, similar to the results of both mixtures utilized in this trial. Additionally, they found that chicory and legumes would have to be reestablished frequently to remain in the mixtures. Similarly, in a review of plot- and pasture-scale experiments, Sanderson et al. (2007) reported that nearly one-half of species in complex mixtures did not persist past the third or fourth yr. The short-lived contribution of chicory to the botanical composition of the complex pastures agrees with previous literature (Belesky et al., 1999; Sanderson et al., 2003; Labreveux et al., 2004; Sanderson et al., 2005). Under grazing, chicory persistence has shown to decrease over time, but can withstand heavier defoliation under rotational grazing as new shoots regenerate from the basal crown (Rumball, 1986). However, spring and autumn grazing management is critical for its persistence over time (Li and Kemp, 2005) and the increased defoliation at the start of the grazing season in 2019 and 2020 may partially explain its lack of persistence. The combination of mechanical topping in 2018 and frequent grazing events may explain the prevalence of red and white clover in the COMP pastures in 2019 and 2020. Frequent

defoliation of forages keeps grass species short, therefore decreasing the shading effect on forage species lower in canopy (Wong and Wilson, 1980; Groya and Sheaffer, 1981; Chiavegato et al., 2015). This perhaps allowed these species to remain competitive as the COMP treatment shifted to grass dominated pastures in 2020. The reduction in red clover from 2019 to 2020 would agree with previous studies that white clover is more long-lived in forage mixtures (Sanderson et al., 2016). The decline in alfalfa concentration was expected for the SIMP forage treatment. In alfalfa-grass mixtures, grasses tend to dominate the mixture over time (Berdahl et al., 2004; Baxter et al., 2017). Alfalfa stands self-thin over time and are susceptible to winter-kill, although grass mixtures can provide some protection (Malhi et al., 2002), and therefore provides grass species more space to proliferate (Aponte et al., 2019).

The forage nutritive quality values reported here are within the range of reported values for cool season forages in the region (Muller and Fales, 1998; Cassida et al., 2000; Soder et al., 2006; Sanderson et al., 2016). Shifts in forage quality mirrored those of botanical composition with treatment paddocks being similar in forage quality in 2020 when both treatments were dominated by grass species. The hypothesis was that the COMP forage treatment would have improved forage quality compared to the SIMP treatment would agree with the IVTDMD48 and ADF values observed in this experiment. Although birdsfoot trefoil did not establish as desired, the high forage chicory concentration in 2018 and clover content in following yrs may explain the difference observed here (Lebreveux et al., 2004; Soder et al., 2006; Mangwe et al., 2020). Additionally, with both grazing treatments being dominated by grass species in 2020 could explain the convergence between the two treatments in nutritive value that yr, particularly in IVTDMD48 in COMP pastures (Deak et al., 2007; Sanderson et al., 2016). Sanderson et al. (2016) reported that digestibility was negatively related to grass percentage and neutral detergent

fiber content was positively related to grass content. Similarly, Deak et al. (2007) reported changes in nutritive value in complex mixtures over multiple yrs was explained by proportion of grasses to legumes.

We anticipated that animals grazing the COMP treatment would result in reduced enteric CH₄ emissions compared to those grazing the SIMP treatment. The results of this experiment reject this hypothesis, however there was a tendency for the COMP to have lower enteric CH₄ of a g/d basis. This may partially be explained by the relatively high nutritive value of both treatments. As shown in Table 2 from hand-plucked diet samples during forage intake periods, animals potentially selected high quality diets even during periods of low forage quality. Few studies have considered how species diversity impacts animal performance, as most studies examine mono- or two-species mixtures (Soder et al., 2007; Alemu et al., 2019). However, these results are similar to those reported by Soder et al. (2006) on grazing dairy cows. They tested four forage mixtures with an increasing number of species composition and observed that dairy cows grazing complex mixtures had no decreased performance compared to those of simple mixtures. In a comparison of steers grazing 7- and 12-seed mixtures, Alemu et al. (2019) reported lower ADG values for rotationally grazed steers (0.80 kg/d) and no difference in animal performance between the different mixtures but did between years, similar to this study. Tracy and Faulkner (2006) in a study comparing grazing beef cow-calf pairs on pastures containing 3-, 5- or 8-forage species over 3-yrs and reported no impact of species richness on animal performance.

Enteric CH₄ emissions reported in this study were within the range reported by others examining grazing beef cattle including DeRamus et al. (2003) and Pavao-Zuckerman et al. (1999). McCaughey et al. (1997) evaluated steers grazing alfalfa/grass pasture and found enteric

CH₄ ranged from 171 to 217 g/d, in line with the current study. However, in a similar study comparing yearling beef heifers at similar weights, grazing alfalfa-meadow brome grass (*Bromus biebersteinii*; 40% alfalfa, 60% meadow brome grass) vs. 100% meadow brome grass pastures at similar nutritive values to the current experiment, enteric CH₄ emissions were lower than those reported here (142.8 to 167.6 g CH₄ d⁻¹ for cattle grazing alfalfa-meadow brome grass; Chaves et al., 2006). A potential explanation for this difference between the two studies is the enteric CH₄ values reported here are grazing season means rather than a 5-d sampling period. Enteric CH₄ emissions tended to be lower for COMP, but no difference was observed for EI. The shift in botanical composition we observed and its impacts on rumen function could explain these results. These results are similar to those of Jonker et al. (2018) comparing a ryegrass:white clover mixture, containing both perennial and annual ryegrass species, to a diverse grass, legume and herb mixture. They found no difference in EI between the forage mixtures but there were changes per unit of DM consumed. In this experiment, intake was higher in 2019 and the first sampling period in 2020 for COMP. This could be due to forage diversity, as some research has suggested that increased forage diversity may result in increases in DMI (Wang et al., 2010) and(or) changes in rumen function depending on the forage species present (Jung and Allen, 1995). Additionally, this could indicate that animals grazing SIMP pastures were able to capture similar gains while consuming less forage through more complete fermentation of rumen digestible nutrients. This could have resulted from the grass/legume mixture having reduced rumen passage rate, and therefore more complete digestion of potentially digestible nutrients, which drove enteric CH₄ to be higher per unit of intake but kept performance the same (Jung and Allen, 1995). The high chicory content in yr 1 and high clover content in yrs 2 and 3 for COMP may have resulted in increased rumen passage rate, reduction in rumen degradation, higher DMI

and suppressed methane per unit of intake (Steg et al., 1994; Freudenberger et al., 1994; Navarro-Villa et al., 2011). Navarro-Villa et al. (2011) examined the impact of clover inclusion on *in vitro* methane emissions and found that red clover had reduced emissions per unit of DM incubated compared to perennial ryegrass, but when expressed per unit of DM digested ryegrass had lower emissions, agreeing with Jonker et al. (2018). In a study examining the impact of multiple different forage species on enteric CH₄ production in sheep, Waghorn et al. (2002) hypothesized that the beneficial impact of red clover was due to its lower fiber content and faster rate of passage. Additionally, Aitchison et al. (1986) found that clover hay had significantly faster rates of digestion of DM and NDF compared to grass hay, and that rumen pool sizes were lower for sheep offered clover hay. The 50% inclusion of chicory in yr 1 may have had similar impacts on rumen fermentation as clover (Waghorn et al., 2002; Hristov et al., 2013; Mangwe et al., 2020). Inclusion of forage chicory has inconsistent results on enteric CH₄ (Sun et al., 2012; Williams et al., 2016) and most studies have not found it to be a viable option for CH₄ reduction. Mangwe et al. (2020), in a study examining grazing dairy cows at 50% inclusion level of chicory, observed no changes in DMI compared to those grazing perennial ryegrass, although cows had improved performance, and lower ruminal pH due to increased rumen VFA production. The animals in this experiment grazing COMP in 2018 may have had an altered rumen VFA profile and decreased rumination time which resulted in lower enteric CH₄. Similarly, Waghorn et al. (2002) found that sheep fed forage chicory produced 30% less enteric CH₄, similar to red clover in that study, than those offered alfalfa or a perennial ryegrass:white clover mixture and was hypothesized to be due to the lower fiber content and high digestibility in chicory. One limitation with this experiment was the use of a single GreenFeed™ unit, which inhibited our ability to sample both treatments simultaneously and to directly compare emissions

per unit of intake. Additionally, this sampling structure could be limiting the precision of this experiment when comparing emission rates.

Additionally, while we observed no consistent difference between the two treatments during intake measurement periods, we may not have consistently captured voluntary feed intake from these animals. To test this, we calculated individual animal intake during the sampling periods using equations recommended for yearling cattle by the National Research Council Nutrient Requirements for Beef Cattle (NASEM, 2016; **Table 3.10**). The calculated range of expected intakes was higher than those from measured animals at 7.62 to 11.09 kg DMI d⁻¹ vs. 5.74 to 10.93 kg DMI d⁻¹, respectively. Comparing the treatment, yr, period combinations the lowest values from the dual-marker calculation were increased using the NRC calculation, with the reduction in forage intake in 2019 from the summer to fall sampling period removed. Additionally, the low values observed in 2020 were not present using the NASEM calculation. Utilization of this equation would change our results, notably that treatments did not differ in 2019. Chaves et al. (2006) showed similar results when comparing the alkane method with the Cornell Net Protein Carbohydrate System for predicting intake. These short-term, indirect methods of calculating intake are known to have a high degree of variability due to the use of a marker to estimate fecal output, the reliance on the quality of the collected representative forage sample, and the variance in animal selectivity over a short sampling period (Galyean and Gunter, 2016). This experiment also relied on hand-plucking forage samples which has been shown previously to have operator bias and potentially over-estimate the quality of high-quality forages (Langlands, 1974; De Vries, 1995). In this experiment, the changes in DMI were inconsistent and when DMI was estimated using NASEM (2016) equations there was no difference between the treatments.

3.5 Conclusions

The objective of this experiment was to examine the forage productivity, forage quality, animal performance and enteric CH₄ emissions of two common Midwest grazing forage mixtures: a simple alfalfa:orchardgrass mixture, and a complex forage mixture. The COMP and SIMP mixtures resulted in similar animal performance, forage productivity, but the COMP mixture had lower ADF content and IVTDMD48 over the first 2 yrs of the study. Additionally, animals grazing COMP tended to have lower enteric CH₄ than SIMP on a g/d basis, but this did not result in improved EI. This would agree with previous literature showing that grazing pastures with pastures with differing forage diversity can result in similar animal performance. Additionally, both mixtures had species with poor persistence (alfalfa and forage chicory) that would need to be reseeded regularly to retain them in the mixture. This experiment also serves to supplement the dearth of literature examining animal emissions when grazing complex forage mixtures and continued research is needed to confirm the results of this experiment and to test other producer relevant mixtures.

Figure 3.1: Lake City, MI 30-year average temperature and observed temperatures

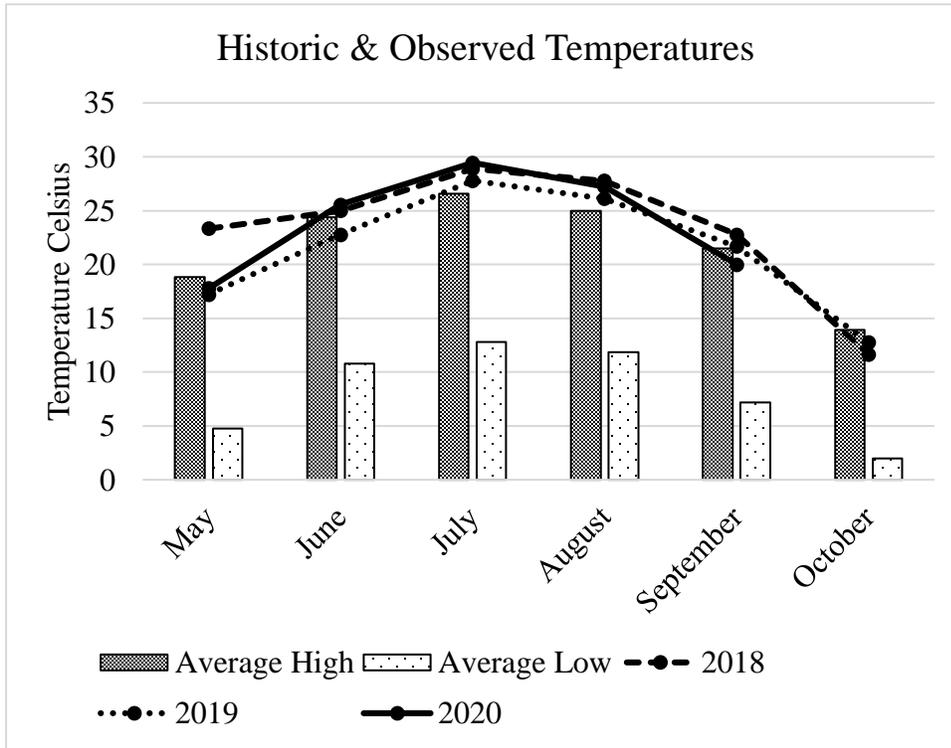


Figure 3.2: Lake City, MI 30-year average precipitation and observed precipitation

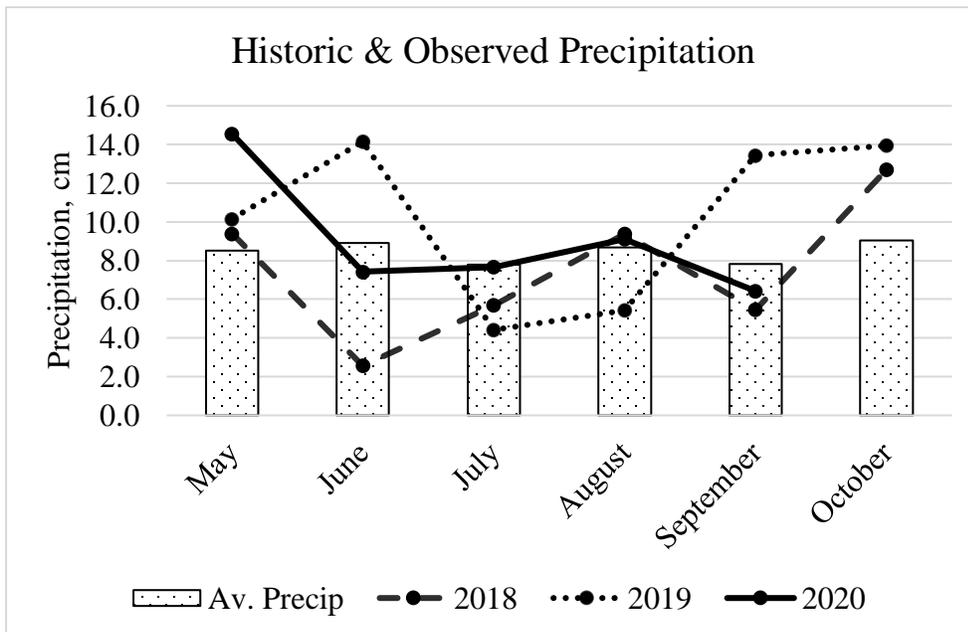


Table 3.1: GreenFeed™ supplement pellet nutritive value by year, %DM

Year	CP, % ¹	aNDF, %	ADF, %	NE _m Mcal/kg	NE _g Mcal/kg	TDN
2018	18.06	45.89	35.25	57.74	31.87	61.44
2019	17.51	50.05	37.17	55.53	29.85	59.94
2020	18.35	51.85	42.03	54.99	29.36	56.16

¹CP = crude protein, aNDF= neutral detergent fiber, ADF= acid detergent fiber, NE_m= net energy for maintenance, NE_g= net energy for gain, TDN= total digestible nutrients; SIMP= simple forage mixture, COMP= complex forage mixture

Table 3.2: Forage nutritive value of composite diet sample each sampling period estimated via hand-plucking

Nutrient, % DM ¹	2018 ²		2019				2020			
	Fall		Summer		Fall		Summer		Fall	
	SIMP	COMP	SIMP	COMP	SIMP	COMP	SIMP	COMP	SIMP	COMP
CP, %	28.19	28.24	20.03	27.6	19.31	24.69	16.63	16.8	24.23	24.82
aNDF, %	31.59	25.78	41.57	29.81	55.72	45.23	55.1	51.51	48.74	42.18
ADF, %	19.2	17.55	26.52	21.04	34.57	28.76	33.83	31.67	24.23	27.55
NE _m , Mcal/kg	1.52	1.56	1.34	1.53	1.18	1.33	1.16	1.2	1.29	1.37
NE _g , Mcal/kg	0.92	0.96	0.76	0.94	0.62	0.76	0.6	0.64	0.72	0.79
TDN	73.94	75.23	68.24	72.51	61.97	66.5	62.55	64.23	63.15	67.44
In Situ digestibility %	79.01	82.15	76.33	86.73	66.11	79.39	71.18	79.16	74.88	68.36

¹ CP= crude protein, aNDF= neutral detergent fiber content, ADF= acid detergent fiber, NE_m = net energy for maintenance, NE_g = net energy for gain, TDN = total digestible nutrients; SIMP= simple forage mixture, COMP= complex forage mixture

² Fall 2018 bolusing period = September 14 through September 18; 2019 bolusing periods = May 27 through June 9 (Summer) and September 5 through September 18 (Fall); 2020 bolusing periods = June 1 through June 14 (Summer) and August 31 through September 13 (Fall)

Table 3.3: Yearly pre- and post-graze forage mass, kg/ha

Pre-graze forage mass kg/ha ^{1,2}						
	SIMP	COMP	Average	SEM	<i>P</i> -values	
2018	2,452	2,354	2,403*	144	Treatment	0.69
2019	2,888	2,949	2,919	137	Year	0.05
2020	2,756	3,004	2,800	153	Treatment*year	0.71
Post-graze forage mass, kg/ha						
	SIMP	COMP	Average	SEM	<i>P</i> -values	
2018	1,489	1,568	1,528 ^b	116	Treatment	0.48
2019	1,970	2,178	2,074 ^a	111	Year	<0.01
2020	2,098	2,118	2,108 ^a	124	Treatment*year	0.84

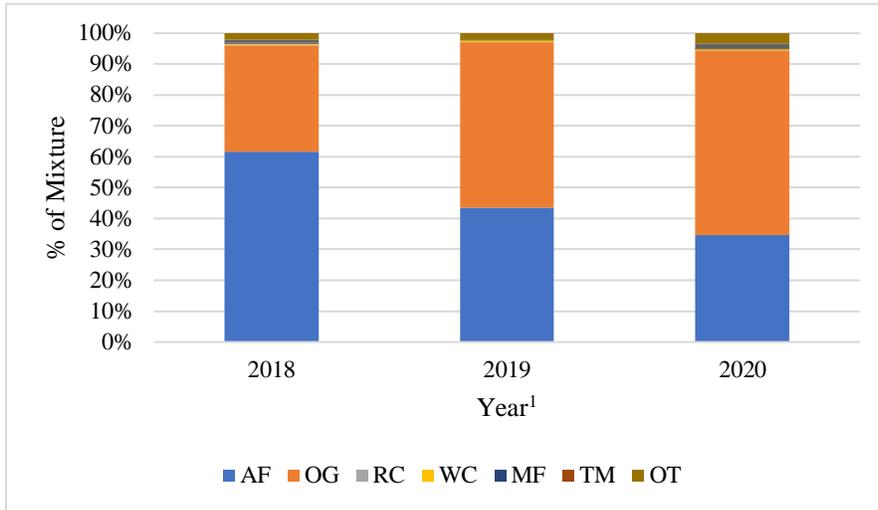
¹SEM= Yearly standard error of the mean; SIMP= simple forage mixture, COMP= complex

forage mixture

² Differing lowercase superscript within row signifies a significant difference ($P \leq 0.05$)

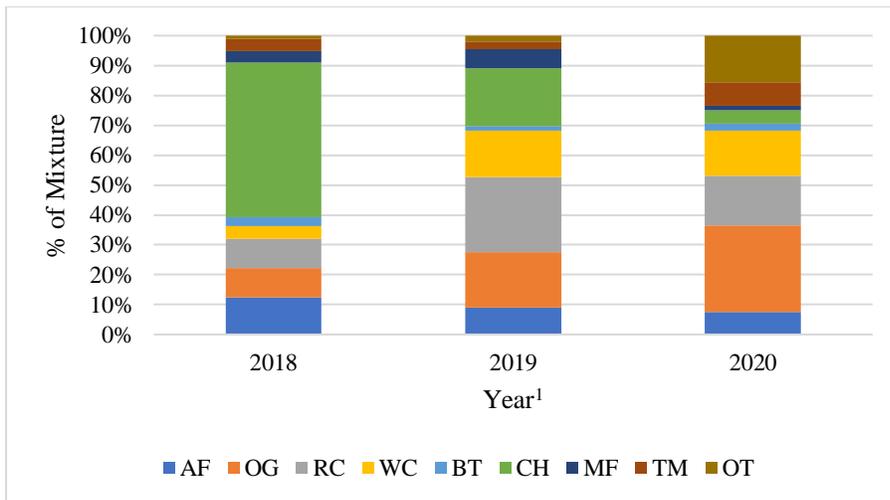
*2018 forage mass tended to be lower than 2019 ($P = 0.06$) and 2020 ($P = 0.10$)

Figure 3.3: Simple forage mixture botanical composition by year, % DM



¹AF= Alfalfa, OG= Orchardgrass RC= Red Clover, WC= White Clover, MF= Meadow Fescue, Tim= Timothy, OT= Other

Figure 3.4: Complex forage mixture botanical composition by year, % DM



¹AF= Alfalfa, OG= Orchardgrass RC= Red Clover, WC= White Clover, BT= Birdsfoot Trefoil, CH= Chicory, MF= Meadow Fescue, Tim= Timothy, OT= Other

Table 3.4: Yearly pre-graze forage nutritive value estimated via near-infrared reflectance spectroscopy, % DM

CP ¹	SIMP ²	COMP	Average	<i>P</i> -Values	
2018	20.44 ^{aA}	18.17 ^{bA}	19.31	Treatment	0.04
2019	19.06 ^{aA}	19.19 ^{aA}	19.12	Year	<0.01
2020	15.42 ^{aB}	15.91 ^{aB}	15.66	Treatment*Year	0.04
Average	18.51	17.55			
aNDF	SIMP	COMP	Average		
2018	44.94 ^{aC}	42.43 ^{aC}	43.68	Treatment	0.06
2019	51.14 ^{aB}	47.99 ^{aB}	49.57	Year	<0.01
2020	55.55 ^{aA}	55.83 ^{aA}	55.69	Treatment*Year	0.07
Average	48.75	50.54			
ADF ³	SIMP	COMP	Average		
2018			32.59 ^B	Treatment	0.02
2019			34.28 ^A	Year	<0.01
2020			35.30 ^A	Treatment*Year	0.82
Average	34.73 ^a	33.38 ^b			
IVTDM48	SIMP	COMP	Average		
2018	75.35 ^{bA}	83.09 ^{aA}	79.22	Treatment	<0.01
2019	77.49 ^{bA}	80.79 ^{aA}	79.14	Year	<0.01
2020	76.38 ^{aA}	77.28 ^{aB}	76.84	Treatment*Year	<0.01
Average	76.41	80.39			
Ash	SIMP	COMP	Average		
2018	8.00 ^{aA}	8.51 ^{aA}	8.25	Treatment	0.14
2019	7.93 ^{aA}	7.27 ^{aB}	7.60	Year	<0.01
2020	6.73 ^{aB}	6.13 ^{aC}	6.43	Treatment*Year	<0.01
Average	7.56	7.30			

Table 3.4 cont.

Lignin	SIMP	COMP	Average		
2018	6.81 ^{aA}	7.28 ^{aA}	7.04	Treatment	<0.01
2019	5.57 ^{bB}	7.08 ^{aA}	6.33	Year	<0.01
2020	5.40 ^{aB}	5.93 ^{aB}	5.67	Treatment*Year	<0.01
Average	5.92	6.76			

¹CP= crude protein, aNDF= neutral detergent fiber, ADF= acid detergent fiber, IVTDMD48= in vitro true dry matter digestibility over 48 hrs

²Differing lowercase superscript withing column signifies a significant difference; Differing uppercase superscript within row signifies a significant difference ($P < 0.05$) ; SIMP= simple forage mixture, COMP= complex forage mixture

³ADF is reported as year and treatment means because of the insignificant interaction

Table 3.5: Forage dry matter intake estimated using the dual marker method, kg DM/head per d

2018 ^{1,2,3}					
	SIMP	COMP	Treatment	<i>P</i> -values	
				Period	Treatment*Period
DMI ⁴	10.93	10.564	0.76	-	-
SEM	0.84				
2019					
	SIMP	COMP	Treatment	<i>P</i> -values	
				Period	Treatment*Period
DMI	6.83 ^b	9.02 ^a	0.01	<0.01	0.28
SEM	0.52	0.54			
	Summer	Fall			
DMI	8.93 ^A	6.92 ^B			
SEM	0.44	0.43			
2020					
	SIMP	COMP	Treatment	<i>P</i> -values	
				Period	Treatment*Period
Summer	6.01 ^{bA}	9.71 ^{aA}	0.14	0.07	<0.01
Fall	7.17 ^{aA}	5.73 ^{aB}			
SEM	0.72				

¹SEM= Standard error of the mean; SIMP= simple forage mixture, COMP= complex forage mixture

²Lower case superscript signifies a significant difference between treatment, uppercase superscript signifies a significant difference between period ($P \leq 0.05$)

³2018 sampling periods: September 14 through September 27; 2019 sampling periods: Summer- May 27 through June 9, Fall- September 5 through September 18; 2020 sampling periods: Summer- June 1 through June 14, Fall- August 31 through September 13

⁴DMI= kg DM forage consumed/head per d

Table 3.6: Gross energy intake from forage estimated using the dual marker method, MJ/head per d

2018 ^{1,2,3}					
	SIMP	COMP	Treatment	<i>P</i> -values	
				Period	Treatment*Period
GEI ⁴	215.49	201.33	0.55	-	-
SEM	16.37				
2019					
	SIMP	COMP	Treatment	<i>P</i> -values	
				Period	Treatment*Period
GEI	129.10 ^b	173.07 ^a	<0.01	<0.01	0.27
SEM	9.88	10.36			
Period	Summer	Fall			
GEI	169.9 ^A	132.27 ^B			
SEM	8.42	8.16			
2020					
	SIMP	COMP	Treatment	<i>P</i> -values	
				Period	Treatment*Period
Summer	114.84 ^{bA}	180.28 ^{aA}	0.04	0.58	0.05
Fall	138.45 ^{aA}	140.34 ^{aA}			
SEM	14.79				

¹SEM= standard error of the mean; SIMP= simple forage mixture, COMP= complex forage mixture

²Lower case superscript signifies a significant difference between treatment, uppercase superscript signifies a significant difference between period ($P \leq 0.05$)

³2018 sampling periods: September 14 through September 27; 2019 sampling periods: Summer- May 27 through June 9, Fall- September 5 through September 18; 2020 sampling periods: Summer- June 1 through June 14, Fall- August 31 through September 13

⁴GEI= gross energy intake from forage in MJ/head per d

Table 3.7: Neutral detergent fiber intake from forage estimated using the dual marker method, kg DM/head per d

2018 ^{1,2,3}					
	SIMP	COMP	Treatment	<i>P</i> -values	
				Period	Treatment*Period
NDFI ⁴	3.45	2.72	0.06	-	-
SEM	0.26				
2019					
	SIMP	COMP	Treatment	<i>P</i> -values	
				Period	Treatment*Period
NDFI	3.27	3.31	0.91	0.13	0.61
SEM	0.24	0.25			
2020					
	SIMP	COMP	Treatment	<i>P</i> -values	
				Period	Treatment*Period
Summer	3.31 ^{bA}	5.01 ^{aA}	0.39	<0.01	<0.01
Fall	3.49 ^{aA}	2.42 ^{aB}			
SEM	0.35				

¹SEM= standard error of the mean; SIMP= simple forage mixture, COMP= complex forage mixture

²Lower case superscript signifies a significant difference between treatment, uppercase superscript signifies a significant difference between period ($P \leq 0.05$)

³2018 sampling periods: September 14 through September 27; 2019 sampling periods: Summer- May 27 through June 9, Fall- September 5 through September 18; 2020 sampling periods: Summer- June 1 through June 14, Fall- August 31 through September 13

⁴NDFI= neutral detergent fiber consumed from forage kg DM/head per d

Table 3.8: Animal performance while grazing treatment paddocks estimated by monthly shrunk body weights

Year	Live weight gain(kg) ^{1,2}			<i>P</i> -value			
	SIMP	COMP	SEM	Treatment	Year	Treatment*Year	Sex
2018	96.77 ^{aB}	101.79 ^{aB}	1.73	0.89	<0.01	0.04	<0.01
2019	128.90 ^{aA}	128.79 ^{aA}	1.50				
2020	104.40 ^{aB}	98.85 ^{aB}	1.50				
Sex	LWG (kg)		SEM				
Heifer	99.10 ^b		1.30				
Steer	120.74 ^a		1.62				
Year	Average daily gain			<i>P</i> -value			
	ADG (Kg) ³	SEM	Treatment	Year	Treatment*Year	Sex	
2018	1.02 ^A	0.03	0.81	<0.01	0.44	0.04	
2019	0.96 ^A	0.03					
2020	0.84 ^B	0.03					
Sex	ADG (Kg)		SEM				
Heifer	0.90 ^b		0.02				
Steer	0.99 ^a		0.03				

¹SEM= standard error of the mean; SIMP= simple forage mixture, COMP= complex forage mixture

²Differing lowercase superscripts signify a significant difference between treatments within year, differing uppercase letters signify a significant difference between year respective of treatment ($P \leq 0.05$)

³ADG= average daily gain in kg/d

Table 3.9: Animal greenhouse gas emissions estimated using the Greenfeed™ emission measurement system

	CH ₄ g/d	SEM ¹	<i>P</i> -value			
SIMP	211.09	5.44	Treatment	Year	Treatment*Year	Sex
COMP	193.37*	5.52	0.06	0.44	0.33	0.90
	CO ₂ g/d	SEM	<i>P</i> -value			
SIMP	7,063.49	169.21	Treatment	Year	Treatment*Year	Sex
COMP	6,934.76	169.12	0.60	0.43	0.64	0.46
	Emission Intensity (g CH ₄ /kg gain)	SEM	<i>P</i> -value			
SIMP	240.89	15.33	Treatment	Year	Treatment*Year	Sex
COMP	227.25	16.09	0.56	0.36	0.52	0.81

¹ SEM= Standard error of the mean; SIMP= simple forage mixture, COMP= complex forage mixture

*COMP tended to have lower CH₄ g/d compared to SIMP (*P* = 0.06)

Table 3.10: Forage dry matter intake calculated using NASEM (2016) equations for growing beef cattle

Summer			Fall		
2018	DMI ¹	St. Dev.	2018	DMI	St. Dev.
SIMP	-		SIMP	10.94	0.73
COMP	-		COMP	11.09	0.93
2019	DMI	St. Dev.	2019	DMI	St. Dev.
SIMP	7.64	0.79	SIMP	9.85	0.79
COMP	7.93	0.61	COMP	10.42	0.69
2020	DMI	St. Dev.	2020	DMI	St. Dev.
SIMP	9.18	0.74	SIMP	7.62	0.77
COMP	9.83	0.47	COMP	8.25	0.45

¹DMI= dry matter intake from forage in kg DM/head per d; St. Dev= standard deviation; SIMP= simple forage mixture, COMP= complex forage mixture

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CHAPTER 4
SOIL GHG EMISSIONS AND C/N CYCLING IN BEEF CATTLE GRAZING
MIXTURES WITH DIFFERING FORAGE DIVERSITY

Abstract

Understanding fates of C/N in grazing environments is an area of ongoing research and understanding how forage diversity impacts the long-term storage of C is crucial for the long-term sustainability of grazing agriculture. Therefore, the objective of this study was to examine how forage diversity impacts soil C/N cycling and soil GHG fluxes. This experiment was conducted over 3 consecutive grazing seasons from 2018 to 2020. Two forage treatments were tested: 1) a botanically diverse, mixed species pasture (**COMP**), and 2) A simple, 50:50 alfalfa:orchardgrass mixture (**SIMP**). Treatments were grazing with 56 red-angus steers and heifers in 4 treatment paddocks per treatment: three 3.2 ha and one 1.6 ha paddock, with 8 and 4 animals each, respectively. Soil organic C (**SOC**) and total soil N (**TSN**) were determined at the beginning at the end of the experiment using the equivalent soil mass approach. At the beginning and end of each grazing season, water extractable C (**WEOC**) and N (**WEON**) was measured and soil CO₂, CH₄, and N₂O fluxes were measured used static chamber methodology. Soil organic C and TSN did not differ by treatment ($P = 0.80$) but did decrease for both treatments from the beginning to end of the experiment ($P < 0.01$). Water extractable organic carbon content was greater at the end of the experiment for both treatments ($P < 0.05$). Both treatments decreased soil CO₂ flux from the beginning to end the grazing seasons (27.30 vs. 17.39 for periods 1 and 2, respectively, across all 3 years; $P < 0.01$) but did not differ between treatments. Soil CH₄ flux did not differ across periods, treatments or year ($P > 0.50$). Soil N₂O flux had period by year interaction ($P < 0.01$), but did not differ between treatments ($P = 0.66$). We hypothesize that legacy management of soils prior to the experiment (tillage in 2017) impacted the long-term soil C and N stocks, and that the similar WEOC:WEON ratio and stocks between treatments partially explains the similarities in N₂O fluxes between the two treatments. The

results of this experiment would indicate that these mixtures have similar impacts on soil C:N cycling when management is equal.

4.1 Introduction

The fate of soil carbon (C) and nitrogen (N) in a grazing ecosystem play a crucial role in the overall C budget of the system and N₂O flux (Allard et al., 2007). Managing grazing ecosystems for increased long-term soil C storage may have the potential to offset some or all emissions arising from grazing ruminants (Teague et al., 2016; Rowntree et al., 2016; Stanley et al., 2018). However, the mechanisms driving soil C storage are not well understood and requires both long- and short-term sampling to understand both labile and long-term C fractions (Milchunas and Lauenroth, 1993; Lavalley et al., 2020). In a meta-analysis, Milchunas and Lauenroth (1993) compared grazed and ungrazed sites for species composition, net primary production, root biomass, and soil nutrients and observed as many positive as negative differences. This led the authors to hypothesize that the local environmental conditions impacted indicator variables rather than management applied. In contrast, Xu et al. (2017) examined above ground biomass conversion into soil C in grazing environments and observed that high producing forage species increased total soil C stocks. However, this C was mostly in labile forms with relatively short turnover rates. It was hypothesized by these authors, as well as Jobbagy and Jackson (2000), that vertical root distribution could alter the depth of soil organic C accumulation and conversion into more long-term C pools. Additionally, in a study examining the behavior of different soil C and N pools, Cotrufo et al. (2019) reported that grassland soils are typically dominated by mineral associated organic matter, which is less susceptible to disturbance and has increase permanence. They hypothesized that for grassland soils to increase the amount of C sequestered, it would require management that increases the particulate organic

matter pool, which is derived from plant litter inputs, but is more susceptible to loss via disturbance.

Soil greenhouse (**GHG**) fluxes in grassland are related to grazing management and are typically observed to be GHG sinks (Chiavegato et al., 2015; Allard et al., 2007; Soussana et al., 2007). Carbon dioxide is exchanged by the soil and existing plant species and can either be a net sink or emission of C, depending on the forage and/or grazing management. Additionally, grasslands, in certain instances, can be a small sink of atmospheric methane (**CH₄**) due to the presence of obligate aerobic methanotrophs located in the upper strata of upland soils (Saggar et al., 2008; IPCC, 2013). Soil CH₄ oxidation is a major sink of atmospheric CH₄ behind oxidation by OH[•] radicals in the atmosphere (Saggar et al., 2008; Hartmann et al., 2011). However, the ecological controls of methanotroph/methanogen activity are still poorly understood, but previous research has suggested that some agricultural soils may be able to offset some CH₄ from manure and part of the enteric CH₄ emissions from grazing ruminants (Saggar et al., 2004; 2007). Grassland soils emit N₂O and these emission rates are influenced by N₂ fixation, amount of excreta, mineralization, and immobilization (Ledgard, 2001). Similar to methane, nitrous oxide is a potent GHG with a global warming potential 265 times that of CO₂ over a 100-yr time frame (**GWP₁₀₀**; IPCC, 2013). Additionally, soil N pools have established relationships with soil C cycling and may have negative feedback on soil CH₄ oxidation, although long term fertilization may be needed for feedback to occur (Dunfield and Knowles, 1995). The N cycle in soils is complex, however, and soil N content was linked to long-term increases in soil C, but this must be weighed against negative consequences of N additions (eg. leaching and emissions; Cotrufo et al., 2019). The additional N via legume-rhizobium symbiosis may increase the amount of N lost via N₂O emissions, thereby offsetting benefits obtained through increased above ground

productivity and potential reductions in enteric CH₄. Therefore, the hypothesis of this project was that a complex forage mixture would result in increased forage productivity via increased forage diversity and thus increase soil C accumulation of more labile C pools. H. Additionally, the diversity of forages would result in more stable GHG compared the a simple (alfalfa:orchardgrass) mixture. The objective of this experiment was to compare soil C/N cycling and GHG fluxes of two grazing forage mixtures with differing foraging diversity.

4.2 Materials and Methods

The use of animals and procedures were approved by the Michigan State Animal Care and Use Committee (Protocol #02-18-019-00).

4.2.1 Pastures and Animal Management

Experimental pastures were located at the Michigan State University Lake City AgBioResearch Center (latitude: 44°18'N, longitude: 85°11'W; elevation 377 m; Appendix A). The predominate soil type is Nester sandy loam containing a 1% to 6% slope that is well drained, at 65% of the study area (Soil Survey Staff, National Research Conservation Service). The rest of the study area was primarily a Kawkawlin loam at a 0% to 3% slope and is somewhat poorly drained (Soil Survey Staff, National Research Conservation Service). Weather data during the grazing periods are recorded in **Figure 4.1** and **4.2** at an on-farm weather station (NOAA, 2020). Experimental treatments were established in the fall of 2017 after termination and tillage of a previous grazing trial. The experiment was conducted over 3 consecutive grazing seasons from 2018 to 2020.

Each treatment contained 3 3.2-ha pastures and 1 1.6-ha paddock with 8 and 4 yearling calves, respectively, assigned to each pasture each grazing season. The first treatment was a

simple (**SIMP**) forage mixture with a target composition of 75% alfalfa (*Medicago sativa* L.) and 25% orchardgrass (*Dactylis glomerata* L.). The second treatment was a complex (**COMP**) forage mixture and was seeded at a rate of 9% alfalfa, 10% orchardgrass, 12% red clover (*Trifolium pratense* L.), 7% white clover (*Trifolium repens* L.), 29% birdsfoot trefoil (*Lotus corniculatus* L.), 12% forage chicory (*Chicorium intybus*), 14% meadow fescue (*Festuca pratensis*), and 7% timothy (*Phleum pratense*). Animals were assigned to either 3.2 or 1.6 ha pastures and allocated 0.2-ha or 0.1-ha sub-paddocks and rotated as detailed in Chapter 2. The grazing season in year one began on June 1, 2018 and ended October 5, 2018. Year 2 began on May 25, 2019 and ended on October 1, 2019, and year 3 started May 30, 2020 and ended on September 19, 2020.

4.2.2 Soil Greenhouse Gas Measurements

At the beginning and end of each grazing season, soil CH₄, carbon dioxide (CO₂) and nitrous oxide (N₂O) emissions were collected from the most recently grazed sub-paddocks for 5-d post-grazing using static chamber methodology (Chiavegato et al., 2015b; Holter, 1997; Appendix A). Sampling periods were: June 4 to June 8, 2018 and October 1 to October 5, 2018 for year 1, May 27 to May 31, 2019 and September 24 to September 28, 2019 for year 2, and June 1 to June 5, 2020 and September 14 to September 18, 2020 for year 3.

Chambers consisted of a stainless-steel ring base and PVC cap. The base had dimensions of 20.3 cm o.d. and a height of 15.2 cm. The PVC cap had dimensions of 23.5 cm outside diameter and height of 9.4 cm. When the cap was placed on the ring for sampling a rubber strap was used to seal the chambers and stop atmospheric air from entering the headspace. A rubber stopper (Molded Thermogreen LB-2 septa, MilliporeSigma St. Louis, MO) was used to seal the

sampling port. The caps contained a 4 mm vent hole to avoid pressure perturbations and subsequent mass flow.

Three pastures per treatment (all 0.2-ha subpaddocks) were selected for soil GHG sampling. Within each 0.2-ha subpaddock, a 10-section grid was used with one chamber randomly placed within each grid section. This grid was used to evenly distribute the 10-static chambers due to the methodology limitations in capturing spatial variability (Chiavegato et al., 2015b). Twenty-four hours prior to sampling, the stainless-steel base was placed (depth of 7.5 cm) to allow soil microbial populations to stabilize. Each d, sampling began at 0900 and, with only 20 static chamber tops available, the order of pasture sampling was randomized each day to minimize temporal variation. Sampling began once metal rings were capped, and gas was sampled from the headspace every 5 min beginning at t 0 for 20-min to calculate gas fluxes. After sampling, the cap was removed so only the base remained at the location for all 5-d. Gas samples were collected using a 20-mL plastic syringe (BD, Franklin Lakes, NJ) and precision glide needles (21 G: BD, Franklin Lakes, NJ). Gas transferred to evacuated 20-mL vials with beveled tops and rounded bottoms (Supelco, Bellefonte, PA), with the vial being filled with two 20-mL samples at each time point. Vials were sealed with 20 mm red rubber septa (MilliporeSigma, St. Louis, MO). Once samples were taken, they were stored at room temperature and transported to Michigan State University Animal Science (East Lansing, MI) for analysis at the conclusion of sampling on d5.

Gas chromatography was conducted immediately after conclusion of each sampling period using a Shimadzu GC-2014 equipped with electron capture and flame ionization detectors (Shimadzu Addison, IL). The ECD temperature was maintained at 325°C and the FID was maintained at 250°C. The column was maintained at 75°C. The carrier gas supply was ultrapure

nitrogen and the flow rate was 44 mL/min with a purge flow of 0.5 mL/min. The GC was equipped with a headspace automatic sampler (COMBI Pal, LEAP Technologies, Carrboro, NC). Calibration curves were generated prior to analysis of each period using a standard gas with concentrations: 3,903 mg/kg CO₂, 20.42 mg/kg CH₄, and 4.015 mg/kg N₂O.

Gas concentration of each sample was calculated as:

$$\text{Equation 1. } \text{GHG}_{\text{conc}} = \text{GHG} \times P \times V_{\text{ch}} / (\text{R} \times \text{AT})$$

GHG represents the gas concentration of each respective gas (CH₄, CO₂, and N₂O), P is the atmospheric pressure at sampling (atm), V_{ch} is chamber plus base volume (cm³), R is the ideal gas constant (atm L mol⁻¹K⁻¹), and AT represents ambient temperature (K) at sampling.

Chambers were randomly assigned to rings each day. This value (GHG_{conc}) was then used to calculate the flux of each gas of each 5-d sampling period by linear regression of the 20 min period.

4.2.3 Soil Sampling

Soil organic C (SOC) and total soil N (TSN) stocks were measured at the beginning of grazing in year 1 and the end of the trial in year 3. Two experimental pastures were chosen from each treatment and eight locations were randomly chosen in each for soil sampling. Sampling locations were randomly selected in a nonstratified approach at eight locations per experimental pasture (Ellert et al., 2002a). In year 1, samples were taken on May 24, 2018 and again in year 3 on August 12, 2020 using a 152.4-cm probe (9110 Ag Probe, AMS Inc., American Falls, SD) to represent beginning and end of the trial. Samples were taken at each location to a depth of 30 cm. Each 30-cm core was divided into 3 depths: 0 to 10 cm, 10 to 20 cm, and 20 to 30 cm. Rocks and roots were removed and weighed and samples were then sieved to pass through a 2 mm screen

(Fischer Scientific, Waltham, MA) and dried at 60°C to a constant weight determine DM composition. Soil bulk density was calculated by dividing the soil dry weight by soil volume (Blake and Hartge, 1986). These bulk densities were then used to calculate SOC and TSN stocks.

Next, in a 20 mL scintillation vial, half the vial was filled with dried soil and ground using a hex tumbling bar on a SampleTek vial rotator (Model 200: Lawrenceburg, KY). Samples were then transported to Michigan State University for later analysis. One subsample per depth was used for total organic C and N analysis via combustion (Leco cn928 Combustion Analyzer, LECO Corp., St. Joseph, MO) at the University of Missouri Soil Health Assessment Center (Columbia, MO). Total C is reported as total organic carbon as previous research at this location has determined no non-organic C (Chiavegato et al., 2015a). An additional 4 g subsample was taken from the 0 to 10 cm depth and used for water extractable soil organic C (**WEOC**) and N (**WEON**: Haney et al., 2012) analysis using a vario TOC select (Elementar, Langensfeld, Germany). The 4 g sample was weighed into a 50 mL centrifuge tube and 40 mL of deionized water was added to the centrifuge tube. Tubes were then placed on a rotary shaker table (Waverly Scientific, Waverly, IA) for 10 minutes, followed by centrifugation (Waverly Scientific, Waverly, IA) at 626 g for 5 min. The supernatant was then filtered through whatman 2V filter paper (GE Healthcare, Buckinghamshire, UK) and transported to Michigan State University for analysis. As the WEOC and WEON pool are more labile, at the end of year 1, beginning and end of year 2, and the beginning of year 3, additional soil cores from the same experimental units were taken using a hand corer (2.22 cm x 53.34 cm: AMS Inc., American Falls, ID) and samples were analyzed for WEOC and WEON at the 0-10 cm layer.

Changes in soil nutrient concentration were calculated using the equivalent soil mass procedure described by Ellert et al. (2002a). This adjustment utilizes a reference soil mass (RM)

which is the smallest soil mass at the prescribed depth from all sites across sampling periods.

The calculation is as follows (for SOC):

$$\text{Equation 2: } \text{SOC}_{\text{FD}} = \sum C_i \times \text{BD}_i \times L_i \times 0.1$$

Where SOC_{FD} is soil organic carbon at a fixed depth (Mg/ha), C_i is the concentration of C at depth i (mg/g dry soil), BD_i is the calculated bulk density (g/cm^3) at depth i , and L_i is the length of the core at depth i (cm). The utilization of bulk density from the actual cores were utilized rather than a reference soil core to minimize spatial variability as recommended by Ellert et al. (2002a). Next the mass of the soil was calculated as:

$$\text{Equation 3: } \text{SM} = \sum \text{BD}_i \times L_i \times 100$$

Where SM is the soil mass (Mg/ha), BD_i is the bulk density (g/m^3) at depth i , and L_i is the length of the core at depth i (cm). The next step was the calculated of the excess soil mass at depth i :

$$\text{Equation 4: } \text{Mex} = \text{SM} - \text{RM}$$

Where Mex is the excess soil mass (Mg/ha), SM is the soil mass to a fixed depth (Mg/ha), and RM is the reference soil mass (Mg/ha). Lastly, Mex is then used to adjust the SOC_{FD} by :

$$\text{Equation 5: } \text{SOC}_{\text{FM}} = \text{SOC}_{\text{FD}} - \text{Mex} \times C_{\text{dl}} / 1000$$

Where SOC_{FM} is the soil organic carbon at a fixed mass (Mg/ha), Mex is the excess soil mass (Mg/ha), and C_{dl} is the carbon concentration at the deepest depth (mg/g dry soil). This same calculation was utilized for TSN.

4.2.4 Statistical Analysis

Soil GHG flux was analyzed as a completely randomized design using PROC MIXED (SAS, v. 9.4, SAS Inst. Inc., Cary, NC) with pasture as the experimental unit. Variance was

tested using the Levene's test (Levene, 1960) and residual plots were examined for deviations from normality (Kravchenko and Robertson, 2015). As expected, N₂O flux exhibited unequal variance but residuals did not display severe skewedness and variance was allowed to vary as no tested transformation improved both the unequal variance and residual plots (de Klein et al., 2020). The final model was:

$$y = \mu + \tau_i + \lambda_j + \gamma_f + \kappa_l + \lambda\tau_{ij} + \gamma\tau_{if} + \lambda\gamma_{jf} + \tau\lambda\gamma_{ijf} + P_{m(n*\tau*\kappa)} + v_{in} + \delta_{jfn} + e_{ijflm}$$

Where μ is the overall mean, τ_i is fixed effect of treatment i , λ_j is the fixed effect of year j , γ_f is the fixed effect of period f , κ_l is the fixed effect of day l , $\lambda\tau_{ij}$ is the interaction between year and treatment, $\gamma\tau_{if}$ is the interaction between treatment and period, $\tau\lambda\gamma_{ijf}$ is the interaction between treatment, year and period, $P_{m(n*\tau*\kappa)}$ is the random effect of replicate nested within pasture, treatment and day, v_{in} is the random interaction between pasture and year, δ_{jfn} is the random interaction between year, period, and pasture, and e_{ijflm} is the residual term. Soil temperature at 10cm (°C), precipitation (mm), RH (%), and air temperature (°C) were used as explanatory variables. These variables were recorded at an on-farm weather station (Great Lakes Bioenergy Research Center, 2020).

Soil organic carbon, TSN and carbon:nitrogen ratio analyzed as a completely randomized design using PROC MIXED with pasture as the experimental unit. Pasture nested within treatment and replicate nested within pasture was included as random terms. Year and treatment and the interaction along were included as a fixed effect. Water extractable organic C and WEON was analyzed as a completely random design with pasture as the experimental unit and period were included as a repeated measure. Pasture and replicate nested within pasture were included as the random term. Tested fixed effects were treatment, year, period and the

interactions. Means were separated using the LSMEANS statement with a Tukey adjustment. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

4.3 Results

4.3.1 Soil Bulk Density

As expected, soil bulk density (g/cm^3) differed by depth and year ($P < 0.01$; **Table 4.1**) but did not differ significantly by treatment ($P = 0.26$). Bulk density increased by depth but no treatment by depth interaction was observed ($P = 0.35$). Bulk density was 0.92 , 1.11 , and $1.24 \pm 0.02 \text{ g/cm}^3$ at depths of 0 to 10, 10 to 20, and 20 to 30 cm, respectively, across year. In 2020, bulk density was significantly higher compared to 2018. In 2020, the bulk density was 1.13 compared to $1.04 \pm 0.02 \text{ g/cm}^3$ in 2018.

4.3.2 Soil Carbon and Nitrogen Results

To put soil C and N on an equal basis, SOC_{FM} based on bulk density of the soil cores was adjusted using the equivalent soil mass equation (Ellert et al., 2002a). Soil organic carbon concentration of the soil decreased from 2018 to 2020 ($P < 0.01$; **Table 4.2**) for both treatments. There was no treatment by year interaction or treatment effect of SOC concentration (Mg/ha ; $P > 0.48$). The SOC stock decreased from 48.00 ± 3.34 in 2018 to 45.52 ± 3.34 in 2020. Total soil N decreased from 2018 to 2020 ($P = 0.01$; Table 3) for both treatments, but there was not treatment by year interaction or treatment effect ($P > 0.90$). The TSN stock declined from 4.10 ± 0.19 in 2018 to 3.92 ± 0.19 in 2020. The C:N ratio had a tendency for a treatment by year interaction ($P = 0.08$; Table 3). Soils in the SIMP treatment had no change in C:N ratio, 11.61 ± 0.73 in both 2018 and 2020. However, soils in the COMP treatment tended to have a reduced C:N ratio in 2020 compared to 2018 ($P = 0.02$), going from 11.46 ± 0.73 in 2018 to 11.19 ± 0.73 in 2020.

Soil organic C and N was impacted by depth ($P < 0.01$; **Figure 4.3 and Figure 4.4**). Carbon content was not different between the 0 to 10 cm and 10 to 20 cm soil layer but was significantly lower in the 20 to 30 cm soil layer. Nitrogen content followed the same pattern with lowest N content being in the 20 to 30 cm soil layer.

Water extractable organic C and N is reported as mg of WEOC or WEON per kg of soil. This soil fraction is more labile and therefore was sampled at the beginning and end of each grazing season in the 0 to 10 cm soil layer. Water extractable organic C content was impacted by a treatment by year by period interaction ($P = 0.04$; **Figure 4.5**). Both treatments increased in WEOC content from beginning to the end of the trial. In 2018, the SIMP treatment WEOC content decreased from period 1 to period 2 ($P < 0.05$), going from 218.56 ± 13.24 vs. 126.32 ± 12.82 mg/kg soil, respectively. The COMP treatment did not differ being 177.45 ± 13.24 and 138.00 ± 13.63 mg/kg soil in periods 1 and 2, respectively, in 2018. The SIMP treatment was similar between period 1 and period 2 in 2019, being 262.19 ± 13.24 mg/kg in period 1 vs. 223.49 ± 13.24 mg/kg soil in period 2. The COMP treatment did not differ between periods in 2019 at 227.62 ± 13.62 and 214.25 ± 13.24 mg/kg soil for periods 1 and 2, respectively. This was similar to 2020 when the COMP treatment was 275.04 ± 13.63 and 260.71 ± 13.24 mg/kg soil for periods 1 and 2 that year. The SIMP treatment was similar between periods in 2020, containing 270.68 ± 12.82 mg/kg soil and 285.78 ± 13.24 mg/kg soil for periods 1 and 2. Both treatments had higher WEOC in period 2 in 2020 than period 2 in 2018 ($P < 0.05$).

Water extractable organic N content was affected by a year by treatment interaction ($P = 0.03$; **Figure 4.6**). There was also a treatment by period interaction ($P = 0.01$; **Figure 4.7**). In 2018, SIMP had similar WEON content than COMP, 34.24 ± 2.91 vs. 26.42 ± 2.76 mg/kg soil, respectively ($P > 0.10$). The SIMP had similar WEON in 2019, 33.62 ± 2.64 mg/kg soil,

compared to 2018 but was higher than COMP that year, 24.44 ± 2.60 mg/kg soil ($P < 0.05$). In 2020, the SIMP again had similar levels of WEON, 35.38 ± 2.56 mg/kg soil, and was similar to COMP that year, 37.65 ± 2.64 mg/kg soil. The SIMP treatment had higher WEON during period 1 compared to COMP, 41.21 ± 2.43 vs. 31.55 ± 2.33 mg/kg soil respectively, across all three grazing seasons. There was not difference between treatments during the second sampling period, 27.61 ± 2.24 vs. 27.46 ± 2.29 mg/kg soil for SIMP and COMP respectively.

The ratio of WEOC:WEON was affected by a treatment by year interaction ($P < 0.01$; **Figure 4.8**) and treatment by period interactions ($P = 0.02$; **Figure 4.9**). In 2018, treatments were not different ($P > 0.10$). Both treatments had higher ratios in 2019 compared with 2018 ($P < 0.05$). The COMP treatment ratio was lower in 2020 than 2019 ($P < 0.05$) and was similar to SIMP. The simple treatment had a lower ratio in period 1 compared to period 2 ($P < 0.05$) The COMP treatment resulted in similar ratios for both periods.

4.3.3 Soil GHG Flux

Soil CO₂ flux differed by year ($P < 0.01$) and period ($P < 0.01$). The flux in 2018 was significantly higher than those observed in 2019 ($P < 0.01$). The lowest CO₂ flux was observed in 2019 and 2020 had an intermediate flux. The three-way interaction between treatment x period x year was not significant ($P = 0.75$). As this was not significant, differences between periods within years were not explored. Overall CO₂ flux decreased from period 1 to period 2 (27.30 ± 2.18 vs. 17.39 ± 2.06 mg CO₂/m² hr⁻¹, respectively; $P < 0.01$) but treatment did not differ within periods ($P > 0.05$; **Table 4.3**). Day of sampling resulted in significant differences in fluxes. The highest flux was observed on d 3 post grazing at 27.66 ± 2.02 mg CO₂/m² hr⁻¹, which was similar to d2 and d1. However, d4 and d5 were significantly lower than d3 ($P < 0.01$; 18.96 ± 2.06 and 16.55 ± 2.05 mg CO₂/m² hr⁻¹, respectively). Soil temperature at 10 cm depth had a tendency to

explain changes in CO₂ fluxes ($P = 0.06$). Air temperature and precipitation had a significant relationship with CO₂ fluxes ($P < 0.01$).

Soil CH₄ flux was relatively small, and was not impacted by treatment, year, period, the interactions, or sampling day ($P \geq 0.23$; **Table 4.4**). Observed fluxes indicated a balance between CH₄ uptake and release by the soils and ranged from 0.42 to -3.44 $\mu\text{g CH}_4/\text{m}^2 \text{hr}^{-1}$. In period 1 the treatments were similar with the SIMP treatment resulting in a flux of $-1.09 \pm 1.89 \mu\text{g CH}_4/\text{m}^2 \text{hr}^{-1}$ compared to $0.42 \pm 1.88 \mu\text{g CH}_4/\text{m}^2 \text{hr}^{-1}$ for the COMP treatment. Similarly, period 2 had a flux of $-0.38 \pm 1.89 \mu\text{g CH}_4/\text{m}^2 \text{hr}^{-1}$ for the SIMP treatment and $-3.44 \pm 1.88 \mu\text{g CH}_4/\text{m}^2 \text{hr}^{-1}$ for the COMP treatment. Methane flux variation was not explained by any tested environmental variables, perhaps due to the small flux observed.

Soil N₂O flux values are reported here as $\mu\text{g N}_2\text{O}/\text{m}^2 \text{hr}^{-1}$. The flux had a significant year by period interaction ($P < 0.01$; **Table 4.5**). Within year, fluxes were lower in period 2 compared to period 1 in 2018 ($P < 0.01$). In 2019, fluxes were lower in period 1 than in period 2 ($P < 0.01$; -0.31 ± 1.12 vs. $7.74 \pm 1.19 \mu\text{g N}_2\text{O}/\text{m}^2 \text{hr}^{-1}$). The flux in period 1 in 2019 was lower compared to the flux in 2018 ($P < 0.01$) but was similar for period 2. In 2020, the flux in period 1 was similar to fluxes in 2019 and 2018 ($P \geq 0.23$). There was a significant impact of day on N₂O flux ($P < 0.01$) with the flux on d 2 being significantly higher than the flux on d 4 and d 5 (8.00 ± 0.93 vs. 4.41 ± 0.89 and 4.04 ± 0.89 , respectively). Fluxes were impacted by precipitation, temperature, and relative humidity ($P < 0.01$).

4.4 Discussion

The agricultural industry is tasked to supply a growing global population with high quality, nutritious foods, to build more resilient grazing systems to combat increasing climatic

variability and provide ecosystem services to improve environmental quality (Ward et al., 2016; Garrett et al., 2013). One highly valued ecosystem service is the capability of grasslands to store carbon, as they represent the largest global pool of terrestrial carbon, and identifying management interventions to increase the sequestration potential would help meet these demands (Jobbagy and Jackson, 2000; Heidenreich, 2009; Teague et al., 2016). As expected SOC and TSN varied by year of sampling which agrees with previous literature as these stocks display spatial and temporal variability (Chiavegato et al., 2015a; Cannell et al., 1999). Chiavegato et al. (2015a) reported SOC stocks ranging from 42.0 to 63.0 Mg/ha and TSN stocks ranging from 3.44 to 4.85 Mg/ha on the same farm, in the range of those reported here. It was not anticipated that SOC and TSN stocks would decline at the end of the study. That both stocks followed the same pattern was not surprising as C and N are covalently linked in soil OM (Schipper and Sparling, 2011). Carbon losses can occur through several different pathways including respiration, leaching, removal of biomass by grazing animals, decomposition or erosion (Ward et al., 2016). Management practices, therefore, can alter the SOC and TSN stocks by determining the amount and type of organic matter input and the extent of soil degradation (Paul et al., 1997). The original hypothesis for this experiment anticipated that the forage mixture would have increased forage productivity and therefore result in increased soil C accumulation, due to the relationship between above ground productivity and soil C storage (Six et al., 2002). The results of this experiment reject that hypothesis. As we indicated in (Chapter 2), forage productivity did not differ between the two treatments which could partially explain the lack of treatment difference in the SOC and TSN stocks, but not the decline in stocks. One potential explanation for the loss in soil C and N was the legacy of prior management. The forage mixtures were established in the fall of 2017 after tillage to cease a previous grazing experiment on annual

covercrops. Soil nutrient stocks often change slowly and accurate estimates of gain or loss in nutrient levels typically requires long sampling durations on land under consistent management. In this experiment that was not the case, as these treatments were established on replicated research plots where legacy effects can alter the short-term change in nutrient stocks. The management impact is highlighted by comparing bulk density values observed in this experiment to those of Chiavegato et al. (2015a) on the same farm who reported a range of 1.20 to 1.62 g/cm³ over two years for pastures under intensive grazing management. The values reported here are consistently lower at a range of 0.84 to 1.31 g/cm³ and would agree with Franzluebbbers and Stuedemann (2009) that tillage resulted in reduction in bulk density values. Additionally, Cotrufo et al. (2019) highlighted that the particulate associated organic matter is more vulnerable to disturbance and cycles faster than mineral associated organic matter. A potential solution could have been to provide a space-for-time substitution to represent pre-trial conditions (McLauchlan, 2006). Another possible explanation, and most likely a cofactor to the disturbance, is a need for more soil samples at each sampling period (Smith et al., 2020). Soil nutrients are spatially variable, and it is possible that this experiment did not capture that variability adequately sampling two experimental units per treatment, and therefore did not obtain accurate estimates of SOC and TSN (Smith et al., 2020; Minasny et al., 2017). Due to the confounding effects of previous experiments, tillage, and the short-term nature of this trial, more long-term monitoring at this site will be required. As well, expanding sampling onto producer sites, with well documented management, may also be more plausible to alleviate the perpetual legacy effects often seen in replicated plot research.

Both WEOC and WEON pools were within the range expected for pasture soils (Haney et al., 2018). Water extractable pools of organic C and N are labile and represent the microbe-

available soil C and N pools and was suggested as a predictor of N mineralization potential (Castro Bustamante et al., 2016; Haney et al., 2012). The physical disturbance of tillage was shown to increase SOC decomposition rates, particularly that in the labile C pool (McLauchlan, 2006). This would agree with the WEOC and WEON results observed in this trial, where this labile pool increased in concentration as the trial progressed. Tillage alters the physical, chemical and biochemical mechanisms in the soil, increases erosions, and alters aggregate stability (McLauchlan, 2006; Shepherd et al., 2001) but can rebuild after the planted pasture was established. Both treatments had significantly higher levels of WEOC at the end of the experiment compared to the beginning. Additionally, the COMP treatment had significantly higher WEON in 2020 compared to the previous two years. It was hypothesized that these pools can be used as metrics for soil health and can be sensitive to management decisions (Ghani et al., 2003), and these results would indicate that both treatments were beneficial in improving the “quality” of microbial-available C after disturbance, even as total C pools declined (Haney et al., 2012; 2018). The total C pool is largely inactive and does not provide insight into the substrate that is available to microbes to drive the nutrient cycles. The WEOC:WEON ratio was proposed by others to be a more sensitive indicator of management on soil nutrient pools (Haney et al., 2012). The low ratios observed in this experiment would indicate ample potential for N mineralization for both treatments and that pasture soils were high quality.

The C:N ratio in soils have been hypothesized as an indicator of a soil's ability to sequester C (Averill et al., 2014). We hypothesize that the low WEOC:WEON ratio observed during this experiment would indicate that, with extended monitoring, we would see treatments begin building soil C and N after a lag phase following conversion to perennial pasture (Machmuller et al., 2015). In addition to the tillage in 2017, some experimental units utilized in

this experiment were under continuous cropping containing brassica species which may add further legacy effect to the studied soils. Cotrufo et al. (2019) observed that soil C:N ratios are lower in soils dominated by mineral associated organic matter and higher and more variable with particulate organic matter (plant derived organic matter). The C:N ratio observed in the total C and N stocks would indicate the soil organic matter being dominated by the mineral associated pool, which eventually saturates, as expected for grassland soils. However, considering the WEOC and WEON fraction is within the range of healthy pasture soil, and soils can undergo a lag phase after tillage prior to increasing soil C (Machmuller et al., 2015), we postulate that with time the pasture soils will accumulate soil C via particulate organic matter as this pool recovers from disturbance. In support of this theory, Chiavegato et al. (2015a) reported total C:N ratio of 19.4 for the same farm on established pasture-land, which was proposed to indicate C and N immobilization is the predominate process on the part of the farm that was under established management intensive grazing (Haney et al., 2012; Du Preez and Snyman, 1993), and total SOC of 63.0 Mg/ha. That would indicate those soils had higher levels of particulate organic matter than the current experiment and the potential for the current soils to increase total organic C.

Soil CO₂ fluxes were lower than those previously observed on the same farm (Chiavegato et al., 2015b), although differing treatments and time of sampling may explain these differences. Those authors reported average CO₂ fluxes of 171 mg CO₂/m² hr⁻¹, much higher than the range of 17.20-28.07 mg CO₂/m² hr⁻¹ observed in this study. Similar to this study, Chiavegato et al. (2015b) sampled twice per grazing season, but the second sampling period was earlier than the current experiment (starting date of August 13, August 21 and August 26 over 3 successive grazing seasons), and sampled for 14 d compared to 5 in this study. Additionally, the stocking density in that trial was much higher with a range of 32,000 to 112,000 kg BW/ha compared to a

maximum of 2,000 kg BW/ha in the current trial. This results in differing forage utilization rates and trampling effects that could explain part of the differences observed. However, the values reported here were within the lower end of the range reported by Frank and Karn (2003).

Mielnick and Dugas (2000) measured CO₂ flux on a tall grass prairie and reported values similar to those here when in the same temperature range (4.3 to 27.28°C). In this study, we anticipated the CO₂ flux might be more stable in the COMP treatment due to increased forage diversity, but this was rejected. Grazing management was shown to alter soil respiration rates by altering microbial activity based on utilization rates (and its subsequent impact on soil temperature through reduced leaf canopy and light infiltration) and trampling effects (Chiavegato et al, 2015b; Soussana et al., 2004; Bremer et al., 1998). In this experiment stocking rate and density were the same for each experimental unit, although in 2019 and 2020 the COMP treatment was rotated daily compared to every other day for the SIMP treatment at the onset of grazing (Chapter 2). With the observed fluxes being similar, we hypothesize that adoption of forage treatments of similar productivity and quality (Chapter 2), and under similar management and soil characteristics, there is no impact on soil respiration rate. It was posited that pastureland may be a sink of CO₂ during peak biomass growing periods (Frank, 2002; Soussana et al., 2007).

Frank (2002) compared CO₂ flux of a northern prairie to plant western wheat grass and reported daily fluctuations at different points during the growing season. Values from this experiment fall within the range reported for the tall grass prairie (-70 to 189 g CO₂ m² s⁻¹), as well as at similar points in the growing season. Similarly, during periods of CO₂ uptake (June 25th in that study) they found fluxes had increased variation from day-time to night-time with peaks of CO₂ uptake at approximately 1000 hr. This could be a potential explanation for our results, we may have a bias based on time of sampling that resulted in lower fluxes than previous experiments on farm.

The temperate grasses used in this experiment would be expected to have two peak growing periods, the first at the late spring/early summer and a smaller peak in the fall (Sharpe and Rayburn, 2018), which would align with our first sampling period and the second period seeming to fall after that secondary growth peak. This would agree with Soussana et al. (2007) who reported that maximum CO₂ uptake in temperate European grasslands occurred in the late spring/early summer. Precipitation had a positive relationship with CO₂ flux, as expected based on previous literature (Frank, 2002; Mielnick and Dugas, 2000). It was expected that air temperature would impact CO₂ flux, but the negative relationship observed was not anticipated (Frank, 2002; Chiavegato et al., 2015b; Harper et al., 2005). This could partially be explained by the time of sampling, the greatest fluxes are typically observed during hotter months with intermediate soil moisture content (Harper et al., 2005), which would not have fallen into our sampling window, highlighting the limitations of the static chamber methodology. However, variation in climatic conditions and biomass accumulation have been shown to cause variability in CO₂ flux, particularly under grazing (Frank, 2002; Soussana et al., 2007; Harper et al., 2005). The results of this experiment indicate that these pastures post-grazing are a small source of CO₂ during peak biomass accumulation. Additionally, the final sampling period flux observed in this trial being lower than those early in the growing season was expected, as previous literature has shown that CO₂ fluxes in the fall and winter are typically lower (Frank and Dugas, 2001; Frank, 2002; Mielnick and Dugas, 2000).

However, an additional explanation for the CO₂ flux being lower than similar experiments is it could be an artifact of the methodology utilized. The static chamber methodology utilized in the experiment does have known limitations on spatial and temporal flux calculations, which may alter the microenvironment thereby altering soil GHG flux estimates,

technician bias, and is short term monitoring limits interpretations to that snapshot in time and making comparisons across experiments difficult. Differing measuring techniques, such as eddy covariance, that more accurately and precisely monitors the soil:atmosphere interface may help better understand the gas fluxes of these forage mixtures.

The CH₄ fluxes observed in this experiment indicated a near balance between methane uptake and release. This was similar to values reported by Chiavegato et al. (2015b) and Liebig et al. (2010) who both observed grazed pastures emitting and absorbing CH₄ depending on time of year and environmental conditions. Surprisingly, we did not observe an impact of environmental conditions on CH₄ fluxes. However, considering the relatively stable values observed, we may not have had enough variation in flux for an impact to be observed. Oxidation by soil microbes is an important component in the lifecycle of CH₄. Oxidation by -OH radicals in the atmosphere is the main global CH₄ sink, but oxidation of CH₄ by soil microbes is the only other major sink of atmospheric CH₄, estimated to remove 9 to 47 Tg(CH₄) yr⁻¹ (Saggar et al., 2008; Hartmann et al., 2011; IPCC, 2013). The ecological controls of this process are poorly understood, but the majority of soil CH₄ oxidation occurs in upland soils by obligate aerobic methanotrophs. However, soils can either be a source or sink depending on environmental conditions present and biological activity of microbes (Mosier et al., 1998). Microbial activity is dependent on soil diffusion rate, with CH₄ oxidation increasing as less water fills the pore networks and emission rate increasing as moisture content increases (Mosier et al., 1998; Hartmann et al., 2011). Previous experiments have shown that intensely managed agriculture lands have the lowest CH₄ sink potential due to increased disturbance and fertilizer application (N suppression of methanotroph activity; Mosier et al., 1998). This could be a potential explanation of the observed flux rate, as our soils were disturbed prior to experiment initiation

for the current and a previous experiment 3 years prior. We did not measure moisture status of the soil, which could be another explanation observed fluxes. Seasonal variability in soil CH₄ uptake/emission was shown in previous studies (Mosier et al., 1998). When dry soil conditions are present during the summer months, soil methanotroph activity is increased resulting in increased oxidation, and the opposite occurs during cold and wetter months where this activity is suppressed and methanogen activity is higher resulting in emissions (Mosier et al., 1998). Some literature has suggested that agricultural soils may be able to offset part of a forage mixtures enteric methane emissions, but experimental results on this are sparse and inconsistent (Saggar et al., 2004; 2007). The results of this experiment would not suggest that that the forage mixtures utilized on the present soil type would offset part of the enteric CH₄ budget.

The observed N₂O flux in this experiment were within the range of previous experiments (Chiavegato et al., 2015b; Liebig et al., 2010; Jackson et al., 2015) and were similar between the two treatments. Liebig et al (2010) compared native pasture under heavy grazing (1.1 steers/ha) or moderate grazing pressure (0.39 steers/ha) and reported a range of -53.4 to 48.1 μg N₂O/m² hr⁻¹ and -53.1 to 81.5 μg N₂O/m² hr⁻¹ for heavy and moderate grazing, respectively. Similarly, Jackson et al. (2015) measured N₂O flux from cool-season pastures under different management strategies and reported post-grazing pastures could be a source of N₂O emissions, but major emission events were related to significant precipitation events. In this study, N₂O fluxes were associated with precipitation, temperature, and relative humidity. Flux was increased after precipitation events, in agreement with previous experiments (Jackson et al., 2015; Chiavegato et al., 2015b) and decreased slightly with increases in temperature and relative humidity. As we did not measure soil water content, precipitation was used as a proxy and when soil moisture is low, little N₂O is produced via nitrification as nitrate is favored. As moisture content increases

nitrification shifts to denitrification and mineralization of soil organic N is increased resulting in increased N₂O production, until soil moisture reaches field capacity. That N₂O is dependent on the nitrification/denitrification processes in soil, the WEON results in this experiment may help explain why the treatments performed similarly. The WEOC and WEON tests utilized in this experiment have been used as a metric to determine N mineralization potential in soils (Haney et al., 2002). Treatments did not significantly differ in WEON or WEOC indicating the available N pool for transformation (mineralization) was similar between treatments and therefore as environmental conditions favored N₂O flux (ie. precipitation), both treatments would be expected to produce similar fluxes unless management decisions altered soil microbial activity, which did not occur (Granli and Bockman, 1995). Additionally, both treatments generally had similarly low C:N ratios and similar WEOC pools over the trial. Labile organic C and N is thought to be the main energy source for soil microbes that ultimately drives the nutrient cycles (Granli and Bockman, 1995; Haney et al., 2012). Therefore, not only did both treatment soils have similar levels of substrates but similar levels of “fuel” to drive the transformation of N and, ultimately, the production of N₂O when environmental conditions were favorable.

4.5 Conclusions

The results of this experiment indicated that adoption of forage bases of differing diversity, alone, would not cause shifts in soil C:N cycling with management being equal over the short-term. The treatments utilized in this experiment resulted in similar forage productivity and nutritive value (Chapter 2) and did not alter soil GHG fluxes, SOC or TSN, or WEOC and WEON. We hypothesize the legacy effects of prior management (i.e., tillage and prior experiments) influenced changes in soil C:N cycling and environmental conditions explained differences in soil GHG fluxes. However, the limitations of the methodology utilized here (static

chamber methodology) only gave a snapshot in time on soil GHG flux. Additionally, interpretation of these results are limited to the management applied and the soil type present, as soil types have been shown to respond to management differently. The soil C:N pools in the studied soils would indicate that both treatments had similar, high quality nutrients with the potential for additional soil C accumulation through increased particular organic matter. In totality, monitoring experiment indicates that these forage mixtures performed similarly, but the COMP treatment had a tendency to reduce enteric CH₄ which would not be offset by changes in soil GHG fluxes. However, soil C pools move slowly, and the methodologies applied in this experiment, in conjunction with the relatively short-term nature of this experiment, necessitates additional monitoring of these grazing forage mixtures to better understand the long-term tradeoffs between these mixtures. Additionally, the utilization of alternative techniques such as eddy covariance would allow for more robust field level monitoring than was conducted in this experiment

Figure 4.1: Lake City, MI 30-year average temperature and observed temperatures

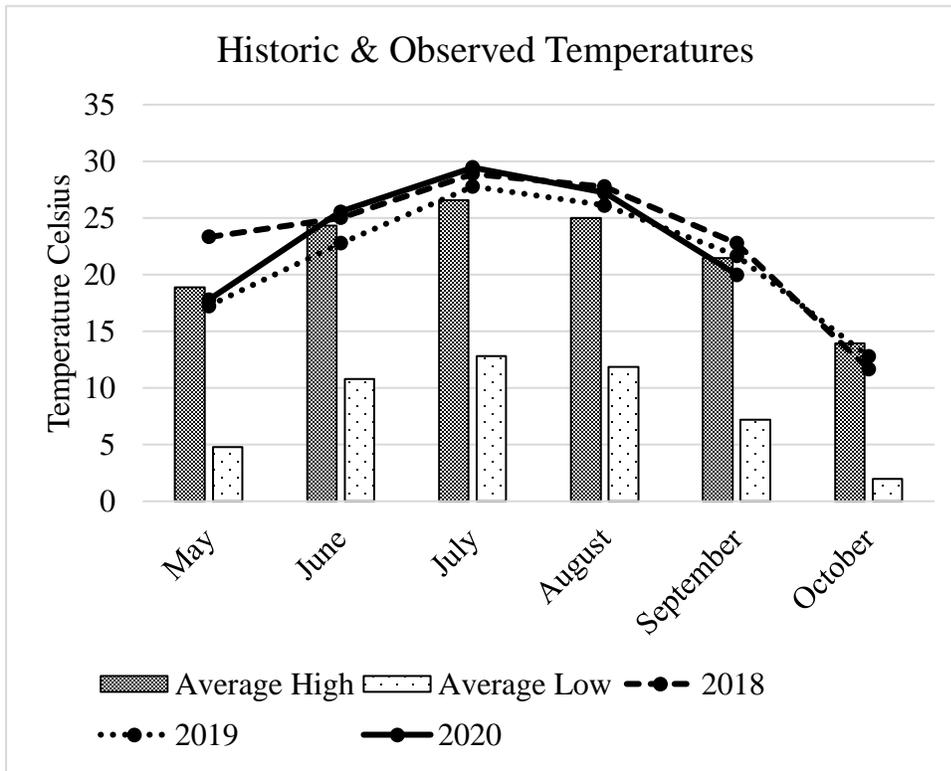


Figure 4.2: Lake City, MI 30-year average precipitation and observed precipitation

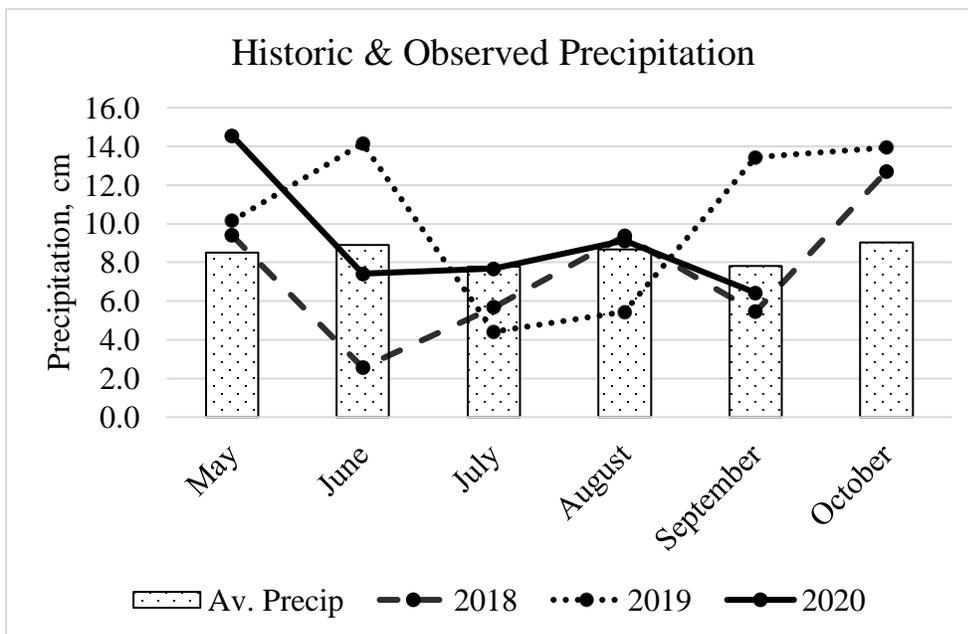


Table 4.1: Soil bulk density, g/cm³, by depth and year

BD g/cm ³	
Depth	Average ¹
0 to 10	0.92 ^c
10 to 20	1.11 ^b
20 to 30	1.24 ^a
SEM	0.02

BD g/cm ³	
Year	Average ²
2018	1.04 ^B
2020	1.13 ^A
SEM	0.02

<i>P</i> -Values	
Treatment	0.26
Depth	<0.01
Treatment*Depth	0.35
Year	<0.01
Treatment*year	0.41

¹Differing lowercase letters indicates a significant difference between depths ($P \leq 0.05$)

²Differing uppercase letters indicates a significant difference between years ($P \leq 0.05$)

Table 4.2: Total soil organic C and N, Mg/ha, adjusted by equivalent soil mass

Total Soil organic C and N Mg/ha					
SOC Mg/ha ^{1,2}					
	Treatment			P-values	
Year	2018	2020		Treatment	0.80
SIMP	48.64	46.75		Year	<0.01
COMP	47.37	44.29	SEM	Treatment*Year	0.48
AVERAGE	48.00 ^A	45.52 ^B	3.34		
TSN Mg/ha					
	Treatment			P-values	
Year	2018	2020		Treatment	0.92
SIMP	4.12	3.94		Year	0.01
COMP	4.07	3.91	SEM	Treatment*Year	0.91
AVERAGE	4.10 ^A	3.92 ^B	0.19		
C:N Ratio					
	Treatment			P-values	
Year	SIMP	COMP		Treatment	0.81
SIMP	11.61	11.61		Year	0.08
COMP	11.46*	11.19		Treatment*Year	0.08
SEM	0.73				

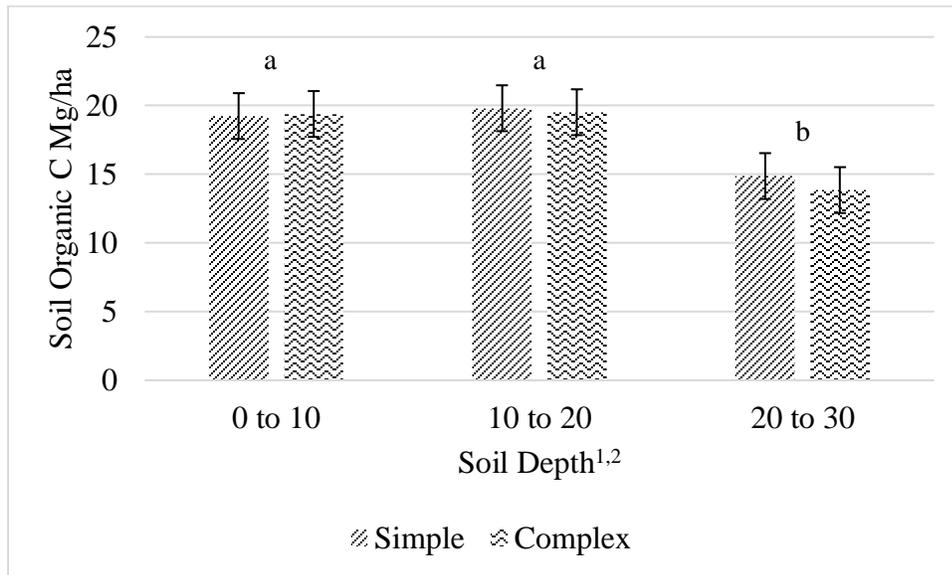
¹Differing letters indicate a significant difference ($P \leq 0.05$) ; SIMP= simple forage mixture,

COMP= complex forage mixture

²Equivalent soil mass equation from Ellert et al. (2002a)

*COMP tended to have a higher C:N ratio in 2018 compared to 2020 ($P = 0.07$)

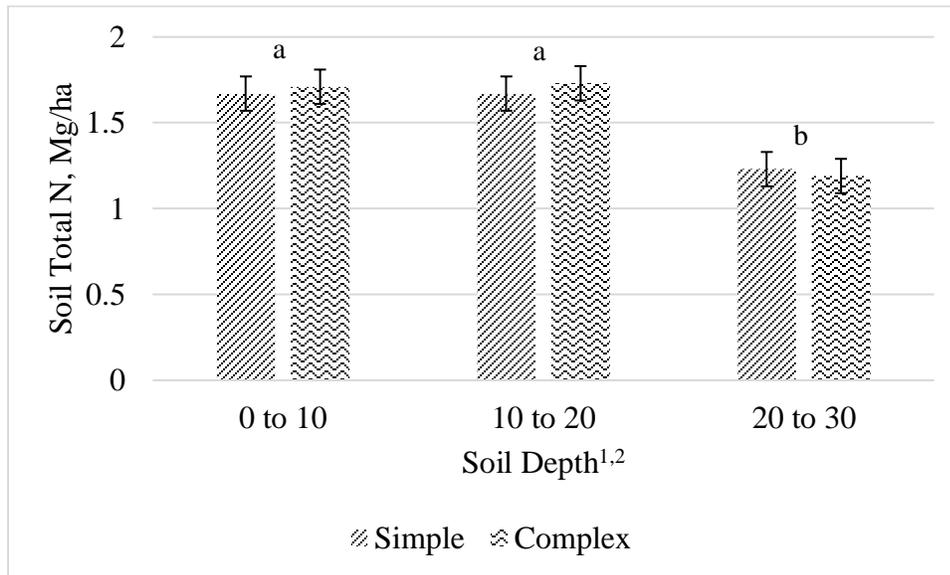
Figure 4.3: Soil organic carbon by depth, Mg/ha, adjusted by equivalent soil mass



¹Differing letter indicates a significant difference between depths ($P \leq 0.05$) ; SIMP= simple forage mixture, COMP= complex forage mixture

²Equivalent soil mass equation from Ellert et al. (2002a)

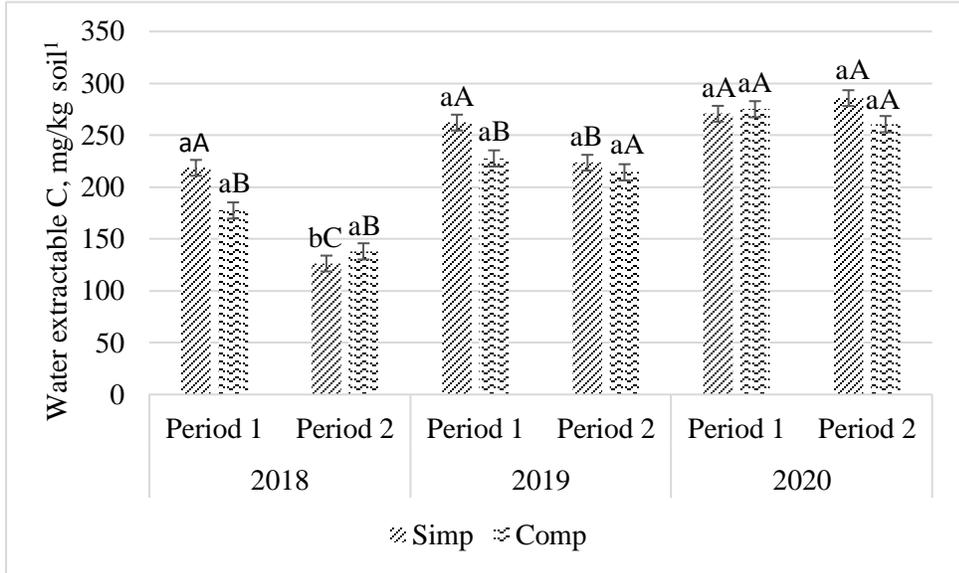
Figure 4.4: Soil total nitrogen by depth, Mg/ha, adjusted by equivalent soil mass



¹Differing letter indicates a significant difference between depths ($P \leq 0.05$) ; SIMP= simple forage mixture, COMP= complex forage mixture

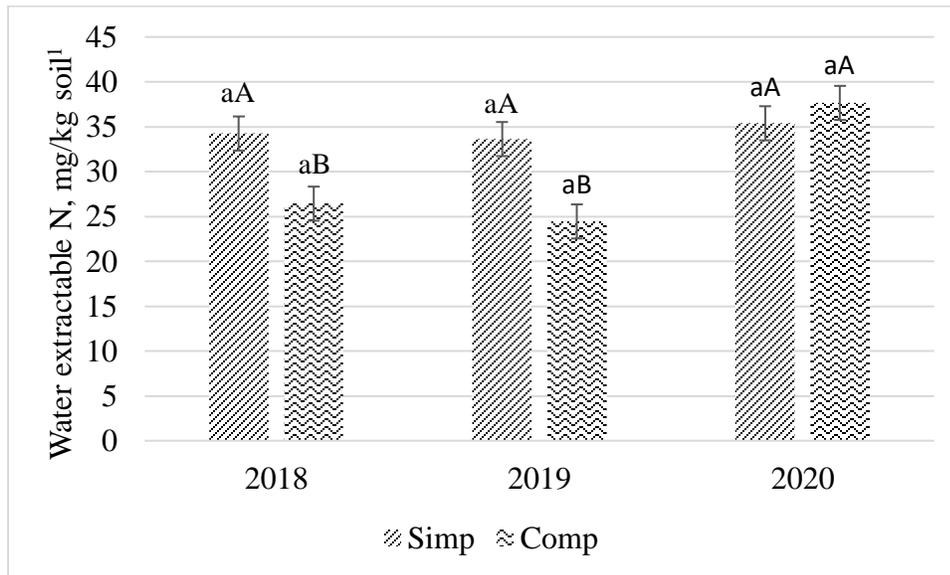
²Equivalent soil mass equation from Ellert et al. (2002a)

Figure 4.5: Water extractable organic C in the top 10 cm of soil, mg/kg



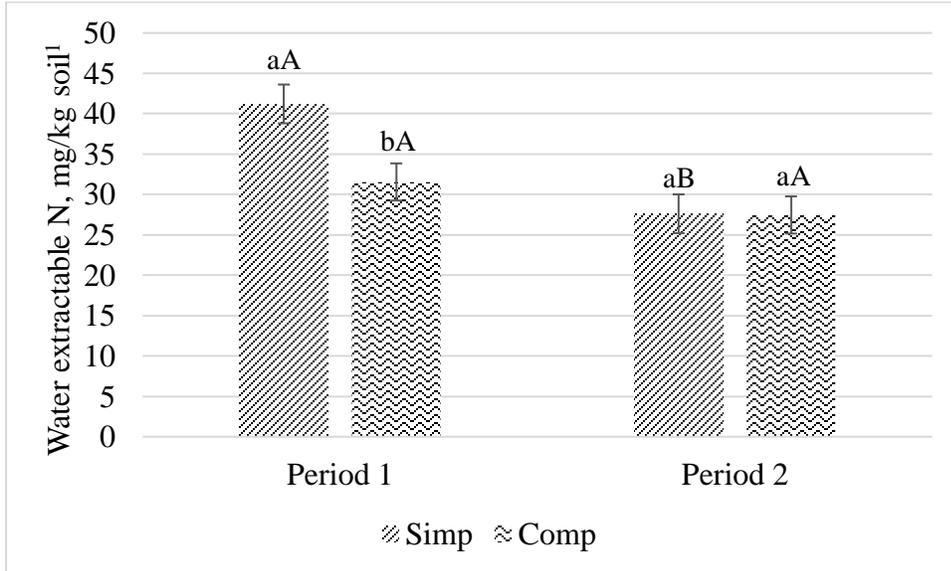
¹There was a significant year by treatment by period interaction ($P = 0.04$). No difference was observed between treatments within period, respective of year; Differing lowercase letters indicates a significant difference between periods within year, uppercase letters indicate significant difference between years ($P \leq 0.05$); SIMP= simple forage mixture, COMP= complex forage mixture

Figure 4.6: Water extractable organic N by year in the top 10 cm of soil, mg/kg



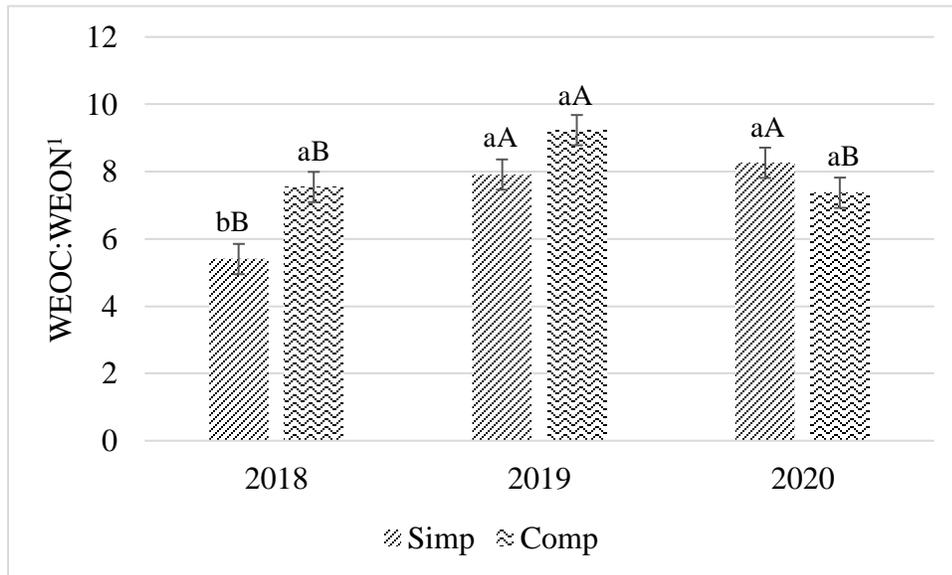
¹There was a significant year by treatment interaction ($P = 0.03$). Differing lowercase letters indicates a significant difference within year, differing uppercase letters indicates a significant difference between year ($P \leq 0.05$); SIMP= simple forage mixture, COMP= complex forage mixture

Figure 4.7: Water extractable organic N by period in the top 10 cm of soil, mg/kg



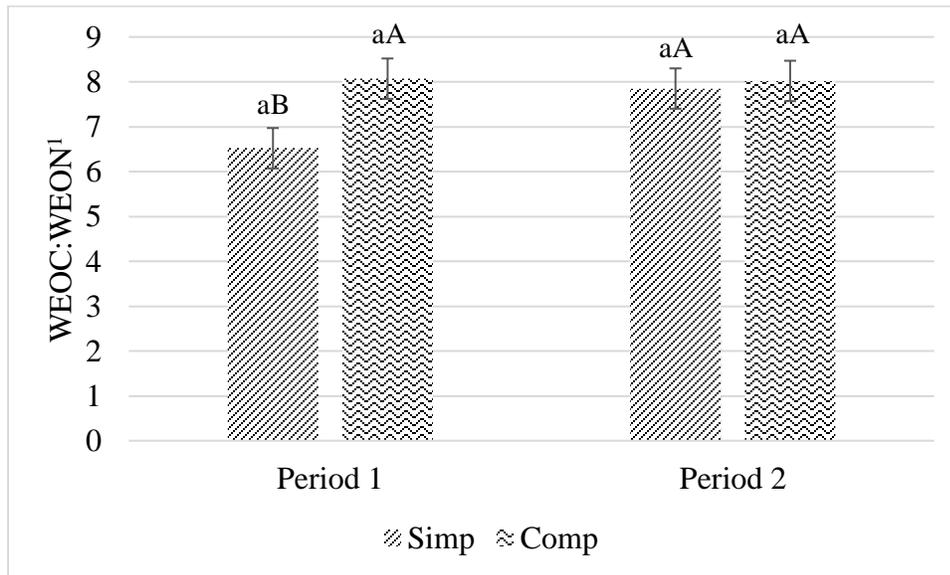
¹ There was a significant treatment by period interaction ($P = 0.01$). Differing lowercase letters indicates a significant difference within period, differing uppercase letters indicates a significant difference between period ($P \leq 0.05$); SIMP= simple forage mixture, COMP= complex forage mixture

Figure 4.8: Water extractable organic C:N in the top 10 cm of soil by year



¹ There was a significant year by treatment interaction ($P < 0.01$). Differing lowercase letters indicates a significant difference within year, differing uppercase letters indicates a significant difference between year ($P \leq 0.05$); SIMP= simple forage mixture, COMP= complex forage mixture

Figure 4.9: Water Extractable organic C:N in the top 10 cm of soil by period



¹ There was a significant treatment by period interaction ($P = 0.02$). Differing lowercase letters indicates a significant difference within period, differing uppercase letters indicates a significant difference between period ($P \leq 0.05$); SIMP= simple forage mixture, COMP= complex forage mixture

Table 4.3: Soil CO₂ flux mg/m² hr⁻¹ by period and year measured using static chambers

	Flux ^{1,2}	SEM	Year	Flux	SEM
Period 1	27.30 ^a	2.18	2018	31.41 ^A	2.56
Period 2	17.39 ^b	2.06	2019	13.49 ^B	2.27
			2020	22.15 ^A	2.67

<i>P</i> -Values		Environmental Variables	
Treatment	0.72	Precipitation (mm)	0.02
Year	<0.01	Soil Temperature at 10 cm (°C)	0.06
Period	<0.01	Air Temperature (°C)	<0.01
Day	<0.01	Relative Humidity	0.57
Treatment*Period	0.82		
Treatment*Year	0.57		
Year*Period	0.44		
Treatment*Period*Year	0.75		

¹Differing letters indicates a significant difference ($P \leq 0.05$); SEM= standard error of the mean

²2018 sampling periods: Period 1- June 4 through June 8, Period 2- October 1 through October 5; 2019 sampling periods: Period 1- May 27 through May 31, Period 2- September 24 through September 28; 2020 sampling periods: Period 1- June 1 through June 5, Period 2- September 14 through September 18

Table 4.4: Soil CH₄ flux $\mu\text{g}/\text{m}^2 \text{hr}^{-1}$ by period averaged across 2018 through 2020 grazing seasons measured using static chambers

	Treatment ¹	
	SIMP	COMP
Period 1	-1.09	0.42
Period 2	-0.38	-3.44
SEM	1.89	1.88

	<i>P</i> -Values	Environmental Variables
Treatment	0.79	Precipitation (mm) 0.42
Year	0.54	Soil Temperature at 10 cm (°C) 0.97
Period	0.65	Air Temperature (°C) 0.48
Day	0.97	Relative Humidity 0.68
Treatment*Period	0.22	
Treatment*Year	0.75	
Year*Period	0.73	
Treatment*Period*Year	0.40	

¹2018 sampling periods: Period 1- June 4 through June 8, Period 2- October 1 through October 5; 2019 sampling periods: Period 1-

May 27 through May 31, Period 2- September 24 through September 28; 2020 sampling periods: Period 1- June 1 through June 5,

Period 2- September 14 through September 18; SIMP= simple forage mixture, COMP= complex forage mixture; SEM= standard error

of the mean

Table 4.5: Soil N₂O flux $\mu\text{g}/\text{m}^2 \text{hr}^{-1}$ by year measured using static chambers

2018	Flux ^{1,2}	SEM	2019	Flux	SEM	2020	Flux	SEM
Period 1	13.44 ^{aA}	1.45	Period 1	-0.31 ^{bB}	1.12	Period 1	8.61 ^{aA}	1.68
Period 2	1.13 ^{bA}	2.33	Period 2	7.47 ^{aA}	1.19	Period 2	5.78 ^{aA}	1.31

<i>P</i> -Values		Environmental Variables	
Treatment	0.66	Precipitation (mm)	<0.01
Year	<0.01	Soil Temperature at 10 cm (°C)	0.31
Period	0.09	Air Temperature (°C)	<0.01
Day	<0.01	Relative Humidity	<0.01
Treatment*Period	0.08		
Treatment*Year	0.91		
Year*Period	<0.01		
Treatment* Period*Year	0.08		

¹Differing lower case letters indicates significant difference between sampling period within year, uppercase letters indicates a significant difference between years ($P \leq 0.05$); SEM= standard error of the mean

²2018 sampling periods: Period 1- June 4 through June 8, Period 2- October 1 through October 5; 2019 sampling periods: Period 1- May 27 through May 31, Period 2- September 24 through September 28; 2020 sampling periods: Period 1- June 1 through June 5, Period 2- September 14 through September 18

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CHAPTER 5
SUMMARY AND CONCLUSIONS

The objective of this dissertation research was to add to our understanding of how management decisions can impact the sustainability of an upper Midwest grazing forage mixtures. Improving the sustainability of beef production can be thought of as: 1) improving its environmental impact, 2) maintaining social license to operate, and 3) making it economically viable. The sector contributing the most to the U.S. beef industry's carbon footprint is via grazing animals, estimated to be 70 to 80% of the total footprint. This dissertation examined this sector through two of the three legs of sustainability: environmental and economic.

Using data collected from 2011 to 2018 at the Lake City AgBioResearch station, we modeled the relationship between cow body size and age on calf weaning weights and economic returns. We conducted a backwards looking enterprise budgeting analysis and forward looking net present value analysis to determine what size cow is economically optimum in the local environment. This is an important decision for producers, as matching cow size to the production environment allows for more efficient utilization of forage resources. However, this can change from region to region depending on local climate and management. Additionally, this may partially meet the goals of sustainable intensification. This concept is that for lands used for agriculture, we should maximize production per unit of land in a manner that meets all three pillars of sustainability. We found, as expected, that heavier cows did in fact wean heavier calves. However, this advantage was small, and lighter body weight cows weaned significantly more of their body weight.

From 2011 through 2018, there was no difference in expected net returns per head between cows of differing body size. However, when this was calculated on per ha basis, lighter weight cows weaned more per ha and for each 22.67 kg increase in cow body size, net returns per ha decreased \$10.27. Next, a net present value, the value of a cow today adjusted for

calculated lifetime returns, was calculated for modeled cow herds beginning at 430.84 kg and increasing in 22.67 kg increments to 634.92 kg. A baseline grazing scenario of 200 d was chosen based on average grazing days for the region. Additionally, a sensitivity analysis was conducted with grazing d being increased or decreased in 5 d increments resulting in a range of modeled scenarios between 175 to 225 grazing d. A grid search was conducted and at the baseline scenario, net present value was maximized at a cow body size of 453.51 kg. However, when grazing days were reduced the 430.84 kg cow size model increased its projected net present value through the 185 grazing d scenario. This may indicate that producers utilizing lighter cows may provide producers protection against adverse weather events and climatic variability. Additionally, utilization of lighter weight cows would maximize both the economic returns and amount of product per unit of land in the upper Midwest. However, what is best for the grazing sector may not be best for downstream sectors such as meat packers and processors. More research is needed to determine the impact that smaller cows would have in the finishing sector.

Research in the final two chapters compares the environmental tradeoffs between two different grazing forage mixtures: a biologically diverse, mixed species pasture and an alfalfa:orchardgrass pasture. Over three grazing seasons, we measured enteric CH₄, forage productivity and quality, soil N₂O, CH₄, and CO₂, as well as soil C and N stocks. We hypothesized that the COMP treatment would have improved forage productivity and quality and this would result in reduced enteric CH₄ emissions. The increased forage productivity was hypothesized to lead to increased soil C sequestration. Additionally, the increased forage diversity would result in more stable soil GHG fluxes compared to the SIMP forage mixture.

Treatments did not differ in forage productivity within years, and only differences between years were observed. Forage quality metrics had a year by treatment interaction for

most nutrients. The COMP treatment had greater IVTDMD48 for the first two years, and over the three grazing seasons had lower ADF content than SIMP. However, both treatments saw reductions in forage quality metrics over the three grazing seasons, perhaps due to both treatments being dominated by grass species by the third year of grazing. Forage DMI was highly variable across and within years with no consistent difference observed between the treatments. We hypothesized that this may have been due to the dual marker method utilized in this experiment. To test this, we calculated forage intake using NASEM (2016) equations for growing cattle and found that lowest values observed in this experiment were lower than calculated values. Additionally, no difference was observed between treatments using the calculated values.

Animal performance did not differ between treatments. Increases in live weight gain between years corresponded to differences in grazing d. Average daily gain ranged from 1.02 to 0.84 kg/d and was greater for steers than heifers. Enteric CH₄ tended to be lower for COMP compared with SIMP, but there was no difference in emission intensity. The botanical composition of the COMP treatment may have resulted in more rapid rate of passage compared to SIMP, but the SIMP treatment possibly resulted in more efficient conversion of consumed feedstuffs into kg of gain.

Total soil C and N were adjusted using equivalent soil mass calculations to account for changes in soil bulk density. We found that both SOC and TSN decreased from the first year of the experiment through the end of the third year. The decline in stocks was not anticipated for this study. We hypothesize that it could be due to prior management of the experimental soils, tillage in fall of 2017 and previous annual cropping, and the need for more soil samples at each sampling period. Soils have considerable spatial variability and it is possible that we did not

capture this variability adequately. Water extractable C, labile pool of soil C, was greatest for both treatments at the end of the study. Similarly, the COMP treatment had higher WEON content at the end of the study. These labile pools have been used as a metric for soil mineralization potential, and these results would indicate that mineralization (ie. that is nutrients available for use) were highest at the end of the experiment. When including the fact that the SOC:TSN ratio ranged from 11:61 to 11.19, there may be the potential for these soils to increase the C pools through increases in particulate organic matter, such as plant derived organic matter.

Treatments did not differ in soil GHG fluxes, but there were differences between years for soil N₂O and CO₂ flux. Soil CH₄ fluxes were generally small and did not have large variability. Precipitation and air temperature both explained considerable amounts of variation in CO₂ and N₂O fluxes. The labile N pools being similar between both treatments may help explain the similarities observed between treatments. Both treatments had similar levels of N available for transformation and similar amounts of “fuel”, WEOC, for microbes.

There is a dearth of studies comparing the environmental tradeoffs of differing grazing studies. The results of this experiment indicated that SIMP and COMP treatments would not have altered environmental footprints over the short-term. However, this experiment is limited by the methodologies utilized and the relatively short duration. Additionally, both treatments were managed the same, therefore it may be possible that under divergent management differences may occur. As new methods for monitoring GHG fluxes of both the soil-plant interface and animals are developed, there is a need for more long-term monitoring.

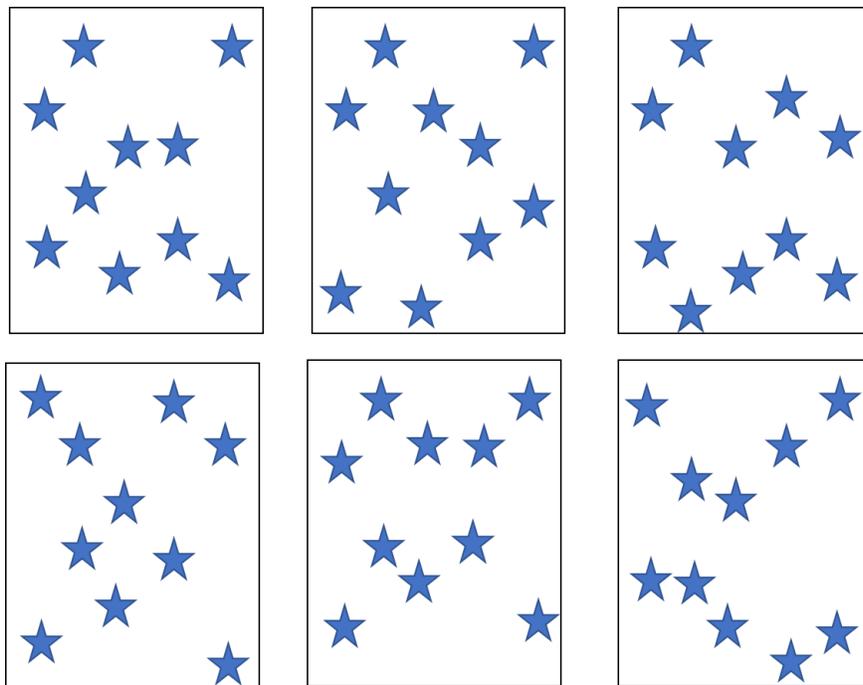
APPENDIX

Figure A.1: Experiment pastures outlined by treatment at the Lake City AgBioResearch Center¹



¹SIMP= simple forage mixture; COMP= complex forage mixture

Figure A.2: Visualization of static chamber placement using a 10-section grid¹



¹Each star represents a chamber placed within a single grid section