

Downstream effects of brown seaweed supplementation in beef cattle diets on greenhouse
gas emissions and coprophagous beetles

by

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Abstract

The Canadian livestock sector is a significant source of greenhouse gas (GHG) emissions. To reduce enteric methane emissions, the sector has started using mitigation strategies, such as dietary interventions. While studies have shown that supplementing beef cattle diets with brown seaweed can reduce enteric methane emissions, the downstream effects of dietary supplementation remain unclear. This thesis explores the downstream effects of supplementing beef cattle diets with brown seaweed on dung and beetle-mediated GHG emissions, as well as on coprophagous beetles' reproductive success and resource use and selection. To assess these effects, dung was collected from beef cattle assigned to four treatment groups based on the dietary level of brown seaweed inclusion (i.e. 0%, 0.5%, 1%, 2%).

Laboratory-based experiments revealed that dietary supplementation, at a rate of 2% of dry matter intake (DMI), reduced dung CH₄ emissions by 49% and CO₂ emissions by 26%, resulting in a 30% reduction in dung CO₂e emissions. While dietary supplementation with brown seaweed reduced dung's baseline CH₄ emissions, the relative beetle-mediated reductions remained identical. This suggests that supplementing beef cattle diets with brown seaweed had no effect on *C. erraticus*' CH₄ mitigation potential, as the smaller absolute effects noted in supplemented dung samples were caused by lower initial CH₄ emissions. Additionally, dietary supplementation had no effect on beetle-mediated CO₂e emissions. Through a combination of field- and laboratory-based experiments, this project found that the inclusion of brown seaweed in beef cattle diets reduced both *Onthophagus nuchiicornis*' resource use for brood ball production and the proportion of major males in beetle offspring. These results suggest that dietary supplementation reduced dung quality. However, when evaluated under field conditions, coprophagous beetles' resource selection was not affected by dietary supplementation.

Across studies, there is a lack of consensus on the impact of dung beetles on GHG emissions from livestock dung. Using a series of laboratory-based experiments, this work shows that methodological decisions strongly influence dung and beetle-mediated GHG emissions. For example, the homogenization of dung prior to GHG sampling, dung's surface-area-to-volume ratio, and dung mass all influence beetle-mediated GHG emissions from dung.

Overall, results from this research reveal the downstream effects of supplementing beef cattle diets with brown seaweed on dung beetle ecology and dung GHG emissions. These results highlight the need to assess the downstream effects of management interventions when trying to determine their ability to improve the environmental sustainability of livestock production.

List of abbreviations used

CH₄: Methane

CO₂: Carbon dioxide

CO₂e: Carbon dioxide equivalents

DMI: Dry matter intake

GHG: Greenhouse gas emissions

GWP: Global warming potential

HCO₃⁻: Bicarbonate

N₂O: Nitrous oxide

SA/V: Surface-area-to-volume

VOC: Volatile organic compound

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Chapter 1: General Introduction

Canada's livestock sector's methane challenge

Methane (CH₄) is a potent greenhouse gas (GHG) with a global warming potential (GWP) 28 times greater than carbon dioxide (CO₂; Myhre et al., 2013). As a short-lived climate pollutant with an atmospheric lifespan of 12 years, reducing CH₄ emissions is necessary to limit near-term temperature rise (Government of Canada, 2022). In 2021, Canada joined the Global Methane Pledge, committing to reduce CH₄ emissions by 30% below 2020 levels by 2030 (Government of Canada, 2022). To achieve this goal, it is necessary to evaluate strategies that can help reduce emissions from Canada's three largest methane-emitting sectors (i.e. oil and gas (38%), agriculture (30%), and waste (28%); Government of Canada, 2022).

On a global scale, livestock is projected to account for 40-78% of CH₄ emissions by 2100 (Harmsen et al., 2020). In 2020, the agricultural sector was responsible for 30% of Canada's CH₄ emissions (Government of Canada, 2022), with beef production accounting for 71% of these emissions (Government of Canada, 2022). Methane emissions from the livestock sector are primarily from enteric fermentation (86%) and the decomposition of dung and manure (14%; Government of Canada, 2022). In response to the need to reduce agricultural CH₄ emissions, the Reducing Enteric Methane Emissions from Beef Cattle (REME) protocol was introduced (Government of Canada, 2023). This protocol aims to incentivize producers to adopt strategies, such as dietary reformulation, to reduce enteric CH₄ emissions from the beef sector (Government of Canada, 2023).

Enteric methane production and mitigation strategies

Enteric fermentation is a digestive process during which methanogenic archaea, single-celled microorganisms in the rumen, help ruminants digest cellulose-rich feeds (Mottet et al., 2017). The process of methanogenesis takes place during the digestion of these cellulose-rich feeds (Thauer, 2012; K. Wang et al., 2018). During this process, methanogenic archaea and protozoa produce CH₄ as a by-product of the reduction of carbon dioxide (CO₂) and bicarbonate (HCO₃⁻) as described in the Wolfe cycle: $4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$ and $\text{HCO}_3^- + 4\text{H}_2 \rightarrow \text{H}^+ \text{CH}_4 + 3\text{H}_2\text{O}$ (Wolin et al., 1963; Thauer, 2012; K. Wang et al., 2018). Several strategies have been suggested to mitigate enteric methane emissions, including the use of methanogenic inhibiting feed additives (Króliczewska et al., 2023).

Methanogenic inhibiting feed additives reduce enteric methane emissions through the alteration of biochemical processes occurring in the rumen (Callaway et al., 1997; Kumar et al., 2013). Methanogenic inhibitors include feed additives such as saponins, tannins, and nitrocompounds (e.g. 3-nitrooxypropanol). Saponins and tannins consist of plant-derived organic compounds that reduce CH₄ emissions through the alteration of microbial populations in the rumen (Patra & Saxena, 2009). Methanogenic-inhibiting nitrocompounds are primarily short-chain aliphatic chemical compounds containing a nitro group (NO₂; Gutierrez-Bañuelos et al., 2008). These nitrocompounds reduce CH₄ emissions by acting as alternative electron acceptors, thereby inhibiting H₂ and formate oxidation (Gutierrez-Bañuelos et al., 2008; Zhang et al., 2018).

Mitigating methane emissions through dietary supplementation with brown seaweed

Supplementing cattle diets with seaweed can also reduce enteric CH₄ emissions. When included in beef cattle diets at a rate of 1% dry matter intake (DMI), a locally harvested brown seaweed reduced both CO₂ (-11%) and CH₄ (-8%) emissions (Borzouie et al., manuscript in preparation). Brown seaweeds, including kelp (Laminariales) and rockweed (Fucales), contain phlorotannins (McAllister et al., 1994; Vissers et al., 2018). These polyphenolic compounds found exclusively in brown seaweeds inhibit the ruminal bacterial activity associated with fibre and starch digestion and alter the composition of the rumen's microbiome, resulting in the reduction of enteric methane emissions (McAllister et al., 1994; Vissers et al., 2018; Santos et al., 2019; Pandey et al., 2022). While the effects of seaweed-based methanogenic inhibitors on enteric emissions are well studied, and the likelihood of adopting brown seaweed in beef cattle diets appears favorable, their impact on dung GHG emissions remains unclear. Given the contribution of dung decomposition to agricultural emissions, it is important to examine the effects of supplementing beef cattle diets with brown seaweed on dung GHG emissions.

Cattle dung decomposition and its contribution to GHG emissions

When assessing GHG emissions from the agricultural sector, it is important to consider the emissions resulting from the decomposition of dung and manure, as they account for approximately 14% of the sector's CH₄ emissions (Government of Canada, 2022). In this study, dung refers to the feces or raw excrement of animals, whereas manure describes dung mixed with other organic matter and subjected to various forms of on-farm management (e.g. treatment within deep lagoons). By depositing dung on pasture,

ruminants introduce high levels of nitrogen (N) and carbon (C), thereby stimulating the production of CO₂, CH₄, and N₂O (Saggar et al., 2004).

Fresh dung contains high levels of degradable C compounds that can stimulate CO₂ production (Wang et al., 2013; Zhu et al., 2018), a GHG with an atmospheric lifespan of 50 to 200 years (Inman, 2008). The production of CO₂ is partly caused by heterotrophic respiration, an aerobic process where microbes break down organic compounds in the presence of oxygen, producing CO₂ as a by-product (Billings & Ziegler, 2008; Chen et al., 2011). Heterotrophic respiration is regulated by oxygen availability, which is influenced by factors such as moisture content and temperature (Bauer et al., 2008; Chen et al., 2011). In oxygen-depleted conditions, the anaerobic fermentation of organic matter will occur, resulting in CH₄ production (Saggar et al., 2004), a process that is dependent on the activity of methanogens in the dung (Saggar et al., 2004).

Dung is also an important source of nitrous oxide (N₂O), a relatively long-lived greenhouse gas that remains in the atmosphere for approximately 114 years (IPCC, 2001). The production of N₂O, a potent GHG with a GWP 265 times greater than CO₂ (Myhre et al., 2013), requires both anaerobic and aerobic environments, as it is a by-product of both the nitrification and denitrification process (Robertson & Tiedje, 1987; Saggar et al., 2004, 2013). The microbial production and consumption of N₂O are influenced by factors such as dung temperature, moisture levels, and N content (Monaghan & Barraclough, 1993; Bouwman et al., 2013; Butterbach-Bahl et al., 2013; Broucek, 2017). Therefore, GHG emissions from dung can be influenced by a range of factors, including dung composition (i.e. N and C content), moisture levels, and microbial activity, all of which are influenced by cattle diet (Monaghan & Barraclough, 1993; Jarvis et al., 1995; Yamulki et al., 1999;

Saggar et al., 2004; Bouwman et al., 2013; Butterbach-Bahl et al., 2013; Broucek, 2017; Hahn et al., 2018).

The potential effects of dietary supplementation on dung GHG emissions

Although the effects of supplementing beef cattle diets with seaweed on enteric emissions are better understood (Machado et al., 2018; Ramin et al., 2019; Borzouie et al., manuscript in preparation), there is limited knowledge regarding its impact on dung GHG emissions (Ramin et al., 2023). However, greater research on this topic is needed, as the alteration of cattle diets can influence dung N₂O emissions (Luo et al., 2015), with 70–90% of ingested nitrogen being excreted in the animal's urine and dung (Castillo et al., 2000; Schils et al., 2013). For example, supplementing dairy cattle diets with the brown seaweed *Himantalia elongata* was found to enhance N utilisation efficient, leading to increased total nitrogen levels in dung (Dabiri et al., 2025). Additionally, the alteration of cattle diets can impact dung's C: N ratio, which in turn may impact dung CH₄ emissions (Jarvis et al., 1995; Pelster et al., 2016). Given that CH₄ emissions are negatively correlated with the C: N ratio, an increase in dung DM content, and hence an increase in the C: N ratio may result in reduced CH₄ emissions (Jarvis et al., 1995; Pelster et al., 2016).

Dung GHG emissions are also influenced by dung microbial community composition (Saggar et al., 2004; Jiang et al., 2012; Hahn et al., 2018). While little is known about the effects of supplementing beef cattle diets with brown seaweed on dung microbial composition, altering cattle diets can modify the rumen's microbial composition (Jin et al., 2017; Andrade et al., 2022; Kibegwa et al., 2023). Additionally, supplementing pig diets with brown seaweed can alter the microbial composition in the caecal and colonic sections, which are sections of the hindgut located after the stomach (Reilly et al., 2008; Lynch et

al., 2010). Therefore, by influencing dung nitrogen content and microbial composition, supplementing beef cattle diets with brown seaweed may impact dung CO₂, CH₄, and N₂O emissions.

Coprophagous insect communities in Canada

In addition to potentially impacting dung GHG emissions, supplementing beef cattle diets with brown seaweed may influence coprophagous insect communities. Coprophagous insects, such as dung beetles (Scarabaeidae: Aphodiinae and Scarabaeinae), help maintain pasture fertility by transferring organic matter and nutrients from animal dung to soils (Stevenson & Dindal, 1987). In Canada, cattle dung provides resource and habitat for over 300 species of insects, including, dung beetles, flies, and wasps (Floate, 2023). These insects colonize dung pats through a series of successional stages which are influenced by the age of the dung pat and weather conditions (Hammer 1941; Mohr 1943; Laurence 1954; Yoshida and Katakura 1986; Floate, 2023). Dung pat colonization begins with the arrival of adult flies, who arrive within minutes of deposition (McLintock and Depner 1954). Adult dung beetles then colonize the dung pat, with their colonization peaking within one to five days after deposition (Holter 1975; Lee and Wall 2006; Mohr 1943; Floate, 2023). Parasitoid wasps and predatory beetles then colonize the dung pat (Kessler and Balsbaugh 1972; Mohr 1943). The arrival of soil-dwelling organisms, such as earthworm, springtails, and nematodes, marks the final stage of dung pat colonization (Floate 2023).

Dung beetles (Coleoptera: Scarabaeoidea)

Dung beetles (Scarabaeidae: Aphodiinae, Scarabaeinae, and Geotrupinae) are an important group of dung colonizing insects, with species exhibiting either coprophilous (dung-loving) or coprophagous (dung-feeding) behaviors (Floate, 2023). In Canada, 44 species of dung

beetle are known to utilize cattle dung (Pokhrel et al., 2021). Coprophagous adult dung beetles are filter feeders that consume microorganisms present in the liquid fraction of fresh dung (Holter et al., 2002, 2004). Their larva, on the other hand, feed predominantly on undigested plant fibers (Floate et al., 2023). Dung beetles are classified into three groups based on their nesting behavior: tunnellers, rollers, and dwellers (Cambefort & Hanski, 1991). Tunneling species bury dung directly beneath the dung pat, in small agglomerations of dung commonly referred to as brood balls. Dwellers lay their eggs within the dung pat (Cambefort & Hanski, 1991; Rojewski, 1983), whereas rollers form small dung balls in which they lay their eggs once they have rolled them away from the dung pat (Cambefort & Hanski, 1991; Rojewski, 1983). In Canada, dung beetles' typically produce one generation per year, and exhibit either a unimodal or bimodal pattern of seasonal activity (Floate & Gill, 1998; Kadiri et al., 2014; Bezanson, 2019; Floate, 2023). Dung beetle species who have a bimodal pattern of seasonal activity overwinter as adults and exhibit two peaks of activity per year, one in the spring and one in the fall (Floate, 2023). In contrast, species with a unimodal pattern of activity have a single peak, overwintering in immature stages before completing development and emerging as adults in late spring to early summer (Floate, 2023).

The effects of dietary supplementation with brown seaweed on dung beetles' resource selection

Supplementing cattle diets with seaweed can alter dung nutrients levels, including changes to calcium (-10%), phosphorus (-10%), magnesium (-9%), and iron (-9%; Bassiouni et al., 2013). These changes in mineral composition can affect dung's suitability for larval development as well as its suitability as a feeding resource for adult dung beetles.

Although most dung beetles are generalist feeders, they have shown preference for certain dung types (Dormont et al., 2004, 2007; Whipple & Hoback, 2012; Urrutia et al., 2022). This preference is believed to be influenced by both dung quality and the emission of volatile organic compounds (VOCs; Dormont et al., 2004, 2007; Bogoni & Hernández, 2014). Dung beetles use their olfactory systems to locate dung by identifying specific VOCs (Williams, 1984; Powers et al., 1999; Stavert et al., 2014). These VOCs are produced by microbes during the fermentation of biological macromolecules such as proteins and carbohydrates (Williams, 1984; Powers et al., 1999; Stavert et al., 2014). Although the inclusion of wet distillers grains in cattle diets had no effect on important dung VOCs (i.e., skatole, indole, and phenol; Hales et al., 2012), supplementing cattle diets with brown seaweed can alter dung micro- and macro-mineral composition (Bassiouni et al., 2013). Through the changes to dung nutrient composition, and therefore potentially dung VOC production, supplementing beef cattle diets with brown seaweed may influence coprophagous beetles' resource selection and use.

The impact of cattle diets on dung beetle reproductive success

Changing cattle diets can influence both brood ball production and adult beetle offspring fitness through the alteration of dung characteristics (Edwards, 1991; Madzivhe et al., 2021; Heddle et al., 2024). For example, dung quality can affect the expression of male morphological traits, adult size, and larval development in dung beetles (Kaur et al., 2021; Ridsdill-Smith et al., 1986; Yap et al., 2024). Therefore, given the potential impacts of supplementing cattle diets with seaweed on dung nutrient composition (Bassiouni et al., 2013), and the effects of dietary supplementation on beetle reproductive success (Madzivhe et al., 2021; Heddle et al., 2024), it is important to understand how supplementing beef

cattle diets with brown seaweed might affect dung beetle reproductive success and, by extension, the ecosystem services dung beetles' provide that contribute to maintaining healthy pastures.

Beetle-mediated GHG production

Dung beetles play an important role in ecosystem functioning by promoting ecological processes such as rapid nitrogen mineralization rates (Nichols et al., 2008), secondary seed dispersal (Nichols et al., 2008), and improving soil aeration and water porosity (Mittal, 1993; Nichols et al., 2008). While the effects of dung beetles on GHG emissions vary across studies, it is generally accepted that dung beetles can reduce dung CH₄ emissions (Penttilä et al., 2013; Iwasa et al., 2015; Piccini et al., 2017). This reduction in CH₄ emissions has been attributed to dung beetles' tunneling behavior, which aerates dung pats, resulting in the reduction of anaerobic conditions and the presence of GHG-producing microbes (Penttilä et al., 2013).

Understanding the inconsistencies in beetle-mediated GHG emissions

Although dung beetle presence influences dung GHG emissions, the significance and direction of these effects vary across studies (Penttilä et al., 2013; Iwasa et al., 2015; Hammer et al., 2016; Slade et al., 2016; Piccini et al., 2017; Evans et al., 2019; Fowler et al., 2020; Verdú et al., 2020). These differences may be attributed to methodological choices, such as dung pat size, which has ranged from 300 g (Piccini et al., 2017) to 1.5 kg (Evans et al., 2019) across studies, experimental location (laboratory-based vs field-based), and dung beetle species richness (single species vs. 9 species).

Dung manipulation (e.g. homogenization to minimize heterogeneity) prior to GHG sampling may explain some of these noted inconsistencies. For instance, dung homogenization, and the resulting aeration of dung pats, was predicted to result in the reduction of CH₄ emissions (Fowler et al., 2020). This reduction in dung CH₄ emission may weaken the beetle-mediated reduction in CH₄ emissions, particularly if prolonged homogenization further reduces anaerobic conditions. As a result, the magnitude of beetle-mediated CH₄ reduction may be smaller in homogenized dung samples, contributing to variability across studies. While evaluating this theory, it was found that non-homogenized dung emitted 1.5 times more CH₄ than homogenized samples; however, this difference was not statistically significant (Fowler et al., 2020). Given the number of uncontrolled variables, such as dung pat age, it is important to further evaluate the effects of dung homogenization on GHG emissions under more carefully controlled conditions.

Dung mass and shape can also influence GHG emissions (Saggar et al., 2004; Wang et al., 2013; Sordi et al., 2014; Zhu et al., 2018). For instance, changes in dung mass can affect the availability of accessible carbon substrates and promote prolonged moisture retention (Sordi et al., 2014; Wang et al., 2013; Zhu et al., 2018). By doing so, dung mass can influence oxygen availability, thereby impacting both CO₂ and CH₄ emissions (Sordi et al., 2014; Wang et al., 2013; Zhu et al., 2018). For example, smaller dung pats retained 36% less moisture when compared to dung pats 16 times larger (Du et al., 2021). Dung shape (i.e., surface area) has been found to affect moisture retention and crust formation, both of which can impact CH₄ emissions (Husted, 1994; Yamulki et al., 1999; Mazzetto et al., 2014; Mori & Hojito, 2015; Cardoso et al., 2019). Crust formation alone can reduce CH₄ emissions by up to 12 times (Husted, 1994), with dung moisture content and temperature

strongly influencing crust development (Akbar et al., 2015). Given, the observed inconsistencies in beetle-mediated dung GHG emissions, it is essential to further evaluate how different methodological approaches can impact beetle-mediated GHG emissions from cattle dung.

Final objectives and chapter breakdown

Through a combination of laboratory- and field-based experiments, my project aims to assess the downstream effects of supplementing beef cattle diets with a brown seaweed. Specifically, it aims to determine the impact of brown seaweed supplementation on dung GHG emissions, coprophilous beetles' resource selection, as well as dung beetles' resource use and reproductive success. These studies are complemented by an additional experiment that investigates the inconsistencies in beetle-mediated GHG emissions reported across studies. Collectively, this project aims to provide new insights into the broader environmental effects of methane mitigation strategies in the livestock sector.

In Chapter 2, I examine the impact of supplementing beef cattle diets with brown seaweed on dung GHG emissions, as well as on beetle-mediated GHG emissions. I also evaluate the effects of dung homogenization on GHG emissions. Through a combination of laboratory-based experiments, I found that supplementing beef cattle diets with brown seaweed reduced overall dung emissions, leading to a 49% reduction in CH₄ and 26% reduction in CO₂ emissions. In addition to this, dung homogenization was found to reduce CH₄ emissions through the aeration of dung samples, with homogenized dung samples emitting 1.46 times less CH₄ when compared to non-homogenized samples.

To better understand the inconsistencies observed in beetle-mediated dung GHG emissions across studies, Chapter 3 evaluates how experimental practices influence beetle-mediated GHG emissions. By manipulating dung mass and surface-area-to-volume (SA/V) ratio, I found that changes in dung mass (100 g vs 400 g) significantly affected beetle-mediated CO₂ and CO_{2e} emissions, with beetle presence leading to a stronger increase in CO₂ and CO_{2e} emissions in larger dung pats. Additionally, the interaction between dung mass and SA/V ratio, as well as beetle presence and dung SA/V ratio, significantly affected CH₄ emissions. Regardless of dung mass, a reduced SA/V ratio (i.e., non-flattened dung pats) resulted in higher CH₄ emissions on a per gram of dung basis; however, these effects were less pronounced in dung samples of greater mass. Additionally, beetle presence in flattened dung pats (i.e. reduce SA/V ratio) resulted in a greater reduction in CH₄ emissions.

To further assess the downstream effects of methane mitigation strategies in ruminant farming, Chapter 4 examines how supplementing beef cattle diets with brown seaweed impacts coprophilous beetles' (Coleoptera: Hydrophilidae, Scarabaeidae, Staphylinidae) resource selection as well as *Onthophagus nuchicornis*' resource use and reproductive success. Dietary supplementation influenced both *Onthophagus nuchicornis*' reproductive success and dung utilization. In a no-choice experiment, higher inclusion rates of brown seaweed reduced the proportion of major males. In addition to this, when given a choice between dung of varying levels of brown seaweed inclusion, beetles consistently preferred using dung from the lower supplementation groups for brooding purposes.

To further explore the experimental findings presented in this project, Chapter 5 examines the downstream effects of supplementing beef cattle diets with brown seaweed, as well as the influence of experimental practices on the quantification of beetle-mediated GHG

emissions. The results show that the benefits of dietary supplementation extend beyond just reducing enteric emissions, contributing to significant reductions in dung GHG emissions. However, changes in the proportion of major males in dung beetle offspring, as well as the reduction in dung use for brooding purposes suggest that dietary supplementation may have negatively affected dung quality by altering its nutritional composition. Additionally, the observed effects of experimental practices on GHG emissions emphasize the importance of carefully considering experimental practices when interpreting results or comparing results between studies. Specifically, dung mass, SA/V ratio, and homogenization were all found to significantly influence dung GHG emissions.

Chapter 2: Mitigating greenhouse gas emissions from beef cattle dung through dietary supplementation with brown seaweed

Abstract

The livestock sector is a significant contributor to methane emissions in Canada. While supplementing beef cattle diets with brown seaweed can reduce enteric methane emissions, the downstream effects of dietary supplementation are unknown. Therefore, this study examines the effects of supplementing beef cattle diets with brown seaweed on dung and beetle-mediated GHG emissions. Additionally, to better understand how experimental practices affect the quantification of beetle-mediated CH₄ emissions, I evaluate the impact of homogenization on dung GHG emissions. Through laboratory-based experiments, I found that supplementing beef cattle diets with brown seaweed at a rate of 2% DMI reduced both dung CO₂ emissions (-26%) and CH₄ emissions (-49%), resulting in a 30% reduction in CO₂e emissions. Dietary supplementation also reduced the magnitude of beetle-mediated CH₄ emissions. However, the relative beetle-mediated CH₄ reductions remained identical across treatment groups. This suggests that supplementing beef cattle diets with brown seaweed had no effect on *C. erraticus*' CH₄ mitigation potential, as the smaller absolute reduction noted in supplemented dung samples was due to lower initial CH₄ emissions. Additionally, the homogenization of dung prior to GHG sampling was also found to reduce CH₄ emissions by 46%. These findings highlight that the beneficial effects of supplementing beef cattle diets with brown seaweed extend beyond just reducing ruminant's enteric emissions and reveal that routine methodological practices can impact the quantification of beetle-mediated emissions. Therefore, it is suggested that projects evaluating beetle-mediated GHG emissions should carefully consider how methodological choices can influence results, and where possible, methods should be standardized or clearly reported to improve comparability across studies.

Introduction

The Canadian livestock sector is responsible for over half of the agricultural sector's greenhouse gas (GHG) emissions, primarily in the form of methane (CH₄; Environment and Climate Change Canada, 2023), a potent GHG with a global warming potential (GWP) twenty-eight times greater than that of carbon dioxide (CO₂; Myhre et al., 2013). Ruminants contribute to these emissions through enteric fermentation, a digestive process taking place in the rumen (Gibbs et al., 2000). Enteric fermentation accounts for 86% of the livestock sector's CH₄ emissions (Government of Canada, 2022). To mitigate these emissions, dietary strategies targeting enteric methane emissions have been explored (McGinn et al., 2019; Roque et al., 2019; Suybeng et al., 2020). For example,

supplementing beef cattle diets with brown seaweed can reduce enteric methane emissions (Borzouie et al., 2025, manuscript under review). This reduction in enteric methane emissions is likely due to the seaweeds' phlorotannin content, which can alter the rumen's microbial community composition (Wang et al., 2008; Vissers et al., 2018; Pandey et al., 2022; Huang et al., 2023).

While reducing enteric methane emissions is essential, it is also important to assess the downstream effects of dietary supplementation on dung GHG emissions, as manure and dung decomposition account for 14% of the livestock sector's CH₄ emissions (Bowen & Dahal, 2024). The deposition of fresh cattle dung introduces high levels of nitrogen (N) and carbon (C) to pasture systems, thereby stimulating GHG emissions (Saggar et al., 2004). Emissions resulting from dung decomposition are influenced by a range of factors, including dung composition (Amaral Júnior et al., 2025), dung microbial community composition (Cai et al., 2017; Hahn et al., 2018), and the presence of coprophagous insects in dung pats (Penttilä et al., 2013; Hammer et al., 2016; Piccini et al., 2017; Evans et al., 2019).

Dietary supplementation has been found to alter dung GHG emissions. For example, supplementing grazing beef steers' diets with maize grain caused a 57% reduction in dung CH₄ emissions, while doubling dung N₂O emissions (Lombardi et al., 2022). These changes in dung GHG emissions were attributed to the increase in supplemented dung's N and dry matter (DM) content (Lombardi et al., 2022), as CH₄ emissions are negatively correlated with dung C content and C:N ratio (Jarvis et al., 1995; Pelster et al., 2016). Additionally, these changes to dung composition can impact dung beetle resource use and selection

(Kasper et al., 2012; Stavert et al., 2014), thereby impacting beetle-mediated GHG emissions.

Coprophagous insects, such as dung beetles (Scarabaeidae: Aphodiinae, Scarabaeinae, and Geotrupinae), are important members of pasture ecosystems. Through their feeding and nesting behaviours, dung beetles effectively breakdown dung pats (Macqueen & Beirne, 1975) and provide several ecosystem benefits, such as secondary seed dispersal and soil fertilization (Nichols et al., 2008). While it is well established that dung beetles can alter dung CH₄ emissions, the direction and magnitude of these effects vary across studies (Penttilä et al., 2013; Iwasa et al., 2015; Piccini et al., 2017; Evans et al., 2019; Fowler et al., 2020). Fowler et al. (2020) hypothesized that homogenizing dung, a practice commonly used to standardize samples, could impact our ability to quantify beetle-mediated CH₄ emissions by reducing the abundance of methanogens in dung through the aeration of dung pats (Fowler et al., 2020). However, a field-based study, conducted by Fowler et al (2020), found dung homogenization to have no significant effect on dung CH₄ emissions. These results may potentially result from the high experimental variability caused by site-based vegetative differences, as well as varying GHG chamber and dung volumes (Fowler et al., 2020). This experimental variability highlights the need for a lab-based study with standardized experimental variables to better assess the effects of dung homogenization on CH₄ emissions.

It is also important to evaluate the effects of dietary supplementation with brown seaweed on beetle-mediated GHG emissions. Supplementing cattle diets with seaweed also impacts the micro- and macro-mineral content of the dung (Bassiouni et al., 2013), thereby potentially impacting dung GHG emissions (Bassiouni et al., 2013). Therefore,

supplementing beef cattle diets with brown seaweed could influence beetle-mediated GHG emissions, with possible additive or synergistic benefits resulting from the combination of dietary supplementation and dung beetle activity.

Therefore, this chapter aims to: 1) evaluate the impact of adding brown seaweed to beef cattle diets on dung GHG emissions, 2) assess the effects of dung homogenization on dung GHG emissions under laboratory conditions, and 3) examine how the brown seaweed feed additive influences beetle-mediated dung GHG emissions.

I hypothesize that 1) the addition of brown seaweed to beef cattle diets will increase dung CO₂ emissions while reducing dung CH₄ emissions, due to the anticipated increase in dung dry matter content, 2) dung homogenization will reduce CH₄ emissions through the aeration of dung pats, and 3) the relative impact of dung beetles on CH₄ reduction will be smaller when feeding on dung from beef cattle fed the brown seaweed supplement.

Methods and materials

Two complementary experiments were performed under controlled laboratory settings to examine the effects of supplementing beef cattle diets with a brown seaweed feed additive on dung GHG emissions. In the first experiment, I evaluated how dung homogenization and the inclusion of brown seaweed to beef cattle diets affected dung GHG emissions. In the second, I assessed how the biological activity of the dung beetle *Colobopterus erraticus* in combination with dietary supplementation affected dung GHG emissions.

Beef cattle diets and dung collection

Fresh dung samples were collected from a group of ten beef heifers at the Nappan Research Farm (43°39.1935' N, 79°22.9911' W). The heifers were randomly assigned to one of two treatment groups based on their level of seaweed supplementation. Heifers in the control

group received no seaweed supplementation (0% of dry matter intake (DMI)), while those in the supplemented group received 2.0% seaweed supplementation on a dry matter basis (2% DMI). Over the 90-day period prior to sampling (31 August 2023 to 27 November 2023), all heifers were fed a basal diet consisting of 75.6% grass silage (DM), 20% barley (DM), and 4.4% soybean (DM). The supplemented group additionally received 2% DMI inclusion of a brown seaweed additive, which was purchased from North Atlantic Organics (Atlantic GRO ®; Tignish, PEI, Canada). The mineral composition of Atlantic GRO ® is provided in Table 2.1. The seaweed was added to the supplemented diet using a serial addition method. Starting with the control group’s feed, a measured amount of brown seaweed was gradually added to the basal diet mixture to meet the brown seaweed percentage requirements of the 2% DMI treatment group. The heifers had not received antibiotic or antiparasitic treatment throughout the feeding trial.

Table 2.1 Mineral content of North Atlantic Organics’ brown seaweed feed supplement.

	Ca%	Mg%	K%	Na%	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Mo (ppm)
As fed	2.49	0.68	0.6	0.171	1,000	26	13	303	0.3
DM	2.79	0.76	0.67	0.191	1,130	30	15	340	0.4

calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), and molybdenum (Mo)

On 27 November 2023, dung was collected directly from the rectum of the five heifers in the two supplementation groups, with 2.5 kilograms of dung from each group portioned into 100-gram dung pats. The samples were kept on ice for transport to Dalhousie’s Agricultural Campus. Upon arrival, one kilogram of dung from each group was stored at -21°C to evaluate the effects of dietary supplementation on beetle-mediated GHG emissions. The remaining two kilograms were immediately used to determine the effects of the brown seaweed feed additive and homogenization on dung GHG emissions.

Subsamples of the two treatment groups were sent to the Nova Scotia Department of Agriculture for nutrient analysis (Table 2.2).

Table 2.2 Composition analysis of dung samples for the control group (0% DMI) and the supplemented group (2% DMI).

Treatment	Nutrients				C:N ratio
	DM (%)	N (%)	C (%)	NH ₄ -N (%)	
Control (0%)	13	2.02	41.98	0.29	20.79
Supplemented (2%)	14.13	1.96	43.25	0.22	22.1

dry matter (DM), nitrogen content (N), carbon content (C), ammonium-nitrogen content (NH₄-N)

Experiment 1: Effects of a brown seaweed supplementation and homogenization on dung GHG emissions

To examine the effects of supplementing beef cattle diets with brown seaweed, as well as dung homogenization on GHG emissions, a randomized 2-level factorial design was used to measure the emission of CO₂, CH₄, and N₂O from dung samples. This 2-level factorial design paired two feed inclusion levels (control (0%) vs. supplemented (2%)) with two homogenization treatments (with homogenization vs. without homogenization). Dung samples were homogenized for 30 seconds at 10-second intervals using an electric power drill with a paint mixer attachment. These pairings created four treatment combinations: supplemented group with (2H) and without (2NH) homogenization, and a control group with (0H) and without (0NH) homogenization. Each combination was replicated five times (n=5), resulting in a total of 20 mesocosms (N=20).

Mesocosms consisted of 2.5 L cylindrical high-density polyethylene (HDPE) containers, with 2-mm drainage holes at the bottom (Priority Plastics, Grinnell, IA, US). Each mesocosm was filled to a depth of 13 cm with 2.5 kg (\pm 41 g) of coarse silica sand (Model #8526, Shaw Resources, New Brunswick, Canada) and 500 mL of water. At time zero

(t=0), 85 grams of dung were added to each mesocosm, which were then sealed with a 2-mm fiberglass mesh, allowing for gaseous exchange with the surrounding air (see Appendix A).

Experiment 2: The effects of dietary supplementation with brown seaweed on beetle-mediated GHG emissions

A second 2-level factorial design was used to examine how the supplementation of beef cattle diets with brown seaweed influenced dung beetle-mediated GHG emissions. The experiment followed a two-level factorial design with supplementation levels (supplemented vs. control) and the presence or absence of the dung beetle *Colobopterus erraticus* (Linnaeus, 1758) as factors. This resulted in four treatment groups consisting of: (1) supplemented dung with *C. erraticus* (2B), (2) supplemented dung without *C. erraticus* (2NB), (3) control dung with *C. erraticus* (0B), and (4) control dung without *C. erraticus* (0NB). Each combination was replicated five times (n=5), resulting in a total of 20 mesocosms (N=20).

To conduct this experiment, 200 *Colobopterus erraticus* beetles were collected from Woolies of Upperbrook Farm in North River, Nova Scotia. *C. erraticus* is a non-native dung beetle, measuring between 6-8 mm in length, that was introduced to eastern Canada from Europe prior to 1940 (Floate, 2023). This tunneling species flies to fresh dung pats, where it buries dung in tunnels beneath the pat for feeding and nesting purposes (Yamada et al., 2007; Estes et al., 2013; Floate, 2023). To collect the beetles, sheep dung was hand-searched for *C. erraticus* which were then placed in 1.3 L cylindrical high-density polyethylene (HDPE) containers (Priority Plastics, Grinnell, IA, US). Dung samples were removed from the freezer 48 hours prior to the start of the experiment and homogenized for a 30 second period at 10 second intervals. The 2.5 L mesocosms (Priority Plastics,

Grinnell, IA, US) were filled with 2.1 kg (\pm 260 g) of coarse silica sand (Model #8526, Shaw Resources, New Brunswick, Canada) and 500 mL of water. At the start of the experiment ($t=0$), 85 g of dung was added to each mesocosm, with twenty randomly allocated *C. erraticus* being added to the ten mesocosms belonging to the 0B ($n=5$) and 2B ($n=5$) treatment groups. To prevent beetle escape while allowing for gaseous exchange, the mesocosms were covered with a 2-mm fiberglass mesh.

GHG sampling procedure

Gas samples for both experiments followed identical sampling procedures. In experiment one, samples were collected on seven occasions over a 21-day period (at days 0.5, 1, 3, 5, 7, 10, and 21) from 28 November 2023 to 18 December 2023. In experiment two, samples were collected seven times over a 21-day period (days 0.5, 1, 3, 5, 7, 10, and 21) from 13 June 2024 to 3 July 2024. Air temperature was monitored throughout the sampling period using a data logger (Kestrel DROP D2HS Heat Stress Monitor).

The greenhouse gas sampling protocol and flux calculations followed Dalhousie University's "Standard Operating Procedure for Greenhouse Gas (CO_2 , CH_4 , and N_2O) Flux Measurement" (Burton & Janes, 2013). Sampling began at time $t=0$, when a laboratory air sample was collected using a 20 mL syringe and immediately transferred into a 12 mL pre-evacuated exetainer for future analysis. The measured concentration of each gas from this sample was later used as the first data point in the linear regression to calculate the gas flux in each mesocosm. Afterward, an airtight lid with a septum was securely placed on the first mesocosm, and 60 mL of laboratory air was injected into the controlled environment using a 60 mL syringe. After a one-minute interval, sampling proceeded to the adjacent mesocosm. A 20 mL syringe was used to collect gas samples

from the mesocosm at 10, 20, and 30 minutes, which were then injected into pre-evacuated exetainers (12 mL).

Once sampling was completed, the airtight lids on the mesocosms were replaced with the mesh lids, and the mesocosms remained in place until the next sampling period, or until the end of the experiment. The concentrations (ppm) of CO₂, CH₄, and N₂O from each sample were measured using a Bruker SCION SQ 450 Gas Chromatograph (SCION Instruments Canada, Edmonton, AB) equipped with three detectors, electron capture (ECD), flame ionization (FID) and thermal conductivity (TCD), along with a Combi-PAL autosampler. Linear regression was used to calculate gas fluxes, requiring linearity in the data. In cases where the data were not linear ($r^2 < 0.85$), the sampling point that deviated most from the trendline was excluded, thereby reducing the number of sampling points included in the linear regression to three. Gas fluxes were calculated based on the change in gas concentration in the mesocosm headspace (7.5 cm) over time (in g K⁻¹ min⁻¹; Burton & Janes, 2013). The fluxes were normalized by the average dry mass of the dung pats. The cumulative flux for each mesocosm over the 21-day sampling period was expressed as the area under the curve. The GWP impact factors for a 100-year time for each gas (GWP_{100-CH₄} = 28; GWP_{100-N₂O} = 265), as per the fifth IPCC report (Myhre et al., 2013), were used to convert the total emissions per mesocosm into CO₂-equivalents (CO₂e). CO₂e allows for the descriptions of the different GHGs in a common unit allowing for the easy comparison of emissions across sectors (Brander & Davis, 2012). The cumulative fluxes over the 21-day period were used as the response variables in the subsequent analyses.

Statistical analysis

Statistical analyses were conducted using linear regression models to evaluate the effects of beef cattle diet supplementation with brown seaweed on dung and beetle-mediated GHG emissions as well as the effects of dung homogenization on dung GHG emissions. Models were implemented in R version 4.5.0 (R Core Team, 2024), using R studio version 2024.09.0. Separate models were created for each response variable (i.e., CO₂, CH₄, N₂O, CO₂e).

Two distinct sets of linear regression models were constructed. The first set included dung homogenization and dung treatment group as predictor variables, whereas the second set included beetle presence and dung treatment group. Interactions between the predictor variables were considered, including those between dung treatment and homogenization, and dung treatment and beetle presence. Model selection was assessed using a stepwise logistic regression with backwards selection from the MASS package (Venables & Ripley, 2002). Akaike Information Criterion (AIC) was used to identify the best-fitting model based on the lowest AIC value. Model assumptions, including normality, homoscedasticity, linearity, and independence were assessed by analyzing the models' residual plots. To control for Type I errors, p-values were adjusted using Benjamini and Hochberg's (1995) control for false discovery rates (Benjamini & Hochberg, 1995). The model's R-squared value was used to assess its explanatory power.

Results

Experiment 1: Effects of a brown seaweed supplementation and homogenization on dung GHG emissions

Carbon dioxide (CO₂) emissions: The inclusion of the feed additive in beef cattle diets significantly reduced cumulative CO₂ emissions from fresh dung over the 21-day sampling

period ($F_{(2,17)} = 9.525$; $P < 0.001$; $R^2 = 0.53$). Dung samples from the supplemented group ($0.629 \pm 0.045 \mu\text{g CO}_2 \text{ g}^{-1}\text{s}^{-1}$) emitted 26% less CO_2 compared to dung from the control group ($0.845 \pm 0.026 \mu\text{g CO}_2 \text{ g}^{-1}\text{s}^{-1}$; Figure 2.1). However, homogenizing dung prior to gas sampling did not significantly affect CO_2 emissions ($F_{(2,17)} = 9.525$; $P = 0.26$; $R^2 = 0.53$; Figure 2.1).

Methane (CH_4) emissions: Both the feed additive ($F_{(2,17)} = 37.55$; $P < 0.001$; $R^2 = 0.82$;) and dung homogenization ($F_{(2,17)} = 37.55$; $P = 4.09\text{e-}4$; $R^2 = 0.82$) significantly reduced CH_4 emissions. Dung samples from the supplemented group ($2.69 \pm 0.283 \text{ ng CH}_4 \text{ g}^{-1}\text{s}^{-1}$) emitted 49% less CH_4 than those from the control group ($5.22 \pm 0.388 \text{ ng CH}_4 \text{ g}^{-1}\text{s}^{-1}$; Figure 2.1). Homogenizing dung prior to sampling resulted in the homogenized group emitting 46% less CH_4 ($3.21 \pm 0.384 \text{ ng CH}_4\text{g}^{-1}\text{s}^{-1}$) when compared to the non-homogenized group ($4.70 \pm 0.564 \text{ ng CH}_4\text{g}^{-1}\text{s}^{-1}$; Figure 2.1).

Nitrous oxide (N_2O) emissions: Neither the feed additive ($F_{(2,17)} = 0.02$; $P = 0.850$; $R^2 = 0.02$) nor homogenization ($F_{(2,17)} = 0.02$; $P = 0.913$; $R^2 = 0.02$) significantly affected nitrous oxide emissions (Figure 2.1).

Carbon dioxide-equivalents (CO_2e) emissions: The feed additive significantly reduced CO_2e emissions ($F_{(2,17)} = 10.77$; $P < 0.001$; $R^2 = 0.56$). Dung from the supplemented group ($0.693 \pm 0.057 \mu\text{g CO}_2\text{e g}^{-1}\text{s}^{-1}$) had CO_2e emissions that were 30% lower than emissions from the control group ($0.991 \pm 0.035 \mu\text{g CO}_2\text{e g}^{-1}\text{s}^{-1}$; Figure 2.1). Homogenization had no significant effect on CO_2e emissions ($F_{(2,17)} = 10.77$; $P = 0.2838$; $R^2 = 0.56$).

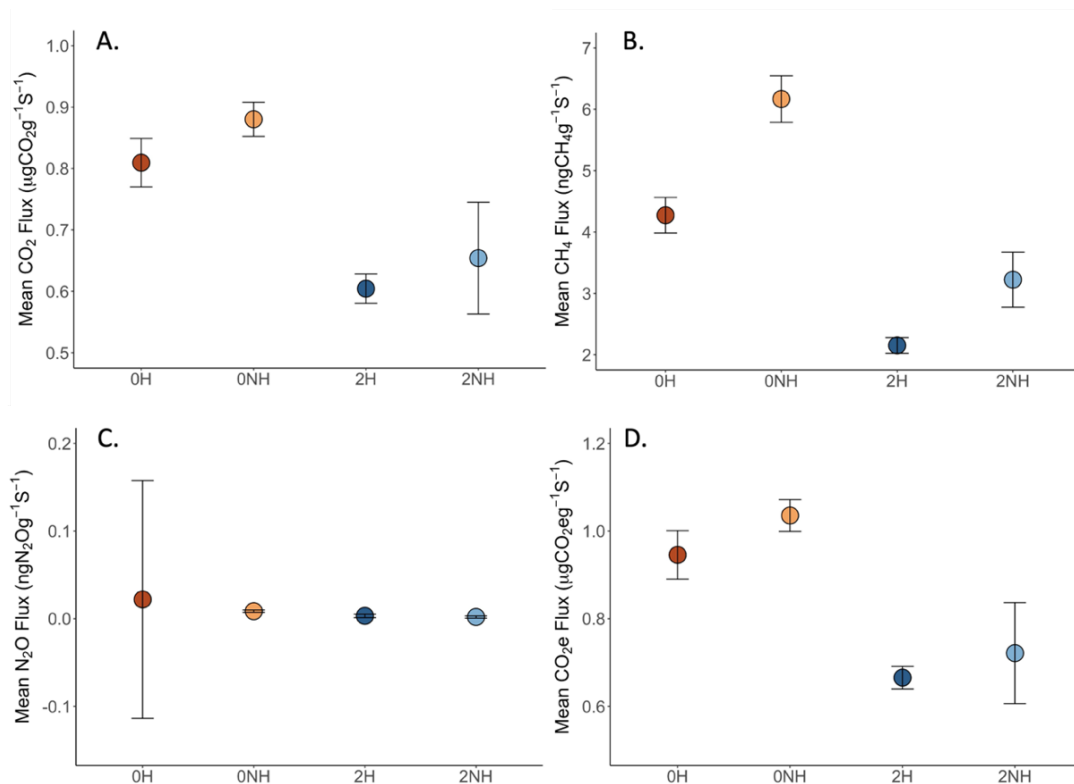


Figure 2.1 Mean and standard error carbon dioxide (A), methane (B), nitrous oxide (C), and carbon dioxide-equivalent (D) emissions from fresh dung samples. Treatment pairings (control dung with homogenization (0H), control dung without homogenization (0NH), supplemented dung with homogenization (2H), and supplemented dung without homogenization (2NH)), respective mean emissions are represented, with orange circles representing the control group and blue circles representing the supplemented group. Dietary supplementation significantly reduced CO₂ (-26%), CH₄ (-49%), and CO₂e (-30%) emissions, while homogenization significantly reduced CH₄ emissions (-46%). Darker colors represent homogenized dung samples, while lighter colors represent non-homogenized samples.

Experiment 2: The effects of dietary supplementation with brown seaweed on beetle-mediated GHG emissions

Carbon dioxide (CO₂) emissions: The interaction between the feed additive and beetle presence was not significant ($F_{(3,16)} = 1.88$; $P = 0.2776$; $R^2 = 0.26$; Figure 2.2). However, supplementing beef cattle diets with brown seaweed weakened the beetle-mediated increase in CO₂ emissions. In the control group, *C. erraticus* presence led to a 16% increase in emissions, while in the supplemented group, *C. erraticus* presence resulted in only a 4% increase (Figure 2.2).

Methane (CH₄) emissions: There was a significant interaction between the supplementation of beef cattle diets with brown seaweed and beetle presence on cumulative CH₄ emissions ($F_{(3,16)} = 68.86$; $P = 1.47e-05$; $R^2 = 0.93$). Regardless of the treatment group, beetle activity reduced dung CH₄ emissions (control group = 90% reduction; supplemented group = 91% reduction). While the relative reduction in CH₄ emissions was the same across treatment groups, the presence of *C. erraticus* in the control group led to a greater absolute reduction in emissions compared to the supplemented group. In the supplemented group, beetle presence reduced emissions by 0.143 ng CH₄ g⁻¹s⁻¹, compared to 0.539 ng CH₄ g⁻¹s⁻¹ in the control group. Therefore, the addition of brown seaweed to beef cattle diets reduced the magnitude of the effects of *C. erraticus* on dung CH₄ emissions (Figure 2.2).

Nitrous oxide (N₂O) emissions: The addition of the brown seaweed feed additive did not significantly influence beetle-mediated nitrous oxide emissions from beef cattle dung ($F_{(3,16)} = 3.02$; $P = 0.8635$; $R^2 = 0.36$; Figure 2.2).

Carbon dioxide-equivalents (CO₂e) emissions: The interaction between the feed additive and beetle presence did not significantly affect overall CO₂e emissions from beef cattle dung ($F_{(3,16)} = 1.94$; $P = 0.1269$; $R^2 = 0.27$; Figure 2.2).

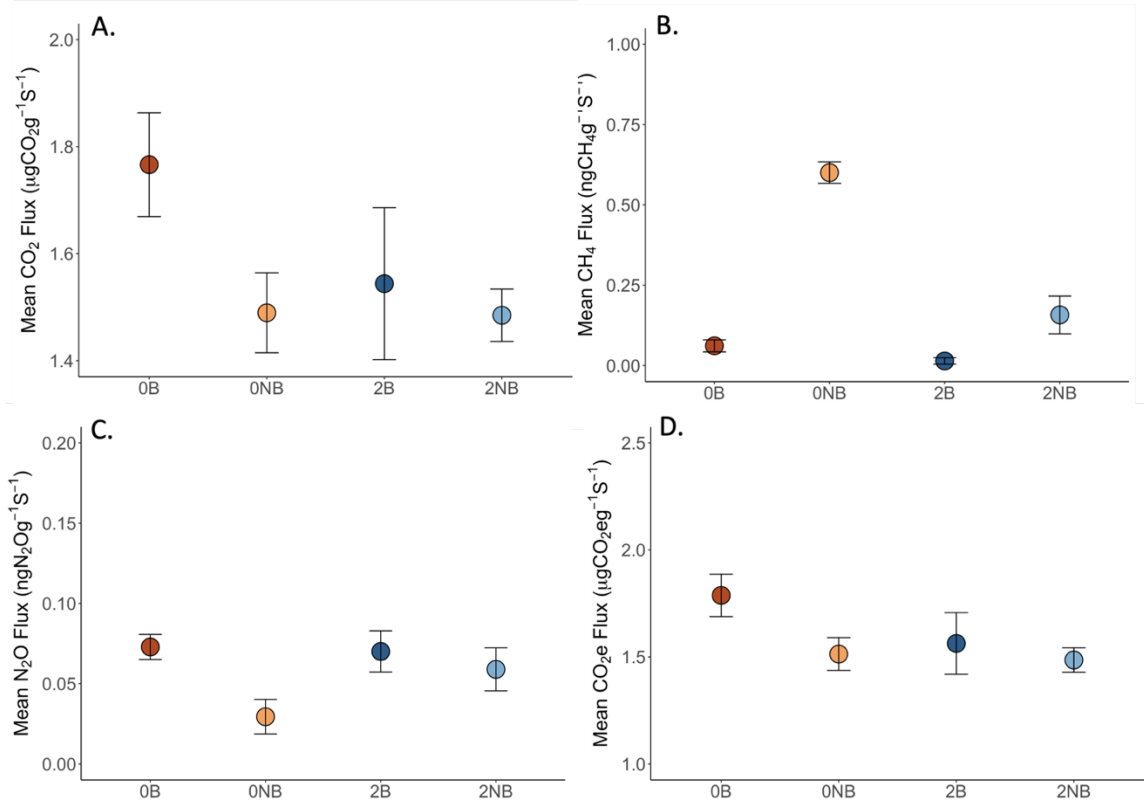


Figure 2.2 Mean and standard error carbon dioxide (A), methane (B), nitrous oxide (C), and carbon dioxide-equivalent (D) emissions from thawed dung. Mean emissions are shown for different combinations of dung feed treatment groups and beetle presence are represented: supplemented dung with *Colobopterus erraticus* (2B), supplemented dung without *C. erraticus* (2NB), control dung with *C. erraticus* (0B), and control dung without *C. erraticus* (0NB). Mean emissions are represented by circles, with orange circles indicating the control group and blue circles representing the feed additive treatment group. Darker colors represent beetle presence and lighter colors represent beetle absence. A significant interaction between beetle presence and dietary supplementation was found for CH_4 emissions, with beetle presence resulting in stronger beetle-mediated reductions in control dung than in supplemented dung.

Discussion

Supplementing beef cattle diets with brown seaweed led to a significant reduction in dung CO_2 , CH_4 , and CO_2e emissions, but had no effect on N_2O emissions (Figure 2.1). The homogenization of dung prior to GHG sampling significantly reduced CH_4 emissions, but had no effect on CO_2 , N_2O or CO_2e emissions (Figure 2.1). Additionally, supplementing beef cattle diets with brown seaweed significantly reduced the magnitude of beetle-mediated effects on dung CH_4 emissions (Figure 2.2).

The effects of brown seaweed on dung GHG emissions

In this study, I hypothesized that supplementing beef cattle diets with brown seaweed would increase CO₂ emissions while reducing CH₄ emissions. Although the results rejected the null hypothesis, which stated that dietary supplementation would have no effect on dung emissions, the direction of the effects of dietary supplementation on dung CO₂ emissions was not as expected. The greater dry matter (DM) content and C:N ratio observed in dung from supplemented treatment groups (Table 2.2) was expected to raise CO₂ emissions, as higher DM content stimulates both microbial activity (Haynes & Williams, 1993) and microbial mineralization processes (Flessa & Beese, 2000; Jiang et al., 2012). The increased C: N ratio was predicted to reduce CH₄ emissions, as CH₄ emissions are negatively correlated with the C: N ratio (Jarvis et al., 1995; Pelster et al., 2016). Despite the favorable conditions for CO₂ production, dietary supplementation reduced both dung CO₂ and CH₄ emissions, suggesting that these reductions may be associated with decreased microbial activity.

Previous studies have demonstrated that modifying cattle diets can alter the composition of dung microbial communities (Jin et al., 2017; Andrade et al., 2022; Kibegwa et al., 2023). For example, supplementing dairy cattle diets with 4 g/d/head of active dried yeast altered both the population size and composition of fecal methanogenic archaea (Jin et al., 2017). However, to my knowledge, no study has investigated the effects of supplementing beef cattle diets with brown seaweed on dung GHG emissions or the composition of the fecal microbial community. Ramin et al (2023) found that supplementing dairy cattle diets with *Asparagopsis taxiformis*, a red seaweed containing the secondary metabolite bromoform, had no effect on dung CH₄ emissions or fecal microbial communities. This

lack of effect may be due to the rapid degradation of bromoform in the rumen, preventing its excretion in feces (Muizelaar et al., 2021; Romero et al., 2023; Cowley et al., 2024). In contrast, brown seaweeds contain phlorotannins (Chkikvishvili & Ramazanov, 2000) and polysaccharides (Chandini et al., 2008), two compounds containing antimicrobial properties (Nagayama, 2002). These compounds are resistant to digestion and absorption (Deville et al., 2004) and have been shown to alter microbial composition in the cecum and colon when used as dietary supplements (Reilly et al., 2008; Lynch et al., 2010). The composition and abundance of the microbial community within the rumen influence GHG emissions, as microbial respiration is an important source of CO₂ (Billings & Ziegler, 2008) and methanogenic potential is closely tied to microbial community composition (Hahn et al., 2018). Therefore, changes to dung's microbial community composition, or to dung microbial activity, may explain the observed reductions in both CO₂ and CH₄ emissions.

Although the results support that supplementing beef cattle diets with brown seaweed altered dung microbial activity or community composition, it had no effect on dung N₂O emissions (Figure 2.2). However, N₂O emissions from dung in this study were low. Although dung is an important source of N₂O, N₂O-N losses from urine (0.56%) are greater than those from dung (0.19%), due to urine containing more readily available forms of N (Yamulki et al., 1998; Saggar et al., 2004). Therefore, the low N₂O emissions noted in this study may be explained by the dung sample's anaerobic environment and the lack of more readily available forms of N for denitrification processes in dung samples. Therefore, the reductions in both CO₂ and CH₄, along with no significant change to N₂O emissions resulted in an overall decrease in GHG emissions.

The effects of homogenization on dung GHG emissions

Dung homogenization was hypothesized to reduce CH₄ emissions by promoting oxygenation. As CH₄ is produced in anaerobic environments, homogenization is expected to reduce CH₄ production by creating more aerobic conditions. Although past research found that homogenized dung emitted 1.5 times less CH₄ than unmixed samples, this reduction was not statistically significant (Fowler et al., 2020). Similarly, my study found that homogenization reduced CH₄ emissions, with non-homogenized dung emitting 1.46 times more CH₄ than homogenized samples. However, in my case this relationship was statistically significant, likely due to my experiment occurring under controlled laboratory conditions.

The difference in the statistical significance of the effects of homogenization on dung CH₄ emissions may be attributed to unmeasured sources of variation in the Fowler et al. (2020) study, such as dung mass. In their study, unmixed dung mass was derived from dung volume, as opposed to directly weighting dung mass, which likely introduced greater variation by reducing measurement precision and accuracy. Additionally, Fowler et al. (2020) introduced variation in dung pat age as unmixed dung pats were not collected directly from the cattle. However, despite the difference in statistical significance, both studies have demonstrated that homogenizing dung prior to GHG sampling reduces cumulative CH₄ emissions.

The effects of supplementing beef cattle diets with brown seaweed on beetle-mediated GHG emissions

The inclusion of brown seaweed in beef cattle diets was hypothesized to weaken the beetle-mediated reduction in CH₄ emissions. It was further hypothesized that the presence of *C.*

erraticus in the mesocosms would lead to a greater increase in CO₂e emissions in supplemented dung, due to the weakened beetle-mediated reduction in CH₄ emissions. Ultimately, the addition of brown seaweed to beef cattle diets was found to reduce the magnitude of *C. erraticus*' effects on dung CH₄ emissions (Figure 2.2), while having little impact on CO₂, N₂O, or overall CO₂e emissions. Although, the magnitude of beetle-mediated effects on dung CH₄ emissions was reduced in supplemented dung pats, the relative beetle-mediated reductions remained identical across treatment groups. This suggests that supplementing beef cattle diets with brown seaweed had no effect on *C. erraticus*' CH₄ mitigation potential, as the smaller absolute effects noted in supplemented dung samples were caused by lower initial CH₄ emissions.

Previous studies have shown that dung beetle presence typically increases CO₂ emissions (Penttilä et al., 2013; Iwasa et al., 2015; Evans et al., 2019) due to increased beetle respiratory activity, enhanced microbial respiration (Stevenson & Dindal, 1987), and the creation of beetle-made macropores within the dung pat (Evans et al., 2019). In my study, although not statistically significant, the beetle-mediated increase in CO₂ emissions was weaker in dung from the supplemented treatment group. This finding aligns with the previous hypothesis that dietary supplementation suppressed dung microbial activity, primarily in the form of methanogenic activity. Although one would expect a weakened beetle-mediated increase in CO₂ emissions in dung with suppressed microbial activity, changes in dung moisture dynamics can also affect GHG emissions (Evans et al., 2019). Therefore, further research is necessary to confirm this hypothesis and clarify the underlying mechanisms, as dung beetles can alter GHG emissions not only through

microbial stimulation but also through physical alterations of the dung environment, resulting in shifts in microbial activity.

These results demonstrate that the inclusion of brown seaweed in beef cattle diets reduced the magnitude of beetle-mediated effects on dung CH₄ emissions (Figure 2.2), however this effect was caused by a reduction in initial dung pat CH₄ emissions rather than in the change in beetle-mediated effects. Dung beetle presence has been found to consistently reduce dung CH₄ emissions, (Penttilä et al., 2013; Iwasa et al., 2015; Hammer et al., 2016; Fowler et al., 2020), due to the increased aeration of dung pats caused by beetle tunneling activity (Penttilä et al., 2013). The addition of the feed additive to beef cattle diets led to a reduction in dung CH₄ emissions, thus weakening the effects of beetle-induced aeration. Although the use of the feed additive reduced the magnitude of beetle-mediated effects on dung CH₄ emissions, the treatment group pairing both *C. erraticus* presence and supplemented dung had numerically lower CH₄ emissions than other treatment groups, whilst dietary supplementation had no effect on beetle-mediated CO₂e emissions.

Limitations and future directions

Results from this study suggest that the antimicrobial properties of brown seaweed resulted in the reduction of dung microbial activity, leading to a reduction in cumulative GHG emissions. However, this study did not directly assess the effects of dietary supplementation on the dung microbial community composition or activity. Therefore, further research is needed to understand the effects of the feed additive on dung microbial composition using methods such as 16S rRNA Amplicon Sequencing (Ramin et al., 2021). Given the microbial interactions between dung and soil (Aarons et al., 2009; Cai et al., 2017), it is also important to evaluate the effects of the feed additive on dung GHG

emissions under field conditions, where these interactions may lead to increased microbial activity (Aarons et al., 2009; Cai et al., 2017).

Although the supplementation of beef cattle diets with brown seaweed was found to have no effect on dung N₂O emissions, the headspace volume of the closed chambers in relation to dung volume used in this project may have affected my ability to detect any changes in N₂O production. While widely practiced as a methodology, the use of closed chambers to measure N₂O emissions has been shown to reduce to goodness of fit (R²) in linear regressions when the measured emissions are below 5 g N₂O-N ha⁻¹d⁻¹ (Lammirato et al., 2018). Therefore, the use of closed chambers in combination with the small magnitude of N₂O emissions measured in this study may have contributed to greater variation in the data, impacting model fit and obscuring the effects of dietary supplementation on dung N₂O emissions. Other potential limitations of this study should be acknowledged, including the freezing of dung prior to GHG sampling. Although freezing dung samples was necessary to align dung collection from the feeding trial with dung beetle activity, freezing dung samples before GHG analysis can affect microbial activity. The effects of freeze-thaw cycles have been studied in soils, where microbial activity (Tian et al., 2018; Rooney et al., 2022) and GHG emissions were altered (Osei et al., 2024). Notably, these cycles led to increased CO₂ and N₂O emissions within the first 21-days of the freeze-thaw cycle (Osei et al., 2024). Therefore, although sample treatment was standardized across samples, it is important to recognize that the transport of dung on ice and the freeze-thaw cycle may have influenced the results.

Conclusion

Cattle dung is an important source of GHG emissions in the Canadian livestock sector. While previous research has shown that supplementing beef cattle diets with brown seaweed can reduce enteric methane emissions, the present study reveals that these benefits extend to emissions from cattle dung. The findings from this research show that supplementing beef cattle diets with brown seaweed can reduce dung emissions while having no effect on beetle-mediated CO₂e emissions, revealing that dietary supplementation with brown seaweed is a viable strategy for reducing emissions across the livestock sector.

Dung homogenization led to a reduction in cumulative CH₄ emissions over the 21-day sampling period, which is important to consider when assessing beetle-mediated GHG emissions. Results from this study highlight the need to further examine how experimental procedures and variables can impact the quantification of beetle-mediated emissions. Therefore, this study not only advances our understanding of brown seaweed's potential to reduce emissions but also offers insights into how dietary supplementation can help achieve emissions reduction targets beyond enteric emissions.

Chapter 3: Experimental methodologies matter when measuring beetle-mediated GHG emissions from dung

Abstract

The livestock sector contributes approximately 15% of global greenhouse gas emissions, with the decomposition of dung on pasture being an important source of CH₄ and N₂O emissions. Dung beetles (Coleoptera: Scarabaeoidea) can influence dung decomposition processes, thereby impacting dung GHG emissions. However, research findings from existing literature on beetle-mediated GHG emissions are inconsistent, varying both in terms of the magnitude and direction of the noted effect. These inconsistencies may result from variations in experimental practices, including differences in study duration and experimental settings. In this study, I evaluate how dung mass and surface-area-to-volume (SA/V) ratio influence beetle-mediated emissions. Using a laboratory-based experiment, I found that the alteration of dung mass can significantly impact beetle-mediated CO₂ and CO₂e emissions. The presence of the dung beetle *Onthophagus nuchicornis* in mesocosms containing the 100 g dung pats increased CO₂ emissions by 29% per gram of dung, while their presence in mesocosms containing the 400 g dung pats increased emissions by 64% per gram of dung. In addition, the interaction between dung SA/V ratio and dung mass as well as dung SA/V ratio and beetle presence significantly impacted CH₄ emissions. Flattening 100 g dung pats resulted in a 44% reduction in CH₄ emissions, while flattening 400 g dung pats reduced CH₄ emissions by 11%. Additionally, beetle presence in flattened dung pats resulted in a 78% reduction in CH₄ emissions, whereas their presence in non-flattened dung pats only resulted in an 8% reduction CH₄ emissions. These findings reveal the importance of methodological decisions for understanding the relationship between dung beetle activity and GHG emissions from dung pats.

Introduction

Globally, the livestock sector is responsible for approximately 15% of greenhouse gas (GHG) emissions (Bellarby et al., 2013; Gerssen-Gondelach et al., 2017). The decomposition of dung on pasture is an important source of emissions (Jarvis et al., 1995) and can be influenced by various abiotic and biotic factors including temperature, moisture, and dung beetle presence (Maljanen et al., 2007; Lin et al., 2009; Maljanen et al., 2012; Penttilä et al., 2013).

Dung beetles (Scarabaeidae: Aphodiinae, Scarabaeinae, and Geotrupinae), a group of coprophagous insects, play an important role in dung pat decomposition in pasture systems (Stevenson & Dindal, 1987; Lee & Wall, 2006). Their tunneling activities aerate dung pats,

impacting aerobic respiration processes and influencing GHG emissions (Breymeyer et al., 1975; Stevenson & Dindal, 1987; Penttilä et al., 2013). In a study that combined field measurements with lifecycle analyses, the activity of dung beetles in dung pats was found to reduce GHG emissions at both the dung pat and pasture level, by 7 and 12% respectively (Slade et al., 2016).

Despite studies finding that dung beetles can impact dung GHG emissions, they differ with respect to direction and magnitude of beetle-mediated GHG emissions. Although most studies agree upon the effects of dung beetle's tunneling activity on dung pat aeration, only a few studies have found beetle presence to reduce dung CH₄ emissions (Penttilä et al., 2013; Iwasa et al., 2015; Hammer et al., 2016; Slade et al., 2016; Fowler et al., 2020; Verdú et al., 2020). In contrast, other studies found dung beetle presence to result in increased CH₄ emissions (Piccini et al., 2017; Evans et al., 2019).

In addition to the observed impacts of dung beetle presence on CH₄ emissions, the aeration of dung pats can significantly increase N₂O emissions (Penttilä et al., 2013; Iwasa et al., 2015; Fowler et al., 2020). It has been suggested that beetle-induced aeration of dung pats increases NO₃⁻ levels, which in turn increases N₂O emissions through denitrification (Kazuhira et al. 1991). While some studies found beetle presence to increase N₂O emissions (Penttilä et al., 2013; Iwasa et al., 2015; Fowler et al., 2020), others observed a reduction (Piccini et al., 2017). Inconsistencies surrounding beetle-mediated CO₂ emissions follow similar trends, with some studies reporting beetle presence to increase CO₂ emissions (Evans et al., 2019; Iwasa et al., 2015), while others report a significant reduction in CO₂ emissions (Penttilä et al., 2013; Piccini et al., 2017; Fowler et al., 2020). These

inconsistencies are not well understood, highlighting the need to further evaluate the effects of the experimental practices used to measure beetle-mediated emissions.

Studies evaluating the effects of dung beetles on dung GHG emissions vary in terms of study duration (e.g. 7 days vs. 56 days), beetle species composition (single species vs. 9 species), location (i.e. laboratory-based vs. field-based), and dung mass (300 g vs. 1.5 kg). Time since deposition explains over 60% of the variation in CH₄ fluxes, with it lowering CH₄ emission by 4.98× over the first 5 days (Fowler et al., 2020). On the other hand, greater dung mass has been hypothesized to stimulate CO₂ and CH₄ production by increasing the availability of accessible carbon substrates and promoting prolonged dung moisture retention (Sordi et al., 2014; Fowler & Mullens, 2016; Zhu et al., 2018; Du et al., 2021). Both dung moisture and C substrate content can influence dung degradation, as well as microbial diversity and richness (Zhang et al., 2025), and therefore affect GHG emissions (Li et al., 2024). Although previous research found dung mass to have no effect on GHG emissions (Zhu et al., 2018), the impact of dung mass on beetle-mediated emissions remains unexplored. Changes in dung mass can impact beetle-mediated emissions by altering dung moisture retention and C substrate availability.

This study seeks to evaluate how the interaction between dung shape and mass affect beetle-mediated emissions. Specifically, it examines how the manipulation of dung surface-area-to-volume (SA/V) ratio and dung mass influences GHG emissions. I hypothesized that increased dung mass will enhance the effects of beetle presence on GHG emissions, as it increases available carbon substrate and dung moisture retention, thereby stimulating CO₂ and CH₄ production. However, increased SA/V ratios (i.e. a flattening of the dung pat, while maintaining a consistent mass) will lessen the expected impact of

beetles on GHG emissions by creating less favourable conditions for CH₄ production via increased crust formation rates.

Methods and materials

Cattle diets and dung collection

Fresh dung was collected from five dairy cows at Dalhousie University's Ruminant Animal Centre (43°39.1935' N, 79°22.9911' W). Prior to dung collection, the cattle grazed on pasture and were fed a total mixed ration (TMR) consisting of corn silage (21%), grass silage (62%), and concentrate (17%). The cows did not receive any antiparasitic treatment, but were treated year-round with monensin, an ionophore known to improve animal performance and modify rumen bacterial populations (Duffield et al., 2008). On 22 July 2024, twelve kilograms of dung were collected directly from the dairy cows and stored in the laboratory at 4 °C until needed (< 12h). Prior to setup, 10 kg of dung were homogenized for 30 seconds at 10-second intervals using an electric power drill with a paint mixer attachment.

Experimental design

To examine the effects of dung SA/V ratio and dung mass on beetle-mediated GHG emissions, a randomized 2 x 2 x 2 factorial design was used. This approach allowed for the evaluation of the effects of the three predictor variables —dung mass, dung SA/V ratio, and beetle presence — as well as their interactions, on the cumulative emissions of CO₂, CH₄, and N₂O. This experimental design combined two surface-area-to-volume treatments (high vs low) with two dung masses (100 g vs 400 g), each tested in the presence and absence of dung beetles. The SA/V ratio of dung pats was adjusted by flattening the dung pat prior to sampling, resulting in an increased diameter and reduced thickness. This

resulted in four combinations of treatment pairings using 400 g dung pats: flattened (400-SA/V(High)) and unflattened (400-SA/V(Low)), each tested in the presence (400-SA/V(High)-B; 400-SA/V(Low)-B) and absence (400-SA/V(High)-NB; 400-SA/V(Low)-NB) of dung beetles (Figure 3.1). Another four combinations of treatment pairings were created using 100 g dung pats: flattened (100-SA/V(High)) and unflattened (100-SA/V(Low)), also tested in the presence (100-SA/V(High)-B; 100-SA/V(Low)-B) and absence (100-SA/V(High)-NB; 100-SA/V(Low)-NB) of dung beetles. Each treatment pairing was replicated five times (n=5), yielding a total of 40 mesocosms (N = 40). However, one 400-SA/V(Low)-B replicate was excluded from analysis due to experimental error, resulting in a final number of N = 39 mesocosms.

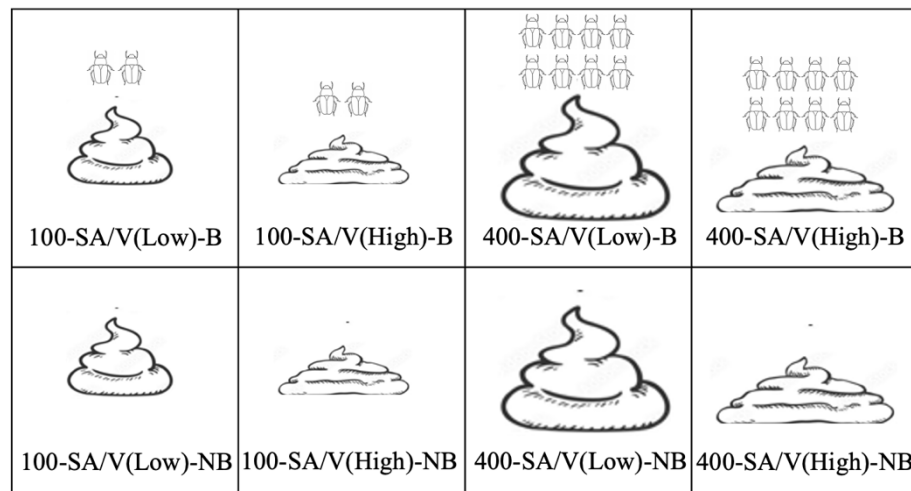


Figure 3.1 Visual representation of the four treatment combinations used in this study both sampled in the presence and absence of dung beetles. Changes in dung pat size reflects variations in dung mass (100 g vs. 400 g), while changes in dung shape indicates changes to the SA/V ratio (SA/V (High) vs. SA/V(Low)). Beetle presence in mesocosms is represented by the beetle symbols.

On July 22, 2024, c. 300 dung beetles of the species *Onthophagus nuchicornis* were collected at Arbour Ridge Farm (45°21.8208' N, 63°13.8036' W). *O. nuchicornis* is a common non-native dung beetle species that was first reported in Canada in 1945 (Hatch,

1971). This tunnelling dung beetle species can be found across both Canada and the United States, representing about 40% of total trap catch in both Alberta and Michigan (Floate et al., 2017). The beetles were collected by hand-searching horse dung and were placed in 1.3 L cylindrical high-density polyethylene (HDPE) containers (Priority Plastics, Grinnell, IA, US). They were held in containers for 24 hours, with RO-water-moistened paper towel and horse dung *ad libitum*.

Mesocosms consisted of 7.5 L plastic containers (PR80001MHP09, Canadian Tire, Nova Scotia, Canada), each filled to a depth of 13 cm with 6.5 kg (\pm 253 g) of coarse silica sand (Model #8526, Shaw Resources, New Brunswick, Canada). Sand was then moistened with 750 mL of water. On 23 July 2024, at time zero ($t=0$), either 400 g or 100 g of dung were added to each mesocosm, depending on the treatment group of the experimental unit. To maintain consistent SA/V ratios across samples, dung pats were formed using 500 mL (1346891, Kirkland Signature, Nova Scotia, Canada) and 227 mL (11115001806, Becel, Nova Scotia, Canada) plastic containers. These containers were used as molds to shape the dung pats into standardized shapes. Once all dung pats were formed, those in the high SA/V ratio treatment group were flattened with a spatula, increasing dung pat diameter and decreasing dung pat thickness. Dung pat thickness was measured to ensure consistency of the flattening treatment across replicates within the same treatment group. The average dung pat diameter, thickness, and SA/V ratio for each treatment group is presented in Table (3.1). Five dung beetles (2 males, 3 females) were then placed in mesocosms containing 100 g of dung, while 20 beetles (8 males, 12 females) were placed in mesocosms containing 400 g of dung, such that the beetle-to-dung mass ratio remained constant. Mesocosms were

sealed with a 2-mm fiberglass mesh, ensuring the beetles remained in the mesocosms while allowing for gaseous exchange with the surrounding air.

Table 3.1 Summary of the dung pat dimensions at $t=0$, including the average dung pat thickness (mm), diameter (mm) and surface-area-to-volume (SA/V) ratio for each treatment.

	Mean thickness (mm)	Mean diameter (mm)	Mean SA/V ratio
100-SA/V (High)	5 ± 0.47	102 ± 7.6	0.45 ± 0.04
100-SA/V (Low)	44 ± 2.2	70 ± 0.87	0.10 ± 0.01
400-SA/V (High)	6 ± 2.3	171 ± 2.1	0.37 ± 0.02
400-SA/V(Low)	59 ± 6.2	110 ± 1.4	0.10 ± 0.03

GHG sampling

Gas samples were collected seven times over a 21-day sampling period (on days 0.5, 1, 3, 5, 7, 10, and 21) from 23 July 2024 to 13 August 2024. Air temperatures were monitored throughout the 21-day sampling period using a data logger (Kestrel DROP D2HS Heat Stress Monitor). Gas sampling took place over two sampling rounds per sampling period, with gas samples extracted from half the mesocosms per round. The gas extraction procedure and greenhouse gas flux calculations followed Dalhousie University’s “Standard Operating Procedure for Greenhouse Gas (CO₂, CH₄, and N₂O) Flux Measurement” (Burton & Janes, 2013). At the start of each sampling round ($t=0$), a 20 mL syringe was used to collect an air sample, which was then injected into a pre-evacuated exetainer (12 mL). The gas concentrations from this sample acted as the initial data point in the linear regression to calculate gas flux. Once the lab air was sampled, the mesh lid on the first mesocosm was replaced with an airtight lid fitted with a rubber septum. After securing the lid, 60 mL of laboratory air were injected into the mesocosm using a syringe (60 mL). This procedure was repeated at one-minute intervals until all mesocosms had been injected with

60 mL of air. Following this, three gas samples were collected from each mesocosm at 10-minute intervals (10, 20, and 30 minutes) using a 20 mL syringe. The gas samples were then injected into pre-evacuated exetainers (12 mL). Once the first round of sampling was completed, the airtight lids were replaced with their original mesh lids and the second round of greenhouse gas sampling was performed following the same procedure.

The concentration (ppm) of each gas (CO₂, CH₄, and N₂O), was measured using gas chromatography. The Bruker SCION SQ 450 Gas Chromatograph (SCION Instruments Canada, Edmonton, AB), equipped with three detectors, electron capture (ECD), flame ionization (FID) and thermal conductivity (TCD), along with a Combi-PAL autosampler was used. The gas flux was calculated using linear regression, with the four gas samples taken per mesocosm (0, 10, 20, 30 minutes) used to fit the linear regression line. If the relationship between the data points was non-linear, one sampling point was excluded. When two or more sampling points had to be removed to achieve linearity, the replicate was dropped from analysis. Gas fluxes were calculated as the change in gas concentration in the headspace (11 cm) over time per gram of dung (Burton & Janes, 2013). All fluxes were normalized by the average dry mass of the dung. Once fluxes had been calculated, the cumulative flux for each mesocosm over the 21-day period was determined as the area under the curve. To facilitate comparison of emissions across mesocosms, total emissions were converted to CO₂-equivalents (CO₂e). The global warming potential (GWP) impact factors for a 100-years' time for each gas (GWP_{100-CH₄} = 28; GWP_{100-N₂O} = 265), as per the fifth IPCC report (Myhre et al., 2013), were used to convert each gas to CO₂e. The cumulative flux of each gas and the CO₂e of each mesocosm were used as response variables in the subsequent analyses.

Statistical analysis

Statistical analyses were conducted using linear models implemented in R version 4.5.0 (R Core Team, 2024), using R studio version 2024.09.0. The significance level was adjusted using Benjamini and Hochberg's (1995) control for false discovery rates (Benjamini & Hochberg, 1995). The model's explanatory power was assessed using the adjusted R-squared value.

Four linear models were developed to analyse the effects of dung pat mass and SA/V ratio on beetle-mediated greenhouse gas emissions. The measured cumulative flux of each gas (i.e. CO₂, CH₄, N₂O, CO₂e) acted as the response variable. Dung mass (100 g or 400 g), the SA/V ratio (high or low), beetle presence (present or absent), as well as the interactions between these three variables were included as model terms. Model complexity was evaluated using the Akaike Information Criterion (AIC), with the best-fitting model selected based on the lowest AIC value. A stepwise function with backward selection, guided by AIC, was employed to identify the optimal combination of model terms. The final model for each gas is presented in Table (3.2).

Table 3.2 Summary of the model terms used to evaluate the effects of dung mass (Mass), surface-area-to-volume (SA/V) ratio, and beetle presence (Beetle) on the cumulative flux of the greenhouse gases of interest. The table includes model terms, Akaike’s Information Criterion (AIC) values, and adjusted R² values. AIC measured the relative quality of statistical models by balancing model fit and complexity, with lower values indicating better model performance. Adjusted R² values are provided to show the proportion of variance explained by each model. The notation of ‘:’ denotes interaction effects between variables. The significance level was adjusted using Benjamini and Hochberg’s (1995) control for false discovery rates (Benjamini & Hochberg, 1995).

Dependent Variable	Model terms	AIC value	Adjusted R²
CO₂	Beetle + SA/V + Mass + Beetle: Mass + SA/V: Mass	-25.491	0.79
CH₄	Beetle + SA/V + Mass + Beetle: Mass + SA/V: Mass + Mass: Beetle + SA/V: Mass: Beetle	114.94	0.85
N₂O	Beetle + SA/V + Mass	126.41	0.10
CO₂e	Beetle + SA/V + Mass + Beetle: Mass + SA/V: Mass + Mass: Beetle + SA/V: Mass: Beetle	-17.480	0.79

Results

Carbon dioxide (CO₂) emissions: Following the stepwise model simplification, the final model included the three main effect variables (Beetle, SA/V, and Mass) as well as two interaction effects (SA/V: Mass and Beetle: Mass). The model output is presented in (Table 3.3).

Table 3.3 Carbon dioxide model output showing the estimate coefficient and P-value for the five terms included in the model: beetle presence (Beetle), dung surface-area-to-volume (SA/V) ratio, dung mass (Mass), the two-way interaction occurring between beetle presence and dung mass (Beetle: Mass) and dung SA/V ratio and dung mass (SA/V: Mass). The significance level was adjusted using Benjamini and Hochberg’s (1995) control for false discovery rates (Benjamini & Hochberg, 1995).

Model term	Estimate coefficient	P-value
Beetle	1.6186	< 0.001*
SA/V	1.2575	< 0.001*
Mass	-0.7387	0.054
Beetle: Mass	1.3574	0.004*
SA/V: Mass	-0.5746	0.192

The interaction between beetle presence and dung mass significantly affected CO₂ emissions ($F_{(5,33)} = 29.22$; $P = 0.004$), while the second interaction term did not significantly influence the final model ($F_{(5,33)} = 29.22$; $P = 0.192$; Figure 3.2). The presence of beetles in mesocosms containing 400 g of dung resulted in a greater increase in CO₂ emissions compared to the addition of beetles in the 100 g dung mass mesocosms (Figure 3.2). The presence of beetles in mesocosms with the 100 g dung pats increased the mean CO₂ emissions by 29% per gram of dung, while their presence in mesocosms containing the 400 g dung pats resulted in a 64% increase in CO₂ emissions per gram of dung (Figure 3.2).

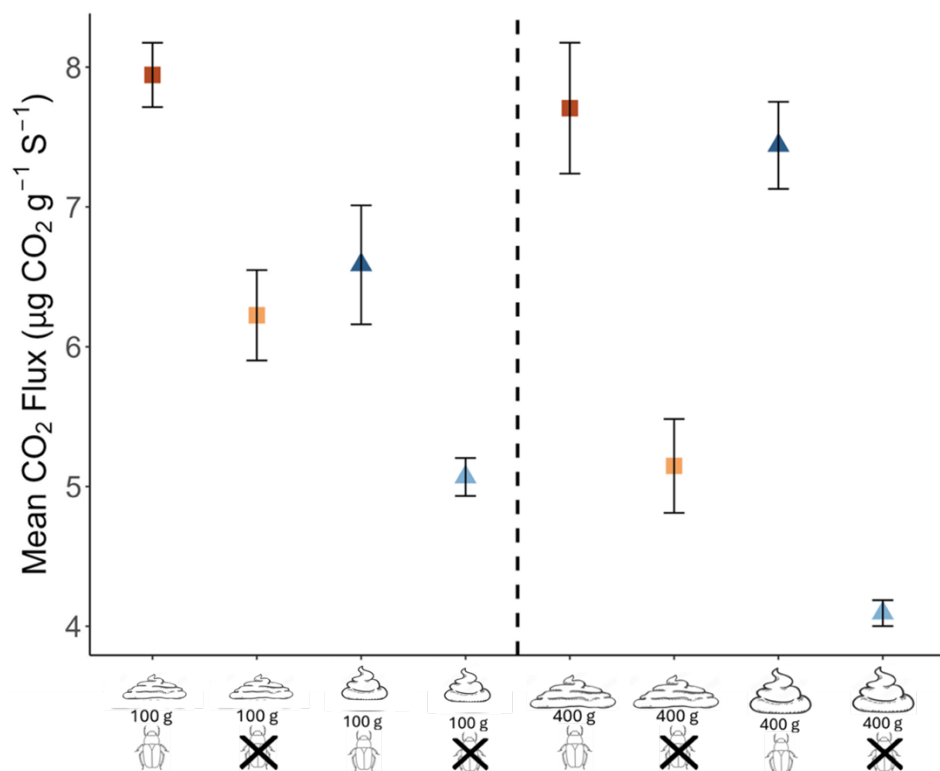


Figure 3.2 Mean \pm standard error of carbon dioxide (CO_2) emissions per gram of fresh dairy cow dung. Mean emission values for the 100 g dung mass treatment are shown to the left of the dotted line, and those for the 400 g dung mass treatment are shown to the right. Dung samples with a higher surface-area-to-volume (SA/V) ratio are represented by orange squares, and those with a lower SA/V ratio are represented by the blue triangles. Darker colors indicate beetle presence, while lighter colors indicate beetle absence. A significant interaction between beetle presence and dung mass was observed, with beetle-mediated CO_2 increases larger in the 400 g dung treatments than in the 100 g treatments.

Methane (CH_4) emissions: Following the stepwise model simplification, the three main effect variables (Beetle, SA/V, and Mass) as well as four interaction terms (Beetle: Mass, SA/V: Mass, Mass: Beetle, and SA/V: Mass: Beetle) were included in the final model (Table 3.4).

Table 3.4 Methane model output showing the estimate coefficient and P-value for the seven terms included in the model: beetle presence (Beetle), dung surface-area-to-volume (SA/V) ratio, dung mass (Mass), the two-way interactions between beetle presence and dung mass (Beetle: Mass), dung SA/V ratio and beetle presence (SA/V: Beetle), and dung SA/V ratio and dung mass (SA/ V: Mass), as well as the three-way interaction between dung SA/V ratio , dung beetle presence, and dung mass (SA/V: Beetle: Mass). The significance level was adjusted using Benjamini and Hochberg’s (1995) control for false discovery rates (Benjamini & Hochberg, 1995).

Model term	Estimate coefficient	P-value
Beetle	-6.1651	0.020
SA/V	-12.5146	< 0.001*
Mass	0.5688	0.823
Beetle: Mass	6.5907	0.074
Beetle: SA/V	-7.4614	0.045*
SA/V: Mass	15.6177	< 0.001*
Beetle: SA/V: Mass	-9.4519	0.075

The three-way interaction between beetle presence, SA/V ratio, and dung mass had no significant effect on CH₄ emissions ($F_{(7,31)} = 31.69$; $P = 0.075$; Figure 3.3). The interaction between beetle presence and dung mass did not significantly impact CH₄ emissions ($F_{(7,31)} = 31.69$; $P = 0.074$; Figure 3.3). However, the interaction between the SA/V ratio and dung mass ($F_{(7,31)} = 31.69$; $P < 0.001$), as well as between beetle presence and SA/V ratio ($F_{(7,31)} = 31.69$; $P = 0.045$) significantly impacted CH₄ emissions per gram of dung (Figure 3.3). Through this experiment, it was found that changes to the dung pat’s SA/V ratio had a stronger effect on dung of lower mass. Increasing the SA/V ratio, and hence the flattening of the dung pat, reduced CH₄ emissions by 44% in the 100 g dung treatment, while increased SA/V ratio only reduced emissions by 11% in the 400 g dung pat treatment group (Figure 3.3). Additionally, beetle presence was found to result in a stronger reduction in CH₄ emissions in flattened dung pats, with beetle presence resulting in a 78% reduction in CH₄ emissions in flattened dung pats and an 8% reduction in CH₄ emissions in non-flattened dung pats (Figure 3.3).

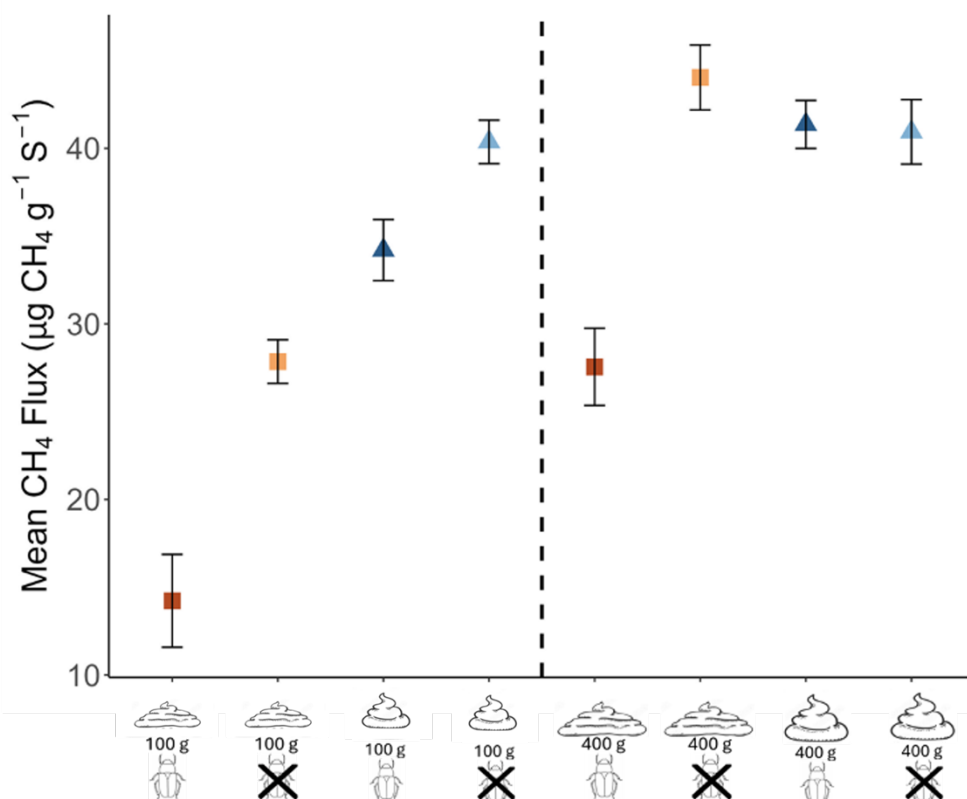


Figure 3.3 Mean \pm standard error of methane (CH_4) emissions per gram of dung, with the emissions from the 100 g dung mass treatment group to the left of the dotted line, and those of the 400 g dung mass treatment group to the right. Dung samples of higher surface-area-to-volume (SA/V) ratio are represented by orange squares, whereas blue triangles represent dung samples of lower SA/V ratio. Darker colors represent beetle presence, while lighter colors represent beetle absence. A significant interaction between SA/V ratio and dung mass was observed, where increased SA/V ratios had stronger effects on dung of smaller masse.

Nitrous oxide (N_2O) emissions: The stepwise function removed all interaction terms from the analysis, with the final model for nitrous oxide including only the three main effect variables. Neither dung mass ($F_{(3, 35)} = 0.5356$; $P = 0.321$), SA/V ratio ($F_{(3, 35)} = 0.5356$; $P = 0.511$), nor dung beetle presence ($F_{(3, 35)} = 0.5356$; $P = 0.746$), significantly affected N_2O emissions (Figure 3.4).

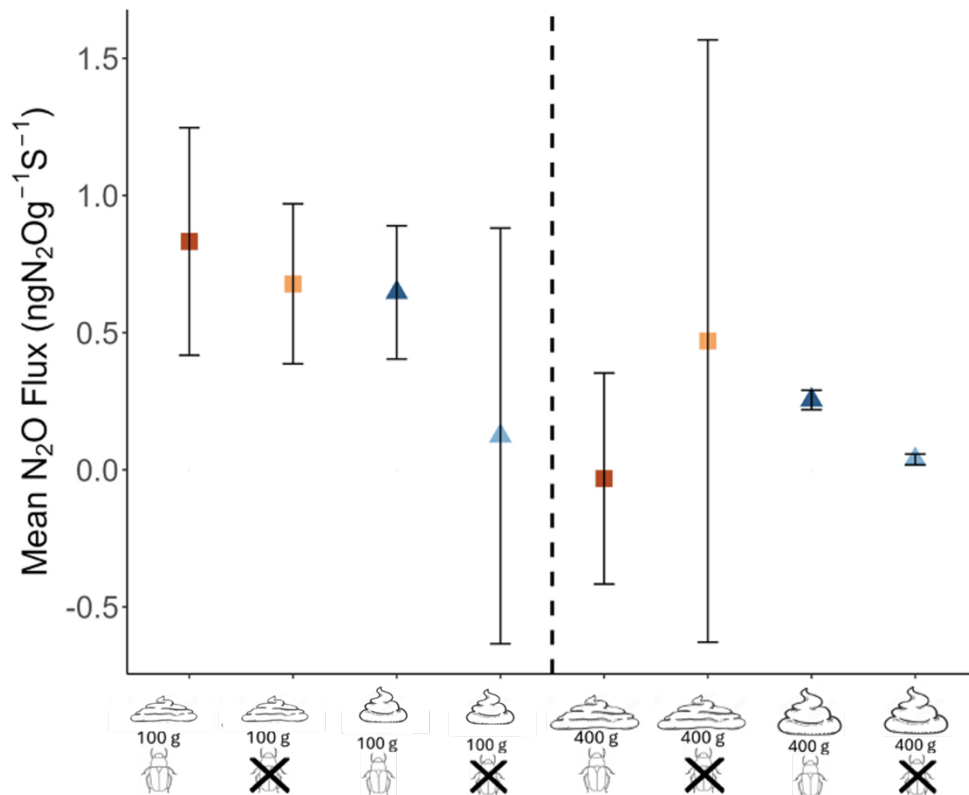


Figure 3.4 Mean \pm standard error of nitrous oxide (N_2O) emissions per gram of fresh dairy cow dung, with the emissions from the 100 g dung mass treatment group to the left of the dotted line, and those of the 400 g dung mass treatment group to the right. Dung samples of higher surface-area-to-volume (SA/V) ratio are represented by orange squares, whereas blue triangles represent dung samples of lower SA/V ratio. Darker colors represent beetle presence, while lighter colors represent beetle absence.

Carbon dioxide-equivalents (CO_2e) emissions: The final model included the three main effect variables (Beetle, SA/V, and Mass) as well as all four interaction terms (Beetle: Mass, SA/V: Mass, Mass: Beetle, and SA/V: Mass: Beetle) The model's output is presentend in Table 3.5.

Table 3.5 Carbon dioxide-equivalents model output showing the estimate coefficient and P-value for the seven terms included in the model: beetle presence (Beetle), dung surface-area-to-volume (SA/V) ratio, dung mass (Mass), the two-way interactions between beetle presence and dung mass (Beetle: Mass), dung SA/V ratio and beetle presence (SA/V: Beetle), and dung SA/V ratio and dung mass (SA/V: Mass), as well as the three-way interaction between dung SA/V ratio, dung beetle presence, and dung mass (SA/V: Beetle: Mass). The significance level was adjusted using Benjamini and Hochberg’s (1995) control for false

Model term	Estimate coefficient	P-value
Beetle	1.5176	0.001*
SA/V	1.1565	0.011*
Mass	-0.9745	0.030
Beetle: Mass	1.8290	0.005*
SA/V: Beetle	0.2020	0.740
SA/V: Mass	-0.1031	0.866
SA/V: Beetle: Mass	-0.9895	0.263

The three-way interaction between beetle presence, SA/V ratio, and dung mass had no significant effect on CO₂e emissions ($F_{(7, 31)} = 20.93$; $P = 0.263$; Figure 3.5). Neither the interaction occurring between SA/V ratio and beetle presence ($F_{(7, 31)} = 20.93$; $P = 0.740$; Figure 3.5) nor SA/V ratio and dung mass ($F_{(7, 31)} = 20.93$; $P = 0.866$; Figure 3.5) significantly affected CO₂e emissions. However, the interaction between dung mass and beetle presence significantly impacted CO₂e emissions ($F_{(7, 31)} = 20.93$; $P = 0.005$), with beetle presence resulting in a greater increase in emissions in dung pats of higher mass (Figure 3.5). Beetle presence in the 400 g dung treatment increased CO₂e emissions by 46%, whereas their presence in smaller dung pats increased CO₂e emissions by 17%.

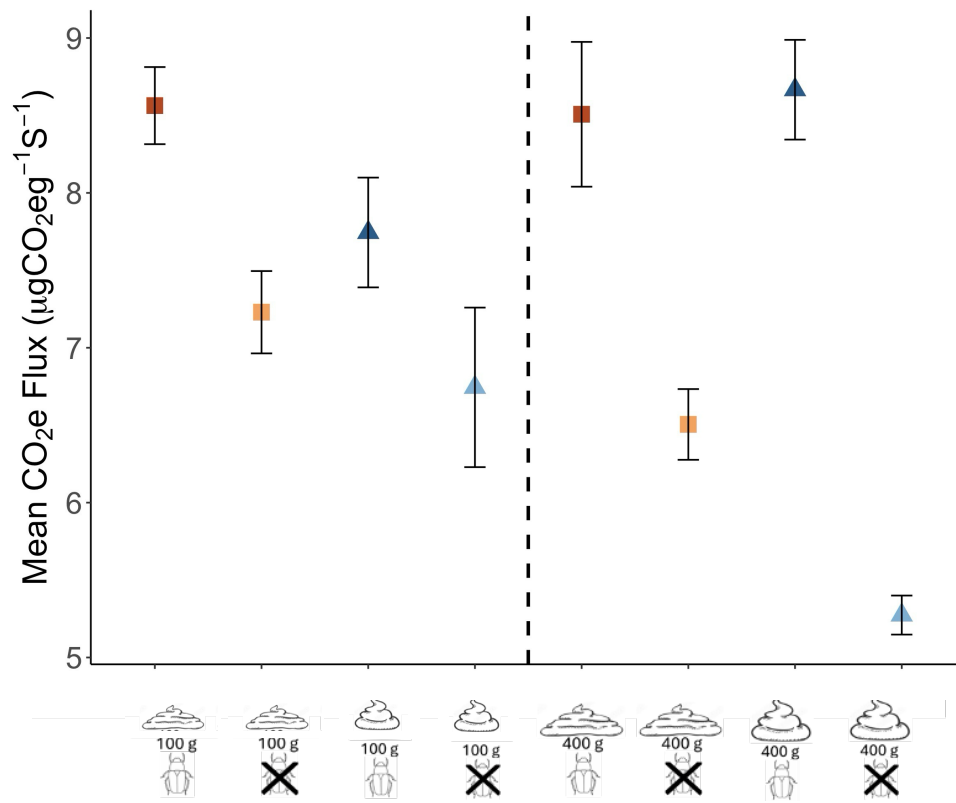


Figure 3.5 Mean \pm standard error of carbon dioxide- equivalent (CO_2e) emissions per gram of dung. Samples from the 100 g dung mass treatment are found on the left of the dotted line, and those of the 400 g dung mass treatment on the right. Orange squares represent flattened dung samples of higher surface-area-to-volume (SA/V) ratio, whereas blue triangles represent dung samples of lower SA/V ratio. Darker colors represent beetle presence, while lighter colors represent beetle absence.

Discussion

The alteration of dung mass significantly affected beetle-mediated CO_2 and CO_2e emissions, with dung of greater mass strengthening the beetle-mediated increase in CO_2 and CO_2e emissions (Figure 3.2 & 3.5). Additionally, the interaction between dung mass and SA/V ratio, as well as the interaction between beetle presence and dung SA/V ratio significantly impacted CH_4 emissions. However, neither dung mass, SA/V ratio, nor beetle presence impacted N_2O emissions.

The combined impact of dung mass and dung beetles on CO₂ and CO_{2e} emissions

Beetle presence increased both CO₂ and CO_{2e} emissions, however this effect was more pronounced in mesocosms containing larger dung pats (Figure 3.2 & 3.5). In this study, changes in CO₂ emissions were the primary driver of variations in CO_{2e}, with CO₂ accounting for approximately 85% of the total cumulative emissions in smaller dung pats and 84% in larger dung pats. Dung beetle presence promotes CO₂ emissions by increasing bacterial abundance (Breymer et al., 1975), beetle respiration (Verdú et al., 2020), and through the formation of macropores, which have been found to facilitate gas transport in dung (Evans et al., 2019; Iwasa et al., 2015). Given that a constant beetle-to-dung mass ratio was maintained, an increase in dung mass and consequently, beetle abundance, would result in greater CO₂ emissions. However, by calculating emissions per gram of dung, I controlled for the expected effects of increased beetle and microbial abundance, as well as the increased availability of carbon substrate in larger dung samples (Sordi et al., 2014; Zhu et al., 2018). Therefore, the influence of dung mass on beetle-mediated CO₂ emissions can be attributed to the interactive effects occurring between beetle-made macropores and dung mass.

Changes in dung mass can significantly affect dung moisture retention, with smaller dung pats experiencing greater moisture loss (Fowler & Mullens, 2016; Du et al., 2021). Dung moisture levels have been found to affect the importance of beetle-made macropores on CO₂ production, with CO₂ emissions in dung of higher moisture content being heavily dependent on gas transport processes (Evans et al., 2019). Beetle-made macropores can increase gas transport in porous media (Perret et al., 1999) and are hypothesized to influence CO₂ emissions by aerating the dung interior (Penttilä et al., 2013; Piccini et al.,

2017; Evans et al., 2019). Therefore, the more pronounced effects of beetle presence on CO₂ emissions observed in larger dung pats may result from the greater moisture retention observed in dung pats of greater mass (Du et al., 2021). Therefore, it is suggested that larger dung mass strengthened the impact of beetle-made macropores on CO₂ emissions (Evans et al., 2019).

These results suggest that research evaluating beetle-mediated CO₂ emissions will likely observe more pronounced effects when using dung pats of greater mass. These findings are particularly important when assessing the impact of beetle-mediated emissions at the pasture or lifecycle level. In their study, Slade et al. (2016) used 1.2 L dung pats. Given that the average dung pat size from cattle on pasture is approximately 3 L (Finn & Giller, 2000), one can expect beetle presence in pasture systems to have stronger effects on CO₂ emissions than predicted. Therefore, we can expect a stronger beetle-mediated increases in both CO₂ and CO_{2e} emissions at the dung pat, pasture, and lifecycle level.

The effects of dung SA/V ratio on dung CH₄ emissions

Results from this project reveal that dung CH₄ emissions are influenced by dung SA/V ratio's interactions with both dung mass and beetle presence. Overall, a reduced SA/V ratio (i.e., non-flattened dung pats) resulted in higher CH₄ emissions on a per gram of dung basis. However, these effects were less pronounced in dung samples of greater mass (Figure 3.3). Additionally, the presence of *O. nuchicornis* in mesocosms lead to a significantly greater reduction in CH₄ emissions in dung pats of a greater SA/V ratio (Figure 3.3).

The high moisture content of freshly deposited dung provides an ideal microhabitat for methanogenic microorganisms (Mazzetto et al., 2014; Mori & Hojito, 2015). Both dung

moisture content and crust formation influence CH₄ emissions, with increased crust formation and reduced dung moisture content leading to lower CH₄ emissions (Husted, 1994; Yamulki et al., 1999; Mazzetto et al., 2014; Mori & Hojito, 2015; Cardoso et al., 2019). The formation of crust on dung surfaces alone can reduce cow dung CH₄ emissions by up to 12 times, showing greater effects at lower temperatures due to surface crust being less permeable (Husted, 1994). Crust formation on cow dung pats begins shortly after deposition (Laubach et al., 2013), in the peripheral regions of dung pats, and typically requires 2–3 weeks to reach the inner portions of the pat (Akbar et al., 1995). The process of crust formation creates two distinct dung layers. The first layer is a very dry external crust which grows in thickness with time and slows the release of both CO₂ and CH₄ (Akbar et al., 1995; Shine et al., 2022). The second layer is the protected interior, which contains greater moisture levels (Shine et al., 2022). By flattening dung, and thereby increasing the SA/V ratio, I increased dung's surface area, and thereby, the proportion of dung that is exposed to environmental conditions and susceptible to crust formation and initial moisture loss.

The interactive effects of dung SA/V ratio and mass on dung CH₄ emissions

In this study, flattened dung pats of smaller mass (100-SA/V(High) = 0.45 ± 0.04) exhibited a greater mean SA/V ratio compared to larger flattened dung pats (400-SA/V(High) = 0.37 ± 0.02 ; Table 3.1). The higher SA/V ratio in smaller dung pats likely resulted in the accelerated formation of crust. The formation of crust likely resulted in dung pat moisture loss in peripheral regions, but once formed, the crust increased moisture retention in the inner layer. Consequently, it is suggested that smaller dung pats exhibited a more significant shift from CH₄ production to CH₄ oxidation when flattened, as it reduced the

area of the inner layer. CH₄ oxidation, a process carried out by methanotrophs (Bowman, 2006), is the oxidation process in which CH₄ and O₂ are converted to methanol, then further oxidized to formaldehyde, formate, ultimately producing CO₂ and H₂O as byproducts (Malyan et al., 2016). Therefore, due to an increased SA/V ratio, dung pats of smaller mass experienced greater rates of CH₄ oxidation, resulting in weaker CH₄ emissions. The observed interactive effects between dung SA/V ratio and mass on dung CH₄ emissions may explain some of the inconsistencies between previous studies, especially when evaluating inconsistencies regarding the magnitude of beetle-mediated effects on CH₄ emissions. These results suggest that standardizing dung pat shape is especially important when working with smaller dung samples. Therefore, these results highlight the need to standardize dung pat shape and size, to help reduce variation between measured effects, as increased variability can reduce the significance of findings and obscuring the overall impact of dung beetles on CH₄ emissions.

The effects of dung SA/V on beetle-mediated dung CH₄ emissions

Beetle presence in flattened dung pats led to a greater reduction in CH₄ emissions (-78%) compared to beetle presence in non-flattened dung pats (-8%). In studies where beetle presence was found to increase dung CH₄ emissions, the effect was attributed to greater moisture availability in the dung (Evans et al., 2019) or in the brood balls (Piccini et al., 2017). As mentioned earlier, dung pats with a higher SA/V ratio were expected to retain higher moisture content, as the protected interior layer, which contains greater moisture levels (Shine et al., 2022), will consist of a greater proportion of the dung volume in relation to the crusted peripheral layer.

Therefore, it is likely that the higher moisture content in non-flattened dung pats allowed *O. nuchicornis* to tunnel through more anaerobic environments, thereby releasing CH₄ that had accumulated in these oxygen-poor environments (Evans et al., 2019). The interactive effect of dung SA/V ratio and beetle presence on dung CH₄ emissions highlights the importance of controlling for and reporting on dung pat shape, as dung pat shape may result in weakened reductions or even increased beetle-mediated dung CH₄ emissions.

Study limitations and challenges in detecting treatment effects on N₂O emissions

Both crust formation and moisture loss can reduce N₂O emissions (Cardoso et al., 2016). However, neither dung mass, SA/V ratio, nor beetle presence impacted N₂O emissions (Figure 3.4). Although beetle presence increased N₂O emissions (Figure 3.4), this increase was not statistically significant, potentially due to high levels of variation within the data. The closed chamber method employed in this study is commonly used when measuring N₂O emissions (Denmead, 2008; Hensen et al., 2013; Pavelka et al., 2018). However, when measuring N₂O flux values of lower magnitudes (< 5 g N₂O-N ha⁻¹d⁻¹) this method may result in reduced goodness of fit (R²; Lammirato et al., 2018). Therefore, the small magnitude of the N₂O flux values measured in this study may have contributed to greater variation in the data, possibly obscuring the effects of these variables on N₂O emissions. In future studies, to address this issue, it is recommended to use smaller chambers to increase N₂O concentrations relative to the chamber's headspace area (Lammirato et al., 2018).

Conclusion

Dung beetles can influence dung GHG emissions, but studies often differ in the direction and magnitude of these effects (Penttilä et al., 2013; Iwasa et al., 2015; Hammer et al.,

2016; Slade et al., 2016; Piccini et al., 2017; Evans et al., 2019; Fowler et al., 2020; Verdú et al., 2020). The results from this study reveal that experimental practices, such as dung mass and SA/V ratio, can impact GHG emissions, thereby affecting the measured beetle-mediated emissions. These findings highlight the importance of experimental practices when evaluating beetle-mediated emissions and the significance of biotic organisms in shaping emission scenarios for life cycle assessments. These results also highlight the importance of using larger quantities of cow dung, such that study outcomes will more accurately reflect real-world conditions. Additionally, more detailed reporting of dung pat shape should be included in published literature to facilitate the comparison of beetle-mediated emissions across studies.

Chapter 4: Brown seaweed supplementation affects dung use and attractiveness to coprophagous beetles

Abstract

Cattle dung deposited on Canadian pasture systems supports a diverse insect community, including dung beetles (Coleoptera: Scarabaeoidea). Dung quality and the composition of volatile organic compounds are important factors influencing dung beetles' resource selection for both brooding and feeding purposes. Additionally, dung's mineral composition can impact beetle offspring fitness. The inclusion of brown seaweed in cattle diets can reduce enteric methane emissions; however, its downstream effects on coprophagous beetle communities is poorly understood. Therefore, this study evaluates the effects of supplementing beef cattle diets with brown seaweed on dung beetle resource use and reproductive success, as well as its effects on coprophilous beetles' resource selection. Results from this study showed that dietary supplementation with brown seaweed reduced dung attractiveness to dung beetles. When given a choice, beetles preferred to brood using dung from cows fed with a lower quantity of brown seaweed. Beetles produced, on average, 35% more brood balls using dung from the lower seaweed supplemented treatment group. However, dietary supplementation with brown seaweed had no effect on the attractiveness of dung to coprophilous beetles under field conditions. In a no-choice laboratory test where beetle's offspring could complete development, supplementing beef cattle diets had no statistically significant impact on any reproductive endpoints. However, dietary supplementation resulted in reduced proportions of major males. Due to the observed effects of dietary supplementation on brood ball production and the proportion of major males, it is suggested that supplementing beef cattle diets with brown seaweed reduced dung quality, highlighting the importance of evaluating how changes in livestock diets can impact coprophagous beetle communities.

Introduction

In Canadian pasture systems, cattle dung provides resources and habitat for over 300 species of arthropods, including dung beetles (Scarabaeidae: Aphodiinae, Scarabaeinae, and Geotrupinae; Floate, 2023). Dung beetles are a group of predominantly coprophagous insects that feed on dung (Floate, 2023), with adult beetles feeding on the dead and living microorganisms present in the liquid fraction of dung, while larvae bulk feed on the solid fraction (Halffter & Matthews, 1966; Halffter & Edmonds, 1982). The introduction of various dung beetle species to North America, through both intentional and accidental means, has resulted in the accelerated degradation of cattle dung on pasture (Pokhrel et al., 2021). *Onthophagus nuchicornis*, a Palearctic dung beetle species first reported in Canada

in 1945 (Hatch, 1971), is an abundant tunnelling species, representing about 40% of total trap catch in both Alberta and Michigan (Floate et al., 2017).

Although dung beetles are often considered generalist feeders capable of feeding on a variety of resources (e.g. dung of animals with varying diets), they can exhibit traits of “choosy generalism” (Dormont et al., 2004, 2007; Verdú et al., 2007; Urrutia et al., 2022). Dung nutrient quality, proximity, and olfactory cues, specifically volatile organic compounds (VOCs; Dormont et al., 2004, 2007; Bogoni & Hernández, 2014) can impact dung beetle resource selection.

Dung beetle species are classified into three groups based on their nesting behaviors: tunnellers, rollers, and dwellers (Cambefort & Hanski, 1991). Tunneling species bury dung by creating tunnels directly beneath the dung pat in which they build nests and lay their eggs (Rojewski, 1983). *Onthophagus nuchicornis*, a tunnelling species, creates nesting chambers up to 20 cm deep (Rojewski, 1983; von Lengerken, 1954). Adult beetles build agglomerations of dung, called brood balls, at the end of tunnels. A single egg is laid inside each brood ball, and the larva will complete development within the brood ball. Therefore, the brood ball will constitute the entirety of the nutrition available for the larva to complete development (Rojewski, 1983; Floate, 2023). Thus, the appropriate selection and provisioning of dung by adult dung beetles is essential for larval development (Heddle et al., 2024; Yap et al., 2024).

Dung characteristics, such as moisture and nitrogen content, can influence dung beetle brood ball production and offspring size (Edwards, 1991; Heddle et al., 2024). Dung quality can also influence the expression of male morphological traits (House & Simmons,

2007; Toh et al., 2022; Yap et al., 2024). Male *Onthophagus* spp. possess horns on their head or thorax, which can manifest in two phenotypes: major and minor morphs. Major males have significantly longer horns compared to minor males, whose horns are rudimentary (Laini et al., 2022; Moczek & Emlen, 1999), with male horn length in *Onthophagus* spp. increasing with greater dung quality (House & Simmons, 2007; Toh et al., 2022; Yap et al., 2024).

The alteration of livestock diet can alter both dung nutrient quality and dung volatile composition (Dadour & Cook, 1996; Perera et al., 2022). Although dietary supplementation with brown seaweed can reduce enteric methane emissions (Machado et al., 2014; Kinley & Fredeen, 2015), little is known about its effects on coprophagous insect communities. The inclusion of brown seaweed in cattle diets can enhance the mineral content of animal feed (Hamed et al., 2015; Morais et al., 2020). However, it can also negatively impact nitrogen degradability (Belanche et al., 2016) and starch digestibility (Wang et al., 2008), resulting in increased dung nitrogen and organic matter content. Considering the effect of livestock diet on dung volatile production and dung quality, there is reason to believe that supplementing beef cattle diets with brown seaweed may influence dung beetle resource selection and reproductive success.

In this chapter, I examine how supplementing beef cattle diets with brown seaweed impacts coprophagous beetles. Through three complementary experiments, I ask:

- 1) Does supplementing beef cattle diets with brown seaweed affect coprophilous beetles' resource selection under field conditions?
- 2) Do dung beetles show a preference for brooding purposes when presented with dung from beef cattle who had been fed differing quantities of brown seaweed?

3) Does supplementing beef cattle diets with brown seaweed affect dung beetle reproductive success?

Methods and materials

Dung collection and nutritional content

Dung samples were collected from sixteen beef heifers at the Nappan Research Farm (43°39.1935' N, 79°22.9911' W). The animals did not receive any antibiotic or antiparasitic treatments throughout the feed trial. Their basal diet consisted of 75.6% grass silage (DM), 20% barley (DM), and 4.4% soybean (DM) in addition to varying levels of brown seaweed inclusion rates depending on the treatment group to which the heifers had been assigned. Beef cattle were randomly assigned to four treatment groups, each receiving a different level of seaweed supplementation. The brown seaweed additive, produced by North Atlantic Organics (Atlantic GRO ®; Tignish, PEI, Canada), was selected based on an *in vitro* study using dairy rumen fluid (Kinley & Fredeen, 2015). This study suggested that the additive could reduce methane production by up to 16% *in vitro* (Kinley & Fredeen, 2015). However, as these results had not been validated *in vivo*, a subsequent study aiming to determine the optimal dose of the feed supplement was conducted (Borzouie et al., manuscript in preparation).

The heifers were fed a specialized feed mixture for 110 days (31 August 2023 to 15 December 2023), followed by a 24-day period during which they were taken off the diet, and then were returned to the specialized diet for an additional 35 days prior to dung collection (8 January 2024 to 11 February 2024). The control group received no seaweed supplementation on a dry matter basis (0% DMI), the second group received 0.5% seaweed supplementation (0.5% DMI), the third group received 1% supplementation (1% DMI),

and the final group received 2.0% supplementation (2% DMI). Seaweed was combined with the feed mixture using a serial addition method (Borzouie et al., manuscript in preparation). Starting with the 0.5% seaweed supplementation group, seaweed was gradually incorporated into the basal diet to attain the desired concentrations for each treatment group.

A total of 8 kg of dung were collected directly from animals in each supplementation group on 25 January 2024 and 31 January 2024. Samples were kept on ice for transport to Dalhousie’s Agricultural Campus. Upon arrival, 500 g subsamples from each treatment group were sent to the Nova Scotia Department of Agriculture for analysis (Table 4.1), while the remaining samples were stored at -21 °C for later use.

Table 4.1 Composition analysis of dung samples from the four brown seaweed supplementation treatment groups (i.e., control, 0.5%, 1%, 2% DMI).

	DM (%)	pH	N (%)	C: N	Ca (%)	K (%)	P (%)	Mg (%)
Control	13.00	7.02	2.02	20.79	0.471	1.972	0.528	0.291
0.5%	13.06	6.91	1.99	21.14	0.496	1.877	0.525	0.295
1%	13.38	6.71	2.20	19.15	0.553	1.871	0.601	0.354
2%	14.13	6.84	1.96	22.10	0.455	1.445	0.442	0.290

dry matter (DM), nitrogen content (N), carbon to nitrogen ratio (C: N), carbon content (C), potassium content (K), phosphorus content (P), magnesium content (Mg)

Experiment 1: Evaluating the effects of beef cattle diet supplementation with brown seaweed on dung beetle resource selection under field conditions

To assess dung beetle resource selection in field, a study was conducted on the campus farm at Dalhousie University Agricultural Campus (45°22. 17.94" N, 63°15. 29.268" W). The study area consisted of an open pasture which had been previously grazed by a herd

of dairy cows. The region has an average summer temperature of 20 °C and an average precipitation rate of 100 mm per month (Pidwirny, 2021). The soil at the study site is moderately coarse and imperfectly drained, classified as a Gleyed Humo-Ferric Podzol soil group (Agriculture and Agri-Food Canada, 2013).

Two days prior to the start of the field experiment, 5 kg of dung from the control (0%) and 0.5% treatment groups, as well as 5.5 kg of the 1% and 2% treatment groups, were removed from the freezer. Dung was left to thaw for 48 hours, after which it was homogenized for 30 seconds in 10-second intervals using an electric power drill with a paint mixer attachment. On 27 June 2024, baited traps were prepared by adding 150 g of coarse silica sand (Model #8526, Shaw Resources, New Brunswick, Canada) to 1.4 L aluminium pie plates perforated with 8 drainage holes at the base. At the field site, the dung was partitioned into 350 g pats and placed on the surface of the sand within the traps. Baited traps containing dung from the control and 0.5% treatment groups were replicated 14 times ($n = 14$) due to limited dung availability, while the 1% and 2% treatment groups were replicated 15 times ($n = 15$), resulting in a total of 58 baited traps ($N=58$). Baited traps were randomly distributed across the pasture using the “Create Random Points” geoprocessing tool in ArcGIS Pro (Figure 4.1). The number of points was set to 58, representing the number of baited traps to be placed in the field, with a minimum distance of 3 m between sampling points. The traps were left in the field for a 48-hour period.

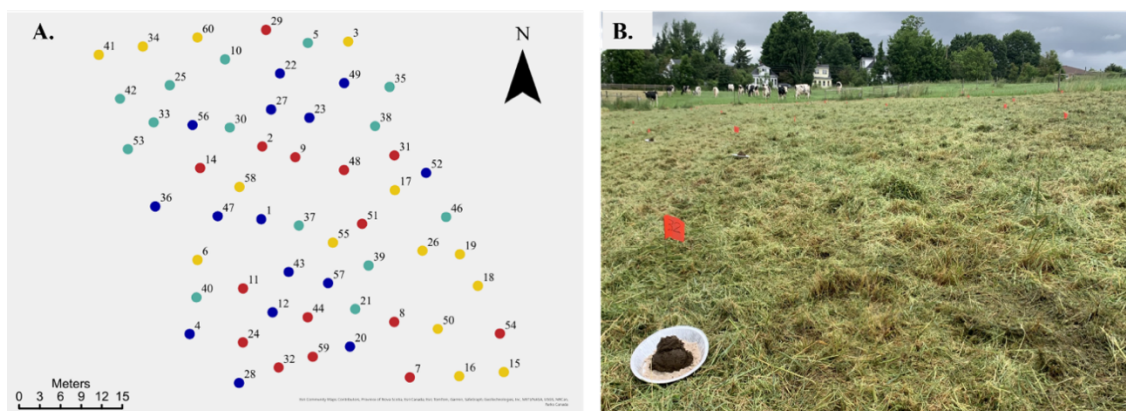


Figure 4.1 (A) Map showing the random distribution of the 58 baited traps across the sampling site. The dung treatment groups are distinguished by point color: light blue points represent baited traps containing control dung, yellow points indicate traps containing dung from the 0.5% treatment group, red points correspond to traps containing dung from the 1% treatment group, and the dark blue points represent traps containing dung from the 2% treatment group. All sampling points are separated by a minimum distance of 3 meters. (B) Picture of the field set-up, showing a baited trap in the lower left-hand corner.

On 29 June 2024, 48 hours after having set the baited traps, flotation was used to extract beetles from the dung pat. To perform flotation, a 7.5 L bucket was filled with 5.5 L of water. Dung pats were removed from the traps and placed into the bucket. The contents of the bucket were stirred to break up the dung and help dislodge clods. All beetles exiting the dung were removed from the water surface until none were observed for up to 5 minutes and preserved in 70% ethanol. All beetles, except for those in the *Staphylinidae* family, were identified to the species level using the following taxonomic keys: Britton (1956), Smetana (1978), van Berge Henegouwen & Foster (2019), Allegro (2021), Floate (2023). Total trap biomass was determined by measuring the dry weight (g) of the sample after the beetles were air-dried for 24 hours at 21°C.

Experiment 2: Effects of beef cattle diet supplementation on dung beetle resource use under laboratory conditions

To assess resource use in a controlled laboratory environment, 300 *Onthophagus nuchicornis* beetles were hand-collected from horse paddocks at Arbour Ridge Farm,

Salmon River, Nova Scotia, Canada (45°21'49.248" N, 63°13'48.216" W), on 14 June 2024. Prior to the experiment, beetles were maintained in 1.3 L cylindrical high-density polyethylene (HDPE) containers (Priority Plastics, Grinnell, IA, US) for 24 hours with RO-water-moistened paper towel, and fed horse dung *ad libitum*. At the start of the experiment ($t=0$), each treatment group was randomly assigned a specific glitter color — green (0%), red (0.5%), purple (1%), and silver (2%). The frozen dung was thawed for 48 hours, at which point following the methods of Lewis et al. (2023), 75 grams of glitter was incorporated into 1.8 kg dung to allow us to determine what dung source was selected for brooding (Figure 4.2).

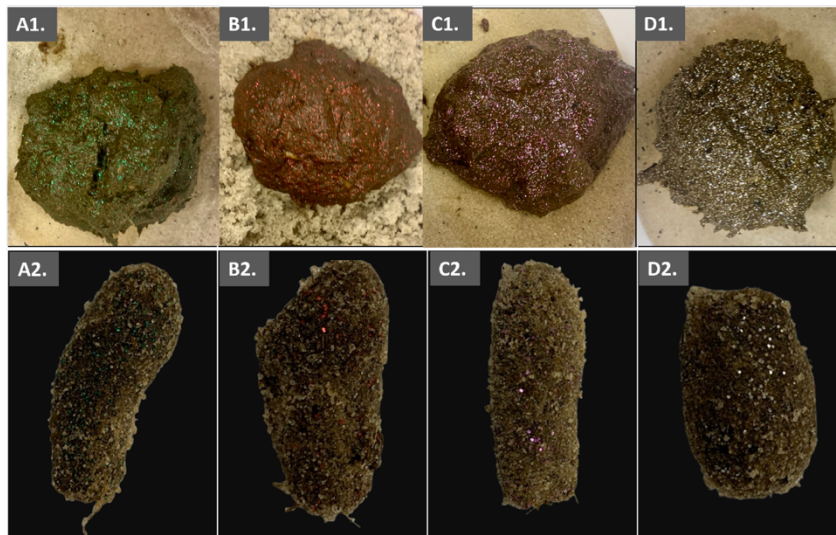


Figure 4.2 A1-D1: Dung pats resulting from the combination of dung from each treatment group with its associated glitter color. **A1** represents the control dung treatment mixed with green glitter; **B1** represents dung from the 0.5% treatment group combined with red glitter; **C1** represents dung from the 1% treatment group mixed with purple glitter; and **D1** represents dung from the 2% treatment group combined with silver glitter. **A2-D2:** Brood balls produced by *O. nuchicornis* beetles during the 10-day experimental period, illustrating the incorporation of glitter from the dung.

Mesocosms were constructed using 7.5 L plastic containers (PR80001MHP09, Canadian Tire, Nova Scotia, Canada), and were filled to a depth of 13 cm with 6.3 kg (\pm 195 g) of coarse silica sand (Model #8526, Shaw Resources, New Brunswick, Canada) moistened

with 750 mL of water. Two 100 g dung pats from different treatment groups were paired in each mesocosm (0 vs. 0.5%, 0 vs. 1%, 0 vs. 2%, 0.5 vs. 1%, 0.5% vs. 2%, and 1 vs. 2%) and placed c.10 cm apart on the sand surface. Each treatment pairing was replicated six times ($n = 6$), resulting in a total of 36 experimental units ($N = 36$). At the start of the experiment (15 June 2024; $t=0$), eight *O. nuchicornis* beetles (5 females, 3 males) were selected at random and added to each mesocosm, following which the mesocosms were sealed with a 2-mm aperture fiberglass mesh lid. Mesocosms were left undisturbed for a 10-day period at an average laboratory temperature of 20 °C. To ensure an accurate brood ball count, it was essential for brood balls to remain intact. Therefore, the 10-day brooding period was implemented based on the developmental rate of *Onthophagus* spp., as some species can pupate in as little as 17 days (Schwab et al., 2017). On 25 June 2024, brood balls were retrieved by sifting the mesocosm sand through a 1 x 1 cm plastic sieve. The number of brood balls and their associated glitter color as well as brood ball mass were recorded to identify the preferred treatment group for brooding.

Experiment 3: Evaluating the impact of dietary supplementation in beef cattle on dung beetle reproductive success

To assess the effects of supplementing beef cattle diets with brown seaweed on dung beetle reproductive success, I used a no-choice test performed under laboratory conditions. Fresh horse dung from Arbour Ridge Farm (45°21'49.248" N, 63°13'48.216" W), was hand-searched on 13 June 2024 to collect 140 *O. nuchicornis* beetles. Beetles were held in 1.3 L cylindrical high-density polyethylene (HDPE) containers (Priority Plastics, Grinnell, IA, US) for 48 hours with RO-water-moistened paper towel, and fed horse dung *ad libitum*. Approximately 500 g of dung from each treatment group were removed from the freezer to thaw for 48 hours. Following this the dung samples were homogenized for a 30-second

interval prior to forming dung pats. Each treatment group was replicated five times ($n= 5$), resulting in a total of twenty mesocosms ($N = 20$), consisting of 2.5 L cylindrical high-density polyethylene (HDPE) containers (Priority Plastics, Grinnell, IA, US) with 2-mm drainage holes, filled to a total depth of 13 cm with 2.3 kg (± 91 g) of coarse silica sand (Model #8526, Shaw Resources, New Brunswick, Canada) and 400 mL of water. On 15 June 2024, at time zero ($t=0$), 100 g of dung were added to each mesocosm, along with 7 *O. nuchicornis* beetles (4 females, 3 males). Throughout the experiment, ambient air temperature was maintained at an average of 20 °C. Mesocosms were inspected daily for beetle emergence. The total number of beetles emerging was recorded for both the F0 and F1 generations (beetles emerging after a 9-week period were classified as F1 generation), and the offspring survival rate (Alive: Dead) was documented based on the beetle's condition at emergence. Beetle mass (g), average pronotum width (mm), male morphology (major or minor), and sex were recorded for F1-generation beetles.

Statistical analyses

Experiment 1: Evaluating the effects of beef cattle diet supplementation with brown seaweed on dung beetle resource selection under field conditions

The effect of supplementing beef cattle diets with brown seaweed on species abundance and richness was evaluated using a generalized linear model with a negative binomial error distribution to account for overdispersion. The effect of the additive on beetle biomass was assessed using a generalized linear model with Gaussian distribution. Model assumptions, including normality, homoscedasticity, linearity, and independence, were assessed by analyzing the models' residual plots. Adjusted p-values using Benjamini and Hochberg's (1995) control for false discovery rates (Benjamini & Hochberg, 1995) were applied to control for Type I errors.

Experiment 2: Effects of beef cattle diet supplementation on dung beetle resource use under laboratory conditions

To assess the effects of dietary supplementation on dung beetle resource use under laboratory conditions, Pearson's chi-squared tests were used, with the total count of brood balls from each treatment group used as the response variable. Brood balls formed from two different dung types (as indicated by mixed glitter colours) were not included for further analysis. A generalized linear model with a Gaussian distribution was used to assess the effects of the feed additive on the average brood ball mass.

Experiment 3: Evaluating the impact of dietary supplementation in beef cattle on dung beetle reproductive success

To assess the effects of the inclusion of brown seaweed in beef cattle diet on dung beetle reproductive success, the total F1-generation emergence count was analyzed using a generalized linear model with a Poisson error distribution. Male morphology (Major: Minor), sex ratio (Female: Male), and survival rate were assessed using a generalized linear model with a binomial error distribution. To determine the impact of seaweed feed inclusion on beetle morphology, I ran separate generalized linear models with Gaussian error distribution for male and female beetles. For each sex, I ran three separate models to determine the effects, using: mean beetle mass (g), mean pronotum width (mm), and mean time to emergence (days) as dependent variables. Benjamini and Hochberg's (1995) control for false discovery rates (Benjamini & Hochberg, 1995) was applied to control for Type I errors.

Results

Experiment 1: Evaluating the effects of beef cattle diet supplementation with brown seaweed on dung beetle resource selection under field conditions

A total of 265 coprophilous beetles, representing eleven species, were collected from 58 baited traps. The species abundance across the four treatment groups is presented in Table 4.2. Overall, the supplementation of beef cattle diets with brown seaweed had no effect on the abundance of dung beetles captured in baited traps ($X^2 = 0.092$; $df = 1$; $P = 0.775$; Table 4.2). The feed additive did not affect trap biomass ($X^2 = 0.0013$; $df = 1$; $P = 0.1211$) or species richness ($X^2 = 0.328$; $df = 1$; $P = 0.56546$).

Table 4.2 Total abundance of the 11 captured coprophilous beetle species by brown seaweed supplementation treatment group (i.e., 0%, 0.5%, 1%, 2% dry matter intake). Species feeding behaviours are listed: omnivores (O), coprophages (C), or predators (P). Biodiversity metrics, including species richness and Shannon diversity index by treatment group (i.e. 0%, 0.5%, 1%, 2%).

Family	Species name	0% (n= 14)	0.5% (n= 14)	1% (n= 15)	2% (n= 15)
<i>Hydrophilidae</i>	<i>Cercyon haemorrhoidalis</i> ^O	8	10	4	6
	<i>Cercyon lateralis</i> ^O	35	28	12	23
	<i>Cercyon pygmaeus</i> ^O	2	1	1	3
	<i>Sphaeridium lunatum</i> ^O	5	0	4	1
	<i>Sphaeridium scarabaeoides</i> ^O	11	10	3	14
<i>Scarabaeidae</i>	<i>Aphodius ater</i> ^C	2	1	1	1
	<i>Colobopterus erraticus</i> ^C	0	1	2	1
	<i>Onthophagus nuchicornis</i> ^C	4	0	2	6
	<i>Otophorus haemorrhoidalis</i> ^C	0	0	1	1
	<i>Teuchestes fossor</i> ^C	0	0	0	1
<i>Staphylinidae</i>	<i>Staphylinidae</i> spp. ^P	22	1	21	16
Total		89	52	51	73
Biodiversity metrics	Species richness	1.93	1.43	1.40	2.07
	Shannon diversity index	0.457	0.379	0.399	0.519

^O: Omnivores (coprophagous adults, predaceous larvae)

^P: Predators

^C: Coprophages

Experiment 2: Effects of beef cattle diet supplementation on dung beetle resource use under laboratory conditions

In total, the 36 mesocosms produced 755 brood balls. All but two (753/755) brood balls were of a single glitter color, with the remaining two being of mixed glitter colors from the 0.5% and 1% treatment groups. The feed additive significantly influenced *O. nuchicornis*' resource use, with beetles producing on average 35% more brood balls using dung from the lower seaweed supplemented treatment group (Table 4.3). However, the feed additive had no effect on brood ball mass ($X^2 = 0.512$; $df = 1$; $P = 0.377$).

Table 4.3 Total number of brood balls produced from both high and low dung treatments in each dung pairing, along with their corresponding chi-square (X^2) values and p-values. Each pairing has 1 degree of freedom. In four of six trials, beetles showed a significant preference to brood using dung from cows fed the lower rate of seaweed inclusion. In the remaining two trials, beetles did not demonstrate any preference.

Dung pairings	Brood ball count		X^2	P-value
	low treatment group	high treatment group		
0% vs. 0.5%	67	43	5.24	0.0221*
0% vs. 1%	77	43	9.63	0.0019*
0% vs. 2%	84	74	0.63	0.4263
0.5% vs. 1%	72	59	1.29	0.2560
0.5% vs. 2%	72	39	9.8108	0.0017*
1% vs. 2%	84	41	14.792	0.0001*

Experiment 3: Evaluating the impact of dietary supplementation in beef cattle on dung beetle reproductive success

A total of 132 beetles emerged from the twenty mesocosms. The feed additive had no effect on total beetle emergence ($X^2 = 0.789$; $df = 1$, $P = 0.378$), or on the mean time to emergence of either female ($X^2 = 3.3$; $df = 1$, $P = 0.827$) or male beetles ($X^2 = 3.88$; $df = 1$, $P = 0.695$). Brown seaweed supplementation rates had no impact on the beetle offspring survival rate ($X^2 = 0.1098$; $df = 1$, $P = 0.744$).

The feed additive had no effect on the beetle sex ratio ($X^2 = 1.0975$; $df = 1$, $P = 0.29468$). Although not a significant effect, the addition of brown seaweed to beef cattle diets did impact male morphology ($X^2 = 6.068$; $df = 1$, $P = 0.0336$; Figure 4.3), with increased feed inclusion rates resulting in smaller proportions of major males (0% = 0.55, 0.5% = 0.33, 1% = 0.06, 2% = 0.11). The feed additive had no significant effect on pronotum width for either female ($X^2 = 0.007$; $df = 1$; $P = 0.723$) or male beetles ($X^2 = 0.044$; $df = 1$; $P = 0.257$). The supplementation of beef cattle diet had no significant effect on the mass of either female ($X^2 < 0.001$; $df = 1$; $P = 0.291$) or male beetles ($X^2 < 0.001$; $df = 1$; $P = 0.275$).

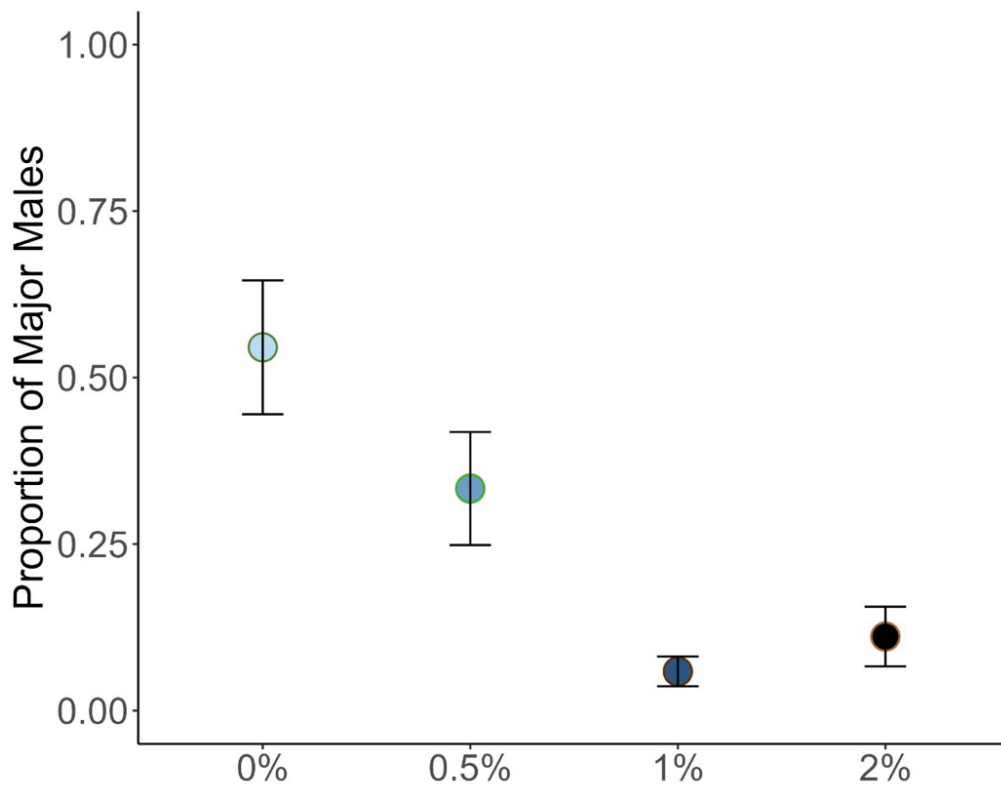


Figure 4.3 Proportion and standard error of major males in each supplementation group. Cattle in the control group (0%) received no dietary supplementation. The 0.5%, 1%, and 2% groups received brown seaweed supplementation at rates of 0.5%, 1%, and 2% of dry matter intake (DMI), respectively.

Discussion

The inclusion of brown seaweed in beef cattle diets significantly reduced resource use, with *O. nuchicornis* preferring to brood using dung from the lowest supplementation treatment group (Table 4.3). However, coprophilous beetles showed no preference when selecting dung resources for feeding purposes under field conditions. The inclusion of brown seaweed in beef cattle diets had little impact on dung beetle reproductive success, with only male morphology being impacted. Results from this project showed that higher levels of brown seaweed resulted in lower proportions of major males (Figure 4.3).

Diet supplementation alters dung beetle resource use

Dung beetles (Scarabaeidae: Aphodiinae, Scarabaeinae, and Geotrupinae) are selective generalists, showing preferences for specific dung types (Dormont et al., 2004, 2007; Whipple & Hoback, 2012; Urrutia et al., 2022). However, they can reproduce using a variety of resources (Indigo et al., 2025). Dung resource selection is influenced by both dung quality and dung volatiles (Dormont et al., 2004, 2007; Bogoni & Hernández, 2014). While the inclusion of brown seaweed in beef cattle diets had minimal impact on dung nutrient content, increased levels of seaweed inclusion resulted in greater dung dry matter content (Table 4.1). Increased dung moisture, and hence reduced dung dry matter content, enhances nutrient availability, resulting in greater brood ball production (Kaur et al., 2021). Therefore, the increased dung dry matter content, caused by higher supplementation rates, may have reduced dung's suitability for brood ball production.

Aside from dung quality, dung volatiles can influence dung beetle resource selection (Dormont et al., 2004, 2007). VOCs serve as olfactory cues that allow beetles to find ephemeral resources (dung) that are patchy in space and time (Dormont et al., 2004, 2007).

The results from the laboratory experiment suggest that dung from lower seaweed-supplemented treatment groups emitted more attractive blends of VOCs, leading to greater resource use. The emission of dung volatiles is affected by both dung microbial activity (Stavert et al., 2014; Köninger et al., 2021; Haider et al., 2024) and moisture content (Dillon & Dillon, 2004; Stavert et al., 2014). The inclusion of brown seaweed in beef cattle diets likely impacted dung volatiles through two mechanisms: increased dung dry matter content (Table 4.1) and altered dung microbial communities. Brown seaweed contains two compounds exhibiting antimicrobial properties, phlorotannins and polysaccharides (Chkikvishvili & Ramazanov, 2000; Nagayama, 2002; Chandini et al., 2008). These compounds alter the microbial community composition in the cecum and colon when incorporated into pig diets (Reilly et al., 2008; Lynch et al., 2010). Therefore, dietary supplementation may have altered dung microbial community composition, thereby impacting the production of dung volatiles. Additionally, the noted increase in dry matter content in supplemented dung inhibits VOC emissions, as higher moisture content, and hence reduced dry matter content, results in greater VOC production (Kasper et al., 2012; Stavert et al., 2014). In this study, *O. nuchicornis* preferred to brood using dung from lower supplemented treatment groups. Therefore, changes in dung quality and the production of less attractive dung volatiles may explain *O. nuchicornis*' preference to brood using dung of lower seaweed-supplemented treatment groups.

Comparing dung beetle preferences in laboratory and field

The coprophilous beetles collected in the field-based experiment consisted of dung generalists (Smetana, 1988; Gordon, 2006; Sladeczek et al., 2013; Levesque & Levesque, 2017) and generalist predators that feed on a variety of insects (e.g., *Staphylinidae* spp;

Thayer, 2016; Stocker et al., 2022). However, only 24 of the 265 beetles collected in this experiment consisted of dung beetles, whilst most beetles collected consisted of predacious (60/265) or omnivorous beetle species (181/265). Although the laboratory-based experiment found that *O. nuchicornis* preferred to brood using dung from lower seaweed supplemented treatment groups, the coprophilous beetles collected in the field-based experiment exhibited no resource preference. Although few studies have assessed dung beetle preference under both field and laboratory conditions, prior research suggests that coprophagous beetles exhibit stronger resource preference in laboratory settings (Giménez Gómez et al., 2021). The inconsistencies observed between the two preference experiments conducted in this study may be due to the experimental design used in the field-based experiment. The baited traps employed in the field allowed beetles to move freely between traps and feed on preferred dung types, but did not measure brood ball production (only two species captured are classified as paracoprids). Therefore, the field-based experiment assessed coprophilous beetles' resource selection for feeding purposes rather than their resource use for nesting. Although little is known about dung beetle resource selection for nesting (deCastro et al., 2023), it is suggested that beetle resource preference differs for nesting and feeding purposes (Kerley et al., 2018). Consequently, results from these two experiments may not be directly comparable. However, when considered together, they provide insights into coprophilous beetles' dung resource selection at different stages of their life cycle. Additionally, the low colonization rates observed in this study may have limited the experiment's ability to assess the effects of dietary supplementation on coprophilous beetles' resource selection.

In addition to dung quality and volatile emissions, feeding preferences can be influenced by factors such as predation and competition (Schmitz et al., 1997). Under field conditions, increased competition has been found to alter dung beetles' olfactory response to dung volatiles, with greater insect diversity leading to reduced dung beetle attraction (Dormont et al., 2010; Manning & Ford, 2016). For instance, *Aphodius constans* preferred dung samples in which other beetles of the same species were present (Dormont et al., 2010). However, this same species was repelled when beetles of different species were present in dung samples (Dormont et al., 2010). Similarly, the dung beetle *Aphodius fossor* demonstrated sex-specific attraction to dung resources, with female beetles avoiding dung colonized by other females while preferring dung colonized by males (Manning & Ford, 2016). Therefore, although the laboratory-based experiment indicated that dung from lower supplemented treatment groups was of higher quality, the presence of other insects in dung samples, the low colonization rates observed in this study, and the potential difference in resource selection for feeding vs. nesting purposes may help explain the variation in resource preference observed between the two experiments.

Brown seaweed supplementation alters the proportion of major males in *Onthophagus* beetles

Although not significant, supplementing beef cattle diets with brown seaweed reduced the proportion of major males in the F1-generation, with higher feed inclusion rates leading to a greater reduction in major male abundance. Male horn length is an important morphological trait as it influences mating behaviours in *Onthophagus* spp. (Moczek & Emlen, 1999). Major males defend tunnel entrances containing breeding females, while minor males use sneaking behaviours to access and mate with females inside the tunnels (Moczek & Emlen, 1999). Male horn length in *Onthophagus* spp. is influenced by

nutritional conditions during larval development, which is determined by both the quantity and quality of dung provided (Emlen, 1997; Moczek, 1998; Yap et al., 2024). However, dung quality has the greatest effect on male horn length (Yap et al., 2024). Therefore, the results suggest that higher inclusion rates of brown seaweed in beef cattle diets reduced dung quality, and although the feed additive had little effect on dung beetle reproductive success, it did influence male horn length, which could impact male mating behaviours.

Although the existence of a positive sigmoidal-shaped relationship between beetle body size and horn length has been observed (Laini et al., 2022), changes in the proportion of major males did not coincide with significant changes to male beetle pronotum width in my study. While the mean pronotum width of the F1-generation followed a similar trend to male horn length, only a moderate correlation existed between the two variables ($r = 0.54$). Despite the lack of a strong correlation, results from this study align with previously noted relationships between pronotum width and horn length (Moczek, 1998; Moczek & Emlen, 1999; Laini et al., 2022).

Study limitations and the need of future work to better understand dung beetle resource use

This study revealed that supplementing beef cattle diets with brown seaweed reduced dung beetle resource use for brooding purposes under laboratory conditions but had no effect on resource selection under field conditions. However, it is important to note that dung beetle resource use was assessed only in *O. nuchicornis*, a species that was found in low abundance under field conditions, with only 12 individuals being collected in across the 58 baited traps. Another limitation of the field study was the lack of consideration of beetle sex when identifying dung beetles. Given that dung beetle resource attraction is influenced by beetle sex (Salomão et al., 2020, 2021), assessing sex discrepancies between treatment

groups would have been valuable. In addition to this, evaluating resource use in the field using similar methodologies to those applied in the laboratory could help differentiate between resource selection for feeding and nesting.

While freezing dung prior to conducting the field experiment was necessary to align dung collection with dung beetle activity, it may have altered VOC emissions. Although microbial integrity is generally preserved when fecal samples are frozen, it can influence microbial metabolic activity (Chan et al., 2015; Fouhy et al., 2015), and therefore VOC emissions (Berkhout et al, 2016). As a result, the findings of this study may not fully represent how supplementing beef cattle diets with brown seaweed affects coprophilous beetles' selection of freshly deposited supplemented dung on pasture. Finally, considering the impact of the feed additive on male horn length, it would be important to evaluate how the additive influences male mating tactics and its effects on subsequent generations of beetles.

Conclusion

The two laboratory-based experiments revealed that supplementing beef cattle diets with brown seaweed can influence *O. nuchicornis*' resource use and male morphology. Specifically, when given a choice, *O. nuchicornis* preferred to use dung from lower seaweed supplementation treatment groups. Although not a significant effect, higher levels of seaweed supplementation resulted in reduced proportions of major males, suggesting that the feed additive reduced dung quality. However, under field conditions, dietary supplementation had no effect on the resource selection of coprophilous beetles for feeding purposes. This lack of observed effect may have been caused by unaccounted for environmental conditions experienced under field conditions as well as the low abundance of dung beetles collected in this experiment. This discrepancy between the effects of dietary

supplementation on dung beetle feeding and nesting resource selection highlights the need to evaluate the effects of dietary supplementation on resource selection for brooding purposes under field conditions. Although supplementing beef cattle diets with brown seaweed can reduce enteric emissions (Borzouie et al., manuscript in preparation), little research has been done to evaluate its effects on coprophilous beetles. Results from this study highlight the importance of assessing the effects of dietary supplementation on coprophilous beetles' nesting behaviours and reproductive success.

Chapter 5: General discussion

The Canadian livestock sector

While the Canadian beef industry is a significant contributor to national GHG emissions, it is a pillar of the country's agricultural sector, generating CAD\$10.2 billion in farm sales annually (Statistics Canada, 2022; Aboagye et al., 2024). Canadian beef producers are working to reduce the industry's environmental footprint, having already achieved notable reductions in GHG emissions (-15%; Legesse et al., 2016), ammonia emissions (-17%; Legesse et al., 2018a), land use (-24%; Legesse et al., 2016), and water use (-20%; Legesse et al., 2018b) per kg of beef since 1981. Dietary supplementation with brown seaweed is one way through which the livestock sector can reduce enteric emissions. However, before its widespread adoption, it is important to consider the potential downstream effects of supplementing beef cattle diets with brown seaweed. In Chapter 2, I found that supplementing beef cattle diets with brown seaweed at a rate of 2% DMI reduced CO₂e emissions by 30% (Figure 2.1). In Chapter 4, it was found that dietary supplementation reduced *O. nuchicornis*' dung use for brooding purposes (Table 4.3) and reduced the proportion of major males in beetle offspring (Figure 4.3).

Seaweed additive and dung GHG

In Chapter 2, I found that dung from the seaweed-supplemented treatment group had higher dry matter content compared to dung from the control group (Table 2.1). This increase in dry matter content is expected to reduce CH₄ emissions (Jarvis et al., 1995; Pelster et al., 2016) while increasing CO₂ emissions (Haynes & Williams, 1993; Flessa & Beese, 2000; Jiang et al., 2012). However, in this study, a simultaneous reduction in both CO₂ (-26%) and CH₄ (-49%) emissions was observed. This reduction in both CO₂ and CH₄ emissions

may be explained by changes in dung microbial community composition or activity, as increased methanogenic potential is known to be linked to microbial community composition (Hahn et al., 2018), while CO₂ emissions are driven by microbial mineralization processes (Flessa & Beese, 2000; Jiang et al., 2012). However, future research should assess the effects of brown seaweed's phlorotannin content on dung microbial community composition as it was not measured within my research. Overall, the results from this research suggest that the beneficial effects of supplementing beef cattle diets with brown seaweed extend beyond reducing enteric GHG emissions (Borzouie et al., manuscript in preparation), resulting in a 30% reduction in dung CO₂e emissions.

The reduction in dung CH₄ emissions from supplemented dung samples resulted in the reduction of the magnitude of beetle-mediated effects on dung CH₄ emissions (Figure 2.2). When evaluating beetle-mediated emissions at both the pasture and dung pat levels, it was found that beetles led to a 7% and 12% reduction, respectively, in CO₂e emissions, primarily through a reduction in CH₄ emissions (Slade et al., 2016). Given that beetle presence in supplemented dung pats resulted in similar CH₄ and CO₂e emissions over the 21-day period, it can be said that dietary supplementation had no impact on overall beetle-mediated dung GHG emissions.

The downstream effects of diet supplementation on coprophagous beetles

While supplementing beef cattle diets with brown seaweed reduced GHG emissions from dung, the overall impact of the feed additive on coprophagous beetles appear less positive. Results from Chapter 4 revealed that dietary supplementation reduced both the proportion of major males in beetle offspring and adult beetle dung use for brooding purposes (Table 4.3; Figure 4.3). Results showed that *O. nuchicornis* preferred to brood using dung from

lower seaweed-supplemented treatment groups (Table 4.3). While *O. nuchicornis* can reproduce using a variety of resources (Indigo et al., 2025), dung beetles exhibit clear preferences for certain dung types (Dormont et al., 2004, 2007; Whipple & Hoback, 2012; Urrutia et al., 2022). Given that both horn length and dung use are influenced by dung quality (Emlen, 1997; Moczek, 1998; Yap et al., 2024), results suggest that dietary supplementation with brown seaweed reduced dung quality.

However, when assessing the effects of dietary supplementation on coprophilous beetles' resource selection under field conditions, no significant impact was observed. While unaccounted for environmental conditions may have altered coprophilous beetles' resource selection, the low colonization rate observed in this study likely limited this experiment's ability to assess the effects of dietary supplementation on coprophilous beetles' resource selection. Therefore, to clarify the effects of dietary supplementation on coprophilous beetles' resource selection, future studies should reconsider the experimental design used in this project and adopt methods that promote higher colonization rates. For example, baited pitfall traps could be used alongside the flotation method. Baited pitfall traps prevent beetles from leaving the trap, often resulting in increased beetle abundance (Southwood, 1978). Although it was not a significant effect, it is important not to underestimate the observed effects on male horn length. The observed reduction in the proportion of major males in dung beetle populations may influence paternal provisioning tactics (Cook, 1988; Hunt & Simmons, 1998) and male mating behaviors (Moczek & Emlen, 1999), thereby affecting subsequent generations of dung beetles.

A need for future research

The observed effects of supplementing beef cattle diets with brown seaweed on the proportion of major males highlight the need for future research. Previous research has shown that male *Onthophagus spp.* ' horn length can influence paternal provisioning tactics (Cook, 1988; Hunt & Simmons, 1998) and male mating behaviors (Moczek & Emlen, 1999). Therefore, given the effects of dietary supplementation on male horn length, it will be important to evaluate the impact of the feed additive on dung beetle reproductive success over multiple generations, especially as little is known about its effects on the lifespan or reproductive development of the F1-generation. Considering the hypothesized effects of the feed additive on dung quality, it would also be important to evaluate the effects of dietary supplementation on dung's effectiveness as a natural soil amendment.

Limiting the effects of variations in experimental practices on study outcomes

While multiple studies have shown that dung beetle presence alters dung GHG emissions, the magnitude and direction of these effects vary across studies (Penttilä et al., 2013; Iwasa et al., 2015; Hammer et al., 2016; Slade et al., 2016; Piccini et al., 2017; Evans et al., 2019; Fowler et al., 2020; Verdú et al., 2020). The results from my study suggest that these observed inconsistencies may be attributed to differences in experimental practices. In Chapter 2, it was found that homogenizing dung prior to sampling led to a 46% reduction in CH₄ emissions (Figure 2.1). Results from Chapter 3 showed that dung mass can significantly influence beetle-mediated increases in both CO₂ and CO_{2e} emissions (Figure 3.2; Figure 3.5). The interaction between dung mass and SA/V ratio can significantly affect dung CH₄ emissions, with changes in SA/V ratio having a more pronounced effect in dung pats of a lower mass. Additionally, dung SA/V ratio was found to significantly impact

beetle-mediated CH₄ emissions, with beetle presence causing a stronger reduction in CH₄ emissions in flattened dung pats. Therefore, these results highlight the potential for experimental practices to influence dung GHG emissions, and thereby potentially altering our understanding of how dung beetles can affect GHG budgeting in pastures.

The tunneling activity of dung beetles can reduce CH₄ emissions by aerating dung pats (Penttilä et al., 2013; Iwasa et al., 2015; Piccini et al., 2017). While commonly used in GHG sampling, dung homogenization is thought to replicate the aerating effects of dung beetles' tunneling behavior, thereby reducing CH₄ emissions from dung pats. In this study, homogenizing dung samples prior to GHG sampling reduced dung CH₄ emissions (Figure 2.1). Therefore, the homogenization of dung prior to GHG sampling weakened beetle-mediated reductions in CH₄ emissions. This finding also raises the question of whether differences in the intensity of dung homogenization across studies could account for some of the observed inconsistencies. Specifically, longer homogenization periods may lead to the greater aeration of the dung pat, thereby weakening the perceived beetle-mediated effect on CH₄ emissions.

Although the inclusion of this experiment in Chapter 3 would have been more fitting, the noted inconsistencies regarding beetle-mediated effects on dung GHG emissions were only observed following the completion of Chapter 2's experimental procedures. Given that evaluating the effects of dung homogenization on dung GHG emissions was an initial goal of my thesis, this experiment was included in Chapter 2 rather than Chapter 3.

Dung mass was also found to influence beetle-mediated CO₂ emissions (Figure 3.2). The presence of dung beetles in dung pats has been associated with increased CO₂ emissions

due to increased bacterial abundance (Breymer et al., 1975), beetle respiration (Verdú et al., 2020), and the formation of beetle-made macropores (Iwasa et al., 2015; Evans et al., 2019). In this study, the effects of increased dung mass on beetle-mediated CO₂ emissions are attributed to the greater importance of gas transport processes in dung pats with higher moisture content (Evans et al., 2019). Dung pats of greater mass retain more moisture compared to those of smaller mass (Fowler & Mullens, 2016; Du et al., 2021). Consequently, the formation of beetle-made macropores in dung pats of greater mass resulted in greater beetle-mediated increases in CO₂ emissions. Given that dung beetle presence has been found to reduce CO₂ emissions in dung samples weighing between 300 g and 1.3 kg (Penttilä et al., 2013; Piccini et al., 2017; Evans et al., 2019), dung mass alone may not account for all the inconsistencies observed across studies. However, the results from this study highlight the importance of dung mass on study outcomes.

Additionally, the results presented in Chapter 3 revealed that the interaction between the dung SA/V ratio and dung mass significantly impacted dung CH₄ emissions (Figure 3.3). In this study, flattening dung pats, thereby increasing their SA/V ratio, was found to reduce CH₄ emissions regardless of dung mass. However, this effect was more pronounced in smaller dung pats (Figure 3.3) due to faster degradation and crust formation (Dickinson & Craig, 1990). Crust formation begins in the peripheral regions of dung shortly after deposition (Akbar et al., 1995; Laubach et al., 2013) and gradually extends inwards, slowing the release of CH₄ and reducing dung moisture retention (Akbar et al., 1995; Shine et al., 2022). By flattening dung samples, I increased their surface area, and therefore the proportion of the dung pat susceptible to crust formation. Given that crust formation can reduce CH₄ emissions (Husted, 1994; Mazzetto et al., 2014; Mori & Hojito, 2015; Cardoso

et al., 2019), the higher mean SA/V ratio observed in dung pats of lower mass could explain the observed interaction between dung mass and SA/V ratio. Although the interaction between dung SA/V ratio and dung mass did not affect beetle-mediated CH₄ emissions, these results suggest that dung SA/V ratio can potentially alter the magnitude and statistical significance of beetle-mediated CH₄ emissions.

Finally, results from Chapter 3 revealed that dung SA/V ratio significantly influences beetle-mediated CH₄ emissions. More precisely, flattening dung pats increased the magnitude of *O. nuchicornis*' effects on dung CH₄ emissions. Beetle presence in mesocosms containing flattened dung pats resulted in a 78% reduction in dung CH₄ emissions, whereas their presence only reduced CH₄ emissions by 8% in non-flattened dung pats. The effects of dung SA/V ratio on beetle-mediated CH₄ emissions are suggested to be associated with dung moisture retention. Flattened dung pats, with an increased SA/V ratio, have lower moisture retention due to greater rates of crust formation (Akbar et al., 1995; Shine et al., 2022). However, dung pats of lower SA/V ratio have an inner layer of greater volume, resulting in prolonged periods of moisture retention (Akbar et al., 1995; Shine et al., 2022) and reduced oxygen availability. Therefore, it is suggested that beetle burrowing activity through non-flattened dung pats allowed for the release of CH₄ that was previously stored in these oxygen-poor environments (Piccini et al., 2017; Evans et al., 2019).

The results from Chapter 2 and 3 reveal that experimental practices can impact the magnitude and direction of beetle-mediated dung GHG emissions. For example, dung mass and SA/V ratio can directly affect beetle-mediated emissions, and help explain inconsistencies surrounding the magnitude of beetle-mediated effects on dung CO₂ and

CO₂e emissions as well as the direction and magnitude of beetle-mediated effects on dung CH₄ emissions. Additionally, results revealed that dung mass and shape can impact the magnitude of beetle-mediated effects on dung CH₄ emissions. Although this project explores the impacts of some experimental procedures and factors on beetle-mediated dung GHG emissions, other experimental factors should be studied. For example, greater research on the effects of dung moisture content on beetle-mediated emissions would be important due to its impacts on beetle-mediated CH₄ emissions (Piccini et al., 2017; Evans et al., 2019). Although the results from this study do not explain all inconsistencies observed between published literature, they highlight the effects of methodological practices on beetle-mediated GHG emissions, as well as the need for further research evaluating these effects.

Conclusion

Supplementing beef cattle diets with brown seaweed is an effective strategy for reducing enteric methane emissions (Borzouie et al., manuscript in preparation). The results from this study demonstrate that, under laboratory conditions, the beneficial effects of dietary supplementation extend downstream, as supplementing beef cattle diets reduced dung CO₂e emissions by 30%, indicating that dietary supplementation is a useful tool for reducing GHG emissions from the Canadian livestock sector.

However, supplemented dung was suggested to be of lower quality due to the observed reductions in both the proportion of major males in *O. nuchicornis*' offspring as well as the noted reduction in dung use. Considering the effects of dietary supplementation on the proportion of major males, and possibly *Onthophagus* spp. male mating behaviors, it would be of interest to evaluate how dietary supplementation impacts future generations of *O.*

nuchicornis. Although the results from this study support the use of brown seaweed supplementation as a viable strategy to help reduce emissions in the livestock sector, they also indicate the need for further research into the long-term effects on coprophagous insect communities.

Additionally, this project found that procedural decisions (e.g. dung mass and shape) can influence beetle-mediated GHG emissions, and therefore our understanding of GHG dynamics in agricultural systems. The observations from this study highlight the need for greater research exploring the effects of experimental practices on beetle-mediated GHG emissions. Additionally, the results highlight the importance of carefully reporting on experimental practices to help facilitate the interpretation of study outcomes and the comparison of findings across studies. Overall, the results from this research improve our understanding of the downstream effects of supplementing beef cattle diets with brown seaweed, while also revealing how experimental practices, such as dung homogenization, shape, and mass, can complicate cross-study comparisons and influence interpretations of beetle-mediated emissions.

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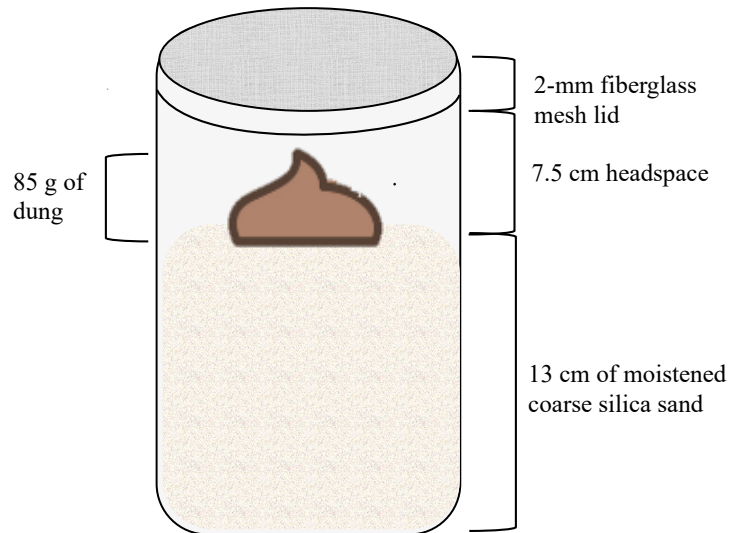
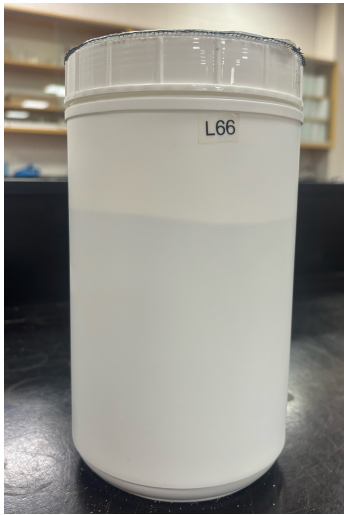
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Appendix



Appendix A. Experimental mesocosm set-up of the 2.5 L cylindrical high-density polyethylene (HDPE) containers, filled to a depth of 13 cm with 2.5 kg of coarse silica sand moistened with 500 mL of water, resulting in a 7.5 cm headspace. At the start of the experiment, 85 grams of dung was added to the mesocosm, which was then sealed with a 2-mm fiberglass mesh lid.